

**Byproducts from Agriculture and  
Fisheries**

# Byproducts from Agriculture and Fisheries

Adding Value for Food, Feed, Pharma, and Fuels

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## Preface

Several countries and scientists continue to grapple with what to do with the byproducts generated from agriculture and fisheries production. Byproducts constitute an enormous amount of opportunities that have been under-exploited. In recent years, several innovative approaches have been developed to convert these by-products into value-added products for food, feed, pharmaceutical, and fuel applications.

This book is organized into three parts. The first part is related to animal byproducts and their application in microbial enzyme production, bioenergy, feed, and bioactive peptides. In the second part, recent developments in the conversion of plant-based byproducts into biofuels and enzymes are illustrated, alongside explanations of their use as biopesticides, biofertilizers, contaminant remediation agents, and other products.

The last and concluding part discusses an often-overlooked component in addressing utilization of agriculture and fisheries byproducts: constraints. In addition to the question of commercial value, several constraints may hinder the commercial processing of these byproducts to value-added products. These include policies and economic, transportational, technical, and managerial limitations.

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# 1

## An Introduction to Agricultural and Fishery Wastes

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### 1.1 Introduction

The term agricultural and fishery waste is broadly used to describe waste material generated from various farming and fishery operations. These activities include livestock cultivation, dairy farming, poultry egg production, wild fish harvest and aquaculture, as well as fruits and vegetable farming. Some of the produce derived from the activities listed above and their transformation into shelf stable forms co-produce byproducts that are considered either unsaleable or unfit for human consumption. The waste is further classified as either natural (organic) and non-natural types (Ashworth and Azevedo 2009). The natural waste includes agricultural and food industry refuse (manure and wastewater from farms, slaughterhouses, and fish processing plants), and residues derived from the growing and processing of raw agricultural and fishery products (such as livestock, fish, dairy products, eggs, fruits, and vegetables). The non-natural types include plastic packaging waste, fertilizer run off and other agrochemicals such as pesticides discharged into the environment, discarded pesticide containers, farm vehicle and fishing fleet parts such as tires and batteries, and others like oil residue and oil containers, old machinery, and much more.

Agricultural and fishery wastes occur in several forms – as solids, liquids, or slurries; and they constitute a significant proportion of the global agricultural and fishery harvest. These abundant wastes produced by the agricultural and fishery sectors have been variously estimated at over 30% of the global agricultural and fishery productivity. They are invariably high in moisture and nutrient contents, which make them highly perishable and prone to degradation by microorganisms and endogenous enzymes. Thus, they pose high environmental pollution potential, if not transformed into stable, value-added forms. Apart from their adverse environmental effects, agricultural and fishery waste also pose specific biohazard threats in the form of animal borne diseases, waterborne diseases, and pathogens (such as bacteria, fungi, molds, and viruses). For example, waste such as

animal manure can expose farm workers to farm dusts to cause them respiratory problems such as occupational asthma. Exposure to farm pesticides and other agro-chemicals can elicit adverse health effects such as eye irritations; disorders of the central nervous system like memory loss, restlessness, and convulsions; as well as chronic diseases like cancers, reproductive and various developmental disorders (Magauzi et al. 2011). Nonetheless, agricultural and fishery wastes are rich sources of useful nutrients and other ingredients that can be recovered and put to profitable use. Thus, agricultural and fishery wastes can have both beneficial and detrimental effects, and sections of this book will address the pros and cons of these waste in today's environment.

Currently, the abundantly produced waste materials are mostly used as animal feed, biogas, cosmetics, fertilizer, and pigments (Jayathilakan et al. 2012). Waste materials associated with various industrial sectors are discussed in some detail in the following sections. In the past, most of the waste was used as fertilizer, feed, dumped in landfills and water bodies, or burnt outright. However, due to increasing food production and current stringent environmental protection standards, the previous ways of managing agriculture and fishery waste are no longer suitable for modern society. In this book, we shall discuss different livestock, fishery and plant byproducts, and some current waste utilization approaches. It is noted here that this chapter is introductory only, and presents a general overview of waste generated by the agricultural and fishery sectors, and some of the ways in which they are utilized.

## 1.2 Livestock Waste

Livestock may be defined in narrow or broad terms. It may be described narrowly to include domesticated animals such as pigs, cattle, goats, sheep, donkeys, horses, llamas, camels, and poultry (e.g. chicken, turkeys, ducks, geese, guinea fowl, squabs); or more broadly to include captive wild animals like deer, elk, monkey, moose, rabbit, buffalo, etc. Either way, livestock mammals produce meat and milk, while poultry produce meat and eggs as food for human consumption. Other commodities derived from livestock rearing include feather, fur, leather, and wool. Some of these animals also provide labor, e.g. the buffalo, camel, donkey, and horse; while others also serve as pets (e.g. birds, cats, dogs, guinea pigs, and horses).

Livestock production for meat, milk, and eggs involves two main phases; viz., raising the animals, and slaughtering the animals. During the raising phase, animals grow to a certain desired body weight in preparation for use as human food; and during the second phase, the animal is killed, cleaned, and cuts into sections according to consumers' needs. As is to be expected, animal manure forms a major agricultural waste when the animal is alive, while body tissues and body parts, as well as wastewater from processing operations form the major agricultural waste of the dead animal. This section deals with the most common livestock byproducts and wastes. Postharvest discards from the slaughterhouse include components such as blood, fatty tissue, hide, viscera, heads, feet, tail, and certain internal organs (e.g. brain, gizzard, heart, and kidney), manure, and wastewater. It is noted here that in some cultures, some of the slaughterhouse discards listed above are coveted, high-priced delicacies, e.g. the blood, brain, heart, stomach, intestine, tongue, kidney, and tail.

### 1.2.1 Manure

Most livestock such as cattle, pigs, poultry, sheep, goats, horses, rabbits, chicken, and turkeys, etc. are raised by large confined animal feeding operations for their meat, milk, or eggs. In the process, these animals, produce a considerable amount of animal manure, also known as animal dung. Manure is a mixture of untreated feces and urine, as well as waste feed, hair and soil. Manures from different animals have different qualities. For instance, sheep and chicken manure have high N and K contents; cow manure has high N and C contents; but pig manure is low in both N and K. Horses are primarily herbivores and cannot digest seeds; thus, horse manure can contain grass and weed seeds (Zhang and Schroder 2014). Thus, the composition of manure varies, and it is influenced by factors such as animal type (i.e. ruminant versus non-ruminant), composition of the feed fed to the animal, nutritional status and age of the animal, and the animals' surroundings.

Manure management entails manure production by the animals, its transfer from the sites of production to collection depots, treatments (by physical, biological, and chemical methods) at the depots to reduce their pollution potential, and holding it in storage facilities for subsequent utilization. The various ways in which livestock manure is utilized include as an energy source for the production of biomethane; or by pyrolysis, gasification, or combustion to generate heat or electricity; as bedding material for animals; as animal feed component; as mulch; or as organic matter and fertilizer for crop and grass production. For example, manure is high in nutrients that plants need, such as N, P, K, Ca, Mg, Fe, and Zn (Combs et al. 1998). Thus, using animal manure as fertilizer furnishes soils with organic matter and improves their physicochemical properties to keep plants healthy and green; and also benefits the soil by controlling soil pH, loosening the soil and increasing water and nutrient uptake, and minimizing runoff and leaching of nitrates into the soil (Ogejo 2015).

### 1.2.2 Blood and Plasma

In the live animal, blood serves as the fluid that transports O<sub>2</sub> and nutrients to cells and body tissues, and simultaneously carries waste materials out of these same cells and tissues. It is dark red in color due to the pigments, myoglobin and hemoglobin, whose central atom, Fe<sup>2+</sup>, enables the uptake and transfer of gases, e.g. O<sub>2</sub> and CO<sub>2</sub>. It is estimated that blood makes up 3–4% of the animal's body weight (Jayathilakan et al. 2012). Furthermore, blood from livestock is high in protein and heme iron and is consumed as food in some cultures. For example, it is used in making products such as animal sausages and blood pudding in some European countries; and as blood curds and blood cakes in some Asian countries (Jayathilakan et al. 2012; Ghost 2001). Plasma from the blood is also used as an emulsifier, stabilizer, colorant, as nutritional additive in foods, or as a foaming agent in baked goods (Silva and Silvestre 2003). Blood also contains the broad-spectrum protease inhibitor,  $\alpha_2$ -macroglobulin, that is used to control muscle food texture deterioration caused by endogenous proteases (Ashie and Simpson 1996, 1994; Sareevoravitkul et al. 1996). Blood from livestock is also put to non-food uses such as water and fat binder by virtue of its transglutaminase component (blood factor XIII), as fertilizer, and as animal feed due to its high vitamin and mineral contents (Silva and Silvestre 2003). The plasma from the blood is also used as a tissue culture media, and as an ingredient for blood agar preparation (Kurbanoglu and Kurbanoglu 2004).

### 1.2.3 Skins

It is estimated that the skins or hides of livestock (excluding poultry) account for about 4–11% of the weight of the live animal (Benjakul et al. 2009). Traditionally, the hides or skins were used as a source of leather and related products (clothing, bags, belts, shoes, automobile seats, etc.), as well as gelatin, glue, sausage casings, and cosmetics. Skins or hides intended for such uses must be promptly treated by air-drying or curing with salt to inactivate microorganisms and endogenous enzymes to avoid decomposition of the tissues. The skin is high in the triple-helical structural protein known as collagen. Gelatin is derived from collagen by chemical or enzymatic hydrolysis (Benjakul et al. 2012). Conversion to gelatin involves the initial removal of non-collagenous protein material, followed by heat treatment and hydrolysis of the heat-denatured collagen into gelatin.

Gelatin has several industrial uses. In the food industry, it is used as an emulsifier or as fat binder in meat products. It is also used as stabilizer in ice creams, yogurt, jellies, and cream pies. Gelatin is also used as a coating or compounding agent in tablets, in ointments and cosmetic products and for making glue. Gelatin has film forming properties, thus it is used to produce biodegradable plastic films, and in wound healing for dressing burns, and as skin graft.

### 1.2.4 Bones

It is estimated that bones constitute about 10–20% of animal carcass (Jayathilakan et al. 2012; MPI 2018). Bones have a high protein content (about 11–16%), which is predominantly collagen and chondroitin-rich proteoglycans. The degree of mineralization of collagen in bones is much higher than that of the skin, which accounts for the more rigid structure of bones versus skins. Thus, bones are also used to make gelatin just like skins. In the household, bones are used to make soups to extract the marrow and flavors for human consumption. The insoluble bone material may be used as pet food for dogs and poultry, or are crushed and used as food nutritive fortifier; or incorporated into animal feed as a nutrient source because of their high content of essential amino acids, vitamins (e.g. B<sub>12</sub>), and minerals (e.g. Ca). Bones are also used as fertilizer because of its high phosphorous content, or as fuel in the form of bone-charcoal obtained after dry distillation. Other uses of slaughterhouse bones include as buttons, knife handles, in soap making, and as a component of porcelain.

### 1.2.5 Internal Organs

The internal organs of livestock include the brain, heart, gizzards, kidneys, liver, lungs, pancreas, rumen, abomasum, stomachs, intestines, tongue, testes, udder, the uterus, and spleen. They are high in nutrients, and have distinct flavors and textures, and are considered as delicacies and are highly prized as food in some cultures. The internal organs of livestock are also used as traditional medicine in many African and Asian countries. Some of the glands such as the pancreas, pituitary, parathyroid, thyroid, kidney, corpus luteum, ovary, and others, secrete hormones that help to regulate metabolism and to treat reproductive problems in women (e.g. progesterone and estrogen from pig ovaries). Other organs such as the brain, hypothalamus, and spinal cord are high in cholesterol which may be extracted and used as an emulsifier in cosmetics and for the synthesis

of vitamin D<sub>3</sub> (Ejike and Emmanuel 2009). Heparin is obtained from the liver and the lungs, and is used as an anticoagulant and blood thinner to prevent blood clotting. The pancreas is a source of the hormone, insulin and glucagon. Insulin regulates glucose metabolism, and is used to treat type 1 diabetes. Glucagon on the other hand is used to increase blood sugar levels, and to treat insulin overdoses or low blood sugar. The pancreas also produces a host of digestive enzymes such as the serine proteases (chymotrypsin and trypsin) and lipases, used in the food, textiles and detergent industries; and in wound healing, etc. The intestines of sheep and calves are used to make surgical sutures, or as sausage casings.

### 1.2.6 Livestock Fat

Animal fats are obtained from the slaughter, processing and packaging of cows, pigs, sheep, chicken, and other animals. Depending on the quality, animal fats may be consumed as human food. The animal fat type fit for human consumption is mostly high-quality lard and high-quality tallow, derived usually by wet-rendering from healthy pigs and healthy cattle or sheep, respectively. High quality lard may be used directly in human food; however, the current trend is to bleach and deodorize it prior to use in food. Lard and tallow were used traditionally for deep frying. However, this mode of use is waning, due to public health concerns. Tallow and lard are currently used as an emulsifier in sausages and other emulsified foods, and for making margarine and shortening (Ghotra et al. 2002). Low-quality lard, and inedible tallow and greases, are produced from the animals usually by dry rendering, and are used in animal feeds, as industrial fats, or as source of energy.

### 1.2.7 Feathers

Feathers are by-products or discards of poultry production. Feathers have the structural protein keratin, as their major component. Feathers have a crystalline structure, and are light in weight, stable, and sturdy. They have a large surface area that enables them to absorb more moisture than cellulose or wool. Feathers are used as decorative material in products such as feather fans, masks, costume accessories, ornaments, earrings, fishing flies, and as flowers. Feathers are also used to produce a range of products such as pillow and mattress stuffing, insulation and padding for fabrics, padding and adsorbent for diapers, high protein animal feed (feather meal), and fertilizer.

### 1.2.8 Egg Shells

Eggs are produced by female birds (and other species) to propagate their kind, or to serve as food. Poultry eggs intended for human consumption are the unfertilized eggs laid by mature female birds, i.e. chickens, or to a lesser extent, other species such as duck, goose, quail. Poultry eggs have shells as outermost covering. The shell constitutes about 9.5% of the total egg weight, and is primarily made of calcium carbonate (98.4%) with some magnesium carbonate (0.8%), calcium phosphate (0.8%), and organic matter (0.4%) mainly protein. There are about 7000–17 000 pore canals per egg for exchange of gases. The pore canals, filled with protein fibers, are sealed by a cuticle layer made of keratin, which can prevent microbial contamination. The color of the egg shell ranges from dark brown

to chalky white, depending on the breed, and is due to pigments called ooporphins that are dispersed on the shell surface.

There are two shell membranes beneath the shell, an inner one and a thicker outer one. Together, they form a ~0.015 mm layer. The membranes are made of keratin and their entangled thread structure represents a primary means of protection against microbial contamination.

Egg shells have many uses. For instance, the shells are crushed and purified as mineral-rich powder that is used as nutritional supplement to fortify baked goods, confectioneries, and beverages. Due to its high Ca content, the crushed shells have also been investigated in the formulation of low phosphate diets for individuals suffering from kidney failure. It is also used as fertilizer and for liming to neutralize very acidic pH soils because of its high  $\text{CaCO}_3$  content.

Another, lesser known application of egg shells derives from their abrasive nature when crushed. Spreading crushed shells on plants keeps slugs off the plants. The abrasive nature of crushed eggshells is also exploited as a facial scrub, and for unblocking clogged sinks and drains. The eggshell membrane found beneath the shell is made up of collagen which is used in the biomedical, cosmetics, and several other industries.

## 1.3 Fishery Waste

Fishery harvesting and processing generate large volumes of waste material that are estimated to range from 35% to 80% of the whole animal. The wastes include by-catch and rejects from fish harvesting, and processing waste generated from de-heading, deskinning, deboning, evisceration, washing, trimming, peeling, and filleting operations. The solid waste material includes heads, bones, cartilages, skin, scales, fins and tails, viscera, hepatopancreas, pyloric ceca, shells, carapace, legs and tails (Ghaly et al. 2013). The soluble waste or wastewater is derived from bleeding, defrosting, cleaning, cooking, and canning operations; and has dissolved salts, pigments, and high protein contents. These wastes all contain useful nutrients and constituents such as enzymes, proteins, fat, pigments, chitins, carotenoid pigments, flavorants, anti-freeze proteins, etc., that may be used to sustain the growth of living organisms, or to produce high value-added products (Ramalingam et al. 2014; Simpson 2007, 2000).

### 1.3.1 Fish Viscera

Fish viscera is made up of parts such as the stomachs, intestines, pyloric ceca, pancreas, and hepatopancreas. They are good sources of digestive enzymes such as proteases and peptidases (e.g. pepsins, trypsins, chymotrypsins, carboxypeptidases, cathepsins, elastase, and erepsin), carbohydrases (e.g. pancreatic amylase, lactase, maltase, and sucrase), lipases (e.g. gastric lipase, pancreatic lipase, phospholipase, and sterol esterase), and nucleases (e.g. deoxyribonuclease and ribonuclease) (Poonsin et al. 2018; Oliveira et al. 2017; Aryee et al. 2007; Noriega-Rodríguez et al. 2009).

As abundantly illustrated by Gildberg et al. (2000), digestive enzymes from fishery waste have a plethora of uses. Some of these uses include for the removal of fish skins, skate wings, and fish scales using a mixture of proteases and carbohydrase from fishery

waste; fish proteases from crab hepatopancreas are used to remove connective tissue associated with fish roe in the production of caviar to improve yield; cod trypsin use for the preparation of carotenoproteins from crustacean waste, and the supplementation of fish fermentation (Poonsin et al. 2018; Gildberg et al. 2000; Haard and Simpson 1994). Other uses of fishery waste enzymes are for the production of cheddar cheese and fish sauce, and for improving the quality of feed fed to farmed fish.

### 1.3.2 Other Fishery Wastes

Fish heads have high protein content, but they are mostly dumped. However, they can be used to produce fish meal, fish protein hydrolysates, fish paste, feed supplement, or fertilizer. Fish skins have high fat and high collagen contents, and are used as sources of gelatin for applications in the food, pharmaceutical, and cosmetic industries. Some fish skins are also used to produce leather for making products like shoes, belts, bags, wallets, purses, and others. Fish filleting leaves a significant part of fish meat attached to the bones as scraps that can be recovered and transformed into products such as fish minces, fish burgers, fish sticks, fish dumplings, and fish hydrolysates, or fish sauce that all have a high mineral content. They are added in canned fish, surimi and a variety of other food-stuff such as soups, pizzas, and pasta.

Fishery waste also find use as fertilizer, because they have high protein content that make them excellent sources of nitrogen. The shells of shellfish also add calcium carbonate as mineral supplement to the soil. Fish and shellfish by-catch and fish harvesting rejects may also be transformed into fish meal for animal feed. This is accomplished via homogenization of the waste, then pressing to expel liquid to recover the residue, when is milled and dried to produce a dark brown powder. Fish oil is a by-product of fishmeal production. After the solids are pressed and milled, the liquid homogenate is centrifuged to separate the oil from the aqueous phase. Fish oil is used in paints, and as feedstock for biodiesel production (Aryee et al. 2011). Wastewater from the fishery sector also contains a large amount of solid organic wastes, which can be fermented into biomethane for use as a household heating fuel (Achinas et al. 2017). The large quantities of effluents or wastewater from the fishery industry have significant amounts of N and P, that make them useful as fertilizer.

## 1.4 Fruit and Vegetable Waste

The harvesting and processing of fruits and vegetables generate large quantities of wastes comprised of harvesting rejects, as well as the unconsumed parts from various processing operations. There are several different fruits, nuts and vegetables; thus, the types and volumes of waste from the different commodities differ. For example, apple processing into juice generates about 11% of seeds and pulp as waste; slicing of papaya produces waste comprised of peels (~8.5%), seeds (~6.5%), pulp waste (~32%); peeling of mandarins co-produces about 16% of peels with the peeled final product; pineapple processing yields about 14% of peels, and about 40% of other waste (e.g. core, pulp, tops) with the finished product (~48%); while processing of mangoes generates about 42% of waste (as peels, seeds, unusable pulp) with the finished product (Sagar et al. 2018). Examples of

fruits produced in commercial quantities include apples, avocado, bananas, cantaloupe, citrus fruits, coconut, coffee, grapes, mangoes, olives, papaya, pears, peaches, pineapples, plums, pomegranate, raspberries, strawberries, and others. Examples of nuts produced in commercial quantities include almonds, Brazil nuts, cashew, chestnut, hazelnuts, macadamia, pecan, pistachio, and walnuts. Examples of vegetables produced in commercial quantities include basil, Brussel sprouts, cabbage, carrots, chives, cucumbers, lettuce, onions, mustard, parsley, peas, potatoes, corn, spinach, Swiss chard, tomato. According to Ajila et al. (2010, 2007) and Schieber et al. (2001), fruits and vegetables commonly generate around 20–30% of waste materials as discards. The processing operations that generate the waste include production of alcoholic and non-alcoholic beverages, canning, production of pasta and vegetable paste and sauces, making of jam and jellies, extraction of vegetable oils, and many more. Some of the waste generated from these commodities includes leaves, peels, pomace, pulp, rinds, roots, skin, seeds, shells, stalks, stems, stones, tubers, and vines (Sagar et al. 2018; Panouille et al. 2007).

#### 1.4.1 Dietary Fiber

Waste materials from fruits and vegetables are rich in dietary fiber, the indigestible portion of plant foods that are considered essential for health. Dietary fiber can enhance bowel movement to prevent or relieve constipation. Because it is not digested or absorbed, it helps in weight management and reduces the risk of non-communicable diseases, such as obesity, diabetes, and cardiovascular diseases. Dietary fiber occurs in fruit waste such as apple and mango peels, apple and red grape pomace, and onions, etc., (Yan and Kerr 2013; Li et al. 2014).

#### 1.4.2 Enzymes

The waste generated from the harvesting and processing of fruits and vegetables are good sources of enzyme. Plant materials have naturally present enzymes such as proteases, amylases, lipases, cellulases, lipoxygenases, peroxidases, and polyphenol oxidases. The proteases break down proteins and long chain polypeptides into low molecular weight peptides and amino acids. Well known plant proteases include the papaya latex enzymes (i.e. papain, chymopapain, and protease III), bromelain (derived from pineapple stem and the pineapple fruit), and ficin (derived from the latex of the fig tree); plant amylases, mainly  $\beta$ -amylases (found in seeds) that function by breaking down polysaccharides into disaccharides; plant lipases (also found in seeds) that act to hydrolyze triglycerides into free fatty acids and glycerol; lipoxygenases (found in seeds like soybeans) that catalyze the addition of O<sub>2</sub> to polyunsaturated fatty acid molecules to produce hydroperoxides; and cellulase (an enzyme complex comprised of exo-1,4- $\beta$ -glucanase, endo-1,4- $\beta$ -d-glucanase, and  $\beta$ -d-glucosidase). Cellulases have been found in a variety of higher plants such as avocado, tomato, pepper, soybean, strawberry, and barley, and they break down glycosidic bonds in fiber (Mojsov 2016; Porta and Rocha-Sosa 2002; Woolley et al. 2001; Ferrarese et al. 1995; Kemmerer and Tucker 1994; Rose and Bennet 1999; Zucker et al. 1985; Hoy et al. 1981).

Fruits and vegetable wastes have also been used as substrates to produce microbial enzymes. For example, banana waste was used to support growth and amylase production by several microorganisms including *Aspergillus niger*, *Aspergillus oryzae*, *Rhizopus*

*oryzae*, and *Bacillus subtilis* (Said et al. 2014). Palm kernel waste was used as substrates for cellulase production by *B. subtilis* and *Bacillus licheniformis* via solid state fermentation (Norsalwani and Norulaini 2012). A combination of bran and citrus hybrid (kinnow) waste, was used to produce cellulase enzymes using *Trichoderma reesei* (Oberoi et al. 2010). Invertase was produced by *Aspergillus niger* and *Aspergillus flavus* using fruit peel, lactose, or sucrose as carbon sources (Mehta and Duhan 2014). Various other fruit and vegetable wastes (e.g. pineapple, orange, and lemon peels) have been used as carbon sources to produce pectinases using *A. niger* and *Penicillium chrysogenum* (Okafor et al. 2010). Other enzymes used in the food industry that have been produced with microorganisms using waste materials fruits and vegetables include proteases, xylanases, and tannases. Many were produced by solid state fermentation using microorganisms *A. niger*, *A. oryzae*, and *Penicillium atramentosum* on waste from tamarind, palm kernel, pomegranate, and leaves from various plants (Varadharajan et al. 2016; Krishna 2005; Kumar et al. 2007). Proteases, xylanases, and tannases are used extensively in food processing to clarify alcoholic and non-alcoholic beverages, and to produce vegetable oils, food thickeners and pigments (Banerjee et al. 2005). Other food industry uses of plant enzymes like the proteases, papain, bromelain, ficin, include their application to tenderize meats. Amylases are widely used in the food industries to produce fruit juices, starch syrup, moist cakes, chocolate cakes, and are used in different processes such as brewing, preparation of digestive aids, and baking (Laufenberg et al. 2009). The enzyme cellulase is used to extract food flavor compounds, natural pigments, essential oils, and polyphenolic compounds from plant food waste such as banana peels, potato peels, and grape pomace (Sagar et al. 2018). Cellulases are also used for the clarification of beverages produced from fruits and vegetables to increase their yields, stability, and visual appeal to consumers. The enzyme invertase is used to convert sucrose to invert sugar, and is widely used in the food industry to produce candies, jam, and confectionery (Panda et al. 2016). Pectinases are applied in the food and beverage industries to facilitate extraction, clarification, and concentration of the finished products. Fruit and vegetable waste enzymes have also been put to non-food uses. For instance, chymopapain has been used to investigate human intervertebral disc degeneration (Wardlaw 2016), and in research studies on rheumatoid arthritis.

### 1.4.3 Polyphenolic Compounds

Other useful biomolecules found in these waste materials include polyphenolic compounds. Examples of these compounds are flavanoids, anthocyanins, stilbenes, lignans, lignins, coumarins, tannins, bioflavonoids, and xanthones (Balasundram et al. 2006), and they occur abundantly in several fruits, nuts and vegetable waste such as the leaves, peels, rinds and seeds. For example, apple leaves contain phenolics like quercetin, chlorogenic acid, catechin, and epicatechin; banana leaves have cyanidin, peonidin, and petunidin; citrus peels and seeds have naringin, hesperidin, and eriocitrin; red beet has ferulic and *p*-coumaric acids; grape seeds have procyanidins; kiwifruit peels have *p*-coumaric acid and caffeoic acid; mango seeds have gallates and gallotannins; carrot pomace has  $\alpha$ - and  $\beta$ -carotenes; garlic has ferulic acid, *p*-coumaric acid and caffeoic acid; tomato skins have lycopene; potatoes have chlorogenic acid, gallic acid, and caffeoic acid; and pomegranate waste contains anthocyanins, ellagitannins, punicalagin, and gallagic acid (Sagar et al. 2018; Balasundram et al. 2006; Friedman 1997; Matharu et al. 2016; Turrini

et al. 2016). Polyphenolics are described as bioactive compounds with a host of biological functions that accrue important health benefits to consumers. Some have antioxidants properties, some have antimicrobial effects, others behave as metal chelators, while some others display enzyme inhibitory effects. Other effects phenolic compounds show include cholesterol lowering, anti-cancer and anti-inflammatory and cardio-protective effects, among others.

#### 1.4.4 Flavor and Aroma Compounds

Flavor and aroma compounds are vital components for applications in several sectors including food and beverages, confectioneries, cosmetics, pharmaceuticals, and health industries. In the food and beverage industry alone, flavor and aroma compounds constitute over a quarter of the global food additives market. Currently, most of the flavoring compounds are produced by extraction from natural raw materials (e.g. plants, animals or microorganisms) or by chemical synthesis. However, recent market trends in response to consumer and regulatory preferences is shifting toward products that can be classified as natural. Nonetheless, natural flavors production by extraction from plants is problematic due to tedious extraction processes, low yields, or possible co-extraction of undesirable components in the raw materials. Thus, production of flavor and aroma compounds is better achieved using microbial biosynthesis or bioconversion. Fruit and vegetable wastes are produced in large quantities, and most of them are rich in nutrients such as carbohydrates, proteins and minerals; thus, they can serve as substrates to support growth and flavor/aroma compounds production by microorganisms. Several microorganisms have been used in extensive studies to produce flavor and aroma compounds using fruit and vegetable wastes as substrates (Longo and Sanromán 2006). Examples of such microorganisms include *A. niger*, *B. subtilis*, *Ceratocystis fimbriata*, *Kluyveromyces marxianus*, *Lactobacillus acidophilus*, *Lactobacillus amylophilus*, *Lactobacillus paracasei*, *Pediococcus pentosaceus*, *Rhizopus oryzae*, and *Zygosaccharomyces rouxii* (Longo and Sanromán 2006; Escamilla-Hurtado et al. 2005; Medeiros et al. 2001; Soares et al. 2000; Christen et al. 2000; Larroche et al. 1999; Bramorski et al. 1998). Some of the wastes that have been used to support the growth and flavor/aroma production by the microorganisms include amaranth waste, apple pomace, cassava bagasse, cocoa husks and cocoa sweatings, coconut fat, coffee husks, garlic skins, licorice root residues, maize cobs, palm bran, rice hulls, soybean hulls and soybean curd residues, sugarcane bagasse, and tobacco waste. The action of the microorganisms on these substrates produced several flavor active compounds such as acetaldehyde, butyric acid, diacetyl, ethanol, ethyl acetate, isobutyl acetate, isoamyl acetate, ethyl-3-hexanoate, lactic acid, and 3-methylbutanol, 6-pentyl-a-pyrone (6-PP), pyrazines (e.g. 2,5-dimethylpyrazine, and tetra methylpyrazine), vanillin and furanones. Some of these compounds were associated with peculiar flavors. For instance, 6-pentyl-a-pyrone (6-PP) is associated with coconut aroma; butyric acid, lactic acid, and diacetyl are associated with dairy aroma; methyl butyrate and geranyl acetate impart fruity aroma; isoamyl acetate elicits banana aroma; methyl butanoate provokes pineapple aroma; vanillin is linked with vanilla flavor; while benzaldehyde contributes to almond flavor. Aroma and flavor ingredients derived from these wastes are used in the food, pharmaceutical, and cosmetic industries (Castilho et al. 2000).

#### 1.4.5 Fruit and Vegetable Wastes as Livestock Feed

Livestock provides the greater bulk of animal food proteins in the form of meat, milk, eggs, and feed. Livestock production requires huge quantities of feed materials to be fed to the animals, and they must be readily available, safe, inexpensive, and replenishable. Fruit, nut, and vegetable production and processing generate huge quantities of wastes throughout the world that is estimated in the million tonnes on a yearly basis (Wadhwa and Bakshi 2013). Most of this waste is dumped in landfills or rivers, causing environmental pollution. However, they are high in nutrients, minerals (e.g. Ca, Co, K, Mn, Mo, Na, P, S, and Zn), and other useful components (e.g. carotenoid pigments, phenolics, etc.), that could be put to profitable use as feed for livestock. Examples of the waste materials that are used as livestock feed include waste from apple harvesting and processing, such as the pomace. Pomace in various forms is included in the diet of cattle and poultry (Ghoreishi et al. 2007). Banana and plantain production produces wastes including rejects, damaged fruits, peels, leaves, stalks, and pseudo stems, that were fed in various forms to livestock. For example, plantain and banana wastes were ensiled with other waste material such as grass, rice bran, molasses, wheat straw, etc., and used as feed for buffaloes, cows, goats, sheep, rabbits, and poultry (Wadhwa and Bakshi 2013; Rohilla and Bujarbarua 2000; Khattab et al. 2000). Tomato harvesting and processing waste include the culls and the pomace. Tomato waste is used in various forms as feed for buffaloes, cattle, goats, pigs, and poultry (Ventura et al. 2009; Bakshi et al. 2014; Sayed and Abdel-Azeem 2009; Sethi 2012). Sugar beet waste were incorporated in feeds for cows, goats, and pigs (Wadhwa and Bakshi 2013). Other fruits and vegetables waste used as livestock feed include cabbage, carrots, cauliflower, chickpeas, citrus fruits, grapes, mangoes, potatoes, radish, and squash. These wastes have been incorporated in various feeds for livestock such as cows, horses, rabbits, sheep, pigs, and poultry and were well accepted by these animals.

### 1.5 Wastewater

The agricultural and fishery sectors are the foremost users of water globally. Water is required for fruits and vegetable farming, livestock rearing, aquaculture, and for growth by all life forms (including insects, worms, bacteria, etc.). This water is derived from rainfall and from freshwater sources such as rivers, lakes, and wells. However, the seasonal nature of rainfall and inclement climatic conditions such as drought, make it necessary to use freshwater sources such as rivers to complement rainfall for irrigation and fish rearing, among others. Fresh water is also needed for handling and processing of agricultural and fishery products. The volume of water required for these activities is tremendous, and puts substantial pressure on freshwater resources. However, these activities also co-produce enormous amount of wastewater that can be treated and recycled to relieve the pressure on freshwater resources. The use of wastewater is associated with both risks and benefits (Toze 2006). The risks with raw wastewater use in agriculture are both chemical and biological in nature. Chemical risks are in the form of heavy metals (e.g. As, Be, Cd, Hg, and Pb), hydrocarbons (e.g. pyrenes, phenanthrene, dioxins, and furans), polychlorinated biphenyls (PCBs) (e.g. arochlor, phenoclor, and clophen), and synthetic pesticides (e.g. insecticides, herbicides, and fungicides such as acephate, propoxur, metaldehyde, diazinon, and malathion). The biological hazards with wastewater use

include various bacteria (e.g. *Escherichia coli*, *Shigella*, *Salmonella*, *Vibrio* and *Campylobacter*, *Helicobacter*, *Arcobacter*, *Staphylococcus aureus*, *Clostridium perfringens*, and *Mycobacterium ulcerans*); viruses (e.g. *Adenoviruses*, *Caliciviruses*, *Noroviruses*, *Reoviruses*, and *Rotavirus*); protozoans (e.g. *Cryptosporidium parvum*, *Giardia intestinalis*, and *Entamoeba histolytica*), helminths (e.g. *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Trichuris trichiura*, *Strongloides stercoralis*, and *Taenia saginata*); and *Schistosoma spp.* (a.k.a., blood-flukes). Biological hazards can have catastrophic consequences in the form of waterborne diseases (such as diarrhea, cholera, gastroenteritis), respiratory tract infections, conjunctivitis and typhoid (Mara et al. 2007). Other illnesses include salmonellosis, dysentery, and Legionnaire's disease (*Legionella pneumophila*). The benefits with wastewater use are as follows: proper treatment of wastewater and its reuse alleviates pressure on freshwater resources considerably; reduces the cost of energy for extracting or pumping groundwater resources for irrigation; and returns naturally present macronutrients (Na, K, and P) and micronutrients (Ca, Mg, B, Mg, Fe, Mn, or Zn) back to the soil to reduce eutrophication of water bodies, and save on fertilizer costs (Barreto et al. 2013; Liu and Haynes 2011; Matheyarasu et al. 2016).

## 1.6 Conclusions

Agricultural and fishery wastes are abundantly available resource materials. They are biodegradable, renewable, inexpensive, and rich in useful nutrients and various other ingredients. The amount of agricultural fishery waste is increasing steadily worldwide in tandem with the increasing production of livestock, fish and shellfish, fruits and vegetables, to alleviate hunger and meet the global food security needs of an increasing global population. The disposal of these wastes by traditional methods poses human safety and serious environmental health problems. Part of the solution to alleviating world hunger would be more efficient transformation of the abundant waste generated from agricultural and fishery harvesting/processing into high value-added byproducts of commercial relevance; instead of producing more foodstuff to generate more waste.

Currently, there is greater awareness of the need to better utilize this abundant waste profitably, and efforts are ongoing world to translate this awareness of the problem into practical reality. Thus, in addition to their traditional use as fertilizer and animal feed, the waste is currently being used as sources of enzymes for industrial biotechnology; for bioenergy such as biodiesel, bioethanol, biomethane, or via other processes to produce heat or electricity. They are also being used as source material for the recovery of various ingredients like bioactive compounds, emulsifiers, stabilizers, and binders, for the food, cosmetic, biomedical and pharmaceutical industries. Others include their use as components of microbial media to support growth of microorganisms to produce various bioingredients.

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## Part I

### On Animal Byproducts

## 2

### Pork Byproducts

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### Abbreviations

EAA	essential amino acids
FAME	fatty acid methyl esters
MUFA	monounsaturated fatty acids
PUFA	polyunsaturated fatty acids
SFA	saturated fatty acids

### 2.1 Introduction

Offal, or edible and inedible animal byproducts, are generally accepted as carcasses, hides and skins, bones, fat and meat trimmings, blood, horns, feet and internal organs (Di Bernardini et al. 2011; Toldrá et al. 2012). Due to strict hygiene considerations and safety concerns, research on animal byproducts suitability regarding nutrients value, cost-effectiveness, environmental considerations and potential risks to consumers' health is ongoing. However, there is a current trend for the effective utilization of animal byproducts in the economic growth of meat industry, including pig industry (Helkar et al. 2016). In fact, millions of tons of slaughterhouse byproducts are produced on an annual basis as an integral part of the meat production chain. These materials are then disposed of or processed and re-used in several different sectors. Solid or liquid byproducts can be found in wastewaters that require treatment because of their high chemical or biological organic demands (Bull et al. 1982; Ursu et al. 2016). In the solid form, such as carcass and skins, byproducts could find valorization as energy vectors after digestion (Hejnfeld and Angelidaki 2009), or can be hydrolyzed by chemicals (Selmane et al. 2008) and enzymes

(Rafieian et al. 2015) into functional proteins. In addition, proteins from red blood cells (Gomez-Juarez et al. 1999) or plasma (Penteado et al. 1979) were found to have suitable functional properties. Even though pork byproducts represent a significant disposal problem for the pig industry, they are also a promising source of bioactive compounds that can be used for their technological or nutritional properties. Depending on the countries, cultures and preferences, edible pork byproducts can be considered as waste material or as delicacies imposing high prices (Nollet and Toldrá 2011). With deeper exploration and development, more animal products of low commercial value could be further transformed into high added-value ingredients, emphasizing a broad of new or improved applications with potential interest in pharmaceutical, cosmetic, and food industries.

## 2.2 Production and Characterization of Pork Byproducts

Pig production is generally the main animal production activity in the different regions of Europe (Aarnink and Verstegen 2007). In addition, pig production represents an important segment of the meat industry throughout the world. Animal's slaughtered for meat products may be divided into four categories: (i) pork (high-value end product); (ii) inedible components that can be used for industrial purposes (e.g. hides, bones, hooves, and blood); (iii) offal and meat meal (low-value components); and (iv) items of no useful purpose (e.g. wool slip, digestive tract content, effluents) that are disposed of as waste (Fallows and Verner Wheelock 1982). The main concern of pig production is the amount of surplus nutrients in excreta and gaseous losses to the environment. Main nutrients of concern are nitrogen, phosphorus, and heavy metals while main gaseous losses of concern are ammonia and methane. Although losses are inevitable to a certain extent, nutrition seems to be a key factor in reducing these losses. Over the last years ago, the meat industry only collected a few products from butchered animals after inspection in the abattoir. Today, this has changed with most of the meat processors increasing the number of animal derived-products available.

The world production of pork edible byproducts in 2004 was 625 million tons, most of it from Asia (50.4%). Europe was the second largest producer with 37.1% of total (Jayathilakan et al. 2012). The dressed carcass or the meat and the skeleton of an animal makes up around two-thirds of the live weight of pig. The remainder of the live weight of an animal can be divided into different product groups known as byproducts or the fifth quarter products. These byproducts derived from slaughter and processing of pigs have been estimated to account for 48–52% of the total live weight (Marti et al. 2011; Jayathilakan et al. 2012; Vernooij 2012). The yield of edible pork byproducts may represent around 6.7% of the pig carcasses weight. The most relevant pork byproducts include blood, bones, hides, skin, lard, feet (pig's trotters), internal organs, and chitterlings (pig's small intestine).

### 2.2.1 Blood

Blood is the first animal edible byproduct obtained after the slaughter of pig, and can represent up to 4% of the live weight of the animal (Bah et al. 2016) or 6–7% of the lean meat content of the carcass (Wismer-Pedersen 1988). Blood is a red fluid, which is made

up of water, cells, enzymes, proteins, and other organic and inorganic substances that can be divided into two fractions, the cellular (rich in red blood cells or erythrocytes, white blood cells or leucocytes and platelets) and plasma fraction (Ofori and Hsieh 2011). Red blood cells have hemoglobin that explained more than 50% of the total proteins present in blood (Liu et al. 1996). It is well known that blood have also high iron content that is useful to combat iron deficiency anemia with some food strategies (Kikafunda and Sserumaga 2005). The plasma (which comprises up to 60%), in turn, contains 6–8% proteins, as albumin, fibrinogen, and globulins. The high nutritional value of blood plasma has been appreciated to introduce this byproduct into a wide range of food products as a highly concentrated and healthy source of proteins (around 15–18% in wet base) with a balanced content of amino acids, particularly in essential amino acids (EAAs). More recently, the recovery and extraction of bioactive molecules from blood represents an important challenge to add economic value and permit new applications for slaughterhouse blood (Bah et al. 2013).

### 2.2.2 Bones, Hides, and Skins

These parts of the animal that are firstly considered to be unfit for human consumption when produced at the slaughterhouse may, after processing, become suitable for human consumption. Bones, connective tissue, skin, and hides, which represent 3–8% of the weight live pig, can be processed to make gelatin and collagen. Collagen is the most abundant protein in mammals with substantial single bond  $-\text{NH}_2$  and single bond  $-\text{COOH}$  groups on its chains. This water-insoluble fibrous protein is the raw material for gelatin production. Gelatin is mainly extracted from skin and bones, but other offal such as lung, tongue, trachea, large blood vessels, or tendons are also sources of collagen (Mullen et al. 2017). Gelatin can be produced by the controlled hydrolysis of collagen and it is used in a wide range of applications.

Eleven percent of pork carcasses are bones, but these values are higher if they include the meat clinging to the bone. Pork made by mechanical deboning produces tissue that is called “mechanically separated,” “mechanically deboned” or “mechanically removed.” Currently, this meat is approved for use in meat products (mixed or used alone) in some countries (Field 1981).

### 2.2.3 Lard

Lard is the major animal fat rendered from fatty tissue of healthy pigs. Lard can be obtained by dry or wet rendering. In the wet rendering process, the fatty tissues are heated in the presence of water, generally at a low temperature (below 49 °C) in order to separate the fat from the protein fraction. The lard obtained from the wet process shows a better quality compared to lard from dry rendering (Jayathilakan et al. 2012). Lard as edible animal fat contributes frequently to energy intake.

### 2.2.4 Feet

The feet of pigs, also known as pig's trotters, are other parts that, after further processing, are suitable for human consumption. These cuts are used in various traditional dishes in many Asian and European cuisines.

### 2.2.5 Internal Organs

Pig offal such as liver, kidney, tongue, heart, and others internal organs have a good nutritional value due to high levels of protein and low fat contents, as well as are good sources of minerals, fatty acids, fiber, essential vitamins, and bioactive compounds (Honikel 2011). For instance, liver constitutes a rich source of fatty acids, iron, zinc, and vitamins B<sub>12</sub> (cobalamin) and A. The liver of a pig weighs on average 1.4 kg and is mostly finely sliced and cooked. Yet, liver is widely minced and incorporated in numerous preparations due to the richness of liver in protein, fat, iron, copper, and vitamins that are important for normal development in humans (Alao et al. 2017). Heart averages 227 g and it is composed of large amounts of iron, selenium, zinc, phosphorus, and vitamins B<sub>3</sub> (niacin) and B<sub>2</sub> (riboflavin). Heart must also be cooked. The tongue of pork weight around 0.3 kg and it is also a good source of protein and vitamin B<sub>12</sub>. Typically, the tongue is boiled, sliced, and marinated after the outer membrane is removed. Pork kidney weighs approximately 110 g and is also widely enjoyed and commonly consumed. The kidney of pork is a significant source of protein and vitamins B<sub>2</sub> and B<sub>3</sub> (Martí et al. 2011).

### 2.2.6 Chitterlings

The dish known as chitterlings is the intestines and rectum of a pig that have to be carefully prepared and cooked due to the possibility of bacterial diseases. Pig intestines are very popular in most pork-eating cultures. Chitterlings are also used as casing for sausages in many parts of Europe.

## 2.3 Nutritional Composition of the Main Edible Pork Byproducts

Pork accounts for about 40% of total meat consumption and ranks first among all meat sources. From a nutritional standpoint, pork is considerably leaner and it is an excellent source of important nutrients. Due to the higher consumer preferences and demand for pork, which is expected to continue to expand (FAO 2009), the amount of pork edible byproducts has also increased. Pig offal also presents high nutritional value (EAAs, fatty acids, minerals, and vitamins) and functional compounds such as bioactive peptides and antioxidants (Aristoy and Toldrá 2011; García-Llatas et al. 2011; Honikel 2011; Kim 2011; Toldrá et al. 2012; Lafarga and Hayes 2014). However, the nutritional composition and the consumption of edible pork byproducts vary worldwide and depend on each particular type of byproduct (Ockerman and Basu 2004; Honikel 2011). For example, pork byproducts like blood, liver, lung, heart, kidney, feet (pig's trotters), and chitterlings (pig's small intestine) are more popular and constitute part of the diet and culinary recipes in countries such as South Africa, Egypt, Italia, Spain, and Asia (Nollet and Toldrá 2011). The consumption of such products is restricted, probably, due to the limited scientific information about the nutritional composition of these edible pork byproducts.

### 2.3.1 Proximate Composition

The energy value and major components of 11 selected pork byproducts are displayed in Table 2.1. The data presents either the range (minimum to maximum) or the mean value of the main constituents. In terms of calories arising from pork byproducts, most of them are rather lean with less than 200 kcal/100 g. Honikel (2011) also reported that lean meat cuts (<2% fat) deliver about 100 kcal/100 g. Small intestine and pancreas present higher energy values that could be attributed to the high fat level in these pork byproducts.

Regarding major constituents in the different pork byproducts, moisture content varies between 75.9% and 82.5%. Small intestine and blood have the highest moisture content (82.5% and 82.1%, respectively), while lung, spleen, stomach, and heart show intermediate values (78.7%, 78.6%, 77.6% and 75.9%, respectively), whereas pancreas and liver contain the lower moisture contents (72.2% and 71.6%, respectively). Total protein in pork byproducts ranged from 10.0% to 28.5%. Brain, heart, kidney, liver, and pancreas show the highest protein contents, whereas the small intestine presents the lowest protein levels. The fat content in offal from pork amounts to about 20.8–27.4%. The small intestine has the highest fat content (23.5%), followed by the pancreas (4.0–15.0%), tongue (13.0%), and stomach (9.5%). In turn, blood, heart, kidney, liver, lung, and spleen are low in fat and comparable to lean meat. Greenfield and Southgate (2003) reported that the major sources of variation in animal products are the proportion of lean tissue to fat, and the proportion of edible to inedible byproducts. Variations in the lean : fat ratio influence the levels of most other nutrients. Although fat intake is essential for human health, because it contributes to energy intake and helps vitamin absorption, a high daily fat intake has been associated with chronic diseases, such as obesity and cardiovascular diseases (Bray et al. 2004). On the other hand, carbohydrates are only present in a few pork byproducts in accountable quantities. The ash content is high in blood, liver, and pancreas (from 1.26% to 1.90%) but relatively low in stomach and small intestine (0.31% and 0.30%, respectively). Thus, facing the nutritional guidelines, that recommend a daily allowance of 2500 kcal, 60 g of protein and 90 g of dietary fat for an adult, a 100 g serving of pig liver would supply 2.6–5.6% of total energy, 32–37% of protein, and 2.7–7.6% of fat.

### 2.3.2 Amino Acid Composition

The relative percentage of essential and non-EAAs in usually consumed pork offal is presented in Table 2.2. The amount of both amino acid classes shows a large variation across the examined pork byproducts. Particularly, brain, liver, and tongue contain the highest levels of the major EAAs, such as histidine, leucine, phenylalanine, threonine, and valine. In addition, small intestine and stomach have the lowest contents of EAA. A similar pattern of amino acid variation can be observed in heart and lung. Moreover, heart along with liver has the highest methionine levels. It is well known that lysine, methionine, and tryptophan are the most limiting amino acids in poor-quality protein sources (Friedman 1996). Pork offal, in general, is a worthy source of essential and limiting amino acids similar to that of muscle proteins (Aristoy and Toldrá 2011).

Concerning non-EAAs, liver has also the highest amounts of these amino acids followed by pancreas and spleen. As reported previously by Seong et al. (2014), the

**Table 2.1** Proximate composition in edible pork byproducts.

	Blood	Brain	Heart	Kidney	Liver	Lung	Pancreas	Small intestine	Spleen	Stomach	Tongue
Energy (kcal/100 g)	70.0	125	115–121	90.0	65.2–140	85.0–98.5	190–199	250	60.5–105	150	180
Moisture (g/100 g)	82.1	—	75.9	—	71.6	78.7	72.2	82.5	78.6	77.6	—
Protein (g/100 g)	17.0–18.5	10.3–22.0	16.8–23.5	15.4–25.4	18.9–22.0	15.0	18.5–28.5	10.0	17.9	16.5	16.0
Fat (g/100 g)	0.4	8.6–9.2	2.7–5.0	2.7–3.6	2.4–6.8	1.8–2.5	4.0–15.0	23.5	0.97–2.5	9.5	13.0
Carbohydrates (g/100 g)	0.1	—	0.5	1.0	3	—	0.5	<0.5	0	<0.5	0.5
Ash (g/100 g)	1.90	—	0.81	—	1.34	0.76	1.26	0.30	1.12	0.31	—

Source: Anderson (1988); Honikel (2011); Mullen et al. (2017); Nollet and Toldrá (2011); Ockerman and Basu (2004); Seong et al. (2014); Souci et al. (2000); USDA (2009); Venegas Fomias (1996).

**Table 2.2** Amino acid composition in edible pork byproducts.

	Blood <sup>a</sup>	Brain	Heart	Kidney	Liver	Lung	Pancreas	Small intestine	Spleen	Stomach	Tongue
<i>Essential amino acids (%)</i>											
Histidine	0.05–0.08	2.7	0.72	2.4	1.1–2.7	0.79	1.1	0.43	0.79	0.66	2.5
Isoleucine	0.09–0.12	4.6	0.76	5.3	1.0–5.1	0.61	0.95	0.46	0.75	0.71	4.6
Leucine	0.12–0.18	8.7	0.71	9.0	1.1–8.9	0.72	1.1	0.50	0.80	0.80	8.0
Lysine	0.15–0.18	7.8–7.9	1.1–8.3	7.2	1.3–7.7	1.2–7.3	1.1	0.67	1.1–7.5	1.1	8.2
Methionine	0.01–0.02	1.9–2.0	0.21–2.6	2.1	0.36–2.5	0.20–1.6	0.42	0.15	0.23–1.8	0.21	2.2
Phenylalanine	0.05–0.08	5.1	0.94	4.7	1.3–4.9	1.6	1.2	1.0	1.1	1.6	4.1
Threonine	0.09–0.15	4.7	0.39	4.1	0.45–4.3	0.22	0.32	0.21	0.32	0.33	4.2
Tryptophan	0.02–0.03	1.3–4.2	1.2	1.3	1.4	0.90	—	—	1.0	—	1.2
Valine	0.19–0.26	5.7	1.5	5.8	2.0–6.2	1.3	2.1	0.88	1.5	1.4	5.2
<i>Non-essential amino acids (%)</i>											
Alanine	0.19–0.29	—	0.74	—	1.1	0.73	0.97	0.52	0.80	0.65	—
Aspartic acid	0.006–0.01	—	1.4	—	1.7	1.1	1.4	0.89	1.4	1.2	—
Arginine	0.14–0.19	—	1.0	—	1.2	0.91	1.1	0.78	1.0	1.0	—
Cysteine	—	—	0.47	—	0.64	0.47	0.53	0.32	0.53	0.37	—
Glutamic acid	0.06–0.08	—	1.5	—	2.1	1.3	1.5	0.81	1.4	1.2	—
Glycine	0.31–0.57	—	0.55	—	0.77	0.48	0.83	0.35	0.57	0.53	—
Proline	—	—	0.78	—	1.1	1.1	0.94	0.69	0.82	1.1	—
Serine	0.08–0.11	—	0.61	—	0.87	0.40	0.81	0.33	0.53	0.51	—
Tyrosine	0.06–0.09	4.2	2.5	3.6	2.9–3.4	2.0	2.3	1.5	2.4	2.3	—

Concentrations in mmol l<sup>-1</sup>.

Source: Aristoy and Toldrá (2011); Cai et al. (1995); Seong et al. (2014); Venegas Fomias (1996).

differences in the types and levels of amino acids may be ascribed to the differences in protein types (e.g. collagen, myofibril protein, etc.) among pork byproducts.

### 2.3.3 Cholesterol Content and Fatty Acid Profile

The cholesterol content, expressed as mg/100 g tissue, on a wet basis, of edible pork byproducts is shown in Table 2.3. Cholesterol levels, as expected, are highest in brain (2000–3100 mg/100 g) compared to the remaining byproducts. Cholesterol is a major structural lipid component of brain cell membranes, accounting for about 20% of the whole body's cholesterol, and plays a central role in the compartmentalization of the plasma membrane and signaling. Cholesterol has been recognized to be utmost important for synaptic transmission, and a link between cholesterol metabolism defects and neurodegenerative disorders, like Alzheimer's disease, Huntington's disease, Parkinson's disease and some cognitive deficits typical of the old age, is well established (Zhang and Liu 2015; Petrov et al. 2016). In addition, cholesterol as a key factor for the functionality of cellular membranes is more dependent on size of cells than on fat content. As shown in Table 2.3, all pork byproducts contain higher cholesterol contents in comparison to pork (around 40–60 mg/100 g) (Reig et al. 2013; Parunovic et al. 2015). The content of cholesterol in the internal organs is higher than in pork since most of the cholesterol is synthesized in liver and kidney as well as in the structural part of brain. Kidney, liver, spleen, and lung present intermediate cholesterol values, ranged from 300 to 405 mg/100 g, while blood shows the lowest cholesterol levels (40 mg/100 g wet weight). According to the nutritional guidelines, the recommended maximum cholesterol intake should be less than 300 mg per day (American Heart Association 2008).

The fatty acid composition of edible pork byproducts is also summarized in Table 2.3. The most representative fatty acids in the selected offal are palmitic (16 : 0) and stearic (18 : 0) acids as saturated fatty acids (SFAs), oleic acid (18 : 1c9), as monounsaturated fatty acids (MUFAs), and linoleic (18 : 2n-6) and arachidonic (20 : 4n-6) acids, as polyunsaturated fatty acids (PUFAs). Similar fatty acid profile in some of these pork byproducts was reported by Seong et al. (2014) and Prates et al. (2011). Besides the order of appearance of major fatty acids in the selected pork byproducts is quite different, the 18 : 1c9 represents 12–46% of total fatty acid methyl ester (FAME), 18 : 2n-6 accounts for 0.6–39% of FAME, the saturated 16 : 0 and 18 : 0 represents 14–34%, and 3.8–27%, respectively, followed by 20 : 4n-6 with 1.1–20%. Brain and tongue have the highest percentages of 18 : 1c9, whereas liver and kidney show the highest percentages of 16 : 0 and 18 : 0. Concerning PUFA, brain displays a distinct fatty acid profile when compared to the remaining organs. The major PUFA in brain is 20 : 4n-6 (9.1–11.0%), followed by docosahexaenoic acid (DHA, 22 : 6n-3) (6.6–8.7%). In contrast, all other pork byproducts reveal higher relative amounts of 18 : 2n-6 and 20 : 4n-6. The percentages of 18 : 2n-6 range from 7.2% in kidney to 39% in blood, whereas the relative amounts of 20 : 4n-6 vary from 1.1% in tongue to 20% in heart and spleen. High amounts of docosapentaenoic acid (22 : 5n-3) is also shown in brain and heart (0.26–4.3% and 1.1–1.9%, respectively). The proportions of n-3 PUFA are generally very low in pork byproducts, except in brain. In agreement, Cordain et al. (2002) stated that brain has the highest proportions of DHA relative to other organ meats. It is well established that tissue fatty acid profile depends on the fat level based on the ratio of triacylglycerols and phospholipids (Wood et al. 2008).

**Table 2.3** Cholesterol content and fatty acid composition in edible pork byproducts.

	Blood	Brain	Heart	Kidney	Liver	Lung	Pancreas	Small intestine	Spleen	Stomach	Tongue
Cholesterol (mg/100 g, wet basis)	40	2000–3100	131–158	319–405	300–368	314–320	193–195	160	360	190	100–116
<i>Fatty acid composition (% FA)</i>											
14 : 0	0.50–0.90	0.35–0.77	0.23–2.4	0.49–1.7	0.48–1.7	1.8	0.94–1.4	1.3	0.9	1.4	1.1–2.0
16 : 0	14–20	18–20	15–23	22–29	16–27	34	21–30	27	28	26	24–27
16 : 1	2.6–3.1	0.82–2.3	0.15–3.0	0.47–3.8	0.39–2.8	0.91	0.31–6.8	0.90	0.53	1.5	0.32–4.0
17 : 0	—	0.26–0.31	0.19–0.54	0.30–0.67	0.37–0.72	—	0.30–0.65	—	—	—	0.33–0.55
17 : 1c9	—	0.12–0.15	0.12–0.22	0.06–0.32	0.18–0.59	—	0.15–0.38	—	—	—	0.33–0.54
18 : 0	3.8–4.6	19–23	12–16	14–17	15–27	14	15–24	17	20	15	11–13
18 : 1c9	29–32	21–28	12–29	17–41	15–34	27	24–33	27	18	36	39–46
18 : 2n-6	32–39	0.60–1.7	21–35	7.2–17	12–17	15	8.6–20	18	13	14	7.9–11
18 : 3n-3	1.2–2.2	0.02–2.3	0.37–2.4	0.17–0.42	0.32–1.2	0.09	0.36–0.77	0.08	0.21	0.07	0.33–0.50
20 : 0	—	0.23–0.31	0.04–0.11	0.15–0.22	0.02–0.05	—	0.19–0.35	—	—	—	0.11–0.20
20 : 1n-9	0.12–0.20	1.2–1.8	0.21–0.56	0.44–0.84	0.19–0.35	0.45	0.46–0.75	0.32	0.30	0.65	1.3–1.6
20 : 2n-6	—	0.10–0.14	0.59–0.89	0.69–0.92	0.23–0.45	—	0.25–0.35	—	—	—	0.53–0.64
20 : 4n-6	4.9–6.2	9.1–11	7.6–20	3.4–19	3.1–17	6.6	1.8–4.9	8.7	20	3.6	1.1–2.2
20 : 5n-3	0.51–0.95	0.07–0.10	0.20–0.49	0.17–0.62	0.07–0.62	0.16	0.07–0.20	0.00	0.75	n.d.	0.03–0.06
22 : 4n-6	0.09–0.15	4.2–5.3	0.88–1.3	0.87–1.8	0.30–1.4	0.54	0.13–0.31	—	—	—	0.36–0.65
22 : 5n-6	—	2.2–3.3	0.18–0.32	0.06–0.15	0.05–0.33	—	0.02–0.05	—	—	—	0.03–0.07
22 : 5n-3	0.37–0.49	0.26–4.3	1.1–1.9	0.53–0.97	0.32–2.4	—	0.15–0.38	—	—	—	0.15–0.29
22 : 6n-3	0.21–0.50	6.6–8.7	0.20–0.56	0.27–1.0	0.10–3.47	n.d.	0.04–0.11	n.d.	0.64	0.00	0.05–0.08
<i>Partial sums of fatty acids (% FA)</i>											
SFA	—	40–43	29–40	38–46	41–46	50	39–55	45	49	43	38–42
MUFA	—	28–32	13–31	19–45	16–40	29	27–40	29	21	39	44–51
PUFA	—	26–29	29–57	11–43	17–40	22	12–23	27	30	18	11–15

n.d. – not detected; FA – fatty acids; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.

Source: Kouba et al. (2003); Prates et al. (2011); Seong et al. (2014); USDA National Nutrient Database for Standard Reference (Release 22, 2009).

The partial sums of fatty acids in pork byproducts are also shown in Table 2.3. Total SFA levels ranged from 29% to 55%, total MUFA vary from 13% to 51% and PUFA ranged from 11% to 57% among the reviewed pork byproducts. It is widely acknowledged that lipids from monogastrics animals have a simpler fatty acid composition with higher proportions of unsaturated and lower amounts of SFA and *trans* fatty acids (TFAs) relative to those from ruminant animals. The relative percentage of TFA in pork byproducts, as expected, is only residual. With exception of heart, pork byproducts are relatively saturated, mainly due to the percentages of 16 : 0 and 18 : 0, which suggest a strong contribution of de novo fatty acid synthesis particularly in brain, liver, lung, small intestine, and spleen. Tongue presents the highest levels of MUFA (mainly 18 : 1c9), followed by kidney and stomach, while heart shows the highest percentage of PUFA. These results are in line with those reported by Prates et al. (2011), who observed similar partial sums of fatty acids in pork offal. In contrast, Seong et al. (2014) reported distinctly higher MUFA (in stomach and heart) and PUFA percentages (liver). Hence, most pork byproducts, with a few exceptions are relatively unsaturated, which mean that the sum of MUFA plus PUFA exceed the SFA content.

### 2.3.4 Vitamins Contents

The values of fat-soluble and water-soluble vitamins in pig offal are summarized in Table 2.4. As expected, the vitamin contents vary widely among the different pork byproducts reviewed. In particular, liver has the highest concentrations of vitamins A (20 000–57 407 µg RE/100 g), D, E and K (5.0 µg/100 g, 0.7 mg/100 g and 30 µg/100 g, respectively), but also of vitamin B<sub>9</sub>, also known as folate (110–1000 µg/100 g), vitamin B<sub>12</sub> or cobalamin (25–40 µg/100 g). Honikel (2011) reported similar vitamin A values for bovine liver (21 000 µg RE/100 g) and pig liver (20 000 µg RE/100 g). The levels of vitamin A in liver exceed 100% of the recommended daily allowance (RDA) of vitamin A. Besides liver, vitamin A is also found in higher concentrations in kidney and pancreas. Vitamin K is rarely mentioned with pork byproducts, except in liver. Minor concentrations of vitamin B<sub>1</sub> (thiamin), vitamin B<sub>2</sub> (riboflavin) and vitamin B<sub>6</sub> (pyridoxine) are available in the selected pork byproducts, although their contents are still higher in liver and kidney. Also liver and kidney contain huge amounts of vitamin B<sub>7</sub>, commonly referred as vitamin H or biotin. Pancreas provides the highest concentrations of vitamin B<sub>3</sub> (niacin) and B<sub>5</sub> (pantothenate) in comparison to all other pork byproducts. Vitamin C is also present in almost of these byproducts, especially in liver and spleen, with up to 25–30 times, respectively, the concentrations in meat (<1 mg/100 g). Overall, liver, pancreas, and kidney of pork are excellent sources of B vitamins. Liver, spleen, and pancreas contain also high amounts of vitamin C. The fat-soluble vitamin A is present in most of the pork byproducts in varying amounts, with higher concentrations particularly in liver, compared to pork, while negligible amounts of vitamin D and K are available in some pork offal.

### 2.3.5 Minerals Contents

The macro- and microelements of pork byproducts are shown in Table 2.5. In general, all pork byproducts have a higher content of minerals than muscular tissue or pork (Ockerman and Basu 2004; Tomovic et al. 2015). The concentration of calcium in

**Table 2.4** Vitamins contents in edible pork byproducts.

	Blood	Brain	Heart	Kidney	Liver	Lung	Pancreas	Small intestine	Spleen	Stomach	Tongue
<i>Fat-soluble vitamins</i>											
Vitamin A (µg RE/100 g)	25	—	2.6–5.0	150	20 000–57 407	tr-13	118	tr-45	tr-73	17	9
Vitamin D (µg/100 g)	0.1	—	0.7	1.0	5.0	—	—	—	—	—	0.6
Vitamin E (mg/100 g)	0.4	—	0.2–0.63	0.2	0.7	—	—	0.18	—	0.04	0.29–0.50
Vitamin K (µg/100 g)	—	—	tr	tr	30	—	—	—	—	—	tr
<i>Water-soluble vitamins</i>											
Vitamin B <sub>1</sub> (mg/100 g)	0.1	0.15–0.23	0.13–0.61	0.26–0.58	0.13–0.31	0.08–0.11	0.10–0.11	0.01–0.07	0.10–0.15	0.05–0.12	0.30–0.49
Vitamin B <sub>2</sub> (mg/100 g)	0.1	0.25–0.28	0.42–1.2	1.7–1.9	0.92–3.0	0.23–0.43	0.22–0.46	0.05–0.10	0.09–0.30	0.10–0.20	0.49
Vitamin B <sub>3</sub> (mg/100 g)	3.5	4.3–4.5	6.6–31	7.5–14	15–28	0.49–3.5	3.5–214	0.10–4.2	3.6–6.0	0.39–4.5	8.0
Vitamin B <sub>5</sub> (mg/100 g)	—	2.8–3.0	2.5–3.0	3.0–3.1	0.90–7.0	0.90–3.7	4.6–15	0.22–1.45	0.73–1.0	1.2–1.6	0.64–2.0
Vitamin B <sub>6</sub> (mg/100 g)	0.01	0.19–0.20	n.d.–0.45	0.44–0.60	0.12–0.70	0.02–0.10	n.d.–0.46	0.01	0.01–0.06	0.02–0.05	0.24–0.35
Vitamin B <sub>7</sub> (µg/100 g)	—	—	4.0–18	30–130	27–50	—	—	—	—	—	2.0
Vitamin B <sub>9</sub> (µg/100 g)	7	6	2–9	42	110–1000	3	n.d.–3	3	4	3	4–8.0
Vitamin B <sub>12</sub> (µg/100 g)	0.6	2.0–2.8	2.4–8.0	6.6–14	25–40	2.5–2.8	6.5–16	0.82	3.3–3.5	0.30–1.0	2.8–3.5
Vitamin C (mg/100 g)	—	14–18	3.0–5.3	12–14	13–25	12–13	15	1.1–4.5	28–30	0.0	3.5–4.4

n.d. – not detected; tr – traces.

Source: Anderson (1988); Honikel (2011); Kim (2011); Nollet and Toldrá (2011); Ockerman and Basu (2004); Seong et al. (2014); USDA National Nutrient Database for Standard Reference (Release 22, 2009).

**Table 2.5** Minerals contents in edible pork byproducts.

	Blood	Brain	Heart	Kidney	Liver	Lung	Pancreas	Small intestine	Spleen	Stomach	Tongue
<i>Macroelements (mg/100 g)</i>											
Calcium (Ca)	7	10	3–6.4	8–11	6–20	7–13	10–22	18–20	6–7.4	10–13	11
Magnesium (Mg)	9	14–15	19–20	17–20	18–30	14–15	15–23	5–12	18–20	18	18
Phosphorous (P) as phosphate	75	280–312	131–220	204–270	288–383	200	235–315	30–119	257–370	139–155	190
Potassium (K)	170	219–260	106–300	178–290	217–320	150–203	200–308	113–115	320–340	178–200	255
Sodium (Na)	210	120–125	54–133	115–190	73–95	150–193	45–85	35–54	85–88	52–121	115
<i>Trace elements (mg/100 g)</i>											
Copper (Cu)	0.15	0.24–0.25	0.31–0.45	0.62–0.80	0.55–6.8	0.12	0.09–0.16	0.14–0.15	0.12–2.7	0.14–0.35	0.25
Iodine (I)	—	—	0.001	0.004	0.010	—	—	—	—	—	0.001
Iron (Fe)	40	1.5–2.4	3.3–4.8	4.9–6.7	19–23	20	2–19	2	21	2	4.5
Manganese (Mn)	0.007	0.001–0.09	0.05–0.06	0.12–0.15	0.12–0.40	0.004	0.15–0.20	0.01	0.05–0.15	0.008	0.500
Selenium (Se)	0.008	0.015–0.016	0.010–0.022	0.190	0.05	0.020	—	—	0.035	—	0.012
Zinc (Zn)	0.3	1.3–1.5	0.18–2.8	2.5–2.8	0.7–9.8	0.15–2	0.38–2.6	0.19–2	0.26–7	0.18–2	2.6

Source: Garcia-Llatas et al. (2011); Honikel (2011); Kim (2011); Nollet and Toldrá (2011); Seong et al. (2014); Tomovic et al. (2011); USDA National Nutrient Database for Standard Reference (Release 22, 2009).

byproducts ranges from 3 to 22 mg/100 g, which is equivalent to pork contents (Honikel 2011). Magnesium content in these byproducts, except for small intestine, is about one to six times higher than calcium. The content of phosphorous as phosphate in pork offal, with the exception of blood, is in the concentration range of potassium, which is, in turn, about one to four times higher than sodium concentration. In pork, potassium concentration is about five times higher relative to sodium (Tomovic et al. 2015).

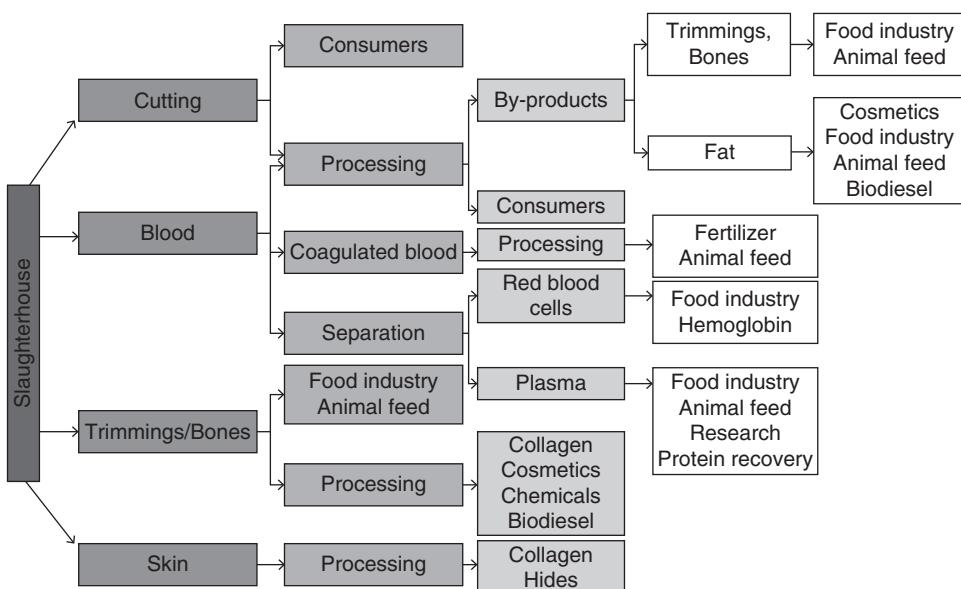
The contents of trace elements in the pork offal are very low, except for iron and zinc. Copper accumulates in liver (0.55–6.8 mg/100 g) but is low in the remaining pork byproducts. Iodine is high in liver, although most of the byproducts present no values. Manganese ranges from 0.001 to 0.5 mg/100 g and it is the highest in liver (0.12–0.40 mg/100 g) and tongue (0.50 mg/100 g), in comparison to all other byproducts. The selenium content varies between 8 and 190 µg/100 g and it is high in kidney. Selenium in pork byproducts is higher than in pork (Honikel 2011). Concerning iron and zinc, iron content exceeds greatly the zinc content in most of the pork offal, such as blood, liver, lung, pancreas, and spleen. By contrary, Tomovic et al. (2015) reported a higher zinc concentration (2.4 mg/100 g) than iron levels (1.4 mg/100 g) for pork. Thus, the concentrations of macro- and microelements in pork byproducts seem to be likely to those of meat, except for iron and zinc.

## 2.4 Applications of Pork Byproducts

Meat waste byproducts constitute about 60–70% of the slaughtered carcass, of which nearly 40% forms edible and 20% inedible (Bhaskar et al. 2007). The pig slaughterhouse produces a large amount of byproducts from animals, such as blood, carcasses, hides, hoofs, heads, feathers, manure, offal, viscera, bones, fat, and meat trimmings. About 15 million tons in the European Union are processed by rendering to produce high quality fats and proteins (Hamilton 2016). These byproducts can be used in many applications, like food, feed and pet food, medical and pharmaceutical, and several others applications (Figure 2.1).

### 2.4.1 Food Applications

Globally, food industry is one of the most important sectors. Byproducts from pigs, and other animal species, are a major concern in the food industry (Mirabella et al. 2014). Thus, efficient utilization of byproducts from food industry can help to reduce the negative costs, decrease environmental pollution and demonstrate sustainability in food industry (Helkar et al. 2016). For example, edible organs and glands, such as brains, hearts, kidneys, livers, melts, sweetbreads, tongues, and chitterlings, are usually sold without significant processing. Intestines and cheek meats are usually processed further, to obtain processed meat products. Extra trimmings and tails are used in soups and bouillons; extra trimmings, blood, stomachs, and intestines can be sausage ingredients or casings. Recently, the use of pork tongue and pork head meat as sausage ingredients has been reported, with no significant differences in some quality characteristics compared to controls (Choi et al. 2016).



**Figure 2.1** Main uses of pork byproducts (adapted from Lafarga and Hayes 2014).

The use of pork byproducts as functional food ingredients is one of the healthy trends in pig industry. The most usually processed and consumed pork offal is liver pâté, foie gras and liver sausages. Other widely available products are black pudding, which is usually made from pig's blood. Pork byproducts have some properties, such as increased water holding and binding, gelling and thickening, which are of relevance in the food industry. Innovation and technical development can promote the utilization of pork byproducts as food ingredients (Helkar et al. 2016). The collagen is a fibrous structural protein and, probably, the animal protein most commonly employed in food production (Mullen et al. 2017). Skin, bones, hide and cartilages are the main sources of collagen, however, other offal such as lung, tongue, trachea, large blood vessels, or tendons are also founts of collagen. Although collagen has a low nutritional value because it lacks EAAs, collagen is also a useful source of bioactive peptides (Saiga et al. 2008; Herregods et al. 2011). The collagen is extracted, hydrolyzed and transformed into gelatin, which is applied to a variety of products like soups, gravies, desserts, or dairy products (Hettiarachchy et al. 2012).

In recent years, much attention has been paid to the generation of peptides with biological activities from food byproducts, including blood (Mirabella et al. 2014). The blood or blood components have a high nutritional value and functional properties that can benefit the food production (Bah et al. 2013). Blood is a rich source of proteins, in particular of hemoglobin, an iron-containing protein (Ofori and Hsieh 2014). About 30% of the slaughterhouse blood produced is used by the food industry (Gatnau et al. 2001), mainly in meat products as a gelling agent and natural colorant. For example, red blood cells, white blood cells and platelets can be used for enhancing the color of sausages, although sensory adverse effects have been described due to the dark color of hemoglobin (Ofori and Hsieh 2011). Numerous societies use whole blood, mainly blood from

pork, as a source of protein in some products, like blood sausages, black puddings, or blood tofu (Bah et al. 2013). More recently, Bah et al. (2016) reported that red blood cells fractions from pig have high antioxidant activity. Likewise, blood has also been marketed for human consumption as a supplement (Bah et al. 2016).

Blood plasma is the second byproduct derived protein most employed as a food ingredient (Mullen et al. 2017). Plasma can be used as it is, or dehydrated for storage as a dry powder (Pares et al. 2014). Porcine plasma, similar to plasma of other mammals has been found to contain both proteinase inhibitors and  $\text{Ca}^{2+}$ -dependent transglutaminase that are responsible for gelation enhancement (Visessanguan et al. 2000). In fact, a combination of porcine transglutaminase, fibrinogen and thrombin was used as binder in restructured meat products (Tseng et al. 2006). Blood plasma presents good properties, such as emulsifying, gelling, foaming and solubility, which it is used in the food industry, mainly as a binder in meat products, egg replacers in bakery, protein-rich pasta, fat replacers or even polyphosphate substitute (Hurtado et al. 2011; Ofori and Hsieh 2011).

#### 2.4.2 Feed and Pet Food Applications

Animal byproducts, like bone, hides and ears, among others, have been traditionally incorporated into pet food formulations as a source of digestible protein, fat and micro-nutrients (Cramer et al. 2007). In fact, meat and bone meal, blood meal, plasma meal, hydrolyzed feather meal, are rich in protein, fat, minerals and trace elements, B vitamins and some fat-soluble vitamins that are crucial in animal nutrition (Pearl 2005; Nollet and Toldrá 2011; Jayathilakan et al. 2012). In addition, meat byproduct protein hydrolysates rich in EAAs can be an alternative to soybean meal outstanding the absence of anti-nutritional factors or allergenic proteins (Martínez-Alvarez et al. 2015). Moreover, hair, nail, feather and outer layer of skin comprising keratin can be also suitable after hydrolysis (Deivasigamani and Alagappan 2008).

Over the last decade, the use of plasma and red cells have also increased, especially in swine feed industry (Gatnau et al. 2001). Approximately 30% of blood derivatives have been used in the pet food industry, mostly in the wet feed, as a gel and water holding agents, and in dry feed ingredients. Whole blood from pig has been added to pet food as a cheap protein source. However, blood can be used to add high value to products as a source of bioactive peptides with antioxidant, antibacterial, and antihypertensive activities or as iron-binding peptides and pre-digested peptides for animal and pet food (Mullen et al. 2015). Some studies have demonstrated that plasma have some benefits, such as increased feed intake, growth performance, decrease diarrhea incidence and protected intestinal function and morphology. A study with spray-dried porcine plasma applied to pigs weaned at approximately 14 or 21 days of age, has shown that plasma is beneficial to young pig performance during the first week after weaning. It was also elucidated that immunoglobulin G (IgG) fraction of plasma was the component responsible for the enhancement in growth rate and feed intake (Pierce et al. 2005).

#### 2.4.3 Medical and Pharmaceutical Applications

In the medical field, edible byproducts, such as organs and glands, removed from livestock at slaughter, have been consumed for medicinal purposes in several countries, like China, Japan, and India, providing hormones and enzymes (Aberle et al. 2001), or used as

a source of particular pharmaceutical substances (Toldrá et al. 2016). For instance, heparin from liver, progesterone and estrogen from ovaries, melatonin from pineal gland and insulin from pancreas (Jayathilakan et al. 2012). Another natural biomaterial used in biomedical applications is collagen, which is the major constituent of connective tissues comprising a family of glycoproteins. Porcine skin articular cartilage and tendon tissues are the main source of collagen. Pork skin can be used as dressing for burns or skin ulcers in humans (Jayathilakan et al. 2012). In addition, protein hydrolysates obtained from collagen can generate peptides against osteoarthritis by accumulation in the joint cartilage (Bello and Oesser 2006).

Other applications in biomedicine are focused on the use of serums vaccines, antigens and antitoxins both derived from animal tissues acquired during slaughter and processing of the animal (Pearl 2005). Purified animal blood is fractionated into numerous products, including thrombin used for blood coagulation agents and skin-graft procedures, fibrin, used in surgical repair of internal organs, and fibrinolysin used to help heal minor burns or as a wound-cleaning agent (Marti et al. 2011). Likewise, certain parts from pigs are used for the insertion of tissue from one species into another. Goodlight (2010) reported that skin, brain, cells, insulin, heart valves and lungs from pigs have been used for human transplants. Moreover, intestines provide surgical ligatures, blood provides albumen, amino acids and fetal serum; bones provide calcium and phosphorus; and other inedible offal provides liver extracts, bile extract, cortisone, heparin, cholesterol, rennet, and pepsin (Marti et al. 2011). Indeed, studies using pig aorta extracts have shown to exert substantial reduction in atherogenic lipoproteins, atherogenic index and total and residual cholesterol (Chernukha et al. 2015).

#### 2.4.4 Other Applications

Nowadays, pork byproducts are used in different industries for their benefits. Blood-derived products are used in agriculture (as fertilizers), cosmetics (as a foaming agents and gelling agent), diagnostics and biotechnology (as reagents). For example, rendered fats from pigs have been increasingly used in cosmetic industry for products, like hand and body lotions, creams, and bath products. In addition, fatty acids from fat have been used in a large quantity of chemical processes, such as rubber and plastic polymerization, softeners, lubricants, and plasticizers (Ockerman and Basu 2006; Toldrá et al. 2012). Other recent applications are targeted toward energy generation. For instance, the thermochemical processing of meat and bone meal (Cascarosa et al. 2012) and the use of animal fats for the production of biodiesel (Baladincz and Hancsók 2015; Adewale et al. 2016).

### 2.5 Concluding Remarks

Pork byproducts are important sources of nutrients and, therefore, the data reviewed here could be of great sense in the promotion of their consumption and contributing to food production sustainability. In general, pork offal provides considerably amounts of vitamins, minerals, proteins and fat, with key PUFAs and amino acids, comparable to those in muscular tissue, which are essential for worthy health throughout life. However,

it is recommended that some of these pork byproducts should be included in our diet only in limited amounts because of the high levels of SFA and cholesterol and their health concerns. In the coming years, the development of novel formulations with pork byproducts is expected. Furthermore, new opportunities for exploiting the inherent value of pork byproducts are required to ensure that this readily available and under-utilized resource can provide compounds with targeted high value and functionality to a multiplicity of industrial activities.

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## 3

### Cattle Byproducts

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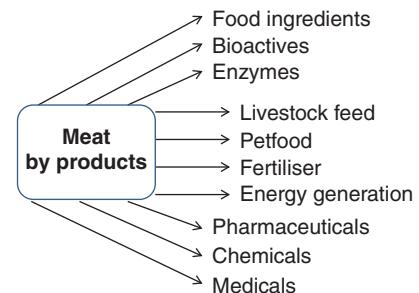
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#### 3.1 Introduction

Cattle are typically raised as livestock animals for producing either beef or veal meat, as well as dairy animals for producing milk and dairy products. Millions of tons of cattle slaughterhouse wastes are produced every day in the world and its disposal constitutes a great problem for processors because of the high treatment costs and the increasingly strict regulations in the majority of countries. Current industrial systems for the treatment of cattle byproducts are in most cases under-utilized and do not cover the treatment costs. Such costs could be balanced if producing added-value products (Toldrá et al. 2012, Mora et al. 2014). Cattle byproducts include a wide variety of products like blood, bones, tendons, meat trimmings, fat or tallow, hides, hooves, horns, internal organs and viscera, and feet. A major division of byproducts consists in edible and inedible byproducts even though sometimes its differentiation depends on a specific culture or country. Edible byproducts are prone to microbial contamination and growth due to their abundance in glycogen and, thus, they must be carefully and hygienically cleaned, handled, processed and refrigerated and cooled quickly (Ockerman and Basu 2004a, 2014). In general, edible byproducts are defined as a food that can be consumed by humans (Ockerman and Hansen 1988). Edible byproducts tend to be consumed in larger proportions in countries with low incomes due to their high nutritional quality to cost ratio. Determined geographic areas prepare delicious recipes with the edible byproducts that are highly valued and appreciated (Toldrá et al. 2016; Ockerman et al. 2017). An additional advantage is that this consumption also contributes to a reduction in residues handling and thus, the environmental pollution problem.

On the other hand, the rest of the byproducts, considered as inedible, may have a variety of applications in fields as diverse as pharmaceutical, medical, chemical, and energy generation. Major applications of cattle byproducts are compiled in Figure 3.1. There are



**Figure 3.1** Main uses and applications of cattle byproducts. Source: Reproduced from Toldrá et al. (2016).

new technologies and uses proposed for such cattle byproducts even though they have difficulties to be implemented due to the need for innovative technology, specific processing methods, and/or appropriate marketing.

### 3.2 Characterization of Cattle By-Products

The yield of cattle edible byproducts is around 12% of the animals' live weight (Ockerman and Hansen 2000). There may be a considerable variation due to the differences in age, sex, live weight, fatness, etc. (Ockerman and Basu 2014). Red viscera usually includes the following edible byproducts including its range of yield as a percentage of the live weight into brackets: liver (1–4.5), heart (0.3–0.5), kidney (0.07–0.24), brain (0.08–0.12), lungs (0.4–0.8), tail (0.1–0.25), tongue (0.25–0.5), and other byproducts like rendered fat (2–11), blood (2.4–6) and white offal that include intestines and stomach (0.75) (Ockerman and Basu 2004b, 2014).

The consumption of cattle edible byproducts constitutes a reasonable way to improve the value of such types of products and contributes to a better sustainable meat industry by reducing the amount of wastes (Ockerman et al. 2017). Some countries may consider a waste an edible byproduct that is highly appreciated in other countries. This is the case of nutritious products such as liver, lung, heart, kidney, brains, spleen, and blood (Anderson 1988, Honikel 2011) and are regularly consumed in many countries (Nollet and Toldrá 2011). The intestines and other areas of the digestive track of cattle are used as casings for traditional sausages and other meat products (Nollet and Toldrá 2011). Trimmings obtained from the deboning of skeletal muscle but also from byproducts like liver, cheek, and tongue are used for sausage production. Soup stock is produced from cooked cracked beef bones and used in cooked products, soups, vegetable dishes, sauces or gravies for nutritional and flavor purposes (Ockerman and Basu 2014). Mechanically separated bovine meat is a typical ingredient in comminuted meat products and also in sausages (Pearl 2014). Examples of typical products derived from cattle edible byproducts are shown in Table 3.1.

Edible byproducts products constitute an excellent source of nutrients, like essential amino acids (Aristoy and Toldrá 2011), minerals and vitamins (Honikel 2011; Kim 2011; García et al. 2011) as detailed in next section.

There are some safety issues when dealing with cattle byproducts. Of course, these types of products must be treated following strict hygiene measures and control. Other

**Table 3.1** Examples of edible meat byproducts and countries where they are typically consumed.

Byproduct	Typical products	Countries
Liver	Splinantero	Greece, Turkey
	Almondega	Portugal
	Kalbsleber	Austria
Spleen	Pani ca meusa (bread with spleen)	Italy
Heart	Cooked and diced	South America
	Gandinga	Caribbean Islands
	Anticuchos	Perú, Bolivia
	Churrasco	Argentina, Brazil
Kidney	Kidney pie	UK
Lung	Bofe	Colombia
Brain	Sesos	South America, Spain
	Mioleira	Portugal
Tongue	Boiled or marinated	South America
	Ingredient in meat products	Spain
	Tacos de lengua	Mexico
Testicles	Criadillas	Spain
Tripe	Lampredotto	Italy
	Trippa	Italy
	Tacos de tripas	Mexico
	Blóomör, slátur	Iceland
	Dinuguan	Philippines
	Korouch	Lebanon
	Callos	Spain
	Cold appetizer	China
	Pieds et paquets	France
Intestines	Casings for sausages	Mediterranean
Sweetbreads	Mollejas	Argentina
Eyes	Tacos de ojos	Mexico
Blood	Morcilla sausage	Spain
	Black pudding	UK
Tail	Rabo de toro (Bull's tail)	Spain
Bones	Gelatin soups	Mediterranean
	Ossobucco	Italy
Tallow	For cooking	Northern Europe

Source: Adapted from Ockerman and Basu (2004a) and Nollet and Toldrá (2011).

recent safety issues are related with the Bovine Spongiform Encephalopathy (BSE), a disease that affects adult cattle and started in 1982 in the UK. BSE belongs to a family of diseases known as Transmissible Spongiform Encephalopathies (TSEs) that are characterized by an increase of abnormal prion proteins in the brain and central nervous system. The Variant Creutzfeldt-Jacob Disease (vCJD) is a TSE disease that affects humans and causes death. This is the reason why particular cattle tissues such as spleen, tonsils, intestine, mesentery, spinal cord, and full head are considered specified risk materials because there is a high probability that they might contain the BSE agent. Therefore,

these risk materials must be removed and destroyed in order to avoid its inclusion in either the human or animal food chains. It must be added that the bones from beef are also considered as specified risk materials in those countries having BSE risk and they are also forbidden in the production of mechanically recovered meat. In the European Union, the Regulation 999/2001 (EC 2001) describes the rules for the prevention, control and eradication of certain TSEs. The European Commission Regulation 853/2004 (EC 2004) describes the specific hygiene rules for the handling of foodstuffs including those of animal origin, while Regulation 1069/2009 (EC 2009) describes the health rules regarding animal byproducts and derived products not intended for human consumption and repealing the animal byproducts. In USA, the Food and Drug Administration (FDA 2004) announced rules to prevent the establishment and spread of BSE, including a prohibition on the use of high-risk, cattle-derived materials that can carry the BSE agent, which are defined as specified risk material. Such risk materials are brain, skull, eyes, trigeminal ganglia, spinal cord, vertebral column, and the dorsal root ganglia of cattle with more than 30 months of age and also the tonsils and the distal ileum of the small intestine of cattle of any age. The ban also included the small intestine of all cattle and the mechanically separated meat from beef.

### 3.3 Nutritional Composition of the Main Edible Cattle By-Products

A better knowledge of the nutritional value of edible byproducts would contribute to an increase in the consumption of these products. The nutritional composition of major edible byproducts is shown in Table 3.2. For instance, liver is rich in vitamins A, B, C, and D, minerals and trace elements like iron, zinc, and copper (García-Llatas et al. 2011; Kim 2011; Martí et al. 2011). The content in selenium is particularly high in liver and kidney, while phosphorus is high in liver, brain, and spleen. Proteins are of high biological value because of the content in essential amino acids, which may be as high as  $72\text{--}82 \text{ mg g}^{-1}$  protein for lysine,  $80\text{--}90 \text{ mg g}^{-1}$  protein for leucine, and  $52\text{--}62 \text{ mg g}^{-1}$  protein for valine (Aristoy and Toldrá 2011). The ratio n-6/n-3 should not exceed 4.0 in accordance to nutritional guidelines (Department of Health 1994) but it is in general much higher than that, except for brain which is 1.3–1.4 and liver which is 4.3–5.5, a little bit higher (Alfaia et al. 2016).

The contents in saturated fatty acids (SFAs) is relatively high in edible beef byproducts, while the contents of n-3 polyunsaturated fatty acids (PUFAs) is low due to hydrogenation of double bonds by rumen microorganisms (Prates et al. 2011; Alfaia et al. 2016). The amount of conjugated linoleic acid (CLA), that has been reported to exert some anticarcinogenic effects and other benefits on the immune system and lipid metabolism (Schmid et al. 2006), is particularly relevant because it is formed by rumen microorganisms in cattle. The CLA content in edible beef byproducts has a considerable variation between animals, ranging from 1.2 to  $10.0 \text{ mg g}^{-1}$  lipid (Prates and Bessa 2009). Within an animal, CLA is highest in the liver, followed by tongue, heart, and kidney (Florek et al. 2012). The most abundant CLA isomer was reported to be c9,t11 isomer (62–84% of total CLA) followed by t7,c9 (3.5–6.3%), except in brain which was t11,c13 (20%), while the other bioactive CLA isomer, t10,c12, was reported to be only in residual percentages

**Table 3.2** Example of proximate composition in major nutrients per 100 g of beef edible byproducts.

	Liver	Heart	Kidney	Brain	Tongue	Spleen	Blood
Energy (kcal)	130	115	100	120	185	110	70
Protein (g)	21	17	16	10.5	16.5	18	16.5
Fat (g)	3.0	5.0	4.0	8.5	13	3.5	0.4
Carbohydrates (g)	5.0	0.5	1.0	<1	0.5	1.0	0.1
Vitamin B1 (mg)	0.3	0.2	0.4	0.15	0.1	0.15	0.1
Vitamin B2 (mg)	3.5	0.45	2.0	0.25	0.25	0.4	0.3
Vitamin B3 (mg)	20	35	9.5	4.5	6.5	8.0	3.5
Vitamin B5 (mg)	7.5	2.5	3.5	2.5	2.0	1.2	—
Vitamin B6 (mg)	1.0	0.3	0.45	0.3	0.15	0.12	0.01
Vitamin B12 ( $\mu$ g)	100	10	30	12	5.0	5.5	0.6
Vitamin A (RE $\mu$ g)	21 000	6	800	—	0	tr	30
Vitamin C (mg)	30	2	15	15	5	45	0
Vitamin D ( $\mu$ g)	1.7	1.0	1.0	—	tr	—	0.1
Vitamin E (mg)	0.7	0.2	0.2	—	0.1	—	0.1
Ca (mg)	7	5	10.5	10	7	6	7
P (mg)	356	210	219	312	175	360	50
Fe (mg)	6.7	4.5	6.5	2.3	2.5	44	50
K (mg)	300	250	230	219	220	320	43
Na (mg)	110	90	178	125	75	80	330
Mg (mg)	35	17	20	15	18	20	3
Zn (mg)	4.0	1.5	2.0	1.0	3.0	4.0	0.5
Se (mg)	15	15	115	—	2	30	15
Cholesterol (mg)	91–140	192–338	100–517	1456–3010	78–171	—	—
$\sum$ SFA (g)	42	27–29	30–31	34–35	35–36	—	—
$\sum$ cisMUFA (g)	12–13	10–11	18–19	24	40–42	—	—
$\sum$ PUFA (g)	33–35	41–42	37–38	22–23	11	—	—
n-6/n-3	4.3–5.5	16.5–22.5	10.3–12.8	1.3–1.4	7.8–10.9	—	—
Total CLA (mg)	9.4	2.3–2.6	10.5	1.2–1.6	11.9–14.9	—	—

Tr: traces.

Source: Adapted from Alfaia et al. 2016; Honikel 2011; Ockerman and Basu 2014; Kim 2011; Bragagnolo 2011.

in beef byproducts (Alfaia et al. 2016). Moreover, the cholesterol content is generally high, especially taking into account the recommended maximum cholesterol intake should be less than 300 mg per day (2015). The cholesterol content is very high in the brain, as it forms part of cell membranes and nerves (Bragagnolo 2011). Tallow is rich in fat and thus, it mainly contributes to energy intake. Bovine blood is made up of plasma

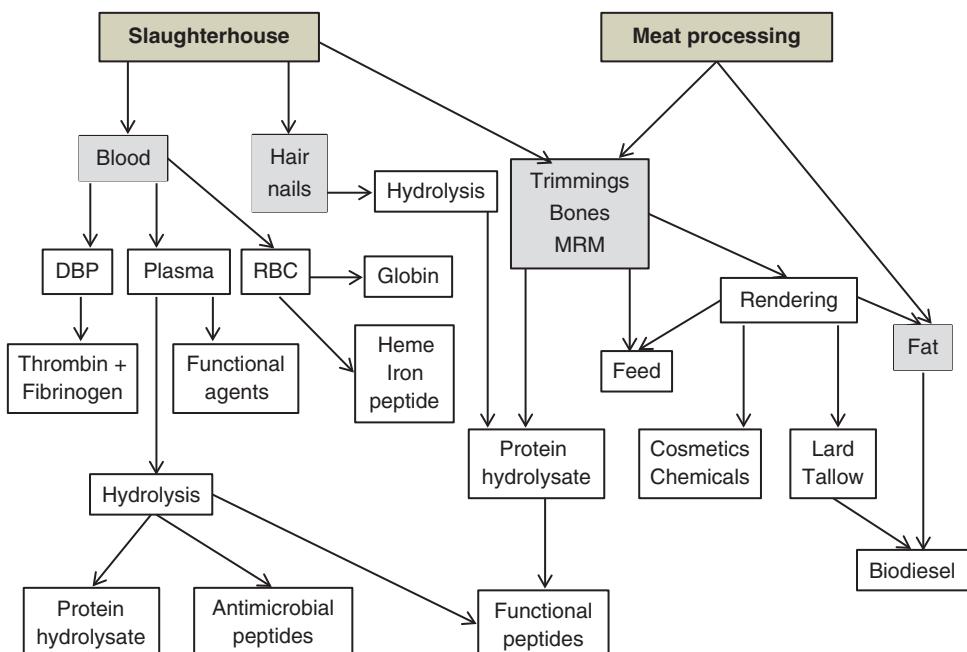
and the cellular fractions (red blood cells, white blood cells and platelets) (Ofori and Hsieh 2011) and is particularly rich in proteins (Duarte et al. 1999).

## 3.4 Applications of Cattle By-Products

The processing of low value cattle byproducts having relevant disposal costs is of primary relevance for sustainability purposes. The main goals are both to obtain a new product with enough added-value to cover all the processing and disposal costs, and to reduce environmental damage. So, recent strategies include the production of foods, feeds, pet foods, functional ingredients or processing aids from edible byproducts or to obtain economic profitability from inedible byproducts (e.g. plastics, pharmaceuticals, energy) (Pearl 2014; Ockerman and Basu 2004b). A diagram showing the main routes for applications of cattle byproducts is shown in Figure 3.2 and briefly described in the next sections.

### 3.4.1 Food Applications

An additional goal for the treatment of cattle byproducts is the production of new products and functional ingredients with a significant added-value in foods (Zhang et al. 2010, Toldrá and Reig 2011). Therefore, the food industry may take profit from improved sensory quality (color, flavor, or texture) through technological functions like water



**Figure 3.2** Flow diagram of main routes for value-addition to cattle byproducts. Source: Reproduced from Toldrá et al. (2012).

bonding agents, protein cross-linking, gelation, foaming and emulsification, flavor enhancement, protein enrichment, etc.

Plasma and cellular fractions are obtained from blood after separation by centrifugation (Ofori and Hsieh 2011). Proteins from the blood plasma fraction have relevant applications in food technology (gelation, foaming, and emulsification) making them interesting added-value ingredients for the food industry (Ofori and Hsieh 2011). For instance, immunoglobulins, fibrinogen and serum albumin that exert gelation and emulsification properties, can be added to food and feed (Cofrades et al. 2000). Plasma proteins may be also used for cross-linking of major proteins (Kang and Lanier 1999) or for enrichment of products like pasta (Yousif et al. 2003). Fibrinogen and the enzyme thrombin are commercially used for binding meat proteins in restructured meat products (Ryan et al. 1999; Lennon et al. 2010). The resulting product has increased hardness and springiness (Herrero et al. 2007).

The cellular fraction is rich in red blood cells because of the heme component of hemoglobin. This gives a dark color but can also impair the flavor of the product (Duarte et al. 1999). Such a fraction can be used for color enhancement of sausages (Ofori and Hsieh 2011), or for replacing fat in meat products (Viana et al. 2005; Hsieh and Ofori 2011). It also exerts a high antioxidant activity (Bah et al. 2016), as well as antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Bah et al. 2016). Heme iron polypeptide can be obtained from the enzymatic digestion of bovine hemoglobin and could be used for improving iron absorption (Nissensohn et al. 2003).

Numerous bioactive peptides produced through the hydrolysis of proteins in meat byproducts by specific commercial proteases, like papain, bromelain, thermolysine, pronase, or proteinase K (Vercruyse et al. 2005), have been reported in recent literature (Toldrá et al. 2016). No more information is given in this chapter, since they are object of another specific chapter in this book (see chapter on bioactive compounds from animal meat byproducts).

Collagen, which is rich in glycine, proline, and lysine but poor in tryptophan and methionine, is a low biological value protein very abundant in cattle byproducts, especially in the skin, bones, and tendons. The controlled hydrolysis of collagen results in gelatin, that has many commercial applications in the food industry such as clarifying agent, stabilizer or protective coating material in bakery, dairy, and the meat industry (Djagny et al. 2001; Ockerman and Basu 2006). Collagen also constitutes an excellent substrate for further hydrolysis into added value bioactive peptides (Jridi et al. 2017). In fact, one of the most popular collagen hydrolysates is used for relieving osteoarthritis in the regeneration of joint cartilages (Bello and Oesser 2006).

Proteins-based foams may be obtained from the animal rendering process after the hydrolysis of bloodmeal or bonemeal (Bressler 2009). Hydrolyzed proteins, rich in small peptides and free amino acids, may be used directly as flavorings or after Maillard reactions with reducing sugars (Baiano 2014; Toldrá et al. 2016).

### 3.4.2 Feed and Pet Food Applications

Rendered animal byproducts have been traditionally used as ingredients in feed and pet food (Murray et al. 1997). In fact, cattle byproducts have been the typical use in feed and pet food, because they could provide protein, fat, minerals, and trace elements, as well as B vitamins and fat-soluble vitamins required in animal nutrition (Pearl 2014). High

quality fats and proteins are obtained by rendering of near 25 million tons per year of animal byproducts in USA and 15 million tons in European Union (Hamilton 2016). Some byproducts, like bone meal, blood meal, plasma meal, and tallow, have been used as raw materials (Alexis and Robert 2004; Pérez-Gálvez et al. 2011). In addition, bone meal ashes obtained by co-incineration (Coutand et al. 2008) or some organs, like heart or lung, after appropriate enzymatic hydrolysis, have also been used as raw materials (Martínez-Alvarez et al. 2015). Proteolysis of meat and bone may increase its utilization and profitability by animals (Piazza and García 2014). However, protein hydrolysates may have an excessive bitterness due to hydrophobic amino acids and the palatability of pet foods needs to be improved by cleaving such amino acids with specific peptidases (Nchienzia et al. 2010). Also, specific microbial cultures are used for the hydrolysis of blood and subsequent generation of concentrates rich in high-quality amino acids (Giu and Giu 2010) or the enzymatic hydrolysis of keratin, a fibrous structural protein present in hair and nails, with keratinase, thus producing crude protein extracts (Deivasigamani and Alagappan 2008; Lasekan et al. 2015).

### 3.4.3 Medical and Pharmaceutical Applications

Skin can be applied to humans for protection in case of burning or skin ulcers (Jayathilakan et al. 2012). Secretions from glands and organs, like heparin from liver, insulin from pancreas, bile from gall bladder, melatonin from pineal gland, and progesterone and estrogen from ovaries, are consumed for medicinal purposes in countries like China, Japan, and India, or used as a source of particular pharmaceutical substances (Nollet and Toldrá 2011; Jayathilakan et al. 2012). Osteoarthritis in the joint cartilage may be treated and alleviated with the consumption of collagen hydrolysates, usually commercialized with added magnesium and hyaluronic acid (Bello and Oesser 2006; Gómez-Guillén et al. 2011). Cosmetics applications like hand and body lotions, creams and bath products may also be obtained from rendered fat.

### 3.4.4 Energy Generation

Biodiesel is being produced in recent years as a valid alternative to diesel fuel without significant modifications in engines. The replacement is justified by being biodegradable, non-toxic, and with a favorable combustion emission profile. This means that significant reductions in carbon dioxide, carbon monoxide, particulate matter, and unburned hydrocarbons may be achieved (Gerpen 2005; Moreira et al. 2010).

Biodiesel may be produced from low cost animal fat byproducts. These fats are transesterified with a low molecular weight alcohol to yield a mixture of fatty acid methyl esters and glycerol as a side product (Bhatti et al. 2008; Moreira et al. 2010). This process may be improved by hydro-oxygenation and hydro-isomerization (Herskowitz 2008), supercritical transesterification (Marulanda et al. 2010) or ultrasounds assisted transesterification (Adewale et al. 2016). However, there are some limitations, like the need for a degumming process (due to the presence of proteins and phosphoacylglycerols), vacuum drying (due to the presence of water), and winterization process or inclusion of additives (due to the presence of SFAs) (Banković-Ilić et al. 2014).

The biogas oil constitutes a second generation of energy produced from biomaterials, where triacylglycerols are converted into a mixture of *iso* and normal paraffin via heterogeneous catalytic hydrogenation (Baladincz and Hancsók 2015).

### 3.4.5 Other Applications

Fatty acids from tallow byproducts are used in many chemical processes, like rubber and plastic polymerization, softeners, lubricants, and plasticizers. Collagen, gelatin and glycerin are also used as ingredients for surfactants, paints, varnishes, adhesives, antifreeze, cleaners, polishes, and pharmaceuticals (Pearl 2014). Rendered fats have been reported to be used as raw material by a recombinant strain of *Ralstonia eutropha* for the production of polyhydroxylalkanoates, a plastic-like biodegradable polymer (Riedel et al. 2015). Detailed information about this application is reported in this book, (chapter “Polymers and adsorbents from agricultural waste”).

A variety of leather-derived products such as shoes, handbags, purses, gloves, fancy leather clothes, and belts, are obtained from cattle hides that represent around 7–8% of the animal’s live weight (Ockerman and Basu 2004b)

Another relevant application is the production of fertilizers with a high content of phosphorus. They are obtained from meat and bone meal through thermochemical processing (pyrolysis, combustion, and gasification) (Coutand et al. 2012). In fact, good mineral fertilizers are obtained with such type of incineration that also allows an efficient energy recovery (Bujak 2015).

## 3.5 Final Considerations

This chapter has presented a brief description of the most usual edible and inedible cattle byproducts and have discussed the specific byproducts regarding major characteristics, yield, and applications in a wide variety of fields, like food, feed, pet foods, medicine, pharmaceutical and chemical, and energy generation. The production of added-value products from byproducts is very important to cover their treatment and disposal costs and to reduce their environmental impact, thus contributing to the sustainability of cattle production.

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## 4

### Byproducts from Dairy Processing

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#### 4.1 Introduction

More than 6 billion people worldwide consume milk and milk products. While the majority of these people live in developing countries, milk and dairy products are major components of the diet in Europe, North and South America, Australia, New Zealand and some Middle Eastern countries. In the last three decades, global milk production has increased by more than 50%, from 500 million tonnes in 1983, to 769 million tonnes in 2013 and about 800 million tonnes in 2015, of which 163, 46, 105, and 31 million tonnes were produced in the European Union, Eastern Europe, North America and Oceania, respectively. World milk production is projected to increase by 177 million tonnes (23%) by 2025, compared to the average production in the base years 2013–2015, and a general expansion in dairy trade is expected over the coming decade (Figure 4.1). The annual growth rate differs for individual dairy products: butter (2.3%), cheese (2.1%), skimmed milk powder (SMP; 2.2%) and whole milk powder (WMP; 1.8%) (OECD-FAO 2016) An overview of the utilization of milk and dairy products in Europe is provided in (Figure 4.2).

Because milk is perishable and its production was, traditionally, seasonal, milk surplus to immediate requirements was converted to more stable products, with some examples being butter or ghee, fermented milk and cheese; smaller amounts of dried milk products were traditionally produced by sun-drying; such products are still important and many new variants thereof have been introduced (Table 4.1). Since the publication of the book *Byproducts from Milk* (Whittier and Webb 1950), enormous developments have taken place in the dairy industry. At that time, the dairy industry was dominated by butter, cheese, liquid milk, and cream. The byproducts were generally used as liquid animal feed or applied to the land as fertilizer. The gradual switch from traditional products to convenience foods posed a new challenge for the food industry, while several new categories of dairy products have been developed during the past 150 years, e.g. sweetened

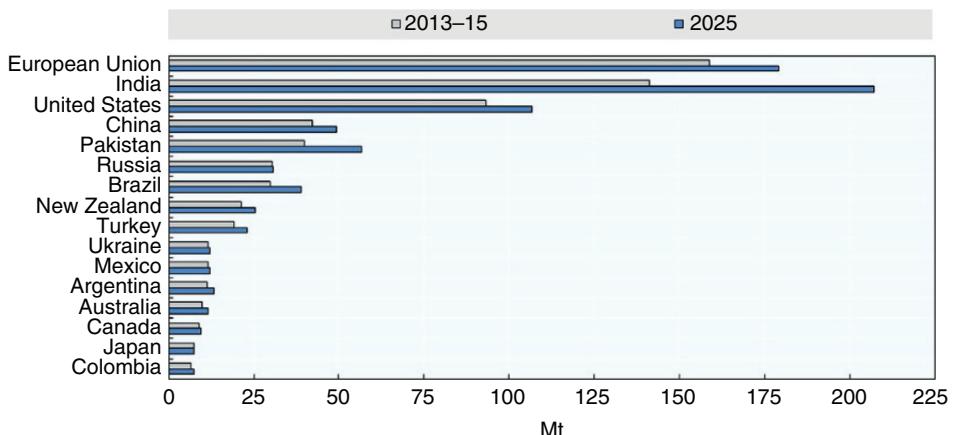


Figure 4.1 Milk production for major countries and regions. Source: From: OECD-FAO (2016).

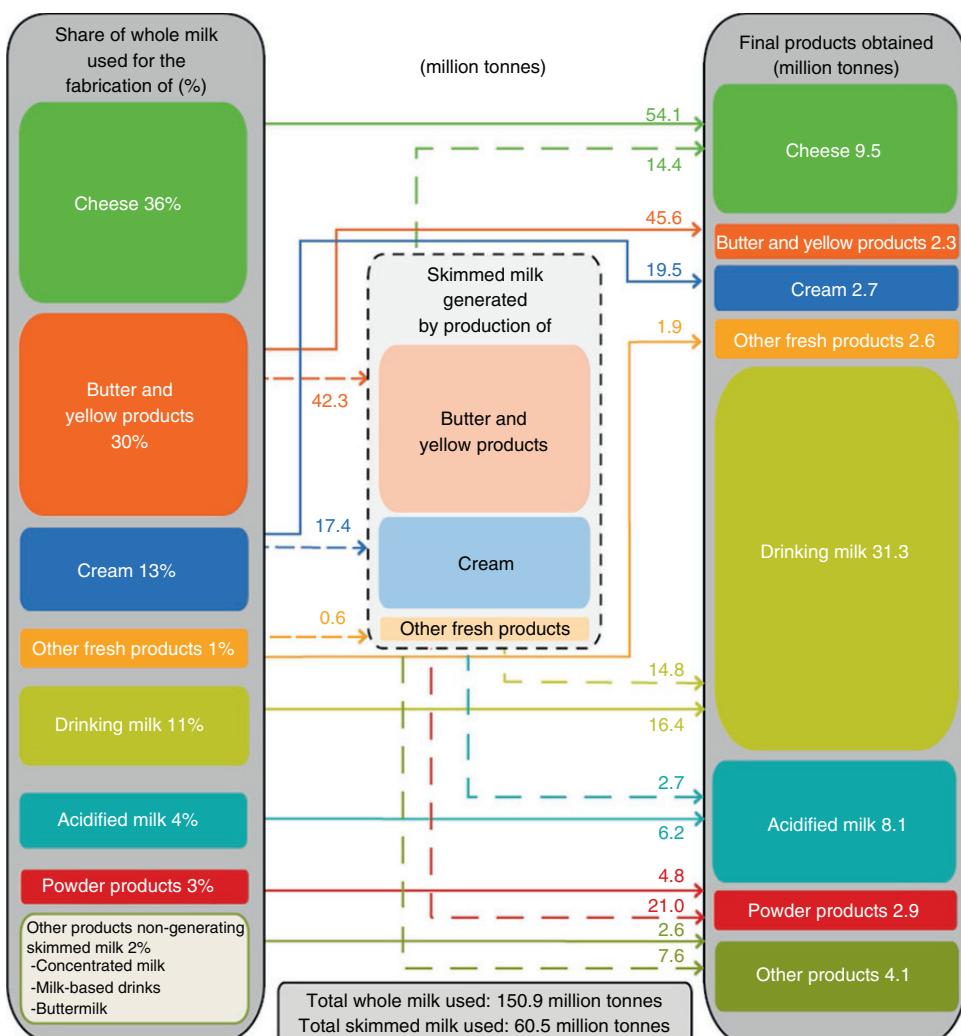


Figure 4.2 Utilization of milk and dairy products in Europe in 2015 (% and million tonnes). Source: From (Eurostat 2016).

**Table 4.1** Diversity of dairy products.

Process	Primary Product	Further Products
Centrifugal separation	Cream	Butter, butter oil, ghee, anhydrous milk fat; creams of various fat content (coffee creams, whipping creams, dessert creams; cream cheeses)
	Skim milk	Powders, casein, cheese, protein concentrates and infant formulae
Thermal processing		HTST or super-pasteurization, UHT-sterilized or in-container sterilized
Concentration, thermal evaporation or membrane filtration		Evaporated or sweetened condensed milk
Concentration and drying		Whole milk powders; infant formulae; dietary products
Enzymatic coagulation	Cheese	1000 varieties; further products, e.g. processed cheese, cheese sauces, cheese dips
	Rennet casein	Cheese analogues
	Whey	Whey powders, demineralized whey powders, whey protein concentrates, whey protein isolates, individual whey proteins, whey protein hydrolysates, nutraceuticals. Lactose and lactose derivatives
Acid coagulation	Cheese	Fresh cheeses and cheese-based products
	Acid casein	Functional applications, e.g. coffee creamers, meat extenders; nutritional applications, cream liquors
Fermentation		Various fermented milk products, e.g. yogurt, buttermilk, acidophilus milk, bio-yogurt
Freezing		Ice-cream (numerous types and formulations)
Miscellaneous		Chocolate products

Source: From: Fox and McSweeney (1998).

condensed milk, in-container sterilized milk, a range of milk powders, ultra-high temperature (UHT)-sterilized milk, ice creams, infant foods, milk protein products, lactose and lactose derivatives.

One of the important developments in dairy technology in more recent years has been the fractionation of milk into its principal constituents, lactose, milk fat fractions, minerals and milk proteins, with the latter serving as the means by which dairy protein-enriched/isolated ingredients are made available; examples include caseins, caseinates, milk protein concentrates (MPCs), milk casein concentrates (MCCs), whey protein concentrates (WPCs), whey protein isolates (WPIs), mainly for use as functional and nutritional protein ingredients, while more recently, some milk protein ingredients, such as lactoferrin, lactoperoxidase, and immunoglobulins have been developed and are marketed as "nutraceuticals," i.e. proteins for specific physiological/nutritional functions.

As a raw material, milk has many attractive features:

- Milk was designed for animal nutrition, and hence, contains the necessary nutrients in easily digestible forms (although the balance is designed for the young of a particular species) and free of toxins.
- The principal constituents of milk (lipids, proteins, and carbohydrates) can be fractionated readily and purified using relatively simple approaches, for use as food ingredients.
- Milk is readily converted into products with highly desirable organoleptic and physical characteristics and its constituents have many desirable, and some unique, physico-chemical functionalities (e.g. gelation and interfacial properties).
- The modern dairy cow is a very efficient convertor of plant material, and over the last 100 years, the productivity of dairy cattle has risen considerably due to scientific advances in many key enabling areas, such as milking equipment, reproductive technologies, nutrition, management, and genetics.
- In terms of the quantity of protein that can be produced per hectare, milk production, is much more efficient than meat production but less efficient than some plants (e.g. soy beans). However, the functional and nutritional properties of milk proteins are superior to those of plant proteins, and since cattle (in particular, sheep and goats) can thrive under farming conditions not suitable for growing cereals or other plants, dairy animals need not be competitors with humans for the use of land.
- One of the limitations of milk as a raw material is its perishability, due to the fact that it is an excellent source of nutrients for microorganisms as well as for humans. However, this perishability is readily overcome by a well-organized, efficient dairy industry.

Milk is probably the most adaptable and flexible of all food materials, as is apparent from Table 4.1, which shows the principal families of milk-based foods – some of these families contain several hundred different products. As the composition, and the quantity, of these primary products usually do not match those of raw milk, this gives rise to byproducts which must be processed. A key objective in processing and fractionating milk into commercially viable, nutritional and techno-functional ingredients and products is to minimize side stream and waste generation. In the dairy sector, given the volume, composition, and chemical/biological oxygen demand of side-streams and byproducts, these must be processed even if they have a negative value (Boer 2014). A byproduct is a secondary product derived from a manufacturing process or chemical reaction. In the context of production, a byproduct is the “output from a joint production process that is minor in quantity and/or net realizable value compared to the main products.” A byproduct can be useful and marketable or it can be considered waste, depending on several factors, including composition, volume, quality and ease of processing. Today, several thousand different product types are produced from milk; these fall into the following principal groups: liquid/beverage milk (40%), cheese (35%), milk powders (15%), concentrated milks (2%), fermented milks (2%), butter (30%, some of which is produced from cream obtained as a byproduct in the manufacture of other products), ice cream, infant formula, cream-based products, protein-rich products and lactose (O’Mahony and Fox 2014).

In the processing of milk, what might be considered a byproduct is arbitrary in some cases, e.g. in the manufacture of butter or butter oil, buttermilk and butter serum are clearly byproducts, whereas, in the separation of milk, either the cream or the skimmed

milk could be the byproduct, depending on the objective of the process; in this article, cream and skimmed milk are considered as byproducts. In the manufacture of butter, butter is clearly the primary product and buttermilk is a byproduct; likewise, in the manufacture of cheese from whole or skimmed milk, cheese is clearly the primary product and whey is a byproduct. Cream is used to produce a range of dairy products such as butter, butter oil, ghee, anhydrous milk fat (AMF), sour cream and cream cheese. As these are products as such, they will not be considered in more detail here, but have been the subject of several relevant publications, namely, Wilbey (2009), Smiddy et al. (2009), Guinee and Hickey (2009), Illingworth et al. (2009) and Mortensen (2009, 2011).

## 4.2 Skimmed Milk-Based Byproducts

### 4.2.1 Skim Milk

Skim milk is a co-product obtained during the manufacture of cream. Skim milk is rich in non-fat solids (i.e. lactose and protein) and has good nutritional value. Skim milk is regarded as a byproduct only when it is not economically used or further processed to derive byproducts like casein and related products, co-precipitates, protein hydrolysates, etc. Skim milk may be pasteurized and sold as a fluid milk product, used in the formulation of infant nutritional products (usually as skim milk concentrate), concentrated and filled with vegetable oils in the production of fat-filled and enriched milk products and is used in standardization of protein content for the manufacture of other dairy products (e.g. cheese) or it may be preserved in a dried powder format. Dried skim milk powder (SMP) is normally produced using spray drying technology, but may be produced by roller drying; SMP has many applications, including, but not limited to, infant nutritional products, recombined milk products, bakery, confectionery and ice cream.

### 4.2.2 Cheese and Fermented Skim Milk Products

Two major families of acid-coagulated cheese are produced from skimmed milk: Cottage cheese and Quarg. A cheese called "Cottage" is produced in many countries but the term usually refers to that which originated in the United States, but is now produced widely. The washed cheese curd is usually dressed with cream to improve its flavor; the typical composition of such a product is 79% moisture, 14% protein, 4% fat and 1% salt. It is particulate and is consumed mainly in salads. American-type Cottage cheese was described by Farkye (2004). The second family is Quarg (Quark) and Quarg-like cheeses, including Bakers cheese, Tvorog, Fromage Frais, Labneh, Petit Suisse, Neuchatel, Skyr, and Queso Blanco. In the production of such products, the curds are generally cooled, optionally blended with cream and/or condiments and packed. In such manufacturing processes, the whey proteins (WPs) are generally lost in the whey, but various modifications (e.g. Centri-whey, Lactal, and Thermo processes) have been developed and implemented to recover the WPs, thereby reducing substantially the generation of a further byproduct stream, while increasing yield of the primary cheese product. In the Centri-whey process, the whey is heated to denature the WPs, which are recovered by centrifugation and added back to the cheese milk. In the Lactal process, the heat-precipitated WPs are allowed to settle and the supernatant decanted; the precipitated

protein is further concentrated by centrifugation and added to regular Quarg to give about 20% protein. In the Westfalia “Thermo process” the milk is heated at ~95 °C for ~3 minutes to denature the WPs and react them with the casein micelles. A fine coagulum is obtained on acidification from which the casein-WPs are recovered by centrifugation. Alternatively, casein-WPs are recovered from heated milk by ultrafiltration (UF) or microfiltration (MF). Undressed Quarg contains ~20% dry matter, ~12% protein, ~2% fat, ~4% lactose plus lactate, and has a pH of ~4.6. Germany is the principal producer of Quarg, but it is also produced widely elsewhere. Probably the principal use is in cheesecake which may be flavored. The production of Quarg and related products was described by Kosikowski and Mistry (1997) and Schulz-Collins and Senge (2004). The types of acid-coagulated and acid-heat-coagulated cheeses and their production were described by Lucey (2011) and (Farkye 2017).

Cultured buttermilk, another product produced from skimmed milk, is an alternative to natural buttermilk, produced mainly in countries where sweet-cream butter is produced. Skimmed milk is acidified by a culture of mesophilic lactic acid bacteria (LAB) and consumed as an alternative to fresh milk. Cultured buttermilk production was described by Libudzisz and Stepaniak (2011).

#### 4.2.3 Caseins and Caseinates

Casein represents ~80% of the total protein in bovine, buffalo, caprine, or ovine milk; it comprises four proteins,  $\alpha_{s1^-}$ ,  $\alpha_{s2^-}$ ,  $\beta$ - and  $\kappa$ -, which occur in milk as large aggregates, micelles, and which may be recovered by isoelectric precipitation at ~pH 4.6, limited proteolysis, ultracentrifugation, UF or MF. The properties of the resultant products, i.e. isoelectric (acid), rennet and micellar casein, are markedly different. Less than 80% of the total protein in milk is recovered in acid or rennet casein, with the whey proteins being lost in the whey. The whey proteins in skim milk may be precipitated with the caseins following heat denaturation (e.g. 90 °C × 10 minutes), by acidification to ~pH 4.6, or by CaCl<sub>2</sub> addition, to yield casein co-precipitate; however, such co-precipitate products have not been as commercially successful as the caseins. Rennet casein is manufactured by drying of the insoluble casein fraction generated during renneting. Although very insoluble, some properties of rennet casein make it suitable for certain food applications, such as the manufacture of analogue cheese, where rennet casein is dispersed in a hot solution of calcium-binding salts before cooling to induce matrix formation (Ennis and Mulvihill 1999; O’Sullivan and Mulvihill 2001). Acid casein is produced from skim milk by isoelectric precipitation of the casein fraction at pH 4.6, typically using hydrochloric acid. The resultant curd is recovered using mechanical separation, washed and dried to produce a powder ingredient. Acid casein is completely insoluble in water, and as a result has a very limited range of applications. It can be used for nutritional supplementation of food products where solubility in water is not a requirement (e.g. protein bars and breakfast cereal).

Acid casein is normally converted to the soluble caseinate forms, for use in a wider range of food applications, by dispersion in water and adjusting the pH to ~6.7 with alkali, usually NaOH, to yield sodium caseinate. KOH, NH<sub>4</sub>OH or Ca(OH)<sub>2</sub> may also be used, giving the corresponding caseinate (Mulvihill and Ennis 2003). In the laboratory, caseinates may be freeze-dried but are usually spray-dried, and to a much lesser extent roller dried, in industrial-scale production. The casein present in rehydrated caseinates is

not micellar, as in milk, MPCs or micellar casein concentrates (MCCs), which results in caseinates having markedly different functional attributes such as poor wettability, high viscosity, excellent heat stability and an ability to remain stable in ethanol solutions.

The major areas of application for casein and caseinate ingredients are food and beverage, industrial, pharmaceuticals and cosmetics, with the food and beverage segment accounting for >70% of the global casein and caseinate market, growing at a rate of 5.0% p.a. (Buyer 2016). The majority of caseinate used commercially in food and beverage applications is sodium caseinate. Casein (probably acid casein) was used as a glue in ancient Egypt, Greece, Rome and China (Soutward 1989). As with many other naturally-produced polymers, such as starch, caseins exhibit excellent adhesive properties, and have been used as one of the major natural adhesive ingredients for thousands of years (Guo and Wang 2016). Around 1960, the situation changed, whereby petroleum-derived products replaced casein in many of these applications as they were cheaper and more consistent. Instead, casein found new applications in the food industry which developed many new products; casein is the functional protein of choice for many food applications, which include beverages, baked goods, coffee creamers, cheese, ice creams, whipped toppings, fudge, meat products, high-fat powders, shortenings, spreads, and nutritional products. The production, composition and functionality of caseins and caseinates have been described by Mulvihill and Ennis (2003), O'Regan and Mulvihill (2011) and Carr and Golding (2016).

#### 4.2.4 Milk Protein Concentrates

MPCs are manufactured from skim milk by ultrafiltration, with or without diafiltration, typically followed by concentration of the total solids using evaporation before spray drying. For the fractionation of milk into casein and whey streams using physical size-based separation (i.e. as opposed to acid- or rennet-induced destabilization of casein micelles), MF membranes, with pore size typically in the range 0.1–0.4 µm are used to generate a casein-rich retentate and a whey protein-containing permeate stream. The casein fraction (i.e. retentate) produced using this approach is referred to as MCC, phosphocaseinate or native micellar casein, while the whey protein fraction (i.e. permeate) is referred to as serum protein, native whey, ideal whey, or virgin whey (Pierre et al. 1992; Kelly et al. 2000; Rizvi and Brandsma 2002; Crowley et al. 2015). Compared with whey obtained as a byproduct of cheese manufacture, MF-derived whey is free of starter culture, rennet enzyme, glycomacropeptide and any colorants (e.g. annatto) that may have been added to the milk in the preceding manufacture of cheese or rennet casein. These differences in composition make this type of whey attractive for formulation of value-added dairy-based products such as protein-rich beverages and infant nutritional formulae (McCarthy et al. 2017).

In the manufacture of MPCs, the lactose: protein ratio decreases as the protein content is increased from ~35% (MPC35) to 80% (MPC80). Higher volume concentration factors during UF, combined with extensive diafiltration, can be used to produce milk protein isolate (MPI) products with a protein content >90%. High-protein MPC and MPI are used as ingredients in a wide variety of food products, ranging from traditional dairy products (e.g. cheese, yogurt) to nutritional beverage formulations (e.g. high-protein beverages for therapeutic use or formulae for lactose-intolerant infants), where their functional attributes (high solubility, contributing opacity, imparting viscosity/

mouthfeel and binding calcium phosphate) and clean label (“milk protein,” “milk protein concentrate”) are desirable.

#### 4.2.5 Micellar Casein Concentrate

When micellar casein (with associated minerals) is separated from the serum proteins in milk, without significantly altering micellar structure, the resultant material is termed an MCC. Less than 10% of the protein (usually 5%) in MCCs is whey protein; however, there is no standard of identity for MCCs and the distinction between MCCs and MPCs is not definitive. The production of MCC involves the use of MF membranes with wider pores than the UF membranes typically used to produce MPCs. The MF process allows whey proteins to be removed in the permeate, along with lactose and other soluble components (Pouliot et al. 1996). Diafiltration with water facilitates further removal of these components, although a certain proportion (~5%) of the whey proteins remains in the retentate with the micellar fraction. The factor which limits whey protein removal may be the progressive growth of a fouling layer comprised of casein micelles, which increases the rejection of whey proteins by a combination of electrostatic and steric repulsion during MF processing (Gésan Guiziou et al. 2013).

When MF is performed at <15 °C,  $\beta$ -casein is depleted from the MCC, with the degree of depletion increasing as temperature is reduced (Crowley et al. 2015). MCC is a useful material for studying casein micelles in their native state and has been used as a model system to study properties relating to the physicochemical aspects of casein micelles (Famelart et al. 1999) and casein micelle structure (Salami et al. 2013; Gonzalez-Jordan et al. 2015). MCCs are also used in sports, clinical, and medical nutrition products and facilitate the development of “slow release” protein formulations for such applications. In addition, MCCs have been used in traditional dairy products such as yogurt and cheese in increasing protein content (Karam et al. 2012), and to encapsulate bioactive substances which are hydrophobic or hydrophilic (Yazdi et al. 2014). Regular MCCs and  $\beta$ -casein-depleted MCCs are major co-products of ideal/native whey and  $\beta$ -casein, respectively, produced by membrane filtration (Crowley et al. 2015). Another active area of application-based research for MCCs is their use as semen-extenders, where they act to preserve the fertility of animal sperm during storage (Batellier et al. 1998; Bergeron et al. 2007). Some researchers have proposed the use of MCCs in liquid concentrates, in gel (Amelia and Barbano 2013) or frozen (Lu et al. 2015) form, which may allow circumvention of issues related to the poor rehydration properties of MCC powders. Compared to MPCs, MCCs are in their infancy and have not yet attracted significant commercial interest from the food industry.

### 4.3 Whey and Whey-Based Products

#### 4.3.1 Introduction

Whey is an aqueous solution containing about 50% of the original nutrients present in milk, such as milk sugar (lactose), serum protein (whey proteins), minerals and all the water-soluble minor components, such as vitamins. In accordance with its origin and processing, whey can be classified as acid whey (acidification), rennet whey (enzymatic

coagulation), cheese whey (enzymatic/acidification) and ideal/native whey (MF). Sweet whey refers to cheese and rennet whey from cheese and rennet casein production, respectively, and acid whey is generated from acid casein manufacture (Bansal and Bhandari (2016). The composition of acid and rennet (sweet) whey differs markedly (Table 4.2). The global production of whey is reported to be ~180–190 million tonnes per year (Mollea et al. (2013), the majority of which is sweet whey, with whey being processed into a wide range of different products (Figure 4.3).

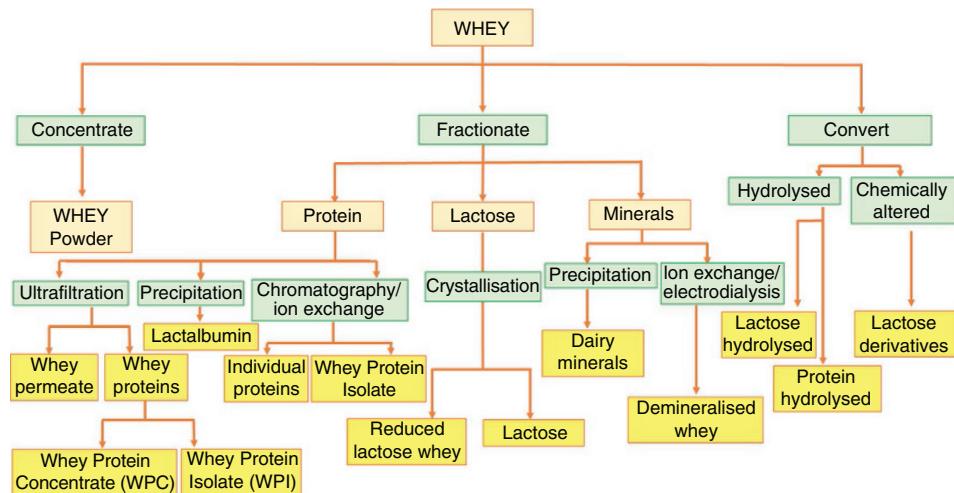
As the largest volume byproduct of the dairy industry, whey represents a disposal/conversion challenge, mainly due to its high lactose content, which is largely responsible for the biochemical oxygen demand (BOD) (30000–50 000 ppm) and its chemical oxygen demand (COD) (50000–80 000 ppm) (Ortega-Requena and Rebouillat 2015). The attitude toward whey and its utilization has changed over the years from being a waste material, to be treated as cheaply as possible (e.g. as pig feed, irrigated on land or dumped into waterways) to a byproduct and currently a value-added raw material. Nowadays, environmental considerations and new technologies make it technologically possible and economically viable to produce a wide range of valuable products from whey (Figure 4.3) with several applications in food, either as functional ingredients or nutritional supplements, and in pharmaceutical applications (Figure 4.4). Whey ingredients

**Table 4.2** Typical composition sweet and acid whey (% by weight).

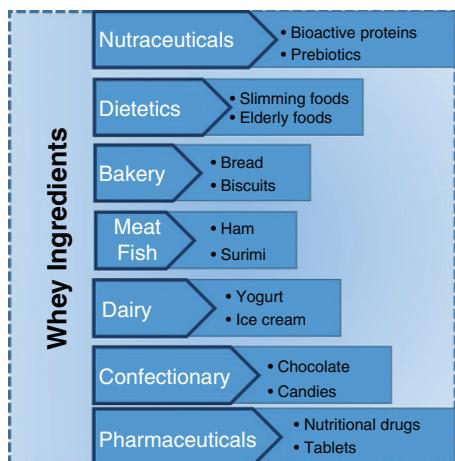
Component	Sweet whey	Acid whey
Fat <sup>a</sup>	0.05	0.03
Lactose <sup>a</sup>	5.0	4.7
Casein <sup>a</sup>	0.10	0.0
Whey protein <sup>a</sup>	0.65	0.57
True protein <sup>b</sup>	0.60	0.60
NPN (non-protein nitrogen) <sup>b</sup>	0.20	0.20
Minerals <sup>a</sup>	0.50	0.80
Calcium <sup>b</sup>	0.035	0.12
Phosphorus <sup>b</sup>	0.040	0.065
Sodium <sup>b</sup>	0.045	0.050
Potassium <sup>b</sup>	0.14	0.16
Chloride <sup>b</sup>	0.09	0.11
Minor components <sup>a</sup>	0.30	0.50
Water <sup>a</sup>	93.4	93.4
<b>Total Solids<sup>b</sup></b>	6.0	6.4
Lactic acid <sup>b</sup>	0.05	0.05
pH <sup>b</sup>	~6.0	< 5.5

<sup>a</sup> From: Mollea et al. (2013).

<sup>b</sup> From: Tetra Pak Processing Dairy Handbook (TetraPak 2017).



**Figure 4.3** Overviewing of processing options for whey and whey products. Source: From: Wisconsin Centre for Dairy Research, University of Wisconsin.



**Figure 4.4** Industrial applications of whey ingredients. Source: Adapted from Ramos et al. (2016).

represent the fastest growing sub category of the dairy ingredients market (Markets (2016), with the market valued at \$45.55 billion in 2015 and projections to reach \$66.11 billion by 2022.

### 4.3.2 Cheese Whey

In 2011, the world production of cheese was approximately 17 million tonnes annually, which equates to an estimated 157 million tonnes of whey (Paterson 2011). Cheese may be produced through the use of enzymes, which coagulate casein, generating sweet whey or by adding acid (e.g. glucono- $\delta$ -lactone, lactic, sulfuric, phosphoric, hydrochloric and

citric) to lower the pH of milk and precipitate the casein, generating acid whey. Sweet whey contains caseino-macropeptides (CMP), produced from  $\kappa$ -casein by rennet action. CMP represents ~20% of the total protein in sweet whey and it is not present in acid whey unless rennet is used in the coagulation process. Cheese varieties from which sweet whey is generated include Cheddar, Mozzarella/Pizza, Swiss and Dutch. Acid whey has a higher level of calcium than sweet whey (Table 4.2); calcium phosphate is more soluble at lower pH; therefore, the lower pH of acid whey will draw more calcium from the cheese curd into the whey than sweet whey. Cheese varieties from which acid whey is generated include Cottage, Ricotta, Quark and cream cheese (Smith 2008). During cheese making, annatto is often added to cheese-milk to confer a yellow/orange color to cheese; however, residual annatto in whey protein ingredients is undesirable in many applications (Kang et al. 2010), with a clear and colorless whey ingredient usually preferred. In addition, there are strict regulatory limits on the concentrations of norbixin, the principal carotenoid in annatto, in ingredients destined for application in infant formulae (Campbell et al. 2014), while the presence of bleaching agents (e.g. benzoyl peroxide or hydrogen peroxide) in whey-based ingredients intended for use in infant formula is also strictly regulated. These regulatory hurdles are accelerating efforts to reduce carry-over of colorant and/or bleach residues into whey intended for use in manufacture of premium nutritional ingredients. These considerations have intensified research into the use of alternative colorants in cheese making such as  $\beta$ -carotene, which is naturally present in milk (Moeller et al. 2014), novel colorant ingredients which associate with the casein protein-based cheese matrix and are not carried over into the whey stream (e.g. ClearWhey from Cybercolors, Cork, Ireland and Whitewhey<sup>TM</sup> from Chr Hansen, Copenhagen, Denmark, respectively), and approaches involving the production of cheese from MF retentates of milk, whereby the native/ideal whey is physically removed upstream of cheese making.

From a valorization point of view, two different options for utilization of cheese whey can be considered: the first one is based on the application of technologies to recover valuable components such as proteins and lactose (Mollea et al. 2013), which is the subject of the next several sections. The second option relies on the application of fermentation processes to obtain value-added products (Prazeres et al. 2012) such as organic acids (e.g. lactic, succinic, and propionic), single cell proteins and oils, biopolymers (enzymes, polyhydroxyalkanoates, exopolysaccharides), and bacteriocins, some of which are discussed in later sections of this chapter.

### 4.3.3 Whey Cream and Whey Butter

Whey cream, a byproduct generated from the processing of whey, is physically removed from fresh whey, by centrifugal separation, to reduce the fat content of whey protein-based products and to improve the efficiency of protein enrichment processes from whey (e.g. ultrafiltration). Whey cream has a similar composition to that of sweet cream, but higher levels of unsaturated fat, biologically active phospholipids (e.g. sphingomyelin) and proteins (e.g. mucins), with the latter components originating from the milk fat globule membrane (MFGM). Therefore, whey cream has the potential as a starting material for the production of bioactive lipid-rich ingredients with interesting nutritional and technological functionalities. Whey butter, which is produced by churning whey cream, has been gaining attention from the dairy industry due to the large volumes of whey and

whey cream produced by the cheese manufacturing industry (Jinjarak et al. 2006). There are currently very few economically attractive uses for this co-product, and it is generally absorbed by the mainstream butter industry, with substantial quantities being converted into butter oil. Any research contributing to better valorization of this product would be of value to butter manufacturers. From the production of whey butter, whey buttermilk (WBM) is generated as a byproduct. Sodini et al. (2006) refers to WBM as a potentially novel ingredient, with better emulsifying properties and lower foaming ability, along with good levels of protein solubility, viscosity, and emulsifying capacity over a pH range of 4–6, compared with both sweet and sour buttermilk. However, WBM is more salty, sour and astringent than sweet cream buttermilk (Olabi et al. 2015).

#### 4.3.4 Whey Powder

Whey powder is a commodity dried whey product produced from fresh whey by concentration of the solids using evaporation, cooling, seeding with lactose crystals, and spray drying of the resultant material. The fresh whey is normally pre-treated using clarification to remove cheese and curd fines, separation to remove fat (i.e. whey cream) and thermalization or pasteurization to inactivate residual rennet and starter culture activity (in the case of cheese and rennet whey). These pre-treatments of fresh liquid whey are generally common for the production of all whey-based ingredients. Whey powder can be difficult to dry, especially acid whey-based powder, due to the high concentration of lactose in all whey powder products, and minerals and lactic acid in the case of acid whey specifically. Crystallization of lactose before spray drying is required as the crystallized form of lactose is considerably more stable during spray drying and produces a less hygroscopic powder with improved yield and better physical stability of the dried powder product. Nanofiltration (NF) is sometimes used to pre-concentrate pre-treated liquid whey prior to evaporation as partial demineralization is achieved in addition to an increase in total solids, helping to improve drying performance of the resultant concentrate. Spray drying, with integrated fluid bed technology is generally used to dry whey powders while belt drying processes are sometimes used also. Whey powders typically contain 11–13% protein, 72–75% lactose and 8–9% minerals and have a wide range of applications in food formulations, e.g. ice-cream, bakery, and desserts due to key nutritional and physicochemical functionalities, such as high solubility, low viscosity and good heat stability.

#### 4.3.5 Demineralized Whey

While whey powder has many applications, one of the compositional aspects which limits its use in more value-added applications is the high (8–9%) mineral content. Demineralized whey is manufactured by removing minerals from whey using one or more technologies including, ion exchange, electrodialysis and NF. Electrodialysis was first introduced for the demineralization of whey in the 1960s, selectively removing sodium and chloride ions, making possible the commercial production of whey protein-dominant infant nutritional products. Ion exchange technology was introduced more recently in the dairy industry for the demineralization of whey as it offers more flexibility to remove minerals (both mono- and divalent ions). NF is increasingly being used to pre-concentrate and partially demineralize liquid whey and many newer commercial whey demineralization plants use combinations of two or more of these technologies. Demineralized whey is an important ingredient in the infant formula industry, where there are

strict regulatory limits on the levels of individual minerals in the final product, while it is also used in other applications (e.g. ice cream, bakery, and confectionery) where the high mineral content of regular whey powder would be an issue. Demineralized whey is available with different extents of demineralization, and demineralized whey 90 (i.e. Demin 90) is the most common form, being used extensively in infant nutritional products, whereby the mineral load of regular whey powder is reduced by ~90% (i.e. from 9% to 1% in the whey powder ingredient).

#### 4.3.6 Whey Protein-Based Products

On a commercial scale, a range of whey protein-enriched products can be prepared from pre-treated liquid whey using different approaches. WPCs are manufactured by UF or combined UF/DF of whey, followed by the concentration of the total solids, either by vacuum evaporation or NF and spray drying. The lactose: protein ratio decreases as the protein content increases from ~35% (WPC35) to 80% (WPC80). Higher protein concentration factors and degrees of diafiltration are required to achieve a higher protein content. High-protein WPCs have increased in popularity in recent years due to the proliferation of nutritional products (e.g. beverages and bars) which are often formulated to be rich in protein and low in carbohydrate. WPIs can be manufactured by membrane separation or ion exchange, in which the proteins are adsorbed on an ion exchange resin, washed free of lactose and salts, and then selectively eluted with acid or alkali. Alternatively, a combined MF/UF/DF process can be used, much like the production of a high-protein WPC except that fat is removed by MF. In such processes, UF is generally used to first concentrate the proteins in liquid whey, thereby reducing the hydraulic load on the MF step. During UF, any residual fat and phospholipid material from the liquid whey is concentrated along with the protein, but is removed subsequently by MF. A second UF step is then used to concentrate the whey further. WPIs with a protein content of 90–95% are available, and are typically used in high-end nutritional products (e.g. sports nutritional supplements). Compositional differences between WPIs produced by ion-exchange or membrane filtration are relatively small, although those produced by membrane filtration generally contain a much higher level of CMP.

The functional properties of WPCs and WPIs are very interesting and render these two types of whey protein ingredients extremely versatile and they support the formulation and development of many food products, as follows (Ramos et al. 2016):

- improve aeration in bakery and confectionary products;
- improve color and taste by interaction between proteins and lactose during thermal processing (Maillard reactions) in candy products (e.g. toffees, caramels, and cooked syrups);
- replace skim milk powder in dairy product formulations (e.g. yogurt, ice cream and milk chocolate drinks);
- improve the quality of meat and fish due to emulsifying, gelatin and hydrophilic attributes;
- develop infant formula with nutritional benefits by adjusting the formula composition to that of human milk; and
- develop dietetic foods with high satiety value – low fat and high protein content, with an excellent amino acid composition.

High-protein WPC and WPI products are key ingredients in several growth areas of the food industry, such as infant formulae, clinical nutrition and sports nutrition. These ingredients contribute the majority of the protein in low-lactose and lactose-free first-stage infant formulae, which have a whey protein: casein ratio similar to human milk (i.e. 60 : 40). In clinical/sports nutrition, WPCs/WPIs are valued for their high concentrations of essential and branched-chain amino acids, and their ability to aid muscle synthesis.

#### 4.3.6.1 Selectively-Enriched Protein Fractions

Enriched fractions of individual whey proteins are attracting increasing interest industrially and are generally prepared from pre-treated fresh liquid whey. WPCs enriched in  $\alpha$ -lactalbumin ( $\alpha$ -la) can be produced in two ways, (i) where  $\alpha$ -la enrichment is achieved by concentrating  $\alpha$ -la resulting in an increased  $\alpha$ -la: $\beta$ -lactoglobulin ( $\beta$ -lg) ratio or (ii) where CMP has been depleted resulting in an increased  $\alpha$ -la level on a weight basis but an unchanged  $\alpha$ -la: $\beta$ -lg ratio. Such ingredients are of interest for use in premium infant formula and medical/therapeutic products (e.g. sleep promotion properties of  $\alpha$ -la).

Lactoferrin and lactoperoxidase are produced from whey using ion-exchange chromatography as these proteins have very high isoelectric points and are positively charged at the natural pH of milk/whey, which allows their selective adsorption/elution from whey (and occasionally, milk) using cation exchange resins. Lactoferrin is a major protein in human milk, where it is present at a concentration approximately 10-fold higher than in bovine milk. Infant formulae supplemented with lactoferrin are sold in Asia (Tomita et al. 2009) and lactoferrin has also found application in cosmetic and health-care applications (El-Loly and Mahfouz 2011). To produce 1 kg of lactoferrin, a large volume of whey (~10 000 l) is needed (Etzel 2004) which means that the final ingredient typically commands a high price (~\$200–300/kg), which in turn limits the potential consumer-base for those formulating food products.

Osteopontin is a minor whey protein which can be enriched in whey using different combinations of MF and ion exchange. This ingredient is considered to have potential health benefits (e.g. bone mineralization) when added to infant formula.

CMP is a valuable ingredient, particularly as a dietary source for sufferers of phenylketonuria (PKU). PKU is characterized by an inability to metabolize the essential amino acid, phenylalanine, which virtually eliminates proteins as a source of amino acids in the PKU diet. Protein substitutes in PKU diets are based mostly on phenylalanine-free blends of amino acids. CMP is free of aromatic amino acids, including phenylalanine, making it a viable alternative to amino acid blends (van Calcar et al. 2009). Large-scale purification of CMP from sweet whey has been achieved, with the most successful methods employing ion exchange of whey (Etzel 1999; McMahon et al. 2006). The whey fraction generated during renneting of MCC is also enriched in CMP.

#### 4.3.7 Lactose and Lactose Derivatives

##### 4.3.7.1 Introduction

Lactose is the principal carbohydrate in the milk of most mammals, at a concentration which varies widely between species. As the principal solid constituent in bovine milk, representing ~35% of the total solids in normal milk, lactose is the principal constituent

in many dairy products, ranging from ~40% in WMP to >80% in demineralized whey powder. Therefore, the properties of several dairy products, especially concentrated and dehydrated products, are dominated by certain properties of lactose, especially its solubility, crystallization behavior, mutarotation properties and its propensity to Maillard browning (McSweeney and Fox 2009).

In the manufacture of cheese, whey represents 85–95% of the initial milk volume. After the removal of valuable whey proteins by UF, the remaining whey permeate can contain up to 85% lactose on a dry matter basis. The recovery of lactose from whey permeate can be not only economically advantageous (by adding value to an underutilized byproduct of whey protein manufacture), but also offers a significant contribution to environmental aspects, since lactose is largely responsible for the high BOD and COD of whey (Geiger et al. 2016). From cheese alone, 157 million tonnes of whey were produced globally in 2011, which roughly represents ~7 million tonnes of lactose. However, Paterson reported a global lactose production of only 0.89 million tonnes the same year, indicating there is a large excess of lactose potentially available as a byproduct (Paterson 2011).

The major commercial organizations in the whey and lactose ingredient industry are the leading dairy and cheese companies in the world such as Lactalis, Friesland Campina, Fonterra, Arla, Saputo, Glanbia, Murray Goulburn, DMK/Wheyco, Leprino, Agropur, Sachsenmilch, Bongrain/Armor Proteines, Sodiaal/Euroserum and Hilmar. Specialist whey and lactose ingredient companies such as Milk Specialties Global, Meggle, Milei, Volac and Carbery also play a similar important role in the global market place. The world's two largest dairy companies (Nestlé and Danone) are rarely actual producers, but they are major end users of whey and lactose ingredients, for applications such as infant formulae.

Besides the production of edible lactose (unmodified), lactose can be modified chemically, enzymatically, or microbiologically into a wide range of derivatives including galacto-oligosaccharides, lactulose, lactitol, lactobionic acid, hydrolyzed lactose, and tagatose, which have found largely *niche* markets with various uses. The chemistry and properties of lactose have been characterized thoroughly, see Fox (2009) and Fox et al. (2015b). The structure and properties of lactose, lactose-based products and their applications and approaches for derivatizing and adding value to lactose, are summarized in the following sections.

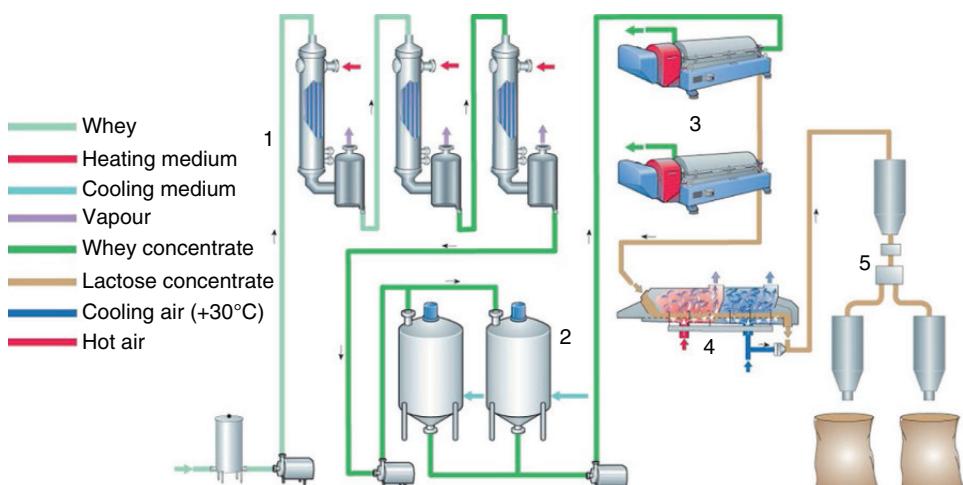
#### 4.3.7.2 Structure and Properties of Lactose

Lactose is a disaccharide consisting of galactose and glucose, linked by a  $\beta$ 1–4 glycosidic bond. The hemiacetal group of the glucose moiety is potentially free (i.e. lactose is a reducing sugar) and may exist as  $\alpha$ - or  $\beta$ -anomers. In contrast with other common sugars, lactose has low solubility; at 20 °C, the solubility of  $\alpha$ -lactose is ~7 g/100 g water while the solubility of  $\beta$ -lactose is ~50 g/100 g water. Because of its lower solubility, the usual commercial form of lactose is  $\alpha$ -lactose monohydrate. Although lactose has low solubility, when in solution it is difficult to crystallize, and, unless crystallization is controlled, will cause a sandy textural defect in liquid dairy products. Lactose is less sweet than the other common sugars, which limits its value as a sweetener, the principal use of sugars, but is beneficial for some applications for which sweetness is undesirable, e.g. as an excipient for medicinal compounds. Since lactose is a reducing sugar it can participate in the Maillard reaction with the formation of brown pigments, (off)-flavors, and a reduction in the level of lysine. The development of color and flavor is desirable in some products

(e.g. to enhance color and flavor development in bakery products), but is undesirable in others, either because of the alteration of color and flavor or because of possible reduced nutritional value due to loss of some amino acids. Caramelization (produced by heating carbohydrates above 110 °C) is generally a desirable process that produces tan to dark brown color, pleasant aromas and flavors, but can result in undesirable sensory attributes as the degree of caramelization increases (Clemens et al. 2016). For further information on the chemistry, structure, nutritional and physicochemical properties of lactose see Fox et al. (2015b).

#### 4.3.7.3 Lactose-Based Products and their Applications

The global market for lactose is projected to exceed 1.3 million tonnes by 2022, driven by the growing popularity of dairy ingredients that improve nutrition, taste, and flavor. Attributes such as water solubility, affordability, and availability, along with its wide range of industry applications and health benefits, have also driven the increase of lactose production and consumption (Global Industry Analysts 2017). Due to its ready availability, whey is still the major source of lactose. The production of lactose essentially involves concentrating liquid whey or UF permeate of whey under vacuum, crystallization of lactose from the concentrate, recovery of the crystals by centrifugation, washing to remove other constituents and drying the crystals (Figure 4.5). The first-crop crystals are contaminated with riboflavin and are therefore yellowish; a higher grade, and hence more valuable, lactose is produced by re-dissolving and recrystallizing crude lactose. Lactose may also be recovered by precipitation with Ca(OH)<sub>2</sub>, especially in the presence of ethanol, methanol, or acetone (Paterson 2009, 2011). Lactose can be converted by chemical reaction, fermentation or hydrolysis into several derivatives, which are addressed in Section 4.3.7.4. The production of edible lactose was described in detail by Paterson



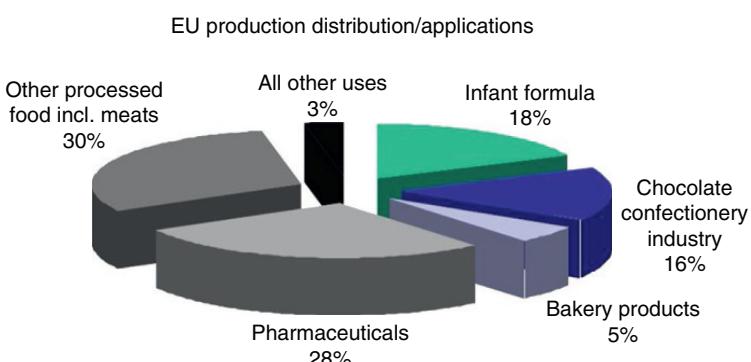
**Figure 4.5** Process line for lactose manufacture. Source: From: Tetra Pak Processing Dairy Handbook (TetraPak 2017). 1-Evaporator; 2-Crystallization tanks; 3-Decanter centrifuges; 4-Fluid-bed dryer; 5-Packing.

(2009), who subsequently reviewed the typical challenges encountered during its production (Paterson 2011).

Lactose products are diverse and the product specificity is linear to the level of added value. The principal applications of lactose are in the food (69%) and pharmaceutical (28%) industries with minor uses in, for example, animal feed and bio-fuel production (3%). In the food industry, lactose is principally used in the processed food (including meats) sector (30%) followed by the infant formulae (18%) and confectionary (16%) sectors (Figure 4.6) (Affertholt-Allen 2007). Lactose has a number of low-volume, specialty applications, for example, as a free-flowing or agglomerating agent, to accentuate/enhance the flavor of some foods, to improve the functionality of shortenings and as a diluent for pigments, flavors, or enzymes (O'Mahony and Fox 2014).

In bakery applications, lactose is used for its resistance to yeast fermentation and hence provides a reducing sugar for the Maillard browning reaction at the surface of baked goods, which facilitates development of a desirable brown crust. In the production of some confectionary products, it is the ability of lactose to make good quality caramel that makes it a more attractive choice than other sugars. Another reason for choosing lactose in baking and confectionary production is that it is not as sweet as sucrose and has better mouthfeel – some of the reasons why it is used extensively in chocolate manufacture. The lactose required for these and other food products needs to be of an edible standard, but does not need to be ultra-pure. This market currently uses around 780 thousand tonnes of lactose per annum (Paterson 2011). The most important application in the food sector is probably in the manufacture of humanized infant formulae based on cows' milk (human milk contains ~7% lactose compared with ~4.8% in bovine milk). The lactose used in this application can be an edible- or pharmaceutical-grade crystalline product or demineralized whey (for physiological reasons, it is necessary to reduce the concentration of inorganic salts in bovine whey) – for further information see Fox et al. (2015b).

The other main use of lactose is in the pharmaceutical industry, which requires high quality, extra-pure lactose, and therefore is more expensive, where it is used as an excipient for making tablets and as a carrier in dry powder inhalers. Lactose has also been used for the production of bio-plastic, namely polyhydroxyalkanoate (PHA) bio-polyesters, which are a group of compostable bio-plastics of increasing significance for numerous



**Figure 4.6** EU market structure for lactose in 2005. Source: From Affertholt-Allen (2007).

industrial applications (Koller et al. 2012). The use of lactose for plastic production was addressed by Ghaffar et al. (2014) and Watanabe et al. (2014).

#### 4.3.7.4 Approaches for Derivatizing and Adding Value to Lactose

Although the demand for lactose has been strong in recent years, it is unlikely that a profitable market exists for all the lactose potentially available from whey. For many years, the most promising idea for converting or adding value to lactose was considered to be hydrolysis to glucose and galactose. Currently, much UF permeate is converted into whey permeate powder and sold as a commodity product, but other modifications are attracting increasing attention (Paterson 2011). Derivatives can be obtained from lactose by chemical, enzymatic, or microbial modifications, including galacto-oligosaccharides, lactulose, lactitol, lactobionic acid, hydrolyzed lactose, and tagatose (Figure 4.7). The areas where current research indicates that significant amounts of lactose might be needed to meet demand are in the production of galacto-oligosaccharides (GOS) for addition to infant formulae to make them more like human milk, and in the production of tagatose, which has potential as a sugar replacer. If the price of lactose remains low, then it is possible that lactose could become an economical substrate for the production of bioethanol for use in transportation fuels or as a substrate for other fermentation products. The lactose market is growing as well as the demand for lactose specialities, which have a higher market value than the current commodity products, e.g. permeate powder and edible lactose (Figure 4.8).

##### 4.3.7.4.1 Enzymatic

Due to the abundance of lactose in whey, one approach to increase the value of whey that has attracted increasing attention, is the bioconversion of lactose to more valuable products using  $\beta$ -galactosidase. The  $\beta$ -Galactosidases ( $\beta$ -Gal; EC 3.2.1.23) catalyze both the hydrolysis and transgalactosylation of  $\beta$ -D-galactopyranosides, including lactose and are widespread in nature. They catalyze the hydrolysis of lactose and are used in the dairy

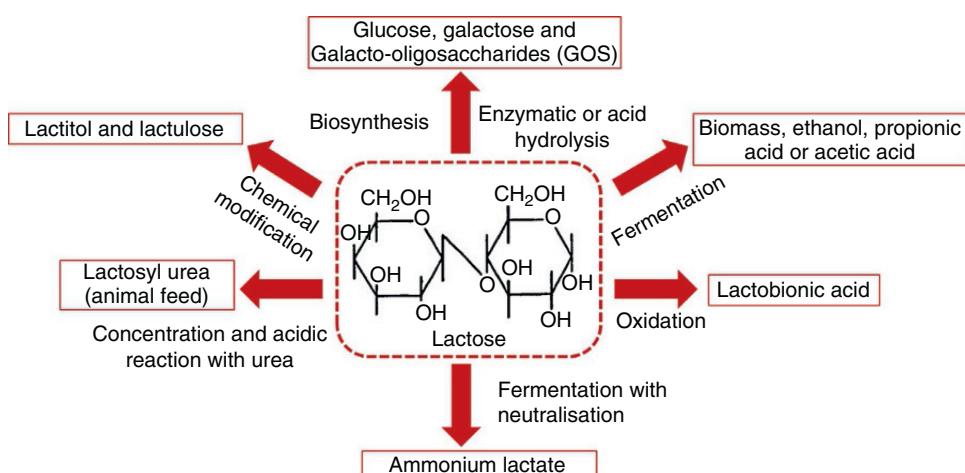


Figure 4.7 Lactose derivatives for food applications.

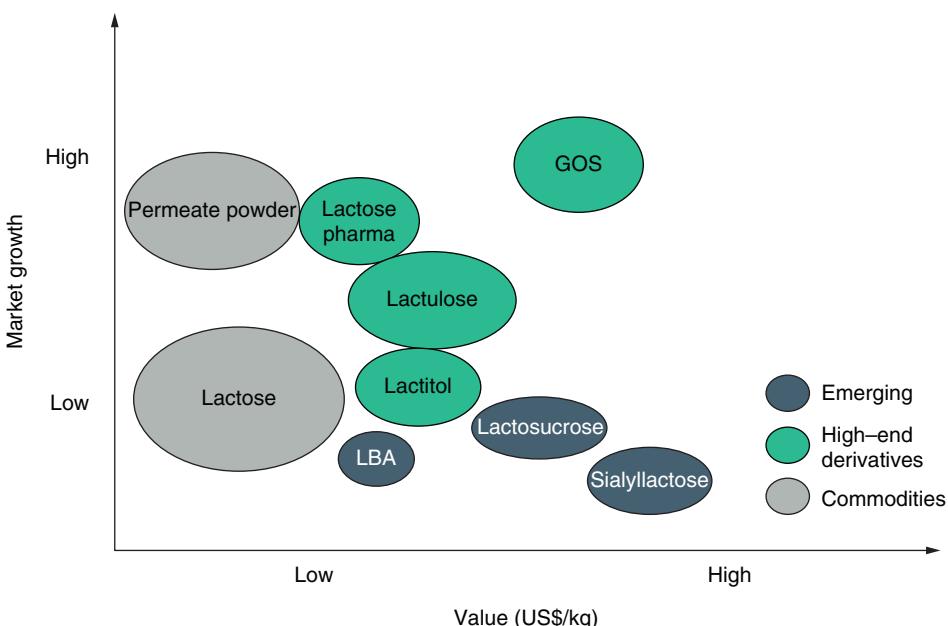


Figure 4.8 Growth opportunities for lactose and derivatives. Source: From Affertholt-Allen (2007).

industry to remove lactose from various products. These enzymes also show transgalactosylation activity, which is of interest because the resulting galacto-oligosaccharides (GOS), are non-digestible carbohydrates with known prebiotic activity (Geiger et al. 2016). Commercial preparations of GOS include Vivinal® GOS, manufactured by Friesland Campina (The Netherlands) and Bimuno®, from Clasado Biosciences (UK).  $\beta$ -Galactosidases can be obtained from various sources, including microorganisms, plants and animals and when they became commercially available, they were considered to have considerable commercial potential as a solution to the “whey problem” and for the treatment of lactose intolerance. In fact, enzymatic hydrolysis of lactose by  $\beta$ -galactosidase has become one of the most popular technologies to produce lactose-reduced milk and related dairy products for consumption by lactose-intolerant people (Husain 2010). An extensive list of bacterial and fungal sources of  $\beta$ -galactosidases, as well as the lactose conversion reaction conditions and yields of GOS, are given in the review by Torres et al. (2010). In addition, extensive literature on  $\beta$ -galactosidases have been reviewed by Playne and Crittenden (2009), Husain (2010), Panesar et al. (2010), Harju et al. (2012) and O’Mahony et al. (2013).

An estimated 70% of the adult human population have inadequate intestinal  $\beta$ -galactosidase activity and are, therefore, lactose intolerant. The problem is particularly acute among Asians and Africans and pre-hydrolysis of lactose was considered to offer the potential to develop new commercial opportunities for dairy products in those regions. Various protocols are available: addition of  $\beta$ -galactosidase to milk in the home, pre-treatment of milk at the factory with free or immobilized enzyme or aseptic addition of sterilized  $\beta$ -galactosidase to UHT milk, which appears to be particularly successful. Glucose-galactose syrups are about three times sweeter than lactose (70% as sweet as

sucrose) and hence lactose-hydrolyzed milk could be used in the production of ice cream, yogurt, or other sweetened dairy products, permitting the use of less sucrose and reducing caloric content. Why the enzymatic hydrolysis of lactose in milk is not used widely and such applications have had limited commercial success, is not clear. One possible reason is that the quality of lactose-free milk can be affected by Maillard reactions due to the presence of reducing monosaccharides, glucose, and galactose, which are significantly more reactive in Maillard reactions than lactose, causing browning, off-taste, and reduction in the nutritive value of milk protein. To avoid these problems, heat treatment of lactose-free milk should be as gentle as possible (Harju et al. 2012). Acid hydrolysis of lactose, as an alternative to the enzyme technology, does not appear practical in untreated whey due to pronounced browning and protein precipitation. Thus, it seems that the treatment of milk with  $\beta$ -galactosidase will be commercially successful only in *niche* markets.

#### 4.3.7.4.2 Chemical

The lactose derivatives, lactulose, lactitol, and GOS find applications in foods and pharmaceutical preparations as prebiotics to promote gut health. Like undigested lactose, these compounds enhance the intestinal absorption of calcium and magnesium. Other lactose-derived compounds, e.g. tagatose and lactobionic acid, have potential applications as a bioactive ingredient in foods. Some of the most interesting derivatives that can be produced from lactose are summarized in brief below. For more detailed information on lactose and lactose derivatives see Schaafsma (2008) and Ganzle (2011).

**4.3.7.4.2.1 Lactulose and Lactitol** Lactulose ( $\beta$ -D-galactosyl-D-fructose) is an epimer of lactose in which the glucose moiety is isomerized to fructose. This sugar, which does not occur naturally, was first synthesized by Montgomery and Hudson (1930). Lactulose is a high added-value product of lactose and it can be produced under mild alkaline conditions via the Lobry de Bruyn-Alberda van Ekenstein reaction and at a low yield as a byproduct of  $\beta$ -galactosidase action on lactose. It is produced on heating milk under sterilizing conditions and is a commonly used index of the severity of the heat treatment to which milk has been subjected, e.g. to differentiate in-container sterilized milk from UHT milk; it is not present in raw or HTST pasteurized milk. Lactulose is not hydrolyzed by intestinal  $\beta$ -galactosidase and hence reaches the large intestine where it can be metabolized by LAB, including *Bifidobacterium* spp., acting as a prebiotic carbohydrate, which stimulates the growth of health-promoting bacteria in the gastrointestinal tract and inhibits the growth of pathogenic bacteria such as *Salmonella*. Lactulose-derived oligosaccharides may form a new group of prebiotics with properties complementary to those of lactulose (Panesar and Kumari 2011). Studies have shown that supplementing preterm infants' feeds with low doses of lactulose might have positive prebiotic effects (Riskin et al. 2010). Lactulose production, purification and potential applications, both for food and pharmaceutical uses were described by Olano and Corzo (2009), Panesar and Kumari (2011) and Aït-Aissa and Aïder (2014). Sitanggang et al. (2016) and Parekh et al. (2016) reviewed lactulose production and significance in milk and milk products.

Lactitol ( $\beta$ -D-galactosyl-sorbitol) is a sugar alcohol produced on reduction of lactose, usually using Raney nickel; it does not occur naturally. It can be crystallized as a mono- or di-hydrate and is not metabolized by higher animals; it is relatively sweet and hence has potential as a non-nutritive sweetener with a wide range of applications in food, namely

to lower the caloric value of products such as jams, marmalade, chocolate, and baked goods. Being non-hygroscopic, it can be used to coat moisture-sensitive foods such as candies. It is claimed that lactitol reduces the absorption of sucrose, reduces blood and liver cholesterol levels, and is anti-cariogenic – for further details please see Fox et al. (2015b). As lactitol is not digested in the small intestine and is fermented by the colonic flora it exhibits a prebiotic effect. Both lactulose and lactitol are widely used in the treatment of patients with hepatic encephalopathy (intoxication of the brain caused by failure of the liver to convert ammonia to urea) and in patients with chronic constipation. Lactitol is positioned mostly in the food sector and used as a bulking agent for sugar-free products. In 2005, 10 000 tonnes of lactitol were produced, worth \$50 million. Danisco (Denmark), Purac (the Netherlands) and Towa (Japan) are among the main producers of lactitol (Affertholt-Allen 2007).

**4.3.7.4.2.2 Lactobionic Acid** Lactobionic acid ( $\beta$ -D-galactosyl-gluconic acid) is produced by oxidation of the free carbonyl group of lactose and it is a relatively new compound that has not yet found application in the European food market. The acid combines a sweet taste, which is very unusual for an acid, with pH-reducing effects. Its lactone crystallizes readily and it has strong mineral-complexing properties, making it suitable for applications as a food ingredient (Schaafsma 2008; Fox et al. 2015b). Lactobionic acid is resistant to digestive enzymes and will be fermented by the intestinal flora, probably exerting prebiotic effects. It is used in preservation solutions for organs (to prevent swelling) prior to transplantation, and in skin-care products (Fox et al. 2015b). Although it is seen as a potential novel food, safety assessment and more clear evidence of mechanism of action are needed, before the compound can be marketed as a food ingredient.

**4.3.7.4.2.3 Lactosyl Urea** Urea can serve as a cheap source of nitrogen for cattle but its use is limited because ammonia is released too quickly, leading to a toxic level of ammonia in the blood. Reaction of urea with lactose yields lactosyl urea, from which ammonia is released more slowly than from urea (Fox et al. 2015b). Lactosyl urea is produced by the nucleophilic reaction of the amine group of urea with the carbonyl functional group of lactose (first step in the Maillard reaction) and it is used as a source of nitrogen in animal feed (Crouquennec et al. 2016).

**4.3.7.4.2.4 Tagatose** Tagatose, a derivative of galactose, is also a relatively new compound, industrially speaking. In 2005, it was approved as a food ingredient in the European Union and now has generally recognized as safe (GRAS) status. Only 20% of tagatose is digested, the remaining 80% is fermented in the colon where it exerts prebiotic effects, favoring the production of short-chain fatty acids and the growth of LAB and has little effect on blood glucose. As a low-calorie sweetener and prebiotic, tagatose can be included in a large variety of products, namely dairy, beverages, confectionary, bakery, health bars, chewing gum, and dietary supplements (Schaafsma 2008). Tagatose is nearly as sweet as sucrose and enhances the flavor contribution of other sweeteners. Tagatose is produced commercially by SweetGredients, a company formed by Arla and Nordzucker (Denmark) (Paterson 2011).

#### 4.3.7.4.3 Fermentation

Lactose, either in dairy permeate or pure lactose solutions, is readily fermented by LAB, especially *Lactococcus* spp. (Liu et al. 2016) and *Lactobacillus* spp. (Maślanka et al. 2015), to lactic acid. Lactic acid is widely used in the production of boiled sweets, pickled foods, and as a raw material in the manufacture of important emulsifiers for the baking industry. It can be used as a food acidulant, flavoring agent (e.g. fruit drinks and desserts), preservative, restricting the growth of microorganisms (e.g. tomato sauce and mayonnaise), chelating agent (e.g. in fats and oils), gelling agent (e.g. pectin in jams), and as a coagulating agent (e.g. acidified cheese and desserts). Lactic acid may also be used as a feed-stock, in the manufacture of plastics, or converted to ammonium lactate as a source of nitrogen for animal nutrition. Lactic acid has been reviewed by Ghaffar et al. (2014).

Propionic acid (PA), produced from lactic acid by the action of *Propionibacterium* spp., has many industrial applications, mainly as a chemical intermediate in the synthesis of cellulose fiber, herbicides, perfumes, and pharmaceuticals. PA is also an important mold inhibitor, and its ammonia, calcium, sodium and potassium salts are used widely as preservatives in animal feed and human foods (Liu et al. 2015). Lactose can also be used as a substrate for *Xanthomonas campestris* in the production of xanthan gum, which has many important rheological and structural applications in dairy (e.g. cheese, cheese products, milk and cream products), bakery products, dressings, table syrups, sauces, gravies, and beverages (e.g. pulp fruit beverages), mainly due to its unique rheological behavior (e.g. mouthfeel and flavor release), stability with salts, resistance to enzymes and water binding properties (Sharma et al. 2006).

For the purpose of alcoholic fermentation, yeasts (i.e. *Saccharomyces cerevisiae*) are usually used since they have a fast fermentation capacity and tolerate high concentrations of ethanol (up to 20% v/v). Since *S. cerevisiae* cannot ferment lactose, the lactose component of whey has to be enzymatically hydrolyzed prior to the alcoholic fermentation. The hydrolysis step is not required if *Kluyveromyces* spp. (e.g. *K. marxianus* var. *marxianus* and *Kluyveromyces fragilis* var. *marxianus*) are employed for the production of bioethanol, as they have the ability to catabolize lactose (Siso 1996; Pesta et al. 2006; Guimaraes et al. 2010; Hadiyanto et al. 2014). Over the past few decades, lactose from whey permeate has been used for bioethanol production in Ireland, New Zealand, Denmark, and in the United States of America (USA) (Guimaraes et al. 2010). In 1976, Carbery Group Ltd. was one of the first Irish dairy companies to use whey permeate from cheese manufacture to produce ethanol, which is used, among other applications, in the production of alcoholic drinks such as cream liquors. Bioethanol produced in this way may also be used for industrial purposes, or as a biofuel, but in most cases is probably not cost-competitive with ethanol produced by fermentation of sucrose or chemically. Most commonly, ethanol is produced from sugar cane or sugar beet, different crops or from cellulosic resources (wooden hydrolysates, agricultural byproducts) (Božanic et al. 2014).

The mother liquor remaining from the production of lactic acid or bioethanol may be further subjected to anaerobic digestion with the production of methane, used as a fuel (Ziemiński and Frąc 2012). Several such plants are in commercial use and Europe has a leading role in the field of biogas production due to the EU policies around renewable energy and in the more specific field of biofuels, where Germany is dominant. In the context of dairy side-streams and byproduct utilization, it is important to consider that many

of the fermentation-based modifications of lactose are probably not economical because lactose is not cost-competitive with alternative fermentation substrates, especially sucrose in molasses or glucose produced from starch; however, some of the scientific advances outlined above show promise in the production of a new generation of products, including energy, from whey (Boura et al. 2017).

#### 4.3.8 Oligosaccharides

In addition to lactose, the milk of most, probably all, species contains other free saccharides, mainly oligosaccharides (OSs), the concentration, proportions, and types of which show large interspecies differences. General reviews on milk OSs include Mehra and Kelly (2006), Urashima et al. (2013) and Oliveira et al. (2015). Among other functions, human milk oligosaccharides (HMOs) play an important role in modulating the epithelial and immune cell responses and contribute to the maturation of the immune system and in the development of the neonatal brain and cognition functions (Bode 2012; Bode et al. 2016). Because, they are not hydrolyzed by human digestive enzymes and are fermented by colonic bacteria with the production of short-chain fatty acids, CO<sub>2</sub> and H<sub>2</sub>, they specifically stimulate the growth and metabolism of intestinal bifidobacteria (Ganzle 2011). Therefore, there have been increasing efforts to mimic HMOs, their structures and especially their health benefits, and considerable interest exists in the development of OS-enriched ingredients for infant nutritional applications in particular.

Given the commercial potential of OSs, several strategies have been investigated to recover, enrich and purify those naturally occurring OSs from the milk of a number of domestic species, namely cow, sheep, and goat, which contain relatively low levels of OSs, compared with human milk. See, for example Urashima et al. (2001), Zivkovic and Barile (2011), Urashima et al. (2013) and Albrecht et al. (2014). During cheese production, almost all the lactose and most of the OSs in milk are transferred into whey, thus whey-based dairy streams represent a potential source of natural milk OSs for food applications (Mehra et al. 2014). Deproteinized and delactosed whey permeate are the two most commonly used starting materials in the development of processes for the recovery of OSs from dairy streams. Some possible approaches for producing OSs similar to those found in human milk, by recovering OSs from cow's milk whey or UF permeate were discussed by Mehra and Kelly (2006) and O'Mahony and Tuohy (2013), and generally involve the application of one or more unit operations including membrane filtration (e.g. nanofiltration) and chromatography (e.g. ion-exchange) for the separation and recovery of OSs from the other principal solid constituents of whey permeate (i.e. lactose and minerals). Similarly, Oliveira et al. (2012b), described a process for the isolation of OSs from caprine whey using membrane technology. In addition to containing about 10 times as much OSs as bovine or ovine milk, the OSs from caprine milk are the most similar (structurally) to those of human milk and have been shown to have prebiotic and anti-infective properties (Oliveira et al. 2012a), offering an alternative to bovine milk-derived OSs. However, the extraction of OSs from natural dairy sources is hampered by substrate availability, variability therein and cost of extraction, and such ingredients are still not commercially available food ingredients, unlike enzymatically-produced galacto-oligosaccharides (GOS), frequently used as prebiotic ingredients in several food formulations, especially infant formula.

## 4.4 Buttermilk

When cream is churned as part of the butter making process, an aqueous phase called buttermilk (BM), and a fat phase (i.e. butterfat) are generated (Conway et al. 2014b). BM is the byproduct of butter manufacture, and butter produced from ripened cream is referred to as natural (conventional) BM. Initially, all, and in fact still most, butter was produced from ripened cream and probably BM was consumed as such, especially when butter making was a farm-based industry. In 2014, nearly 10 million tonnes of butter and ghee were produced globally, an increase of 163 000 tonnes (1.7%) compared with 2013. India remains the largest producer of butter and ghee, contributing 38% of the global production. The European Union was the second largest, averaging 2.31 million tonnes of butter in 2013 (OECD-FAO 2016). Assuming that butter is made from 40% fat cream, about  $10^7$  tonnes of BM are produced annually. Typically, 1000 kg of milk will yield over 45 kg of butter, 2–3 kg of BM powder and a little less than 100 kg of skim milk powder.

The composition of BM varies considerably depending on the butter making technology and seasonality. Typically, natural BM contains lactose (3.5–4.9%), lactic acid (0.5%), nitrogenous compounds (2.7–3.8%), fat (0.3–1.0%) and ash (0.6–0.7%); it contains proteins and phospholipids derived from the MFGM. The composition and properties of BM were described by Sodini et al. (2006) and Vanderghem et al. (2010) and the ratio of casein to whey protein in BM is similar to that of skim milk (Corredig and Dalgleish 1997). Most of the BM produced today is dried and used as a techno-functional ingredient in a wide range of food products (e.g. salad dressings, pasta sauces, chocolate, cheese, ice cream mixes, or yogurt) (Dewettinck et al. 2008; Svanborg et al. 2015; Levin et al. 2016b). A considerable amount of sweet-cream BM is blended with skimmed milk, spray-dried and used as skim milk powder (SMP) substitute or incorporated in fat filled milk powders. The remaining liquid BM, is commonly used as animal feed.

While MFGM material is present in virtually all dairy products containing milk fat, it is naturally enriched in BM (Hintze et al. 2011) as a result of the mechanical destabilization process, which disrupts the MFGM, releasing free fat as the globules coalesce. Currently, BM is the main source of MFGM-derived phospholipids and proteins and is used to produce MFGM-enriched ingredients, thereby increasing the value of BM. The number of scientific publications on the chemistry, fractionation and functionality of BM has quadrupled over the past 20 years (PubMed); the vast majority of these studies have focussed on either fractionating or concentrating various MFGM components. The separation of MFGM material from other dairy constituents, namely proteins, can be achieved by MF, which has been used successfully for the separation and fractionation of milk fat globules (Goudedranche et al. 2000). However, the presence of skim milk solids, especially casein micelles, in this byproduct, restricts the concentration of MFGM, as the MFGM particles and casein micelles are comparable in size. A possible solution to this is to selectively dissociate casein micelles, allowing casein proteins to permeate the MF membrane, along with the whey proteins, lactose, minerals, etc., thereby allowing concentration of the MFGM material in the MF retentate stream. For example, Corredig et al. (2003) used sodium citrate to disperse the casein micelles, thereby decreasing the retention of casein upon MF, which resulted in a phospholipid-enriched retentate. Morin et al. (2007a) produced buttermilk with a lower casein content employing MF, using cream washed with skim milk ultrafiltrate; washing the cream prior to churning yields buttermilk with 74%

less protein than regular buttermilk. Jukkola et al. (2016) used MF for the separation of native milk fat globules from whole milk, which allowed ~90% of milk protein to be removed from the cream prior to butter making; on manufacturing butter using this novel stream, the resulting buttermilk was naturally enriched in MFGM components and was referred to as “ideal buttermilk.”

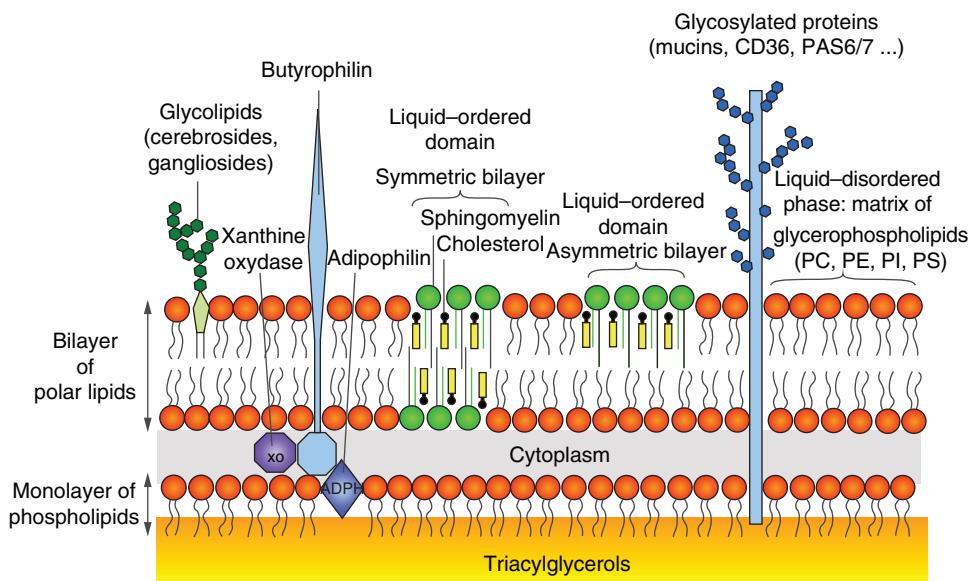
In addition to BM (Sachdeva and Buchheim 1997; Astaire et al. 2003; Morin et al. 2007a), other byproducts have been studied as sources of MFGM material, namely whey BM (Morin et al. 2006) or butter serum (Rombaut et al. 2006a). Morin et al. (2006) showed that whey BM obtained from churning whey cream also represents a valuable source of MFGM components that can be concentrated by MF. However, the low volumes of whey cream produced, and the higher susceptibility to oxidation of whey cream, lead to limited commercial interest in this approach. Rombaut et al. (2006b) demonstrated that butter serum is a more suitable starting material in the isolation of MFGM components than BM, because of its high polar lipid content.

A number of clinical trials support the proposed health benefits of BM (Conway et al. 2013; Conway et al. 2014a) with a limited number of innovative infant nutritional products available commercially, formulated to include MFGM and/or phospholipid materials, mostly isolated from BM (Gallier et al. 2015; Cilla et al. 2016; Claumarchirant et al. 2016). Milk polar lipids could therefore be of economic value industrially after isolation and enrichment from byproducts and marketed as functional food ingredients as they have particular nutritional and functional properties. Research on the recovery and purification of dairy-derived polar lipids, mostly using buttermilk or whey-derived streams as raw material, is very active and this promises to be an interesting area of ingredient development over the next 5–10 years (see Section 4.5.3).

## 4.5 The Milk Fat Globule Membrane

The lipid fraction of milk is a complex matrix composed of tri- (TGs), di- (DGs), and mono-glycerides (MGs), glycolipids (GLs), cholesterol (CH), cholesterol esters (CHEs), free fatty acids (FFAs) and phospholipids (PLs) (Jensen 2002). The milk fat occurs as globules with a nonpolar lipid core composed primarily of TGs and surrounded by the MFGM which contains both phospholipids (PLs) and glycoproteins (Keenan and Mather 2006; Ortega-Requena and Rebouillat 2015) (Figure 4.9). Due to their properties, origin, structure and original function in stabilizing the fat globules in whole milk, MFGM materials are efficient and natural emulsifiers or stabilizers (Singh 2006) and are preferentially enriched in aqueous phases like skimmed milk, buttermilk and butter serum (Rombaut et al. 2006b). Therefore, cream has a polar lipids content (expressed on the basis of total lipid) lower than skimmed milk, just as butter and cheese have a lower polar lipid content than buttermilk and whey (Contarini and Povolo 2013). However, literature values on the composition of the MFGM material are highly variable due to differences in isolation, purification and analytical techniques.

The emulsifying properties of these MFGM materials are strongly dependent on their content and type of polar lipids and proteins, as well as their possible interactions. The starting material, processing, heat treatments and isolation processes have a distinct influence on the composition of MFGM isolates and, consequently, on their



**Figure 4.9** Schematic representation of the milk fat globule membrane. Source: From Lopez (2011). (PC-phosphatidylcholine; PE-phosphatidylethanolamine; PS-phosphatidylserine; PI-phosphatidylinositol).

technological functionalities (Phan et al. 2014). Therefore, knowledge on the origin, composition and properties of the MFGM is crucial in supporting development of new products either with improved nutritional or techno-functional properties and the composition of MFGM materials must be carefully standardized before being used in food products in order to maintain their properties. Details on the origin, nature, composition, structure, nutritional and technological properties of the MFGM were reviewed by Keenan and Mather (2006), Singh (2006) and Dewettinck et al. (2008).

#### 4.5.1 Phospholipids of the Milk Fat Globule Membrane

Milk contains approximately 0.01–0.04% (w/w) of phospholipids (PLs), of which phosphatidylcholine (PC; 35%), phosphatidylethanolamine (PE; 30%), and sphingomyelin (SGM; 22%) together constitute 80–90%; the rest being phosphatidylserine (PS; 3%) and phosphatidylinositol (PI; 5%) (Vanderghem et al. 2010). About 60% of the total PLs are present in the MFGM and the remaining 40% in other membranous material in skim milk, which have been derived from the MFGM (Ortega-Requena and Rebouillat 2015). Phospholipids from milk, colostrum and dairy byproducts were reviewed by Contarini and Povolo (2013) and Verardo et al. (2017).

To date, health authorities recognize only one PL component, choline, as a nutrient. Choline is a major component of PC and SGM, associated with functions such as a methyl donor and a precursor for the neurotransmitter acetylcholine. Infant formulae enriched in MFGM-derived PLs have shown promising results with respect to neurodevelopment (Timby et al. 2014) and defense against infections (Timby et al. 2015). These

health benefits are believed to be strongly associated with choline, provided by the increase of PC and SGM. In this regard, the oil droplets in infant formula enriched in MFGM material, which is still not practiced widely, would be expected to better resemble the structure of those oil droplets found in human milk (Claumarchirant et al. 2016). There has been little research on the requirements for the intake of PLs and an effective dose is very dependent on the type of PL-enriched product (Miraglio 2006). The PL content of dairy products is not only dependent of the raw material/source, but also on the choice of unit operations/processes used in their manufacture. Any treatment that disrupts the MFGM, can affect the distribution and composition of the PLs in the final matrix (Contarini and Povolo 2013).

Despite their modest concentration in the MFGM, PLs are critical in stabilizing milk fat globules against coalescence due to their mixed polar and non-polar structure, making MFGM material an efficient and natural emulsifier (Ortega-Requena and Rebouillat 2015). The use of milk PLs has been reported in a variety of foods such as frozen desserts, bakery products, pumping hams and other meats (Gerdes 2008). Le et al. (2011) used MFGM material (isolated from buttermilk) in formulating yogurts, and concluded that increasing both the polar lipid and protein content, by the addition of MFGM material, not only provided beneficial nutritional properties, but also contributed to the technological properties of the product, such as improved water-holding capacity and increased adhesiveness of the yogurt gels. Normally, the addition level of MFGM required as a technological aid (e.g. emulsifier) is lower than the addition level necessary to support a health effect; therefore, in adding MFGM material to foods to confer various health benefits (e.g. cholesterol-lowering and anti-inflammatory) it is expected that the addition levels would be in excess of that required to confer a technological benefit (Dewettinck et al. 2008; El-Loly 2011; Cilla et al. 2016). Therefore, ingredients enriched in MFGM components have the potential for use as novel food ingredients with technological and biological functionality. Good sources of MFGM-derived PLs are low-fat products such as skimmed milk (11.1–19 g PL/100 g fat), buttermilk (up to 33 g PL/100 g fat) and butter serum (14.8–48.4 g PL/100 g fat) (Pimentel et al. 2016).

#### 4.5.2 Proteins of the Milk Fat Globule Membrane

Depending on the source, 25–70% of the MFGM, on a dry weight basis, consists of proteins (Dewettinck et al. 2008); however, the proteins of the MFGM represent only 1–4% of total milk protein. The most significant MFGM proteins are mucin 1 (MUC1), mucin 15 (MUC15), cluster of differentiation 36 (CD36), butyrophilin (BTN), lactadherin, xanthine oxidoreductase (XOR), adipophilin (ADPH), and fatty acid-binding protein (FABP), with the last three being unglycosylated. The reported composition is highly dependent on the isolation and analytical procedures used, since not all proteins are equally embedded within the matrix of the MFGM (El-Loly 2011). In the MFGM, polar lipids and proteins are closely associated so they will probably co-migrate during dairy processing; therefore, dairy products rich in polar lipids are generally also enriched in MFGM proteins (Dewettinck et al. 2008). Rombaut et al. (2006b) listed the polar lipid content (in g/100 g of product) of various dairy products during processing, butter serum has the highest amount (1.25), followed by cream (0.19), buttermilk (0.16) and skim milk has the lowest (0.02) content. Thus, it can be assumed that the MFGM proteins will follow the same general trend.

To date, important bioactivities associated with MFGM proteins include cell activity and cell growth promotion (Riccio 2004), antiviral, antimicrobial and immune-stimulating effects (Floris et al. 2010) and new-born defense mechanisms (El-Loly 2011). Mucins have been reported to have a role in the prevention of pathogen adhesion to the gut wall (Patton 2001) and lactadherin inhibits rotavirus infection (Kvistgaard et al. 2004). Since, generally, MFGM proteins are absent from infant formulae, supplementing formulae with MFGM proteins may be beneficial; however, it should be realized that specific bovine MFGM proteins, e.g. lactadherin, display less bioactivity than their human counterparts (Kvistgaard et al. 2004). MFGM proteins have been isolated and characterized by Keenan and Mather (2006) and Cavaletto et al. (2008).

#### 4.5.3 Sources and Applications of Milk Fat Globule Membrane-Derived Ingredients

Polar lipids have been used in the food industry for a long time, in applications such as a baking improver to facilitate fat dispersion and as anti-staling agents, as additives to chocolate to reduce viscosity and prevent crystallization, as wetting enhancers for instant products, and as stabilizers for margarine to prevent spattering and browning (Van Nieuwenhuyzen 1976, 1981; Szuhaj 1983; Rombaut et al. 2004). Non-food applications, such as drug delivery vehicles, as fat liquoring for leather softening, as raw materials for the production of ceramides and liposomes (Van Nieuwenhuyzen 1981; Kisel et al. 2001; Guo et al. 2005), have been also reported.

Some milk-derived PL-enriched ingredients are commercially available, such as Lacprodan® PL-20 (Arla Foods Ingredients, Denmark), an MPC ingredient enriched with PL's and gangliosides, which is used, among other applications, in the formulation of infant nutritional products. Some of the benefits of this ingredient, as claimed by the manufacturer, include stability to oxidation, milky taste, and good emulsifying properties, in addition to nutritional benefits, such as its role as a source of choline, PS, and other biologically important lipids. Other examples of commercially available PL-enriched dairy-derived ingredients include the phospholipid concentrates, PC 500™ (25% PLs), PC600™ (75% PLs) and PC700™ (60% PLs) (Fonterra Co.-operative Group Limited, New Zealand) and SureStart™ Lipid 100 (NZMP – Fonterra, New Zealand). The former are believed to be cream-derived ingredients, with PL concentrations more than 5000 times higher than that in raw milk, while the latter is believed to be a MFGM-based complex lipid material arising from the manufacture of AMF from cream.

MFGM materials have been recovered, enriched, and isolated from a range of dairy byproducts, such as buttermilk, butter serum, acid buttermilk, and whey, using various processes, including membrane filtration (Sachdeva and Buchheim 1997; Corredig et al. 2003; Morin et al. 2004; Rombaut et al. 2007) and thermocalcic aggregation (Rombaut and Dewettinck 2007). Rombaut and Dewettinck (2006) also described the separation of milk polar lipids from the serum phase of buttermilk by means of tangential MF and UF approaches, sometimes in combination with the stepwise addition of water (diafiltration), to facilitate further washing out of unwanted components such as lactose, whey proteins, and minerals. However, side streams and byproducts are normally subjected to several unit operations (e.g. heating, homogenization, and evaporation) in the dairy processing industry, leading to differences in composition and technological performance between different MFGM-enriched ingredients (Le et al. 2011). For example, it

has been reported that unit operations such as pasteurization, evaporation, and spray-drying affect the phospholipid content (i.e. PE, PS, and PI) of BM (Morin et al. 2007b).

Of the above byproducts, BM is the most thoroughly studied, and is often used as the starting material for the production of MFGM-enriched ingredients. However, the casein micelles in BM, which are a major solids constituent of BM, can cause difficulties as their diameter is comparable to that of MFGM fragments. Whey BM (Morin et al. 2006), the aqueous fraction obtained on churning of whey cream, and acid buttermilk whey (Rombaut et al. 2007), the aqueous fraction obtained by acidification of sweet-cream buttermilk, have received interest as feed material in the isolation of MFGM using filtration, due to the absence of casein micelles in both materials. Interestingly, Sodini et al. (2006) reported that whey BM has better emulsifying properties and a lower foaming capacity, compared to sweet or sour BM, possibly due to a higher ratio of PLs to protein in whey BM compared with either of the other two streams.

Whey protein phospholipid concentrate (WPPC), a co-product generated as a retentate stream during the MF of whey in the manufacture of WPI, is a relatively new product and does not yet have a standard of identity (Burrington et al. 2014). In 2015, the American Dairy Products Institute (ADPI) published a standard for WPPC composition: a minimum of 50% protein (dry basis), a minimum of 12% fat, a maximum of 8% ash and a maximum of 6% moisture. In 2015, whey powder (WP) remained the dominant (70%) whey product in terms of both volume and value, which together with WPC and WPI, represent a global market value of ~\$4.9 billion. In 2016, the USA alone, produced ~212 225 tonnes of WPC. The general trend in the whey ingredients market is higher growth for higher protein content products, with WPC80 and WPI having the highest growth rates between 2011 and 2015 (Affertholt and Pedersen 2017; USDA/NASS 2017). Considering this, thousands of tonnes of WPPC are generated annually as a co-product of WPI manufacture. Like other co-products, WPPC has a variable composition, and while it is the byproduct of MF-based defatting of whey, it can be manufactured using different technology (i.e. polymeric vs ceramic MF membranes) and processing parameters; thus commercial WPPC products vary widely in terms of chemical composition, and thereby functionality (Burrington et al. 2014).

There are a number of commercially available WPPC-based products, e.g. PRO-Cream (Prinova®, USA), Salibra® 700 (Glanbia Nutritionals, Ireland) and Lacprodan MFGM-10 (Arla Food Ingredients, Denmark). PRO-Cream is a co-product of WPI production and is labeled as a WPC. Lacprodan MFGM-10 is produced from a whey protein fraction with a high concentration of bioactive proteins and lipids, with the ingredient claimed to have a unique protein and lipid profile and including several bioactive compounds, such as lactoferrin, IgG, sialic acid, phospholipids and gangliosides. In addition, Salibra 700 is a value-added WPC ingredient with more than 20% bioactive components derived from whey such as glycomacropeptide (13%), immunoglobulins (5%), lactoferrin (1%) and phospholipids (2%) and can be used in food systems such as yogurt, ice cream, low-fat products, nutritional bars, frozen whipped toppings and nutritional beverages for emulsification, thickening, water-binding and texture stabilization. In addition, blends of WPPC and delactosed permeate (DLP), a byproduct of lactose manufacture, have been used in several food formulations to replace other dairy ingredients, emulsifiers, salt, and eggs in food applications such as ice cream, soups and confectionery products (Bund and Hartel 2013; Levin et al. 2016b). DLP and WPPC have been used in combination as a total replacement for eggs, in cakes, with no change in yield, color, or texture (Levin et al. 2016b).

Lecithin is a food ingredient which is naturally enriched in PLs and is normally derived as a byproduct of vegetable oil (soybean primarily) processing, or from eggs. Lecithin has many technological (e.g. viscosity modifier and emulsifier) (Szuhaj 2003) and nutritional functionalities (e.g. role in neurological development and inflammatory process) (Küllenbergs et al. 2012). It may be possible to use some of the dairy-derived PL-enriched ingredients outlined earlier to replace lecithin in certain food applications, thereby adding value to an existing byproduct of the dairy industry (Zhu and Damodaran 2013). In addition, MFGM is also a natural source of antioxidants, such as vitamin E (Jensen and Nielsen 1996) and riboflavin (Kuchta et al. 2012). Therefore, if MFGM, or its components, could be isolated in greater quantities from additional dairy byproducts, such as whey BM or WPPC, in an industrially and commercially viable way, it has major potential for use as a functional food ingredient in several applications. Further research should focus on the impact of the various processes currently used for MFGM isolation and separation and how changes to the structure of MFGM material/fragments affect their nutritional and functional properties.

## 4.6 Milk and Whey Permeates

### 4.6.1 Introduction

In dairy processing, the term permeate is used to describe the fraction of milk or its derived streams which can permeate through the selectively-permeable membranes used for fractionation, enrichment or purification of target nutrients using pressure-driven membrane filtration processing. The membranes used may be of MF, UF, NF, or reverse osmosis (RO) construction/configuration and the feed material may be milk (usually pasteurized, skimmed milk) or pre-treated (clarified, separated, and pasteurized) whey, thus giving rise to a broad matrix of possible permeate streams. However, the largest by volume, and commercially most significant, permeate streams/products are whey permeate and milk permeate derived using UF membrane technology; these milk and whey permeates are obtained by UF of skim milk and pre-treated liquid whey, respectively.

Permeate is predominantly (>93%) water and contains the low molecular weight water-soluble components (i.e. lactose, minerals, vitamins, non-protein nitrogen) of milk or whey. The UF membranes used for the production of milk and whey permeate generally have a molecular weight cut-off of 5–10 kDa and are intended to retain all the milk/whey proteins in the retentate stream. Fat is also retained by such membranes but the fat content of the feed material (i.e. skim milk or pre-treated whey) is generally maintained low (<0.1%) by the use of centrifugal separation of fat in a pre-treatment step so as to minimize the fat content of the retentate and to minimize fat-based fouling of the membrane filters. MF, NF, and RO permeates of milk and whey are growing in prevalence and commercial significance as part of fractionation (e.g. separation of casein micelles and whey proteins in their native state in skim milk using MF), demineralization (e.g. removal of monovalent ions from whey in the production of demineralized whey) and pre-concentration (e.g. pre-concentration of skim milk prior to evaporation and spray drying) processes, but generally do not contribute directly to the generation of byproducts and therefore they will not be discussed further in this chapter.

## 4.6.2 Milk Permeate

Compared with whey permeate, milk permeate is a relatively new byproduct in the processing of milk. It is normally obtained as a side-stream from UF processing in the production of MPC and MPI ingredients, on-farm concentration of milk and in the pre-concentration of milk intended for cheesemaking. Milk permeate is considered to be a “cleaner” byproduct of milk than whey permeate, as it is physically recovered from milk at an earlier stage in processing and is free from various additives such as, rennet enzyme, pH adjusting aids, color (e.g. annatto) or starter cultures which are commonly used in the production of cheese and casein ingredients (some of which are present in the resultant whey permeate stream). Milk permeate is used mostly in liquid format (normally concentrated from its natural ~6% total solids to 20–25% total solids) for standardizing the protein content of milk powders made from a seasonal milk supply (e.g. in Ireland, the Netherlands and New Zealand), can be dried to produce milk permeate powder. It is also technologically possible to recover lactose from milk permeate, generate a range of lactose derivatives and milk minerals using the approaches outlined above, for the processing of whey permeate; however, this is not usually practiced commercially.

## 4.6.3 Whey Permeate

Whey permeate, as a byproduct of whey processing, has been a dairy processing side stream since the introduction of UF technology in the 1960s for the removal and concentration of proteins from whey. This UF technology-based removal of protein from whey was performed in the early days to reduce the nutrient density and associated BOD/COD of whey being discharged into waterways, but was quickly adopted for the development of WPC ingredients (Section 4.3.6). Whey permeate is now a byproduct of the production of WPC, WPI and some whey protein fractions. The typical composition of whey permeate is 93% water, 6% total solids and 0.6% protein. The volumes of whey permeate generated in the production of WPC and WPI ingredients are relatively large, especially in the production of high protein content WPC/WPI ingredients, particularly with the addition of water to the feed stream, in diafiltration, to wash out more non-protein constituents (lactose and minerals) of whey in the production of such high protein content WPC/WPI ingredients. Whey permeate can be dried to produce whey permeate powder or further processed and fractionated using a number of different approaches to yield various more value-added ingredients as summarized briefly in the following sections:

### 4.6.3.1 Whey Permeate Powder

Whey permeate can be evaporated and dried to produce whey permeate powder. According to the American Dairy Products Institute (ADPI), this byproduct of milk/whey has approximately 3–4% moisture, 76–85% lactose, 8–11% ash and 2–7% protein, and is sometimes referred to as deproteinized whey powder since the whey proteins are removed using UF technology during processing and the nitrogen is predominantly non-protein nitrogen. Due to the high lactose content of the resultant powders, the lactose is normally pre-crystallized (by controlled cooling, seeding and holding) post evaporation and prior to drying to help reduce lactose glass-mediated stickiness during drying and caking, crystallization, clumping of the finished product powders. Research and

development on innovative approaches to the concentration and drying of whey (and milk) permeate is currently very active with a focus on reducing stickiness during drying, increasing the capacity of drying plants and improving the stability of the resultant permeate powders (Tanguy et al. 2017).

#### 4.6.3.2 Lactose

Whey permeate may be evaporated, typically to 60–65% total solids by vacuum evaporation at 70–75 °C and the lactose crystallized by controlled cooling, seeding and agitated storage of the mixture. The recovered (using decanter centrifuge technology usually) lactose crystals are then washed to remove impurities (i.e. minerals and vitamins), dried using fluidized bed drying technology and optionally milled to produce lactose powders of the desired particle size, density, and bulk handling properties for use as an edible ingredient in a range of food applications. The main applications of lactose are in chocolate, confectionery, and in infant formula, and increasingly in the standardization of milk powders (e.g. protein-standardized SMP). Lactose may also be further converted biochemically into a range of commercially important products as described in Sections 4.3.7.3 and 4.3.7.4.

#### 4.6.3.3 Delactosed Permeate

DLP, also referred to as mother liquor, is the side-stream remaining after the physical removal of lactose crystals from concentrated, crystallized whey permeate. It has a substantial concentration of lactose (typically 50–55%) and is enriched in minerals (due to partial removal of lactose by crystallization), compared with regular whey permeate. For each kilogram of milk used for cheese production, close to 0.5 kg of DLP is produced (Bund and Hartel 2013). DLP is currently underutilized in the food industry, with the principal application being as a binder (with some nutritional contribution) in the production of pelletized animal feed products. The excess is typically spread on land, treated as an effluent or used as a feedstock in energy production using anaerobic digestion. Therefore, if DLP could be used in value-added food applications, it would greatly benefit the dairy industry in terms of reduced disposal costs and increased profitability. Development of processes for the conversion of the relatively unstable liquid format into a more stable powder format is an active area of research. DLP is very difficult to dry as the concentrate has a low glass transition temperature and the resultant powder is very hygroscopic and sticky. To the authors' knowledge, there is currently only one DLP powder product commercially available, from Leprino Foods, Denver, US.

The composition and drying properties of DLP have been studied by Liang et al. (2009) and Bund and Hartel (2010); this work showed that the composition (sugar and mineral profiles and lactic acid content), and the ability to dry different DLP materials were influenced by the source of the permeate. DLP has important functional properties that make it a useful ingredient in food applications such as ice cream, soup, and caramel (Levin et al. 2016b). Due to its mineral contribution and salty flavor, DLP (and other permeate materials) have been shown to be effective in reducing the level of sodium in formulated food products (Dixon 2008; Burrington et al. 2014; Smith et al. 2016). In these studies, it was shown that the replacement ratio for whey permeate is roughly 10 g of permeate for 1 g of NaCl, whereas only ~3 g of dry DLP is required to achieve the same sensory properties. DLP, in liquid form, has been shown to extend the shelf-life of fruits and vegetables

(Ahmed et al. 2011, 2012a,b, 2013a,b). The composition and functionality of DLP were described by Levin et al. (2016a).

#### 4.6.3.4 Whey Minerals

Whey permeate is a rich source of dairy minerals, which are incorporated either into products such as whey permeate powder or removed in the manufacture of products such as lactose. Much of the mineral content in whey permeate (especially calcium and phosphate) may be recovered as a discrete ingredient by rendering them insoluble under certain conditions of pH and temperature and physically removing them after precipitation. The resultant material can be washed and dried to produce a powder (e.g. Tru-Cal™, Glanbia Nutritionals, USA), enriched in natural milk minerals for use as a supplement in various food applications (e.g. dairy protein beverages).

#### 4.6.3.5 Ethanol Production

Whey, or more commonly whey permeate, as a rich liquid source of fermentable lactose, can be used for the commercial production of ethanol. This process involves the fermentation of whey permeate using lactose-metabolizing yeast to produce ethanol, which is then recovered by distillation to produce an industrial ethanol product which is used in alcoholic beverages for human consumption, cleaning/sanitation applications and bio-fuel in flexi-fuel vehicles (for further information see Section 4.3.7.4.3).

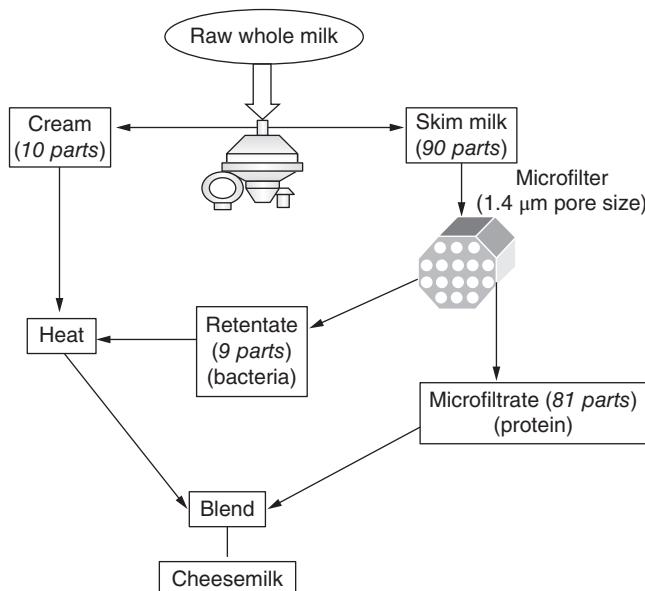
### 4.6.4 Developments in Dairy Permeates

Dairy permeate powders are used in a wide range of food applications and may be used to replace other dairy solids in several food applications, including, but not limited to, bakery and confectionary products. Dairy permeates may also be used in such products to replace sucrose or corn syrups, thereby reducing sugar and salt levels in these applications. Permeate can also be a source of lactose and minerals required for the formulation of nutritional products for the animal feed sector. Such is the extent of recent scientific study and commercial significance of dairy-based permeate powders that the International Dairy Federation (IDF) collaborated with Codex Alimentarius in the development and dissemination of a science-based international standard to clarify and promote the identity, composition, safety and quality of powdered dairy permeates as ingredients in food applications. A key driver of this new standard was that, from a commercial perspective, there was no commonly agreed definition of what constituted a dairy permeate powder, which hindered global harmonization of fair trade practices. The global permeate market has been growing steadily since 2012 but is expected to grow at a moderate rate (4.5%) throughout 2017–2027. This growth can be attributed to the increase in global consumption of food products in which whey permeate has been increasing, namely bakery and dairy products. The market estimation of this segment in 2017 was around \$161 million and is anticipated to reach a value of more than \$250 million by the end of 2027. However, the animal feed segment is expected to be the largest segment with a higher market value and is likely to dominate the global permeate market.

#### 4.6.5 Separator Sludge and Microfiltration Retentates

Heat treatments are commonly applied to minimize health hazards and control bacterial growth during processing of liquid dairy streams. However, these heat treatments almost always affect the flavor and functionality of dairy products treated in this way. Centrifugation, using a bacteria-removing centrifuge, is sometimes used for the physical removal of bacteria and other unwanted contaminants (e.g. somatic cells, hair, dirt etc.) from milk and dairy streams; this process is commonly referred as bactofugation, as a result of the commercial equipment manufactured by Tetra Pak (i.e. Bactofuge™) for such applications. Due to the high microbial load and low volumes produced, this separator sludge byproduct material is normally discarded to the effluent plant.

The decimal reduction is usually low and significant loss of protein takes place with this approach. In that context, MF is increasingly being applied to remove bacteria from milk, especially skimmed milk, as the size range of fat globules and bacteria overlap (Gésan-Guiziou 2017). As an example, Tetra Pak have an MF-based system (Bactocatch®) available for the treatment of milk and other liquid dairy streams, which removes 99.6–99.98% of all bacteria and spores (Figure 4.10). Such technology is used, for example, in the production of extended shelf-life milk and in the removal of spore from milk intended for manufacture of cheese varieties, where it is desirable to avoid late gas blowing, caused by spore-forming bacteria. The resulting retentate byproduct stream contains valuable milk components and is normally subjected to high heat treatment and re-incorporated into the original milk stream or added to another product with less demanding quality criteria, but is generally not fed to the effluent treatment plant.



**Figure 4.10** Process for removal of bacteria from milk by microfiltration. Source: From: (Mistry 2002).

## 4.7 Indigenous Milk Enzymes

The principal indigenous enzymes in milk and their catalytic activity are listed in Table 4.3. Research on the indigenous enzymes in milk dates from 1881 and a very extensive literature has accumulated, which has been reviewed, e.g. (Fox and Kelly 2006a,b) and O'Mahony et al. (2013). At least 60 indigenous enzymes have been reported in normal bovine milk. They arise from (i) the blood via defective mammary cell membranes; (ii) secretory cell cytoplasm, some of which is occasionally entrapped within fat globules by the encircling fat globule membrane (MFGM; see Section 4.5); and (iii) the MFGM itself, the outer layers of which are derived from the apical membrane of the secretory cell, which, in turn, originates from the Golgi membranes; this is probably the principal source of the indigenous enzymes in milk. Thus, most enzymes enter milk due to peculiarities of the mechanism by which milk constituents, especially the fat globules, are excreted from the secretory cells. Milk does not contain substrates for many of the enzymes present, while others are inactive in milk owing to unsuitable environmental conditions, e.g. pH.

Many indigenous milk enzymes are technologically significant from five viewpoints:

- 1) deterioration (lipase (potentially, the most significant enzyme in milk), proteinase, acid phosphatase and xanthine oxidoreductase) or preservation (sulphydryl oxidase, superoxide dismutase) of milk quality;
- 2) as indices of the thermal history of milk: amylase, alkaline phosphatase,  $\gamma$ -glutamyl transferase, lactoperoxidase;

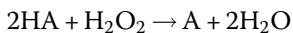
**Table 4.3** Indigenous Enzymes of Significance to Milk.

Enzyme	Reaction	Importance
Lipase	$\text{Triglycerides} + \text{H}_2\text{O} \rightarrow \text{fatty acids} + \text{partial glycerides} + \text{glycerol}$	Off flavors in milk, flavor development in Blue cheese
Proteinase (plasmin)	Hydrolysis of peptide bonds, particularly in $\beta$ -casein	Reduced storage stability of UHT products; cheese ripening
Catalase	$2\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}_2\text{O}$	Index of mastitis; pro-oxidant
Lysozyme	Hydrolysis of mucopolysaccharides	Bacteriocidal agent
Xanthine oxidase	$\text{Aldehyde} + \text{H}_2\text{O} + \text{O}_2 \rightarrow \text{Acid} + \text{H}_2\text{O}_2$	Pro-oxidant; cheese ripening
Sulphydryl oxidase	$2\text{RSH} + \text{O}_2 \rightarrow \text{RSSR} + \text{H}_2\text{O}_2$	Amelioration of cooked flavor
Superoxide dismutase	$2\text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$	Antioxidant
Lactoperoxidase	$\text{H}_2\text{O}_2 + \text{AH}_2 \rightarrow 2\text{H}_2\text{O} + \text{A}$	Index of pasteurization; bactericidal agent; index of mastitis; pro-oxidant
Alkaline phosphomonoesterase	Hydrolysis of phosphoric acid esters	Index of pasteurization
Acid phosphomonoesterase	Hydrolysis of phosphoric acid esters	Reduce heat stability of milk; cheese ripening

Source: From Fox et al. (2015a).

- 3) as indices of mastitic infection: the concentration of several enzymes increases on mastitic infection, especially catalase, *N*-acetyl- $\beta$ -D-glucosaminidase and acid phosphatase;
- 4) antimicrobial activity: lysozyme, lactoperoxidase (which is exploited as a component of the lactoperoxidase-thiocyanate system for the cold pasteurization of milk); and
- 5) as a potential commercial source of enzymes: e.g. ribonuclease, lactoperoxidase.

With a few exceptions (e.g. lysozyme and lactoperoxidase), the indigenous milk enzymes do not have a beneficial effect on the nutritional or organoleptic attributes of milk, and hence their inactivation by heat is one of the objectives of many dairy processes. Milk could serve as a commercial source of several enzymes, but to the best of the authors knowledge, the only commercially produced enzyme is lactoperoxidase. Peroxidases, which are widely distributed in plant, animal, and microbial tissues and secretions, catalyze the following reaction:



where HA is an oxidizable substrate or a hydrogen donor.

Work on the isolation of LPO commenced in the 1920s and LPO was isolated and crystallized in the 1940s. Since then, several improved methods for the isolation of LPO have been published and its characteristics refined. Since LPO is cationic at the pH of milk, as are lactoferrin and some other minor proteins, it can be isolated easily from milk or whey using cation exchange chromatography. There are 10 isozymes of LPO, arising from differences in the level of glycosylation and deamination of Gln or Asn. LPO is synthesized in the mammary gland and is the second most abundant enzyme in milk (next to xanthine oxidoreductase), constituting ~0.5% of the total whey proteins (~0.1% of total protein). LPO binds a  $\text{Ca}^{2+}$ , which has a major effect on its stability, including heat stability; at a pH below ~5.0, the  $\text{Ca}^{2+}$  is lost, with a consequent loss of stability. Apart from its exploitation as an index of flash or super-HTST pasteurization, LPO is technologically significant for the following reasons:

- a) It is a possible index of mastitic infection but is not well correlated with somatic cell count.
- b) LPO causes non-enzymatic oxidation of unsaturated lipids, acting through its heme group; the heat-denatured enzyme is more active than the native enzyme.
- c) Milk contains bacteriostatic or bactericidal substances referred to as lactenins, one of which is LPO, which requires  $\text{H}_2\text{O}_2$  and thiocyanate ( $\text{SCN}^-$ ) to cause inhibition. The nature, mode of action and specificity of the LPO- $\text{SCN}^-$ - $\text{H}_2\text{O}_2$  system has been studied widely.

LPO and thiocyanate, which is produced in the rumen by enzymic hydrolysis of thio-glycosides from *Brassica* plants, occur naturally in milk, but  $\text{H}_2\text{O}_2$  does not. In the presence of low levels of  $\text{H}_2\text{O}_2$  and  $\text{SCN}^-$ , LPO exhibits very potent bactericidal activity; this system is 50–100 times more effective than  $\text{H}_2\text{O}_2$  alone. The LPO system has good bactericidal efficiency for the cold pasteurization of fluids or sanitization of immobilized enzyme column. Indigenous xanthine oxidoreductase, acting on added hypoxanthine, may also be exploited to produce  $\text{H}_2\text{O}_2$ . The bactericidal effects of the LPO- $\text{H}_2\text{O}_2$ - $\text{SCN}^-$  system may be used to cold pasteurize milk in situations where refrigeration

and/or thermal pasteurization is lacking. Addition of isolated LPO to milk replacers for calves or piglets reduces the incidence of enteritis. Where permitted, the LPO system may also be exploited for bleaching colored whey. Milk contains high levels of lysozyme; however, egg white is the main commercial source. Lysozyme is used as alternative to nitrate, e.g. by Italian, Dutch, and Swiss cheese makers, to prevent cheese from blowing by the action of *Clostridium*.

## 4.8 Milk Salts

The salts of milk are mainly the phosphates, citrates, chlorides, sulfates, carbonates and bicarbonates of sodium, potassium, calcium and magnesium. Approximately 20 other elements are present in milk in trace quantities, including copper, iron, lead, boron, manganese, zinc, and iodine. There is no lactate in freshly drawn milk but may be present in stored milk and in milk products. The ash content of bovine milk remains relatively constant at 0.7–0.8%, but the relative concentrations of the various ions can vary considerably. Table 4.4, shows the average concentration of the principal ions in milk, the usual range and the extreme ranges encountered. The latter undoubtedly includes abnormal milk, e.g. colostrum, very late lactation milk or milk from cows with mastitic infection.

The concentration of ash in human milk is only ~0.2%; the concentration of all principal and several minor ions is higher in bovine than in human milk. Consumption of unmodified bovine milk by human babies causes increased renal load and hence demineralized bovine milk or whey should be used in the preparation of infant formulae. Certain of the milk salts, e.g. chlorides, and the salts of sodium and potassium are sufficiently soluble to be present almost entirely in the dissolved phase. But the concentration of others, in particular calcium phosphate, is higher than can be maintained in solution

**Table 4.4** Concentration of milk salt constituents ( $\text{mg L}^{-1}$  milk).

Constituent	Average content	Usual range	Extremes reported
Sodium	500	350–600	110–1150
Potassium	1450	1350–1550	1150–2000
Calcium	1200	1000–1400	650–2650
Magnesium	130	1000–150	20–230
Phosphorus (Total) <sup>a</sup>	950	750–1100	470–1440
Phosphorus (Inorganic) <sup>b</sup>	750		
Chloride	1000	800–1400	540–2420
Sulfate	100		
Carbonate (as $\text{CO}_2$ )	200		
Citrate (as citric acid)	1750		

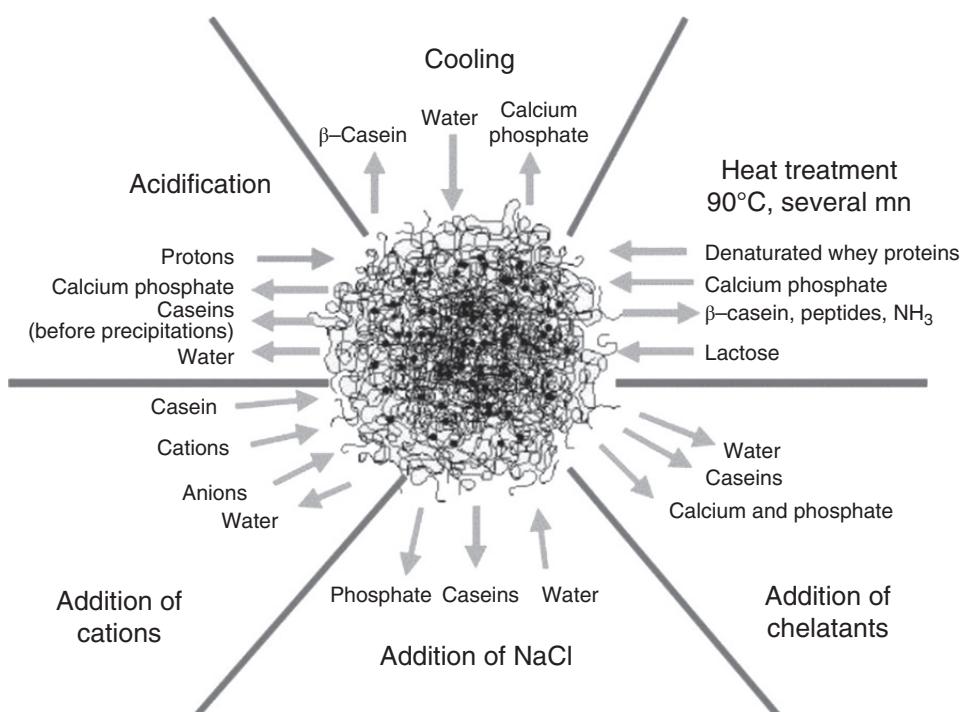
<sup>a</sup> Total phosphorus includes colloidal inorganic phosphate, casein (organic) phosphate soluble, inorganic phosphate, ester phosphate and phospholipids.

<sup>b</sup> Phosphorus (inorganic) includes colloidal inorganic phosphate and soluble inorganic phosphate. From: Fox et al. (2015c).

at the normal pH of milk; consequently, these exist partly in soluble form and partly in an insoluble or colloidal form associated with casein (i.e. as an integral part of casein micelles). Milk salts chemistry, distribution, and technological significance have been reviewed by Holt (1985), Gaucheron (2005) and Lucey and Horne (2009), respectively.

All the major ionic species in milk, with the exception of  $\text{Cl}^-$ , are distributed between the soluble and colloidal phases, but the principal colloidal salt is calcium phosphate; about 67% and 57%, respectively, of the total calcium and phosphate are in the colloidal phase. The colloidal inorganic salts are, therefore, frequently referred to as colloidal calcium phosphate (CCP) although some sodium, potassium, magnesium, and citrate are also present in the colloidal phase. See Fox et al. (2015c). The distribution of salts between the colloidal and soluble phases is influenced by several factors, as summarized in Figure 4.11. The salts of milk are generally recovered as a component of dairy products such as SMP, WMP, WPC, MPC, MCC, etc. In addition to the salts recovered in these products, significant quantities of milk salts are available in low value side-streams of dairy processing, such as whey permeate.

There are a limited number of processes and products developed, and to a limited extent, commercialized, for the recovery of milk salts as ingredients in their own right. These processes normally involve the precipitation and recovery of milk salts from UF permeate of whey, although other dairy processing side-streams and byproducts can also be used as starting material. One option for preparing milk salts exploits the fact that calcium phosphate is inversely soluble with temperature, and careful control of pH,



**Figure 4.11** Schematic representation of the changes that occur in the distribution of salts in milk.  
Source: From: Fox et al. (2015c).

temperature, and time can be exploited to achieve thermocalcic precipitation of salts from dairy processing side-streams such as whey or whey permeate. This precipitated milk salts material can be physically recovered (e.g. using centrifugal technology), washed, and dried to produce powdered milk salts ingredients. Examples of commercially available, natural, milk salt-based products include TruCal® (Glanbia Nutritionals, Ireland), Valio Valsa® (Valio Foods, Finland) and Capolac® (Arla Foods Ingredients, Denmark). The principal applications of such milk salts ingredients are as substitutes for sodium chloride (for example in high-fat spreads, cheese, bread and meat products) and as a natural source of mineral supplements.

## 4.9 Colostrum

Colostrum, the mammary secretion during five to seven days *post-partum*, differs markedly from mature milk. The principal difference is in the concentration of immunoglobulins (Ig) which are about 10% in first bovine colostrum. The type of Ig varies with the species; the colostrum of cattle, goat, sheep and buffalo contains mainly IgG1, with lesser amounts of IgG2, IgA, and IgM. Bovine colostrum has been studied fairly thoroughly and the literature has been reviewed by McGrath et al. (2016). Human colostrum contains mainly IgA, with low levels of IgM and IgG. Ruminants do not transfer IgS to the foetus in utero and the neonate lacks Ig in its blood at birth and is very susceptible to infection. However, the neonate's intestine is permeable to large molecules for some days after birth and absorbs the colostral Ig and rapidly builds up Ig in its blood stream; it produces its own Ig after a few weeks. The modern dairy cow produces much more colostrum than its calf needs or can even consume, and milk is not supplied commercially from farms to creameries for the first several days post calving. Therefore, excess colostrum represents a byproduct of the dairy industry and a small proportion of bovine colostrum is dried and pelleted and fed to orphaned or abandoned calves; and further details on this use for colostrum can be found in O'Mahony et al. (2013). The American Dairy Products Institute has a standard on whole colostrum powder, defined as the product obtained by the drying of colostrum that comes from cows within 48 hours after giving birth. It contains fat (>17.5%), proteins (>40%), carbohydrates (<35%), vitamins and minerals and is used in several food applications such as, beverage bases, dairy product analogs, milk, milk products, nutrition bars and snacks.

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## 5

# Byproducts from Butter and Cheese Processing

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## 5.1 Introduction

Milk is a complex system containing lactose, whey proteins, minerals, and vitamins in solution, lipids in emulsions, and casein micelles in colloidal suspensions. Processing of milk results in a vast array of products containing all or a portion of the components present in milk. From 1976 through 2016, per capita consumption of fluid milk has decreased, while per capita consumption of yogurt and cheese has increased (Figure 5.1) (United States Department of Agriculture 2016).

The processing of butter and cheese results in a majority of the dairy byproducts produced. Skim milk, buttermilk, and whey are the primary byproducts of butter and cheese processing, with casein, whey, and milk fat globule membrane (MFGM) as the major components of these byproducts (Figure 5.2). Lactose is a component of the whey fraction. On a world-basis,  $989 \times 10^3$  tons of butter and  $2430 \times 10^3$  tons of cheese were produced in 2016 (Food and Agriculture Organization 2016). The byproducts of the manufacturer of these products would account for approximately  $1034 \times 10^3$  tons of buttermilk,  $20\,712 \times 10^3$  tons of skim milk, and  $21\,870 \times 10^3$  tons of whey in 2016. Once considered waste products, with technological advances and increased knowledge of the nutritional and functional properties of these byproducts, the effective utilization of these products has increased. In this chapter, we will discuss the composition of the milk, the processing of butter and cheese, and the processing and applications of the primary and secondary byproducts of the dairy industry.

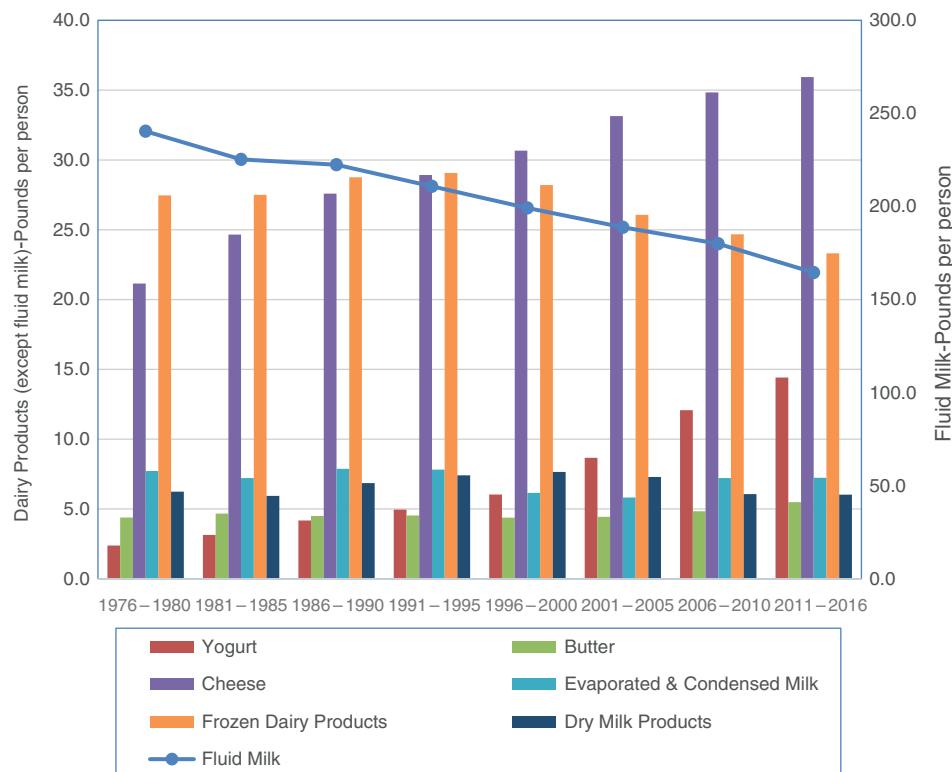
## 5.2 Milk Composition

The composition of bovine milk is dependent on numerous production factors, including diet, breed, stage of lactation, nutritional status, and season (Table 5.1). The major constituents of milk include lactose, lipids, proteins, and salts. Those constituents that are major components of dairy byproducts will be briefly discussed to provide background on their chemical properties.

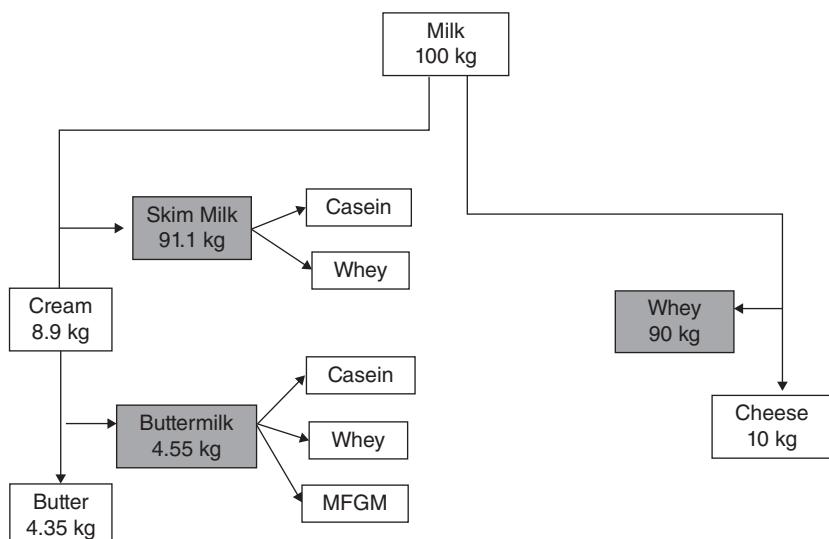
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**Figure 5.1** Per capita consumption of dairy products from 1976 to 2016. Source: Data from United States Department of Agriculture (2016).



**Figure 5.2** Byproducts of butter and cheese processing, showing the approximate yield from 100 kg of milk.

**Table 5.1** Composition of bovine milk from different breeds.

Component	Friesian	Jersey	Holstein-Friesian	Holstein
Fat (g/100 g)	4.47 ± 0.49	5.82 ± 0.39	4.38 ± 0.17	4.08 ± 0.36
Protein (g/100 g)	3.55 ± 0.21	3.98 ± 0.20	3.48 ± 0.06	3.29 ± 0.16
Casein (g/100 g)	2.74 ± 0.14	3.12 ± 0.12	2.72 ± 0.05	2.36 ± 0.09
Lactose	5.04 ± 0.20	5.10 ± 0.12	4.51 ± 0.03	4.59 ± 0.44
Calcium (mg/100 g)	126.2 ± 79.4	149.0 ± 66.1	NR <sup>a</sup>	117.4 ± 7.13

<sup>a</sup> NR – not reported.

Source: Adapted from Auldist et al. (2004), Heck et al. (2009), and Chen et al. (2014).

**Table 5.2** Properties of α- and β-anomers of lactose.

Property	α-Lactose	β-Lactose
Relative sweetness <sup>a</sup>	27	48
Solubility (in water at 20 °C)	7 g/100 ml	50 g/100 ml
Form of crystals	Monohydrate	Anhydrous

<sup>a</sup> Sucrose = 100.

Source: Adapted from Fox and Kelly (2012).

### 5.2.1 Lactose

Lactose ( $\beta$ -D-galactosyl-D-glucose), a reducing disaccharide of glucose and galactose, is the primary carbohydrate present in milk. The  $\alpha$ - and  $\beta$ -anomers of lactose differ greatly in their solubility, crystallization characteristics and relative sweetness (Table 5.2). In aqueous solutions, the lactose will equilibrate in a ratio of 37:63 for the  $\alpha$ - and  $\beta$ -anomers (Fox and Kelly 2012). Lactose is characterized by relatively low sweetness. As a reducing sugar, lactose contributes to browning in baked products.

### 5.2.2 Fat

Bovine milk fat is characterized by a high concentration of short- and medium-chain fatty acids and a low concentration of polyunsaturated fatty acids. The biohydrogenation reactions by bacteria in the rumen contribute to the high content of saturated fatty acids and conjugated linoleic acids (CLAs). Milk fat occurs as globules, with a diameter ranging from 0.1 to 20  $\mu\text{m}$ , surrounded by the MFGM. The outer layer of the MFGM is composed of phospholipids, sphingolipids, and proteins which protect the milk fat from physical or enzymatic damage and helps maintain the emulsion in the milk (Deeth and Hartanto 2009; Dewettinck et al. 2008; Fox and Kelly 2012; Jensen 2002).

**Table 5.3** Protein quality of casein and whey protein as compared to egg.

Measurement method	Casein	Whey protein	Egg
Protein efficiency ratio	2.5	3.2	3.9
Biological value	77	104	100
Net protein utilization	76	92	94
Protein digestibility corrected amino acid score	1.00	1.00	1.00

Source: Adapted from Hoffman and Falvo (2004).

### 5.2.3 Protein

The protein content of milk ranges from 3.2% to 3.5%. Casein and the whey proteins are the major classes of milk proteins, accounting for 80% and 20% of the total protein, respectively. The protein quality of whey protein is higher than that of casein. However, both dairy proteins contain all the essential amino acids, have high digestibility, and are high-quality, complete proteins (Table 5.3). In addition, both casein and whey proteins and peptides have bioactivity (Hoffman and Falvo 2004; Pereira 2014; Smithers 2008).

Four caseins, which differ in their primary structure and degree of phosphorylation, make up the casein micelles. The casein micelles are large (MW  $\sim 10^8$ – $10^9$  Da, 30–300 nm diameter), flexible, amphiphilic molecules, with an open structure and high surface hydrophobicity. The  $\alpha_{s1}$ -,  $\alpha_{s2}$ -, and  $\beta$ -caseins are located on the interior of the casein micelle. The  $\kappa$ -casein is localized on the surface of the micelle, with the hydrophilic C-terminus of the protein providing hydrophilic properties to the casein micelle. Colloidal calcium phosphate crosslinks the casein proteins to maintain the integrity of the casein micelle. The casein proteins are precipitated at their isoelectric point (pH 4.6) or with the addition of the protease, rennet, and aggregate to form the curd of cheeses, but are heat stable (Dalglish 1997; Deeth and Hartanto 2009; Fox and Kelly 2012; O'Mahony and Fox 2013).

The major whey proteins are  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin, accounting for about 50% and 20% of the whey proteins, respectively.  $\beta$ -Lactoglobulin is a highly structured, globular protein (MW  $\sim 18\,300$  Da), rich in sulfur-containing amino acids.  $\alpha$ -Lactalbumin is a compact, globular metalloprotein (MW  $\sim 14\,000$  Da) containing four intramolecular disulfide bonds and rich in tryptophan. Immunoglobulins, lactoferrin, and lactoperoxidase are among the biologically active proteins, present in lower concentrations in the whey. The  $\beta$ -Lactoglobulin and  $\alpha$ -lactalbumin are denatured by heat. In the production of cheeses, casein forms the curd, and whey proteins are solubilized in the whey as a byproduct (Cayot and Lorient 1997; Fox and Kelly 2012; O'Mahony and Fox 2013; Smithers 2008).

### 5.2.4 Minerals

The most abundant minerals that make up the ash component in milk include potassium (136 mg/100 g), calcium (112 mg/100 g), phosphorous (89 mg/100 g), and sodium (53 mg/100 g). Calcium in the milk is present as colloidal calcium phosphate, soluble calcium, and ionic calcium. A significant proportion of the calcium and phosphorous is associated with

the casein micelle as colloidal calcium phosphate. The production of acid-coagulated cheeses, results in the solubilization of calcium and phosphorous from the casein micelle (Deeth and Hartanto 2009).

### 5.3 Processing of Butter and Cheese

Before discussing in depth the utilization of the dairy byproducts, it is important to understand the steps involved in the processing of dairy products. This chapter will focus on two dairy products, butter and cheese, which generate several major dairy byproducts through their production.

#### 5.3.1 Butter

The initial step in the manufacture of butter is the centrifugation of the milk to separate the milk into cream and skim milk. Pasteurized cream with a fat content of at least 35%, is the primary ingredient for the manufacture of butter. Churning the cream disrupts the MFGM, allowing the aggregation of fat globules to form a water-in-oil emulsion. Sweet buttermilk, the aqueous byproduct of butter manufacture, contains caseins and whey proteins, lactose, minerals, and the phospholipids and other components of the MFGM. For every 100 kg of milk, 4.35 kg of butter, and 4.55 kg of buttermilk are produced. The composition of the raw materials and products of butter manufacture is shown in Table 5.4. The range in the composition of the buttermilk is related to the effectiveness of fat removal from the buttermilk following butter manufacture. The presence of MFGM in buttermilk, contributing to a higher content of phospholipids, is one unique aspect that differentiates the composition of buttermilk from that of skim milk (Sodini et al. 2006; Vanderghem et al. 2010).

Skim milk is the initial byproduct of butter manufacture, as the whole milk is separated to produce cream. However, skim and reduced fat milks are also major dairy products. These milks are sold either as fluid milk products, or further processed to produce a variety of dairy products including flavored milk, acidophilus milk, nonfat dry milk, low-fat cheeses, yogurt, and kefir.

**Table 5.4** Composition of raw materials and products of butter manufacture.

	Water (%)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)
Raw milk	87.2–87.4	3.8–4.2	3.2–3.5	4.5–4.9	0.7–0.8
Heavy cream	57.3	36.8	2.2	3.2	0.5
Skim milk	90.9–91.2	0.1–0.2	3.3–3.7	4.4–5.0	0.7–0.9
Butter	16.5	80.5	0.6	0.4	0.2
Sweet buttermilk	88.0–92.0	0.5–1.5	2.4–3.5	3.6–6.7	0.6–0.8
Dried buttermilk	3.0	5.3	32.4	51.3	8.0

Source: Adapted from Chandan (2016) and Vanderghem et al. (2010).

Buttermilk, the major byproduct of butter manufacture, has applications as a dairy ingredient because of its emulsifying capacity and flavor characteristics. However, only a small percentage of buttermilk is consumed directly. The commercial use of buttermilk includes concentration by evaporation and spray drying to form a powder for applications in bakery products, dry mixes, confectionery, and dairy foods (Chandan 2016; Sodini et al. 2006; Vanderghem et al. 2010). The buttermilk may also be fractionated into MFGM, casein, and whey protein fractions using microfiltration to increase the value of the buttermilk (Svaborg et al. 2015). The high biological activity and functionality of the MFGM and other components has contributed to growing interest in the further processing of buttermilk to enhance the utilization of this byproduct.

### 5.3.2 Cheese

Cheese is produced by the coagulation of casein micelles in the milk to form a gel-like curd that entraps fat globules. The casein micelles may be coagulated through the addition of rennet or acid. For each 100 kg of milk, 10 kg of cheese, and 90 kg of whey is produced. The whey is composed of water (93–94%), lactose (4.5–6.0%), proteins (0.6–1.1%), minerals (0.8–1.0%), lactic acid (0.05–0.9%), and fats (0.06–0.5%) (Prazeres et al. 2012).

Approximately 75% of the total production of cheeses, including Cheddar, Swiss, Mozzarella, and Colby, are coagulated with rennet. The active enzyme in rennet is chymosin, which hydrolyzes  $\kappa$ -casein specifically at the Phe<sub>105</sub>-Met<sub>106</sub> bond, releasing the hydrophilic fragment, (glyco) caseinomacropeptide (CMP). Removal of the hydrophilic fragment destabilizes the casein micelles, resulting in aggregation of these proteins to form curds. The whey proteins, CMP, lactose, and calcium salts are separated from the curds into the whey (Boylston 2012; Kelly and Fox 2012). CMP accounts for 15–20% of the protein in sweet whey. The peptide has several potential health benefits, including inhibition of viral and bacterial adhesion, function as bifidogenic factors, suppression of gastric secretions, modulation of immune system responses and inhibition of the binding of bacterial toxins (Kelly and Fox 2012).

Acid-coagulated cheeses, including cottage cheese, cream cheese, and Ricotta cheese, account for approximately 25% of total cheese production. The milk is acidified to the isoelectric point of casein (pH 4.6) through the production of acid by fermentation of lactose to lactic acid by lactic acid bacteria or direct addition of acid. The acid neutralizes the charges on the amino acid side chains and solubilizes the colloidal calcium phosphate, resulting in the aggregation of the casein micelles. The resulting whey contains whey proteins, lactose, and calcium salts (Boylston 2012; Kelly and Fox 2012).

The whey produced from the manufacture of the processing of rennet- and acid-coagulated cheeses was once considered a waste product of cheese processing and either used as animal feed or disposed of as waste. However, the whey is nutrient-rich, with a high biological oxygen demand (BOD), resulting in concerns with disposing this waste stream. Moreover, the identification of important nutritional and functional components within the whey has led to utilization of whey products and components widely throughout the food industry (Prazeres et al. 2012).

The composition of the whey is dependent on coagulation method (Table 5.5). Sweet whey is the byproduct of rennet coagulation, while acid whey is the byproduct of acid coagulation. Sweet whey has a higher pH and lower calcium and lactic acid content

**Table 5.5** Characteristics of sweet and acid wheys.

Characteristic	Sweet whey	Acid whey
pH	>5.6	<5.0
Calcium content (g/100 ml)	0.04–0.06	0.12–0.16
CMP	Present	Absent
Lactose (g/100 ml)	4.60–5.20	4.40–4.60
Whey proteins (g/100 ml),	0.60–1.00	0.60–0.80
Lactic acid (g/100 ml)	0.20	0.64

Source: Adapted from Jelen (2009) and Kelly and Fox (2012).

in comparison to acid whey. The use of acid whey is more limited than that of sweet whey due to the acidic flavor, lower protein content, and higher salt content (Kelly and Fox 2012; Siso 1996).

## 5.4 Dairy Byproducts Applications

The dairy byproducts generated by butter and cheese processing are often further processed to isolate ingredients with enhanced nutritional value and functionality. Byproducts containing MFGM, whey, casein, and lactose are produced in butter processing. Whey and lactose are the major byproducts from cheese processing.

### 5.4.1 Byproducts Containing MFGM

Buttermilk and other dairy products processed from this byproduct of butter processing are unique due to the presence of the MFGM. The MFGM is composed of phospholipids and proteins. The components of the MFGM present in buttermilk have received growing interest due to their emulsification capacity (Corrideg and Dalgleish 1997; Elías-Argote et al. 2013; Sichien et al. 2009) and potential health-promoting effects (Dewettinck et al. 2008; Singh 2006), including antimicrobial and antiviral properties (Elías-Argote et al. 2013).

Buttermilk products have been widely used in the food industry because of their emulsification capacity. The sweet buttermilk powder and cream residue powder, containing elevated levels of MFGM have been shown to improve the stability of sterilized milk products. The effect of these byproducts is attributed to the formation of phospholipid-whey protein interactions which inhibit whey protein aggregation and whey protein-casein interactions. The use of these dairy byproducts containing elevated levels of phospholipids has been suggested as alternatives to non-dairy emulsifiers used in sterilized milk products (Kasinos et al. 2014). The texture of reduced fat Cheddar cheese has been shown to be improved through the addition of buttermilk, as attributed to the emulsification capacity of phospholipids (Raval and Mistry 1999). The presence of phospholipids in buttermilk powders does limit the foaming capacity of these ingredients due to their amphiphilic properties (Sodini et al. 2006).

The emulsifying properties of buttermilk powder is attributed to not only the polar lipids associated with the MFGM, but also the whey proteins and caseins associated with the buttermilk powder. Emulsions prepared with buttermilk whey powder (casein proteins removed through precipitation) were less stable than buttermilk powder. Heat treatment can cause denaturation of the phospholipids, MFGM proteins and whey proteins, resulting in losses in the emulsification properties of the buttermilk powders (Corridge and Dalgleish 1997; Morin et al. 2007; Phan et al. 2014), thus it is important to evaluate the impact of processing on the functional properties of the buttermilk powders.

MFGM can be isolated from caseins and whey proteins in buttermilk using micro- and ultrafiltration techniques. The separation of the MFGM is dependent on pH, temperature, and type and pore size of the membrane. Removal of the caseins prior to filtration through coagulation or dissociation of the casein micelles improves the efficiency of the separation. Several companies have commercialized dairy functional ingredients enriched in phospholipids for applications as emulsifiers (Sichien et al. 2009).

#### 5.4.2 Whey Powders

Although the most conventional source of whey powders is as a byproduct of cheese processing, whey powders can also be produced from skim milk and buttermilk following the precipitation of the casein proteins. In contrast to whey protein products isolated from sweet whey, whey protein products fractionated from buttermilk, skim milk, or acid whey do not contain CMPs (Svanborg et al. 2015). The whey may be processed as whey powder, whey protein concentrate, or whey protein isolate. These powders differ in the content of protein, lactose, fat, and ash (Table 5.6).

The first step in whey processing is the removal of curd particles and fat by centrifugal clarification, resulting in clarified whey. The clarified whey may be blended with natural or concentrated fruit juices to produce whey beverages with a nutritionally beneficial amino acid profile. However, the commercialization of clarified whey is fairly limited. (Kelly and Fox 2012).

Concentration and spray-drying of the whey produces condensed whey and whey powder, respectively. Dry sweet whey is used in numerous products, including bakery products, dry mixes, process cheese foods and spreads, frozen desserts, sauces, meat emulsions, confections, soups, gravies, snack foods, and beverages. Due to the acidic flavor of dry acid whey, applications of dry acid whey are limited to bakery products (Chandan 2016).

However, the applications of whey powder as an ingredient is limited by the high lactose and ash contents in comparison to the protein content. The high lactose content also presents challenges during drying, requiring controlled crystallization of lactose to produce small lactose crystals (Kelly and Fox 2012; Siso 1996). The whey streams may be treated prior to drying to reduce the lactose or minerals content. Reduced lactose whey is produced by partial crystallization of the lactose from the whey stream. Ion exchange or electrodialysis processes are used for the demineralization of whey (Chandan 2016).

To increase the protein content of whey products, purification is needed. Ultrafiltration of the whey concentrates the whey proteins in the retentate, with the lactose, salts and water transferred to the permeate. The retentate may be diluted and the

**Table 5.6** Composition of whey powders.

	Water (%)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)
Whole milk	87.0–87.4	3.7–3.8	3.2–3.3	4.8–4.9	0.7
Whey	93.3	0.25	0.6	4.8	0.54
Dry sweet whey	3–6	0.8–1.5	12–13	70–73	7.5–8.5
Reduced lactose whey	2–3	1–4	18–25	40–60	11–27
Demineralized whey	<3	<1.5	11–13	77–84	1.5–4
Dry acid whey	<3.5	0.8	9–12	65–69	11–12
Whey protein concentrate					
Low-protein	3–5	2–44.0	34–36	44–53	7–8
Medium-protein	3–4	5–6	50–53	35–36	6–7
High-protein	3–4	4–6	59–65	21–11	3.5–4
Very High-protein	4–5	0.3–7.0	72–81	2–13	2.5–6.5
Whey protein isolate	2.5–6	0.1–0.7	89–93	0.1–0.8	1.4–3.8

Source: Adapted from Chandan (2016) and Deeth and Hartanto (2009).

ultrafiltration process repeated to obtain higher protein contents. The resulting whey protein concentrate (WPC) has a protein content ranging from 35% to 80% (Kelly and Fox 2012). Applications of WPC vary depending on the protein concentration. The water binding, fat-like mouthfeel, and gelation properties of 34% protein WPC are beneficial for use in yogurt, bakery mixes, dietetic foods, infant foods, and confections. WPC of 50% or 80% protein form clear suspensions and is used in nutritional drinks, soups, bakery, meat, and dietary foods (Chandan 2016).

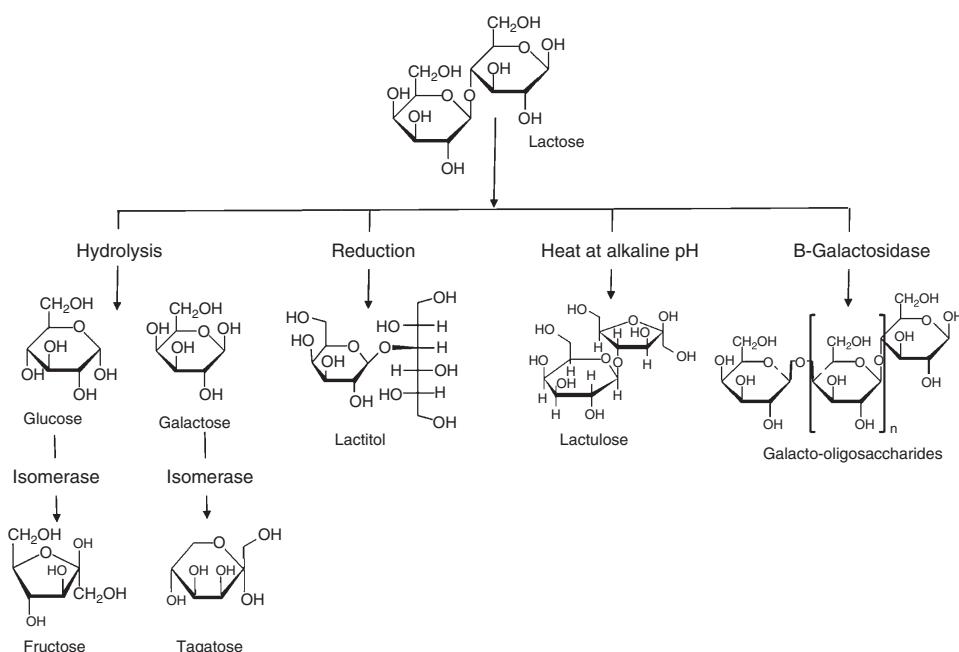
The use of ion exchange technologies to absorb proteins from the whey, followed by selective desorption results in the formation of whey protein isolates (WPIs) with protein contents greater than 88%. Most commercial applications focus on the production of highly purified, unfractionated whey proteins (Kelly and Fox 2012). However, as separation technologies advance, it may be economically feasible to isolate whey proteins with unique nutritional and functional properties (Smithers 2008).

The high protein quality of whey proteins contributes to its applications in sports nutrition and protein supplements. In general, whey proteins are very soluble. In addition to solubility, other functional properties of whey proteins include gelation, foam-formation, and emulsification. The structural characteristics of whey proteins also contribute to the use of whey proteins in edible films and coatings (Ramos et al. 2012). The environment has a significant influence on the functionality of whey powders. Aggregation of the whey proteins occurs at the isoelectric point and with heating, resulting in losses in solubility. Emulsification capacity and foam formation of the whey powders is dependent on the type of the whey powder, pH of the emulsion, and degree of heat treatment (Cayot and Lorient 1997; Hoffman and Falvo 2004; Singh 2009; Smithers 2008).

### 5.4.3 Lactose and Lactose Derivatives

Lactose can be recovered by crystallization from a concentrated (supersaturated, 60–62%, total solids) solution of whey permeate or condensed whey. Due to the low sweetness and low hygroscopicity of lactose, it can be used in food and pharmaceutical applications. Among the applications of lactose in the food industry are uses as an agglomerating/free-flowing agent in foods, to improve the functionality of shortenings in the confectionery industry, as an anti-caking agent in icing mixtures, or as a reducing sugar to enhance Maillard browning (Fox and Kelly 2012). The demand for lactose is significantly less than the lactose produced as a dairy byproduct of cheese and butter processing. The formation of lactose derivatives or hydrolysis products results in products with unique functionality and increased sweetness (Figure 5.3). The relative sweetness and caloric value of lactose, lactose derivatives, and lactose hydrolysis products are compared in Table 5.7. Many of these derivatives are fermented in the colon and function as prebiotics. These lactose derivatives and hydrolysis products provide additional value to dairy byproducts for use in food and pharmaceutical industries.

The hydrolysis of lactose to the monosaccharides, glucose and galactose, results in a syrup with enhanced sweetness for applications in the confectionery and ice cream industries. The hydrolysis of the glycosidic bond can be achieved by either chemical or enzymatic methods. The hydrolyzed syrup can also be consumed by individuals that are lactose intolerant (Gänzle et al. 2008; Kelly and Fox 2012). The sweetness intensity of the syrup can be further increased through the conversion of glucose to fructose by an immobilized glucose isomerase (Siso 1996). The isomerization of galactose results in the



**Figure 5.3** Formation of lactose derivatives from lactose.

**Table 5.7** Relative sweetness and caloric value of lactose, lactose derivatives, and lactose hydrolysis products.

Lactose product	Relative sweetness <sup>a</sup>	Caloric value (kcal g <sup>-1</sup> )
Lactose	20–40	4
Hydrolyzed Lactose	55–70	4
Glucose	60–70	4
Galactose	50–70	4
Fructose	130	4
Tagatose	92	2
Lactitol	30–40	2
Lactulose	40–60	2
Galacto-oligosaccharides	30–60	2

<sup>a</sup> Sucrose = 100.

Source: Adapted from Gänzle et al. (2008), Paterson and Kellam (2009), and Schaafsma (2008).

formation of tagatose. Tagatose has a sucrose-like flavor, reduced caloric value and functions as a prebiotic. Tagatose has received Food and Drug Administration (FDA) approval to be used as a sweetener in a variety of food products (Paterson and Kellam 2009; Schaafsma 2008; Seki and Saito 2012).

The sugar alcohol, lactitol ( $\beta$ -D-galactosyl-D-glucitol) is produced through the chemical reduction of lactose. Lactitol is not digested in the small intestine, but fermented by colonic flora. Additional health benefits of lactitol include reduction of blood cholesterol, reduction of sucrose absorption, and anti-carcinogenic properties (Fox and Kelly 2012; Paterson and Kellam 2009; Siso 1996).

Lactulose ( $\beta$ -D-galactosyl-D-fructose), is produced by heating lactose under slightly alkaline conditions, resulting in the isomerization of the glucose moiety to fructose. Lactulose is a prebiotic and promotes the growth of *Bifidobacterium* spp. in the large intestine, where it is metabolized to lactic and acetic acids. Lactulose also provides a bifidus factor in infant formulas (Fox and Kelly 2012; Paterson and Kellam 2009; Seki and Saito 2012).

Lactitol and lactulose may be used as low-calorie prebiotics in a number of food products. Both derivatives have a caloric value of 2 kcal g<sup>-1</sup> as a result of the metabolism of the colonic fermentation products. Lactitol and lactulose do not contribute to tooth decay and are suitable for use in products such as chewing gum, candies, and ice cream (Paterson and Kellam 2009; Schaafsma 2008).

Galacto-oligosaccharides are oligosaccharides produced from lactose by  $\beta$ -galactosidase. Some galacto-oligosaccharides are found naturally in human and cow milk. These compounds function as prebiotics to provide beneficial effects on the gut microflora, and have been used in infant formula. The prebiotic nature and the low energy value of galacto-oligosaccharides has contributed to growing interest in the application of galacto-oligosaccharides in a number of foods (Paterson and Kellam 2009; Schaafsma 2008; Seki and Saito 2012).

**Table 5.8** Composition of caseins and caseinates.

	Water (%)	Fat (%)	Protein (%)	Lactose (%)	Ash (%)
Acid casein	9.0–9.5	0.8–1.0	88–97	0.1	1.8–1.9
Rennet casein	9.5–11.0	0.8–1.0	85–90	0.1	2.9–8.5
Caseinates (calcium, potassium, sodium)	3–5	0.9–1.5	89–95	0.1–0.2	3.3–5.0

Source: Adapted from Deeth and Hartanto (2009) and Chandan (2016).

#### 5.4.4 Caseins and Caseinates

Caseins may be recovered from buttermilk or skim milk and used as food ingredients. The caseins are precipitated by methods used in cheese processing, either the addition of rennet or acid. The composition of the caseins, particularly the ash content is dependent on the precipitation method (Table 5.8).

Rennet casein is produced by the addition of chymosin to hydrolyze the  $\kappa$ -casein from the micelle, resulting in the precipitation of the casein micelles. Rennet casein is not soluble in water. Most common applications of rennet casein is the production of processed cheese and cheese analogs (Chandan 2016; Deeth and Hartanto 2009; Rollema and Muir 2009).

Acid casein is produced by the acidification of the milk to the isoelectric point of casein. The reduced pH solubilizes the calcium phosphate within the casein micelle, resulting in the dissociation and precipitation of the casein proteins. Treatment of the acid casein with calcium hydroxide, potassium hydroxide, or sodium hydroxide forms calcium caseinate, potassium caseinate, or sodium caseinate, respectively. In solution, calcium caseinate forms a milky cloudy dispersion, while sodium and potassium caseinates form clear solutions (Chandan 2016; Deeth and Hartanto 2009; Rollema and Muir 2009).

Caseins provide good nutritional value and are a good source of high quality proteins, biologically active peptides, calcium, and phosphorous (Hoffman and Falvo 2004). The flexible and amphipathic nature of casein contributes to its excellent emulsification property. Caseins are less effective in foam formation. The flexible structural characteristic of the casein contributes to good foam formation, but the foams are unstable and collapse readily due to limited interactions between casein molecules. The caseins form gels by the addition of acid or proteolytic enzymes, which is the basis for the production of cheese, yogurt, and other cultured dairy products (Dalglish 1997; Singh 2009). Casein-based edible films formed from aqueous solutions have applications in food packaging (Schou et al. 2005).

## 5.5 Conclusions

Dairy byproducts were at one time considered waste products with no economic value. However, through the years, the functionality of these byproducts has been discovered, resulting in their use in many important applications in the food industry. The use of

dairy byproducts enhances the value added to dairy products through processing. With further advances in separation and purification methods, it will be feasible in the future to produce tailor-made' specialty ingredients from dairy byproducts with unique nutritional and functional properties. These dairy ingredients will meet consumers' demands for high quality foods and sustainability of the food supply.

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## 6

### Poultry Byproducts

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#### 6.1 Introduction

Today, the basic issue is not only producing sufficient foods in a profitable manner but also is to do it in a sustainable manner assuring minimal environmental impact. There is a need for high value non-food co-products. The poultry industry creates animal waste from excreta/manure together with waste from poultry and egg processing. These represent a series of environmental problems together with a loss from no producing potential co-products instead of waste products.

Innovative technologies can reduce the input of pollutants on soil and air and re-direct them in the following manner:

- indirectly to food by supplying fertilizer to plants;
- directly to feed of livestock and eventually can supplies and replaces a part of the animal nutrition; and
- supplying value added and culturally sensitive products.

The topics covered attempt to reveal some of the possibilities available for non-food uses of poultry waste, while at the same time reducing the acute environmental problems. Co-products have potentially extraordinary importance in both developed and developing countries. Optimal uses of poultry and other wastes is becoming a national economic issue. This chapter will review the past and current situation together with considering future prospects.

#### 6.2 Chicken Manure/Excreta

There are large volumes of poultry manure produced in the World creating environmental problems. Numerous studies have reported on different uses of poultry manure/excreta. The results have demonstrated that not only is poultry manure not worthless,

but if used well, it can be a very valuable item. More than 90% of poultry manure/excreta is used for agricultural purposes as a fertilizer (Kelleher et al. 2002; Abelha et al. 2003). Today, chicken manure/excreta is used to feed livestock and fish. It is used in a few countries such as India, China, and some African countries to produce biogas for household use. In addition, it is used in some advanced countries, including England, as fuel for modern power plants.

## 6.3 Chicken Manure Types

### 6.3.1 Broiler Manure/Excreta

Chicken manure/excreta has a high nitrogen content containing (White et al. 1944):

- Uric acid – 18–30%
- Ammonia – 17–12%
- Creatine – 2–4%.

It has higher energy than other types of bird manure (Muller 1974). The quality of manure depends on the type of material used for litter as well as the management of manure during the breeding period and storage (Muller 1976).

### 6.3.2 Manure/Excreta from Laying Replacement Pullets

This manure/excreta comes from laying replacement pullets. This manure/excreta contains less litter than that of broiler chickens as less litter is used of pullets.

### 6.3.3 Laying Hens in Colony Systems and Broiler Breeder Litter

These types of manure are collected from floors and nests of colony maintained laying hens or broiler breeders. In general, it contains 16–22% protein and its calcium content is about 6% and its phosphorus in comparison with calcium is low and is about 1.8–2.4% (Flegal et al. 1972; Polin and Chee 1975).

### 6.3.4 Battery Caged Layer Manure/Excreta

Poultry excreta from caged laying hens is relatively pure but containing feathers and broken eggs. It is without litter. Therefore, its nutritive value is higher for use in livestock feeding or as a fertilizer. The following caveat should be followed with this manure. It should not be used for animal feed if the birds are receiving drugs. By drying the manure, its moisture content can be reduced by less than 15%. Care should be taken to ensure it does not contain foreign materials such as rocks, glasses, nails, and wires, etc. if used in livestock feed. Also, its chemical composition should be measured in the laboratory in terms of nutrients such as protein, fat, raw fiber, calcium, and phosphorus (Donaldson 1943).

## 6.4 Methods of Processing Poultry Manure

It is important to collect excreta/manure in a timely but cost effective manner to prevent loss of organic matter including crude protein. At high ambient temperatures and humidities, the rate of decomposition of manure is markedly increased and there is greater loss of nitrogen as inorganic gas (Muller 1956). Therefore, excreta must be processed so that uric acid is either retained or converted into ammonium carbonate which is valuable for plant fertilizers. The following methods are used for treating and storing poultry waste:

### 6.4.1 Stacking Method (Depot)

After the end of the growing or laying period, manure is removed from the house. It can be placed into a specific site in a field adjacent to the poultry house or in the covered area or stored in a cement pit. The extent of aerobic or anaerobic fermentation depends on how long it is stored. The chemical reactions result in changes in the chemical composition of chicken manure and loss of nitrogen. Usually, deposited manure must be mixed regularly. This manure can be used in agriculture (Dana et al. 1978).

### 6.4.2 Drying of Manure

Today, drying poultry manure is common in most countries such as Asian, American, and European countries. It reduces the volume/weight of animal waste by 20–30% (Surbrook et al. 1971). Less space is needed for storage and transportation is cheaper. Other advantages are prevention of propagation of larvae and insects. Manure can be either naturally or mechanically dried. In the natural system, solar radiation reduces manure moisture. Mechanical fertilizer drying is used in modern countries such as Sweden, England, USA, but requires the purchase of equipment (Surbrook et al. 1971). Equipment for mechanical drying is available that also pellets or blocks the poultry waste after drying (Anon 1971a).

### 6.4.3 Ensiling Chicken Manure

Some countries make silage from chicken waste along with other agricultural waste, forage, etc. This can provide livestock feed with 40–65% moisture content and adequate soluble carbohydrates. There is sufficient production of lactic acid and a consequent decrease in pH with ensiled poultry manure with 40% moisture; (Caswell et al. 1975), preserving the nutritive value. In contrast, silage with a moisture content of 22% has reduced uric acid and increased ammonia (Lucas et al. 1975). Ensiling chicken manure is done in combination with rice straw or rice or corn. Ensiling poultry excreta is viewed as acceptable as a proper treatment (Lucas et al. 1975). It is used in large and small farms for animal feed. It can be used in a manner compatible with sound environmental practices and reduces costs in livestock production and aquaculture as feed supplements (Caswell et al. 1978).

## 6.5 Poultry Waste as Cattle Feed

Poultry wastes can be used in cow feed but only if in the dry condition. Dried poultry waste contains the following:

- Dry matter 98.8%
- Nitrogen/crude protein 28.6%
- Fiber 12.4%
- Fat 3% and
- Ash 21.5%.

An example of poultry waste in pelleted livestock feed is the following (Henning and Poppe 1975):

- Cereal 30%
- Dried poultry wastes 30%,
- Beet pulp 14.5%, Straw 25% and
- Vitamin/trace mineral mix 0.5%.

Various feed mixes containing 30% of poultry manure have been fed to dairy cattle experimentally (Henning and Poppe 1975). The results have been promising so far, but some health problems still need to be completely solved. Therefore, the use of poultry dried wastes is recommended only for feeding beef cattle. According to Beseda et al. (1976), feeding poultry wastes causes tension. However, if the diet is properly adjusted, this can be minimized. Dried poultry waste should not form more than 25% of the diets of fattening cattle (Beseda et al. 1976).

### 6.5.1 Broiler Litter in Cattle Feed

Spent broiler chicken litter consists of substrate material together with excreta/waste, spilled feed and feather residue. Its nutritional value depends more on the substrate. Spent broiler litter with sawdust as the substrate contained the following (Sommer and Pelech 1971):

- Dry matter 73.1%
- Digestible nitrogenous materials 83.8% and
- Starch 12.5%.

The digestibility was 46.6% for the spent litter, while in the case of fibers obtained 41.41%. In studies, spent litter was fed to finishing beef cattle in the following proportions (Sommer and Pelech 1971):

- Concentrate – 1.17 kg
- Molasses – 2.27 kg
- Corn silage – 11.63 kg and
- Broiler substrate (ensiled spent litter containing 8–10% of crude protein.) – 4.85 kg.

The average weight gain was 0.936 kg per head for the entire fattening period. There were no adverse effects on the quality of the carcass. Any coccidiostat remaining in the excreta/manure is expected to disappear during fermentation. In another study, cattle

fed with broiler substrate silage together with an oral mix showed the average daily gain of 1.15 kg. In order to prevent the accumulation of undesirable coccidiostat in meat, it removed from the diet 3–4 weeks before the slaughter. The amount of the broiler substrate employed was 3–5 kg per head per day. An appropriate mix of broiler substrate with molasses reduces the amount of grains needed and the cost of the concentrate may be reduced by 25–30% (Greger 1976).

Studies have been performed feeding ruminants with poultry waste to ruminants (dairy, beef cattle, and sheep) and aquatic organisms. Issues related to feeding poultry waste to cattle, the conclusions are based on studies in dairy cattle as follows:

- Before using manure in dairy cattle feed, poultry excreta should be free from pathogens by drying, ensiling, chemical means, etc.
- Processed chicken manure can be used up to the following:
  - 30–25% of the total ration for lactating cows in mid lactation
  - 10–15% of the total ration of high milk producing cows 60% of the protein requirements of a lactating dairy cow with an average production
- If poultry manure is used in dairy cows ration, nutrient requirements are met high energy consumption (molasses, grains, root plants, etc.).
- When a higher percentage of poultry manure is incorporated into the feeds, the more energy-dense components should be added.
- When manure/excreta from broilers and turkeys is used for up to 20% of the total ration, dairy cows need supplementation with calcium and phosphorus. However, the excreta of laying hens is high in calcium, but may need to be supplemented with phosphate.
- While chemical treatment and/or ensiling can greatly reduce off-odors associated with feces, drying is not completely effective. Therefore, poultry dried excreta should be milled so that the particles are mixed well with other concentrates. Adding molasses to concentrate containing poultry dried excreta can make it more palatable.
- There should be a period of adjustment for livestock (Anon 1971b; Surbrook et al. 1971; Henning and Poppe 1975; Beseda et al. 1976; Caswell et al. 1978; Dana et al. 1978; Muller 1980).

## 6.6 Biogas Production of Poultry Manure

Fossil fuels are limited. Energy resources are divided into two categories:

- Renewable
- Non-renewable.

Today we are increasingly recognizing the potential of poultry waste as a renewable energy source for the production of biogas. Optimal use of agricultural and food waste can reduce environmental impacts as well as generating energy. One form of renewable energy is biomass with poultry waste being potentially a source. Anaerobic fermentation of poultry waste generates methane and other flammable gases. When this biogas is collected, a significant amount of usable energy is obtained. Biogas is the gases produced by the digestion of wastes (human, animal, and plant) in the absence of oxygen by anaerobic

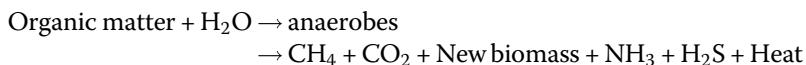
bacteria activity (especially methanogen bacteria). Many of these bacteria have another special feature, that is the ability to digest cellulose. Cellulose is the main constituent of plant fibers. These bacteria are very sensitive to environmental conditions such as temperature, acidity, water content, etc.

Biogas can be used as a source of renewable energy. Biomass consists of all biodegradable components from animal waste, wastewater, agricultural residues (including plant and animal material) and forest product residues together with urban and industrial biodegradable wastes. There is the potential to use large amounts of these materials as a source of energy production. Farmers in North America and Europe have produced biogas from animal, pig, and poultry farms (Salminen and Rintala 2002). There is extensive research to increase biogas production in a cost efficient and competitive manner in advanced countries. Information on biogas in developing countries is limited (Salminen and Rintala 2002).

Biogas can be produced from livestock and poultry manure; the latter including quail waste. Quail meat is a rich sources of protein. Where quail breeding is important, such as in Asia, quail manure can be used as a fertilizer and potentially for biogas production (Nicholson et al. 1999).

## 6.7 Chemical Process of Production of Biogas by Anaerobic Digestion and Its Alternative Applications

Production of biogas involves the degradation of an organic substance under anaerobic conditions by microbial organisms. This leads to the formation of methane together with carbon dioxide:



Organic components of poultry litter are classified into broad biological categories:

- Protein and nitrogenous compounds
- Carbohydrate including cellulose, starch, and sugars
- Fat.

Carbohydrates are a major part of poultry waste. Anaerobic fermentation of poultry waste has two distinct stages (Williams 1999):

*Step 1.* Bacterial hydrolysis of complex components including fat, protein, and polysaccharides to smaller subunits.

*Step 2.* Conversion of the hydrolysis products to gases (mainly methane and CO<sub>2</sub>) by various bacteria including methanogens.

Methane gas from poultry litter can be used to generate electricity.

### 6.7.1 Combustion

Direct combustion of the poultry waste, such as spent litter, has the potential to provide heating for poultry houses and potentially nearby residences. Modern systems have efficient combustion. The heat value of poultry litter is 13.5 GJ per ton which is about half

that of coal. However, this is reduced with increased moisture levels. Poultry waste has a low ashing temperature (Dalólio et al. 2017). This can create problems when using a conventional combustion systems. Parameters to be considered for the efficient operation and design of a combustion center and combustion are combustion temperature, air mixture, and humidity. Production of ash preserves most of the phosphate and all the potash present in the litter (Dalólio et al. 2017). The main nitrogen content is variable with some NOx lost to the atmosphere. The ash is stable, sterile, and easy to transport as an organic fertilizer from conventional poultry litter.

### 6.7.2 Vermiculture or Vermicomposting

There is evidence that vermiculture or vermicomposting can be used to improve poultry manure as a fertilizers. For instance, using *Perionyxceylanensis* with an organic substrate, turkey excreta with cow manure (1 : 1) resulted in a nutrient-rich vermicompost, which can be used as a fertilizer (Jayakumar et al. 2011).

### 6.7.3 Power Generation

Spent poultry litter can be an energy source. There are different types of technologies that are run to convert this biomass to electrical energy. Anaerobic digestion produces biomethane from poultry litter, which in turn generates electricity via turbines. Biogas produced from poultry litter can also be used for heating poultry houses, particularly during brooding (Oliveira et al. 2012).

## 6.8 Solid Waste Systems

Systems for poultry waste treatment include the following:

- Electricity generation by turbines
- Rendering to produce ingredients of livestock feed and fertilizer
- Autoclave incubators garbage used as poultry feed
- Dead embryos and hatchery waste rendered to poultry and livestock feed
- Ensiling/Silage
- Enzyme or sodium hydroxide method
- Composting to reduce pathogens
- Anaerobic digestive systems (Swan 1992; Tchobanoglous and Kreith 2002).

Major environmental contaminants related to poultry production are the following:

- Poultry waste/excreta
- Mortalities
- Feathers.

In summary, there is scope to use poultry waste to produce value-added products such as fertilizer, biodiesel, animal feed, electricity, biogas, bone powder, and biodegradable plastics.

## 6.9 Poultry Feathers

### 6.9.1 Overview

Feathers can be chemically or biologically treated to improve their nutritional value for animal feed. In addition, they can be converted to biodiesel, biodegradable plastics, and organic fertilizers. Chicken feathers contain about 91% protein (keratin), 1% fat, and 8% water. The amino acid composition of chicken feathers shows high levels of serine, cystine, glutamine, and proline and low levels of histidine, lysine, tryptophan, glutamic acid, and glycine. Serine is the most abundant amino acid (16%) in chicken feathers (Kannapan and Bharathi 2012).

Keratin is the insoluble protein of feathers, wool, scales, hair, nails (hard keratin) as well as in the *stratum corneum* of the skin (soft keratin). These particular proteins belong to the group of structural proteins that are highly resistant to physical, chemical, and biological hydrolysis. The mechanical stability and high resistance to proteolytic degradation of keratin is due to the presence of multiple disulfide bonds together with hydrogen bonding, salt association, and interactions.

Chicken feather fiber is composed of alpha helices and beta plate domains. Beta-plates keratin contains higher cystine content than keratin alpha helices (Kannapan and Bharathi 2012). Consequently, there are more disulfide bonds (S-S), which bind the adjacent keratin proteins together. The presence of strong covalent bonds stabilizes the protein structure making it difficult to break (Kannapan and Bharathi 2012).

### 6.9.2 Powdered Feathers

Feathers can be converted to feather powder to be used as a livestock feed or to organic fertilizer. The feed supplement has more than 90% protein content and it is rich in hydrophobic amino acids such as cystine, arginine, and threonine (Tiwary and Gupta 2012). One of the commonly used methods for producing feather powder is a hydrothermal method with feather protein being digested under high pressure at high temperatures. However, the hydrothermal method leads to denaturing and loss of essential amino acids such as methionine, lysine, tyrosine, and tryptophan. This reduces digestibility and gives the product a low nutritional value (Tiwary and Gupta 2012).

### 6.9.3 Chemical Hydrolysis

Chicken feather keratin can be hydrolyzed by the lime method (calcium hydroxide) into a liquid product of amino acids and polypeptides (Coward-Kelly et al. 2006). This can be used as a feed supplement for livestock feed. Treatment at high temperatures (150 °C) results in 80% of chicken keratin being dissolved in 25 minutes. However, a longer reaction time (300 minutes) is required at 100 °C. After three hours of hydrolysis at 150 °C, 95% of the keratin is digested. The following is recommended:

- 100 °C
- 300 minutes
- $\text{Ca}(\text{OH})_2$  0.1 g g<sup>-1</sup>.

allowing about 54% of calcium being recycled. In the rumen fluid, digested keratin solution shows a nutritional value similar to soybean and cottonseed (Coward-Kelly et al. 2006). This process is expensive.

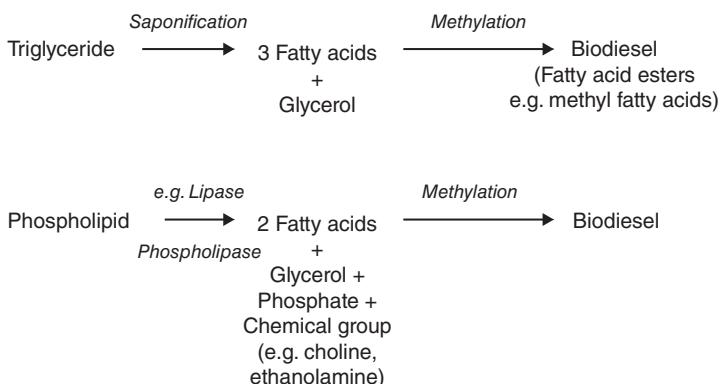
#### 6.9.4 Feather Bioconversion

Feather wastes are used as a protein supplement in the diet as feather powder. While feather waste can be chemically processed at high temperatures to make it digestible, this is expensive. Microorganisms are an alternative approach to increase the nutritional value of feather waste. Feather extract produced by treatment with *Bacillus fermis* has nutritional properties for a feed similar to that of soy protein (Kim et al. 2001). Even though bacterial keratinolytic proteases have the potential for bio-converting of feather, there is a need to improve both enzyme activity and performance for industrial applications (Kim et al. 2001).

Feather ectoparasitic bacteria have been isolated from poultry waste. Three species of *Bacillus subtilis*, *Bacillus pumilis*, and *Bacillus cereus* had marked keratinolytic activity and hence the capability to degrade feathers (Kim et al. 2001). Use of either of latter two keratinolytic proteases has potential. Optimization of conditions are required.

#### 6.9.5 Biodiesel

Poultry processing waste includes feathers, blood and viscera and heads. This is a source of nitrogen for animal feed or as fertilizer. This processing waste contain more than 12% fat (Singh and Singh 2010). This can potentially be used for biodiesel production (Basha et al. 2009; Atabani et al. 2012):



Processes have been developed for the production of biodiesel from feather powder. Fat is extracted in hot water (70 °C) and then saponified and methylated. ASTM analysis confirms that the biodiesel produced from the feather powder had a good quality compared to other biodiesel made from other feed stocks (Song and Guo 2012).

### 6.9.6 Engineered Textiles

Non-woven textiles can be produced using chicken feathers at a relatively low cost (Chinta et al. 2013). There is considerable potential for using chicken feathers to produce textiles (Chinta et al. 2013).

### 6.9.7 Biodegradable Plastics in the Environment

Poultry feather can also be converted into biodegradable plastics by a process called polymerization. Feather keratin is powdered into fine dust and then chemically polymerized at 170 °C into thermoplastics (Thyagarajan et al. 2013). This can be used to produce a variety of products including plastic cups and sheets for furniture production.

### 6.9.8 Fertilizer

Nitrogen fertilizer has been developed from poultry feathers. In this, the keratin fiber is steam hydrolyzed to break di-sulfide bonds and subjected to enzymic hydrolysis using a keratinase from *Bacillus licheniformis* (Choi and Nelson 1996).

Each year, it is estimated that 0.9–1.3 million metric tons of chicken feather from chicken processing are burned, buried, or ground be used in livestock feed or as fertilizer (Thyagarajan et al. 2013). Researchers are seeking better uses for feather waste. It is proposed producing cloth that looks like wool because feathers are mainly made of keratin; this being same the protein as in wool.

Soy protein and chicken feather powder are combined to produce a biodegradable plastics. This was both resistant to moisture but able to breaks down naturally. Richard Pywell at the University of Delaware has employed chicken feathers to produce multiple materials included a printed circuit board featured at an exhibition at the London Science Museum. Feather based “wool” has proposed for the construction of storm-resistant homes (Choi and Nelson 1996).

### 6.9.9 Production of Nano-Particles from Chicken Feathers

Researchers are looking for new ways to recycle and convert feathers to high value added products. In a study, nano-particles have been produced from chicken feathers using an environmentally friendly process that have many applications (Eslahi et al. 2014). The nano-particles exhibited good physical, chemical or thermal properties. This method appears superiority of than other conventional methods is that it used an enzyme hydrolysate and ultrasonic techniques to produce chicken feather nano-particles. There were also reductions in both energy costs and environmental impact. Another advantage is that nano-particles retain the main properties of keratin (Eslahi et al. 2014).

Natural fibers are strong, have high elongation and degradation energies, and are not easily crushed, for instance by conventional milling machines. To produce protein nano-particles, different mechanical methods, including milling have to be used. To increase the efficiency of these methods, fibers are combined before applying mechanical processing together with chemicals. Since these chemicals can be toxic to humans and/or hazardous to the environment, the use of alternative methods is advantageous. To overcome

these disadvantages, enzymic hydrolysis coupled with ultrasound has been used in order to prepare nano-particles from feathers. Based on electron microscopy, the enzyme can penetrate into the feather fiber and destroy non-keratin components. This causes fibrillation resulting in the separation of the micro-fibers (Eslahi et al. 2014). Infrared spectroscopy show no significant changes in the chemical structure of protein fibers after enzymatic hydrolysis and ultra-sonication. Crystallinity is increased with enzymatic hydrolysis and ultrasound waves due to degradation of fibrous amorphous proteins (Eslahi et al. 2014).

The chicken feather nano-particles have a higher thermal stability compared to the primary fibers. The product can be used as following (Eslahi et al. 2014):

- as absorbent to ameliorate contaminations from industrial wastewater; and
- to produce polymer nano-composites used in various textile, automotive and construction industries.

#### 6.9.10 Use of Chicken Feathers to Produce Hydrogen Gas

Chicken poultry waste can be used to produce a material to reduce hydrogen combustion and improve fuel cell efficiency in clean cars. One of the big problems with fuel cells is how to store hydrogen gas without high rates of combustion of hydrogen. Researchers are seeking for materials with a loose bond with hydrogen and reduced its combustion. Chicken feathers heated to 400 °C in the absence of oxygen resulted in carbon porous fibers. When it is cooled to -266 °C, it can store the same levels of hydrogen as carbon nano-tubes. It is suggested that this discovery may be an effective solution for hydrogen storage in vehicle fuel cells.

#### 6.9.11 Ostrich Feathers

The most valuable product of ostriches are their feathers. The qualities of ostrich feathers are unique. Even with the most advanced technology, it is not able to imitate the beauty, elegance and natural excitement of the ostrich feather. High quality feathers are used to make accessories, hats, and clothes. Ostrich feathers are invaluable for cleaning machinery and dusting equipment due to the lack of static electricity (Sales et al. 1999).

Ostrich feathers should be picked every eight months. Over the course of a four to five year life, the quality of the feathers is reduced. The total weight of the feathers of an ostrich at 10–14 months is about 45 g and their length is between 10 and 150 cm (Gunstone and Russell 1954; Sales et al. 1999).

Advantages of ostrich feathers include the following:

- Ostrich feathers are free of static electricity. Thus, they can be used with electronic and computer systems where static electricity is a problem
- Ostrich feathers are used to clean elegant equipment such as cameras, microscopes, computers, decorative items, household equipment, and clothes
- Ostrich main feathers are used to decorate poles at religious ceremonies.

### 6.9.12 Ostrich Feather Categories

Ostrich feathers are categorized as the following:

- *White feathers.* These 24 feathers are arranged in a row on the male ostrich wings;
- *Black feathers.* These are wings feathers in the male that are small and weak feathers lying below the main feathers of wing and tail;
- *Scarce feathers.* There are female wing feathers that are graded as on the light and dark with the white feathers are better than other feathers
- *Dead feathers.* Small feathers under the wings main feathers and the tail of the female ostrich;
- *Silky feather.* A row of feathers under the wings and chest of an ostrich which if belonging to the male ostrich is called black silk and if it belongs to the female, it is called silky-dead; and
- *Body feather.* The ostrich body feathers are black in the male and brown in the female.

#### 6.9.12.1 Tail Feathers

Tail feathers vary from white-brown in the male ostriches to light brown or dark brown in female ostriches.

The length of ostrich feathers is important. Optimally, a feather should be at least 70 cm long and for bodily or silky feather at least 30 cm.

Interest in raising the ostrich has increased, with the number slaughtered greater in recent years. Ostriches are used for meat, skin (leather), feathers, eggs, and specialty oil (Sales et al. 1999). Ostrich oil is widely used in cosmetics as a moisturizer and in the pharmaceutical industry. Ostrich oil contains carotenoids, flavones, polyphenols, tocopherols, and phospholipids in its non-triglyceride portion (Cooper and Horbańczuk 2004). These may have therapeutic benefits including antioxidant properties. Ostrich oil is a high value product in the food industry for humans as well as a supplement for dogs and the cat. The body fat stores is located in the abdomen, chest and back area of the ratite carcass (ostrich, emu, and rhea) (Kawka et al. 2007).

Knowledge on the qualities of ostrich fat is limited. Ostriches have attracted interest as providing high-quality low fat meat, feathers, and eggs. There have been some efforts to determine information on the contents of cholesterol and fatty acids (Horbańczuk and Sales 2001). Supplementation of ostrich feeds with vegetable oils influence meat quality and may impact the oil (Kawka et al. 2007).

## 6.10 Ostrich Oil

Fatty acids are the predominant component of ostrich oil, with a lipid content of 98.8% for subcutaneous adipose tissue, and 98% for retroperitoneal adipose tissue (Abimosleh et al. 2012). Ostrich oil contains the following:

- Oleic acid [omega-9 18 : 1] 42%
- Linoleic acid [omega-6 18 : 2] 21%
- Palmitic acid [16 : 0] 21%.

together with low levels of other fatty acids including 1% linolenic acid [omega-6 18 : 3]. Ostrich oil also contains different amounts of compounds including carotenoids, flavones, polyphenols, tocopherols, and phospholipids in the non-triglyceride group which can have therapeutic benefits including antioxidant properties. Ostriches fed with an unsaturated fat diet (soybean oil) produce oil that contains more unsaturated fatty acids than those a diet rich in saturated fats (Horbańczuk et al. 2008). This indicates that diet can influence the composition of ostrich oil and, possibly, the efficacy of the oil.

For centuries in Egypt, Rome, and Africa, ostrich oil has been used for local relief of dry skin, burns, lesions, contact dermatitis, eczema wounds, psoriasis, sunburn, dry and cracked lips, muscle aches, hair growth, dry hair, bedsore, wrinkles, softening cracked heels, and cuts. Ostrich oil is now widely used in cosmetics and pharmaceutical industries as strong moisturizers (Sales and Horbanczuk 1998; Cooper et al. 2007; Horbańczuk et al. 2007; Poławska et al. 2011).

#### 6.10.1 An Overview of the Biomedical Aspects of Ostrich Oil

Ostrich Oil is rich in polyunsaturated fatty acids (PUFAs); namely linoleic and linolenic acids. These are also considered as essential fatty acids (EFAs). There are many reports about the effects of PUFAs on cell membrane phospholipids, cellular functioning, and tissue protector and cytotoxicity for normal cells (Palanisamy et al. 2011).

Ostrich oil has the ability to penetrate the skin without blocking its pores. This property may be due to the high levels of oleic acid. Thus, ostrich oil can be used as a carrier mediator to aid transfer of pharmaceutical or cosmetic ingredients across the skin. EFAs are known to ensure the proper functioning of the cardiovascular system, fertility, immune system and nervous system. These EFAs are used in the production of phospholipids which are essential for the formation and maintenance of integrity of healthy cell membranes, prevent neurodegeneration and aid brain function and the nervous system (Palanisamy et al. 2011).

#### 6.10.2 Influence on Nervous, Cardiovascular and Immune Systems

EFAs are important nutrients acting as precursors to the group of hormones known as eicosanoids which includes prostaglandins, thromboxanes, and prostacyclines. These help to regulate the central nervous system, blood pressure, heart rate as well as play an important role in the immune system, regulating inflammation and stimulating our body to fight infection. Generally speaking, omega-6 fatty acids are essential for the development of growth, the regulation of metabolism, transport of fatty acids from the liver to the tissue and the maintenance of reproductive performance (Whitehouse et al. 1998).

#### 6.10.3 Effects on Skin and Cancer

Fatty acids at levels found in ostrich oil are increasingly being utilized by the cosmetics industry due to their beneficial effects on the skin. Studies have shown that linoleic acid, when applied topically to the skin, has anti-inflammatory properties, reduces acne, and maintains moisture. EFA deficiency causes inflammation in mice and humans and can be reduced by dermal administration of linoleic acid. Omega-3 fatty acids are also useful to

reduce high blood pressure together with the risk of stroke and arthritis. In addition, omega-3 fatty acids increases survival from autoimmune diseases and cancer preventing (Whitehouse et al. 1998; Palanisamy et al. 2011).

#### 6.10.4 Important Role of Omega 9 Fatty Acids

Omega-9 fatty acids play an important role in inhibiting breast cancer and help to inflammatory responses. Omega 9 fatty acids can support the production of prostaglandins which have multiple biological roles and therapeutic properties. Lipid peroxidation is considered as the main cause of oil oxidation. Peroxidation is common in oils rich in PUFA such as ostrich oil, emu oil, and rhea oil (Palanisamy et al. 2011).

#### 6.10.5 Common Methods of Ostrich Oil Preparation

There are several common ways to produce ostrich oil. In Iran, M-oil is manufactured by the Mobin Tasnim company (Qom, Iran) through a process where the oil become odorless and colorless without affecting its beneficial properties. The common method used to purify ostrich oil is the following:

- The oil is heated to 160 °F (71 °C).
- Mineral silica (1–2%) is added.
- The oil brought to a temperature of 200 °F (93 °C) under vacuum for five minutes.
- The oil in vacuum filtered twice to remove all chemical bleaches and to separate solid fat from pure oil.
- The refined oil is cooled to 98 °F (93 °C) for 24 hours, 74 °F (34 °C) for 24 hours and 60 °F (16 °C) for 24 hours.

Another bleaching process for ostrich oil is performed by adding a minced fat mass into the steaming cooker and then the oil is separated off by hot water at 60–90 °C. The filtered oil is stored under reduced pressure conditions for one day until the odor is removed. The goal of the treatments are to remove impurities from the oil without destroying or damaging any of its beneficial properties (Abimosleh et al. 2012).

Ostrich oil is being investigated for its potential ability for alleviating intestinal disorders. Moreover, there are efforts to assess its effects on physiological/pathological processes such as inflammation and cancer. These *in vitro* screen tests may help prediction of the clinical efficacy of ostrich oil (Palanisamy et al. 2011).

#### 6.10.6 Other Medical Uses of the Ostrich

With the growing economic value of the ostrich, it is important to have more information on the biology of the species. For instance, it is argued that the eye of the ostrich shows close similarity to the human eye and may be used in the corneal transplantation of the ostrich cornea (Abimosleh et al. 2012).

The ostrich tandem foot is also used for human feet grafting and its small brain is also effective in treating Alzheimer's. In fact, Furthermore, the ostrich brain produces a substance that is being studied for the treatment of Alzheimer's disease and other types of dementia (Shanawany 1995).

Ostriches have other advantages. Ancient Egyptians used ostrich eggs for medicinal purposes. The meat has very low fat and cholesterol and is thought of as one of the healthiest red meat available (Bryan 1974). Gelatin from the ostrich bone could be used to make capsules for drug delivery.

#### 6.10.7 Ostrich Skin

Ostrich leather is distinctly different from other leathers in appearance, strength, softness, bending ability, and extreme resistance (Sales et al. 1999). Ostrich leather products include handbags, shoes, boots, coats, and overcoats. European fashion halls have used ostrich leather to make bags for many years. In the United States, this leather was used to produce boots and belts. Due to the unique softness of ostrich leather, some automobile factories or car manufacturers have used ostrich leather in the production of seat covers, dashboards, gearboxes, coated wheels, and even expensive car covers in recent years. The use of ostrich leather for car decoration is more common in Europe.

Manufacturers of sporting shoes employ the strength of this leather and its soft and delicate features. In recent years, ostrich leather has begun to be used in home and office furniture (Sales and Horbaczuk 1998; Cooper et al. 2007; Horbańczuk et al. 2007; Poławska et al. 2011).

### 6.11 Egg Shells

Egg shells are waste from hatcheries and the food industry together with restaurants and homes (Phil and Zhihong 2009). These can be readily collected in large quantities. Problems with egg shell disposal include cost of disposal, odor, and flies (Phil and Zhihong 2009). However, they can be a valuable resource for livestock feed, human foods, building materials collagen production from membranes and artwork (Phil and Zhihong 2009). Using the shells rather than disposing of them reduces the effect on the environment.

Egg shell composition is approximately 98.2%, 0.9%, 0.9% calcium carbonate, magnesium, and phosphate, respectively (Romanoff and Romanoff 1949). The egg shell contains more calcium source than calcium in lime and coral (Romanoff and Romanoff 1949). The eggs shell includes the shell itself together with membranes including the inner and outer membranes. Shell membranes include 69.2% protein, 2.7% fat, 1.5% moisture, and 27.2% ash (Burley and Vadehra 1989). The shell membrane contains about 10% collagen (Froning 1998). Following extraction, collagen has diverse applications in the medical, biochemical, pharmaceutical, food, and cosmetic industries. The values of eggshell and membranes is increasingly recognized. In addition, they may contain useful active biological compounds (Nakano et al. 2003).

#### 6.11.1 Use of Egg Shell

Egg shells contain calcium (~900 mg per egg) together with trace amounts of magnesium, boron, copper, iron, manganese, molybdenum, sulfur, silicon, and zinc. Egg shell is probably the best natural source of calcium being 90% absorbable. Egg shell powder is

made by boiling the egg shells for 5–10 minutes to kill any pathogens, followed by drying and grinding. Egg shell powder can be used as food supplements, for instance, for people with osteoporosis (Schaafsma et al. 2002). Positive effects on femur bone density in women have been reported using egg shell calcium supplements together with magnesium and vitamin D after one year (Rovenský et al. 2003). These findings suggest that postmenopausal women may benefit from high calcium supplementation with egg shell powder.

Extrusion technology has been used to incorporate egg shells into the feed of laying hens (Froning and Bergquist 1990). All type of shell eggs can be used. Waste egg shells can be used as a plant fertilizer because of the calcium content. The production of fertilizer from egg shell is inexpensive and environmentally friendly. Egg shell is an effective source of calcium and alternative to lime (John and Paul 2006).

While lime prevents the soil being too acid, neutral and high calcium overcome acid soils (Holmes and Kassel 2006.).

One study showed that red clover plants grown with ground egg shell fertilizer are more than 10 mm larger than plants grown without egg shells. It has been well-documented that egg shells can be used to increase the content of minerals and expand plants to prevent slurry and scabies. Soil stabilizing agents such as lime are expensive and, therefore, an economically viable alternative is needed (Amu and Salami 2010). Egg shells and membranes are waste materials that can be used as an alternative soil stabilizer. Shell egg powder is not used as a soil stabilizer in most parts of the world such as Hungary, but it could be! Eggshell waste comes from hatcheries, the food industry, restaurants, and homes. Much is presently disposed of in landfills, so there is considerable scope for it to be a resource for value added products such as soil stabilization. The composition of egg shell is about 98.2% calcium carbonate. Egg shell powder improved the plastic index of soil samples (Amu and Salami 2010).

Eggshell powder may be used in construction. Stabilized soil can be used as a bedding material in road construction work. Egg shell powder can be used as an alternative to sand to construct hollow blocks. This is because the powder contains calcium carbonate which gives the blocks hardness and strength (Cecilia et al. 2008). The relative effectiveness of egg shell and sand in hollow blocks have been compared. There egg shell power appeared to be superior (Cecilia et al. 2008). Therefore, egg shell powder may be used to reduce the cost of construction and environments impacts of disposal.

Egg shell are used by artists to make mosaics and texture colors for the three-dimensional effects of artwork (Phil and Zhihong 2009).

### 6.11.2 Use of Shell Membrane

Egg shell membranes contain collagen as demonstrated by the presence of hydroxyproline in the hydrolysate together with biochemical and immunological analysis (Wong et al. 1984). The collagen consists of three types of I, V, and X (Wong et al. 1984; Arias et al. 1992). These types of collagen can have various uses. In comparison to the three collagens in egg shell membranes, there are 25 collagen types in the body (Madison 2011). Collagen is one of the major proteins present in the mammalian body, making

up about 25% of the total protein in the body. Collagen fibers have a high tensile strength. Collagen is widely used in the cosmetics, biomedical and pharmaceutical industries (Jamie 2009). Developments in biotechnology can lead to new uses of collagen in both medicine and industry and considerable potential for expansion.

The sources of collagen has been predominantly pigs and cattle. After the major outbreak of bovine spongiform encephalopathy (BSE) and foot and mouth disease together with autoimmune and allergic reactions, restrictions were introduced on collagen trade. Therefore, safe alternatives are being examined. Collagen in shell membranes have low levels of autoimmune and allergic reactions as well as high bioavailability. Shell collagen is similar to other collagens (Yu-Hong and Yu-Jie 2009) and, therefore, is an alternative to commercial applications in foods, cosmetics and by biomedical and pharmaceutical industries (Yu-Hong and Yu-Jie 2009).

The collagen present in the eggshell membrane can be extracted after digestion with pepsin and acid followed by separation by salt sedimentation (Yu-Hong and Yu-Jie 2009). Collagen along with elastin are integral to the body's connective tissues. Elastin is present where flexibility is required. The combination of collagen and elastin is important in many parts of the body including the lungs, bones, tendons, and blood vessels (Madison 2011).

There is an interest in egg shell membranes as a dietary supplement. Clinical studies have evaluated the safety and efficacy of egg shell membranes as a treatment for pain and non-flexibility associated with connective tissue disorders. A supplement of 500 mg per day from the egg shell membrane for eight weeks was reported to reduce pain and stiffness and improve performance. This improvement was attributed to collagen, glucosamine, chondroitin, and hyaluronic acid content in the egg shell membrane (Ruff et al. 2009).

Collagen supplements is commercially marketed to aid joint mobility. In the food industry, there are multiple applications of collagen, especially after hydrolysis to gelatin (Jamie 2009).

Gelatin is used in the food industry as a bulking, emulsion, and gelatinization agent. Moreover, gelatin or collagen supplements improve the quality of the skin and fingernails (Meier 2006). Skin has a high collagen content but with aging, production of collagen is reduced and the skin is dry with less flexibility. The benefits of collagen supplementation has been accompanied by the development a collagen industry. These collagen supplements have also faced demand for sports nutrition.

It is purported that collagen supplements can increase fat-free muscle, speed up recovery time, repair a joint-damaged joint structure and improve cardiovascular performance in athletes. This is attributable to digestion of collagen providing essential amino acids such as arginine for protein synthesis in muscles, tendons and ligaments during the training and recovery following injury. There are studies where quality of life was improved in cancer patients consuming collagen as a supplement. In many patients with advanced cancers, muscle tissue is lost with the progression of the disease. This is referred to as cachexia. When cancer patients receive hydrolyzed collagen, they showed a wide range of improvements. There is also evidence that collagen supplementation can be helpful in people with chronic arthritis and other conditions. It seems that when there is a physiological demand for new collagen, collagen supplementation may be helpful (Sekine et al. 2001). Collagen is reputedly the oldest adhesive dating back over 8000 years. Collagen has been as an adhesive for musical instruments like violin and guitar and is

often used for repairs. Collagen can easily be softened by reheating, unlike permanent plastic glues (Sekine et al. 2001).

Collagen is used for plastic surgery for beauty and following burns (Meier 2006; Jamie 2009). Collagen can be used to make synthetic skin that replaces skin after burns; synthetic skin being a mixture of collagen, fibroblasts, growth factors, silicones, and glycosaminoglycans. Prior to the development of collagen specific applications, it was not possible to accomplish the needs of victims of fires and of cosmetics (Jamie 2009).

## 6.12 Poultry as an Alternative to Gelatin

Global demand for gelatin is increasing, especially in the food industry and for medicine. The major sources of gelatin is from pigs and cattle with the rest from fish. There are legal, religious and perhaps health issues associated with porcine or bovine gelatin in multiple countries. A particular concern is that products from pigs and cattle gelatin are not permitted by, respectively, Moslems and Hindus. In addition, there can be allergies to porcine, bovine or piscine gelatins (Norizah et al. 2013).

A new potential or re-placement sources for gelatin is chickens using the skin, bone, etc. remaining after further processing. There is limited information on chicken derived gelatin. It has a higher content of glycine, hydroxyproline and proline and has a higher thermal stability than mammals and fish gelatin (Sarabia et al. 2000). Poultry bones, skin and, feet can be processed to poultry and/or bone meal or can be considered as poultry waste. However, there are the challenges of using poultry gelatin at a commercial level.

### 6.12.1 Gelatin Structure

Gelatin is principally derived from collagen. It is primarily used in foods but, in addition, is employed in pharmaceutical, photographic, and technical products. In the food industry, gelatin is used in the following:

- sweets/candy provide chewing ability, texture and foam stabilization;
- low fat spreads to provide firmness and texture;
- baking products as emulsions, gels, and stabilizers; and
- meat products allow water content.

In the human health industry, gelatin is used to make capsules (for pharmaceutical drugs together with vitamins), pills, hemostatic sponges, blood plasma replacements, and suppositories. For the non-food industry, gelatin is used to make films for photography. For all the above purposes, the amount of gelatin used for this worldwide is increasing (Nik-Aisyah et al. 2014).

Gelatin contains high levels of glycine (25%), proline and hydroxyproline. There are two common motifs for amino-acid residues:

- 1) Glycine-Proline-Y
- 2) Glycine-X-Hydroxyproline

X and Y represent any amino-acid other than glycine, proline, or hydroxyproline.

Gelatin contains a low level of cysteine, methionine, and tyrosine. The content of the gelatin amino acid depends on the course raw material. For instance, Nalinanont et al. (2008) reported that the percentages of hydroxyproline varied. There are differences in the distribution of molecular weight due to extraction conditions. Pig and cow skin gelatin are the main sources of gelatin used in the production of foods because these source resources are readily available. Jongjareonrak et al. (2006) argued for the development of alternative sources for pig and cattle gelatin be given priority because of the risk of spreading BSE, commonly referred to as "Mad Cow Disease."

### 6.12.2 Poultry Gelatin

Poultry derived gelatin represents a new type of gelatin that has the following advantages:

- It is acceptable to all major religious groups.
- It has specific properties advantageous to food and/or industrial use.
- There is a ready source from the waste remaining after further processing poultry skin, feet, bones, etc.

Chicken gelatin gel is stronger than bovine gelatin having a much higher real value (0.55) compared to bovine gelatin (0.22) (Norizah et al. 2013). This is because of intrinsic attributes, such as the combination of protein chains, molecular weight distribution, amino acid content and extraction procedure as well as levels and types of collagen.

Chicken skin contains about 5% collagen type I and 25% collagen type III. Chicken skin is mainly used for animal food. In addition, it is used in meat emulsions or as a source of fat, mainly for soup preparation. Poultry skin contains high amounts of fat, but its collagen concentration is low. It is therefore probably as good a source for gelatin compared to other tissues such as legs and feet (Schrieber and Gareis 2007).

Poultry feet (duck and chicken) are another potential source of gelatin production from poultry waste. The massive production of chicken and ducks in Thailand and Malaysia means that there is a lot of feet waste produced and this provides a readily available source of raw materials for the production of gelatin from poultry feet. Limited studies have been reported on gelatin produced from broiler feet (Liu et al. 2001). For example, Lim et al. (2002) reported that collagen extracted from broiler feet has a higher content of hydroxyproline (HYP) and proline (Pro) and show a higher thermal stability than conventional gelatin. Chicken skin gelatin shows similar properties to other gelatin, and it contains a high amount of specific amino acids such as hydroxyproline and proline. Chicken skin gelatin has the highest amino acid. This compound is important for the gel effect and plays an important role in the strength of the gel. It is reported that the collagen of aquatic animals such as fish collagen has a lower amino acid than mammalian collagen.

### 6.12.3 Poultry Gelatin Challenges for Future Commercial Use

Compared to gelatin for cattle, pigs, and fish, the market share of poultry gelatin is still very small.

#### 6.12.3.1 The Limiting Factors for the Gelatin Poultry Industry

The limiting factors for the gelatin poultry industry are:

#### 6.12.3.1.1 Availability of Raw Materials

This should not be a problem as globally, according to the Food and Agriculture Organization (FAO), poultry production is only second to that of pigs and ahead of cattle. However, production of poultry gelatin is limited. A limiting factor is the lack of certification and tracking for poultry raw materials.

#### 6.12.3.1.2 Price

The global popularity of chicken meat is due to price. This would suggest a competitive pricing for chicken gelatin compared to pig/cattle gelatin. In contrast, the price of duck meat is high causing people to be discouraged from buying it. Duck gelatin would be projected to have a high price due to the limited source of raw materials.

#### 6.12.3.1.3 Gelatin Quality

The quality of poultry gelatin would be expected to vary with species, the age of the birds and the relative proportion of skin, feet, and bones used as the source.

#### 6.12.3.1.4 Health Issues

Although there is no dietary issues, there may be health issues by viral contamination with human pathogens such as from avian influenza (e.g. H5N1). However, preparation of gelatin ensures that the product is pathogen free.

### 6.12.4 Prospective for Poultry Gelatin

Global production of poultry gelatin is small but potentially may increase to replace gelatin from conventional sources. We anticipate that poultry gelatin represents an alternative to commercial gelatin. Moreover, poultry gelatin may become a new product that offers unique and competitive properties with other bio-based polymers.

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## Utilization of Egg Byproducts for Food and Biomaterial Applications

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### 7.1 Introduction

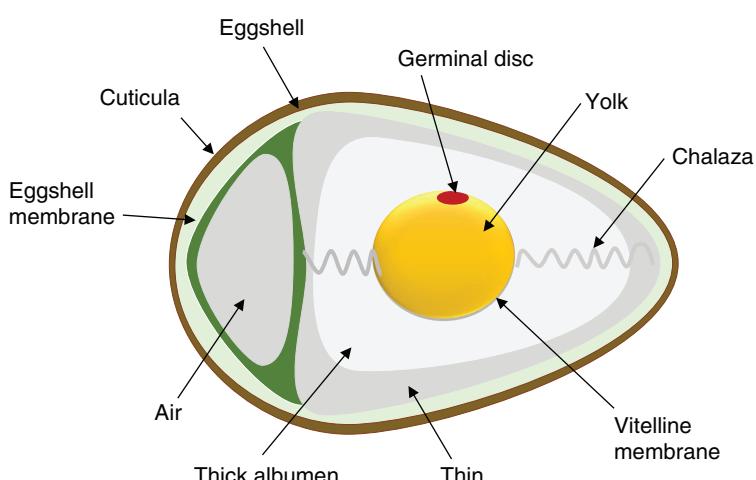
The demand for egg and egg products has increased in many parts of the world (especially in Asia and Africa) and this has led to a surge in global egg production. Eggs are an important part of the human diet, as they provide many essential nutrients, including essential amino acids; eggs are generally high in proteins, low in carbohydrate, and the lipid component comprises mainly of monounsaturated fatty acids (Miranda et al. 2015). Due to their structural and functional properties, egg components are also used by the food industry to develop functional products. Hen egg production more than tripled between 1970 and 2005 (Windhorst 2006). Asia has dominated the egg industry with China accounting for 36.9% of total global egg production, followed by the USA 8.2%, India 5.4%, and Japan 3.8% (Zaheer 2015).

The generation of waste is an ongoing problem globally, especially in the agriculture and food sectors. Egg processing for human consumption leads to the generation of large amounts of byproducts particularly eggshell, which constitutes most of the industrial waste, as well as egg yolk and whites that are discarded due to their "defects." With 70 million tons of egg produced worldwide in 2014, of which 11% of the dry weight is composed of the shell, approximately 8 million tons of underutilized eggshell waste is generated every year (Oliveira et al. 2013). This is a substantial loss of reusable bioresources, in addition to the financial burden it creates for egg producers to dispose of the egg waste in landfill and, in some instances, for composting and use as fertilizer, or in animal feed formulation. These examples represent underutilization of the renewable eggshell byproducts considering their valuable composition (e.g. calcium carbonate and glycoproteins). For the sustainability of the food industry and reduction in environmental problems caused by waste disposal, there is a need to add value to eggshell biomaterials. This can also lead to economic benefit to the egg industry. To date, efforts have been made to transform eggshell waste into materials with functional properties or biological activities.

For instance, peptides derived from eggshell membrane (ESM) proteins have shown antimicrobial, immunomodulatory, antioxidant, and anticancer activities, which are relevant in the prevention and treatment of chronic and infectious diseases (Miranda et al. 2015; Abeyrathne et al. 2013). Carotenoids and lecithin from discarded egg yolk can also be used in food product development as colorant and emulsifying agent, respectively. Furthermore, egg byproducts have been explored for use as biocompatible materials (biomaterials), for replacing non-renewable resources, which can be utilized for food, pharmaceutical and medical purposes. For instance, calcium carbonate, the major component of eggshell, can be used to produce polymer composites such as hydroxyapatite for medical applications such as teeth and bone tissue engineering. This chapter highlights the composition of egg byproducts and processing technologies for transforming the waste and recovery of functional biomolecules and biomaterials for food and non-food applications.

## 7.2 Composition of the Avian Egg

The avian egg comprises of four compartments; a central yolk, albumen (egg white), ESMs and a calcified eggshell (Hincke et al. 2012) (Figure 7.1). The whole egg contains water, lipids, proteins, carbohydrates, and minerals (Abeyrathne et al. 2013). Proteins are mainly found in the egg white and to a lesser extent in the yolk, while lipids are mainly found in the yolk. Understanding the composition of the egg is essential for the separation and application of its byproducts and bioactive components. The egg begins its formation in the ovary and oviduct and acquires its layers as it passes through the tubular organ (Hincke et al. 2012). The main product formed is the egg white, while the egg yolk can be used both as a main product or byproduct, the eggshell is a byproduct that is either discarded or further processed into functional ingredients.



**Figure 7.1** Structure of an egg showing its different parts, including the eggshell (ES) and eggshell membrane (ESM), which are discarded as byproducts of egg processing.

The yolk is a single cell that is suspended by chalazae, which are spiral structures extending from the yolk into the egg white at each end. Chalazae can support and protect the yolk from damage during incubation allowing healthy embryotic development (Itoh et al. 1990). The yolk represents about 29% of the total egg weight; its main components include water, lipids, proteins, minerals, and carbohydrates (Abou-Elezz Fouad Mohammed et al. 2012). Its components are separated by the two main fractions; the dry matter granule and soluble plasma. The total yolk is composed of five main constituents: 68% low-density lipoproteins (LDLs), 16% high-density lipoproteins (HDLs), 10% globular proteins (livetins), 4% phosphoprotein (phosvitin), and 2% minor proteins (Anton 2007). These components are divided into two fractions: granule containing HDL, phosvitin and LDL, and the plasma containing LDL and livetins. The lipid components of the yolk include triglycerides (mainly oleic, palmitic, and linoleic acids) acid, phospholipids such as phosphatidylcholine and omega-3 fatty acids, cholesterol, and carotenoids such as lutein and zeaxanthin (Anton 2007). Significant portion of yolk proteins are found in the form of lipoproteins (HDL and LDL) formed as a result of interaction between proteins and lipids. Other proteins found in the yolk include globular proteins (livetins), phosphoproteins (phosvitins), vitamin-binding proteins, immunoglobulins (IgY) and other minor proteins.

The main product of egg, the egg white, accounts for 63% of its total weight. It is formed during the passage of the yolk past vitelline membrane (VM) and into the oviduct secreting the layers of egg white (Hincke et al. 2012; Nys and Guyot 2011). Four different layers make an egg white, the outer fluid layer, the thick layer, inner thin fluid layer and the shallow dense layer encapsulating the VM of the yolk and attached to the chalazae (Parkinson 1966). Egg white is primarily responsible for providing nutrients to the developing chick and protection against pathogens via antimicrobial proteins (Rehault-Godbert et al. 2010; Alabdeh et al. 2011). Water is the main component of the egg white, followed by soluble proteins, carbohydrates, ashes, and, unlike the yolk, trace amounts of lipids. The major proteins in egg white include ovalbumin, ovotransferrin, ovomucoids, lysozyme and ovomucin while, avidin, ovomacroglobulin, and ovoinhibitor are some of the minor proteins (Abeyrathne et al. 2013).

The eggshell is a highly mineralized structure accounting for 9.3% of total egg composition. Its fundamental functions include protection of the egg from the microbial and physical environment, assisting in the exchange of water and gases during the development of chick embryo, and providing calcium for embryonic development once yolk stores are depleted (Hincke et al. 2012; Nys et al. 2004). The organic matrix membrane and the inorganic matrix shell make up the eggshell structure. ESM are interlaced fiber structures with inner and outer layers that surround the albumin. The fibers of the outer membrane are mineralized, while the inner membrane remains uncalcified (Nys et al. 2004). During mineralization, ESM provides fibrous support.

### 7.3 Processing of Eggs

Eggs were essentially marketed as whole shell eggs for many years but nowadays, those that are not packed for consumption are processed into different products, such as refrigerated liquid, frozen, dried and specialty products. These egg products are used

worldwide in the food, cosmetic and paint industry (Yoo et al. 2009; Ishizuka et al. 1992). Eggs can be separated into whites, yolk, and whole egg at a rate of 32 000 eggs per hour per single row in single-tier egg breaking machine. The drying (i.e. spray) is performed under mild conditions to preserve the quality of the product (Bergquist and Eggs 2007). The use of spray-dried egg yolk in commercial processing is limited because of the heat-induced thermal denaturation of proteins that affect their functional properties. The liquid yolk is then often pasteurized and refrigerated or frozen, although some gelation may occur as a result (Jaekel et al. 2008; Telis and Kieckbusch 2006). In 1990, about 216 million, 390 million, and 661 million pounds of yolks, albumen (whites), and whole dried eggs, respectively were produced in the United States alone (Stadelman 1995). The large amount of eggs broken leaves behind a large quantity of shells, which represent about 11% of the total egg weight.

The eggshell, which is a waste, has two main components: the shell (eggshell, ES) and membrane (ESM). ES and ESM are very different in chemical composition with ES being mostly inorganic while ESM is mostly composed of proteins. The whole eggshell can be milled for various purposes, including the preparation of composite material, absorption of minerals, and use as biomaterials. Ball milling is a common procedure used to bring the shell into fine powders. Many parameters of the milling process (e.g. gravitational acceleration, time) can be varied to obtain powder with different particle sizes and of different morphologies. The time is generally short (2–10 minutes) when the process involves studying mechanochemistry of the grinded shell, compared to hours for other properties (Baláž 2018). Co-milling agents can be used for two main purposes. Gelatin, starch, and polypropylene glycol, for example, are used as protective agents to facilitate the milling process by minimizing wear (Apalangya et al. 2014; Francis and Abdel Rahman 2016; Mondal et al. 2012), whereas phosphoric acid, magnesium oxide, silver nitrate, or polyvinyl are used to react with calcite in the eggshell or with components of the ESM (Wu et al. 2015; Sharafabadi et al. 2017; Baláž et al. 2014). The high mineral content of eggshell makes it useful as a soil conditioner, an additive for animal feed, and as a source of calcium for human nutrition. The eggshell also has other potential applications because of the nature of its proteins (Du et al. 2015).

## 7.4 Extraction and Separation of Bioactive Compounds from Egg Byproducts

Many methods have been used for the separation of compounds, especially peptides, with biological activity from food and other sources. Some common methods include enzymatic hydrolysis, microbial fermentation, solvent extraction and synthesis via chemical, enzymatic or DNA technology methods. The separation of method differs, depending on whether the part of interest is the yolk, egg white, or eggshell.

### 7.4.1 Enzymatic Hydrolysis

Enzymatic hydrolysis is a common process used to breakdown macromolecules in byproducts, thereby facilitating their extraction, separation, and identification. Eggs is mainly composed of proteins, some of which are tightly bound to the shell, and

proteases have been used in the extraction and separation of these components. This process often leads to the production of protein hydrolysates and peptides. Enzymes such as pepsin, bromelain, trypsin, chymotrypsin, papain, and ficin have been used for enzymatic hydrolysis, which is typically followed by separation using membrane and chromatography techniques (Abeyrathne et al. 2013; Mazorra-Manzano et al. 2017). However, due to high cost associated with some enzymes, fermentation using lactic acid bacteria is a cheaper alternative because of the diverse proteases expressed by the cells. Different proteases produce hydrolysates with different functions and biological activities. Novel peptides from yolk proteins have been identified after hydrolysis with pepsin (Zambrowicz et al. 2015) and pumpkin proteinase (Eckert et al. 2014) followed by purification by ultrafiltration, ion-exchange and reverse-phase chromatographic separations. Using response surface methodology, pepsin has been successfully used to solubilize collagen from ESM (Mohammadi et al. 2016), largely due to proteolysis. Furthermore, the separation of eggshell albumen adhering to the ESM was achieved by centrifugation and the remaining eggshell recovered by drying at 80 °C immediately after breaking to avoid microbial spoilage (Derere et al. 2017). Following the drying process, the eggshells are usually processed further before industrial application.

#### 7.4.2 Non-Organic Solvent Extractions

Egg yolk is a complex mixture with two main fractions, a granule and a relatively more abundant water-soluble fraction, plasma. Main components in the granule are the HDL, lipovitellin, and the phosphoprotein phosvitin, while LDL and the globular protein, livetin are the main ones in yolk plasma (Dyer-Hurdon and Nnanna 1993). The two major yolk fractions can be separated by dilution with water, at neutral pH or in sodium chlorine solution followed by centrifugation to remove the insoluble debris (Dyer-Hurdon and Nnanna 1993; Laca et al. 2014).

The separation of ESM from raw eggshell (ES) requires the use of metal chelators such as ethylene diamine tetra-acetic acid (EDTA), in acidic or basic environments. In the study of Ishikawa et al. (1999), ES was kept in 0.5 M HCl overnight followed by mechanical stripping of ESM from the shells. In other works, ES was kept in 0.5 M HCl and then in 0.5 M NaOH for one hour each followed by mechanical stripping (Ishikawa et al. 2002), or by manual stripping after one hour of ES in 5% EDTA, resulting in partial demineralization of ES and loosening of the membrane from the shell (Soledad Fernandez et al. 2001). The ESM is made up of molecules that are not soluble in water. This is due to the presence of cross-linking between thiol groups of keratin and hydroxylysino-norleucine and desmosines (Crombie et al. 1981; Sah and Rath 2016). Soluble proteins in ESM therefore need to be extracted with non-toxic solvents, but not 100% water due to their insolubility (Sah and Rath 2016). For instance, soluble ESM proteins were obtained by performic acid oxidation of ESM followed by pepsin digestion (Takahashi et al. 1996), reductive cleavage of cross-link bonds with 3-mercaptopropionic acid (1.25 N) in the presence of 10% acetic acid (Yi et al. 2003), and dissolution in 5 N beta-thiopropionic acid (Sah and Rath 2016). The preparation of soluble proteins from ESM is important as it facilitates their use in a wide range of applications like foods, biomaterial or tissue engineering.

### 7.4.3 Organic Solvent Extractions

Lipid components of egg yolk are separated from lipoproteins using mainly organic solvents, although a percentage of inorganic solvent can also be used. In fresh yolk, polar solvents such as ethanol are used, as lipids are in lipoprotein forms and contain high amounts of water. However, spray drying the yolk before separation causes free fat to be released from lipoproteins allowing for an easier extraction using a combination of polar and non-polar solvents (Palacios and Wang 2005). Phospholipids have many applications in the food, cosmetics and pharmaceutical industry and their industrial extraction has been intensively studied. Solvent extraction is the primary method used in extracting lipids from fresh egg yolks. Raw egg yolks are first defatted with hexane and dried before the removal of lipoproteins using ethanol (Palacios and Wang 2005). The lipoproteins are then treated with additional ethanol to remove residual proteins from the lipids. The main neutral lipid (NL1), high-cholesterol neutral lipid fraction (NL2), and phospholipids (PLs) are differentiated using hexane extraction for NL1 followed by a combination of acetone/hexane for NL2 recovery (Palacios and Wang 2005). However, these methods can have drawbacks as organic solvents can be detected in the final product, which will lead to differences in the chemical composition of extracts from one batch to another (Laca et al. 2010). Although extraction of lipids using heating methods has been explored, at temperatures of 50–60 °C, heating can denature the protein and therefore making them undesirable in applications where it is important to preserve their native structures (Liot and Anza 2001). Proteins from defatted egg yolk can further be processed and purified for structural and functional studies.

## 7.5 Bioactive Components of the Eggshells and Egg-Yolk

The food and non-food applications of bioactive components in the egg have been explored over the years and continues to gain interest with the advances in processing technology and as the functional food industry expands. It is important to utilize egg byproducts, eggshells, and egg yolk as they can otherwise contribute to environmental pollution and challenges involving high-cost disposal, increase in disposal sites, odor, and abrasiveness (King'ori 2011). Bioactive components found in the eggshell and egg yolks are diverse and they possess emulsifying, coagulating, antimicrobial, antioxidant, antihypertensive and immunomodulatory properties, as well as the ability to enhance overall nutrition and nutrient absorption and bioavailability (Bhat et al. 2015). Several functional foods, biomedical and biomaterial applications that take advantage of the bioactivity of these byproducts, mainly egg yolk and egg shell, are already on the market, or are being developed for commercialization.

### 7.5.1 Antihypertensive Properties

Hypertension is characterized by an abnormally high blood pressure. The renin–angiotensin system (RAS) pathway plays a key role in maintaining blood pressure homeostasis, fluid and salt balance in humans, and as such has been targeted for the development of antihypertensive drugs. Some of the effort focus on components from foods including peptides (Bhat et al. 2015). Hypertension is also a risk factor for

cardiovascular diseases. Many factors can contribute to the prevention of hypertension including the reduction of salt intake, fat, and sugar as well as lifestyle changes (e.g. physical activity, smoking) (Brook et al. 2013). Drugs alternative include egg peptides because of their effect on the RAS system, especially in inhibiting angiotensin converting enzyme (ACE) (Grootaert et al. 2017). Specifically, ACE inhibiting peptides are normally derived from egg white ovalbumin, ovotransferrin, and lysozyme, but some have also been derived from egg yolk proteins after hydrolysis with commercial and non-commercial proteases (Eckert et al. 2014; Yoshii et al. 2001; You and Wu 2011). Yousr and Howell (2015) also reported ACE inhibitory activity ( $IC_{50}$  3.35 mg ml<sup>-1</sup>) for an egg yolk fraction after hydrolysis with pepsin/pancreatin, sequential fractionation by ultrafiltration, and gel filtration. Many studies on ACE inhibitory protein hydrolysates or peptides from eggs have been done *in vitro* and occasionally in rats and humans (Yousr and Howell 2015). Overall, fractionation of the yolk hydrolysate into low molecular weight fraction is important to improve ACE-inhibitory activity.

### 7.5.2 Mineral Binding and Antioxidant Properties

Protein hydrolysates and peptides have shown positive effects on the absorption of minerals *in vitro* and *in vivo*. The chelating ability of these peptides is a key factor in promoting absorption. This chelating property also contributes to their antioxidant properties. Many egg yolk and eggshell hydrolysates have been studied for their mineral binding and antioxidant properties. Phosvitins, phosphorous rich egg proteins can form a complex with metal ions under low ionic strength and in acidic conditions. Feng and Mine (2005) studied these properties and found that phosvitins increased the absorption of iron and calcium through binding and consequently lowering their precipitation. The mechanism involved binding of one phosphate group of the protein to calcium or iron ions (Feng and Mine 2005). In another study, two fractions of yolk hydrolysates were shown to chelate ferrous ions (Yousr and Howell 2015). Ferrous ions are known to act as a catalyst in the generation of free radicals and the initiation of oxidative reactions, and their chelating can lower oxidative stress-related damages in the body. In addition, phosvitin has a better effect than ferritin and transferrin in directly scavenging hydroxyl radicals generated via the reaction of ferrous ions and hydrogen peroxide (Ishikawa et al. 2004). Moreover, fermented ESMs showed dose-dependent antioxidant properties as they scavenged free radicals and reduced ferric ions (Jain and Anal 2017). This scavenging and reducing capacity of ESM hydrolysates can be due to their composition of electron-donating amino acid residues (tyrosine and histidine) as well as their small molecular sizes, which can increase their accessibility to the radicals in solution.

Lutein and zeaxanthin are abundant in the yolk. They are known components of the macular eye pigment and play a role in protecting it from age-related degeneration (Landrum and Bone 2001). Their mechanism of action is associated with singlet oxygen and radical scavenging activity, which leads to a decrease in light-induced oxidative damage. Besides, their ability to absorb blue light plays a role in their antioxidative behavior (Nimalaratne and Wu 2015). Supplementation of lutein to human lens epithelial cells suppressed proteins and lipid oxidation (Gao et al. 2011), while in mice it reduced plasma lipid hydroperoxides, size of aortic lesions, and plasma levels of oxidized LDL in mice and guinea pigs (Kim et al. 2011).

### 7.5.3 Antimicrobial Activity

Bioactive materials with antimicrobial property have been identified in many natural sources including microorganisms, animals, and plants. Hydrolyzed proteins and peptides with antimicrobial activity can be used to inhibit food spoilage bacteria, fungi, and other pathogenic agents (Bhat et al. 2015). The presence of tryptophan and arginine can increase the antimicrobial property of a peptide, because tryptophan promotes the folding of peptides in aqueous solutions, as they are attracted to the membrane resulting in amphiphilic peptides that are easily transported across the cytoplasmic membrane, whereas arginine exerts positive charges on peptides that are then able to form hydrogen bonds with anionic components of bacterial cell wall (Chan et al. 2006). Egg proteins contain many antimicrobial proteins such as lysozyme, avidin, and ovotransferrin (Baron et al. 2016). Another mechanism of action by which proteins and peptides are able to cause microbial cell death is by binding to the lipopolysaccharide cell wall and causing aggregation and destabilization.

Lysozymes are highly concentrated in the egg white but are also found in the eggshell, ESM and the matrix of the eggshell (Hincke et al. 2000). They can damage the bacterial cell wall by attacking the peptidoglycans in the cell wall of mainly gram-positive bacteria such as *Bacillus stearothermophilus*, *Clostridium tyrobutyricum*, and *Clostridium thermosaccharolyticum*. They are known to use bacteriolytic activity to hydrolyze the  $\beta(1-4)$  linkage between N-acetylmuramic acid and N-acetylglucosamine of peptidoglycan (Mine and Kovacs-Nolan 2006). Their effect is known to increase when used with organic compounds such as EDTA, organic acids, and niacin, and they maintain their activity after heating and in solution of pH 4.5–7.0 making them applicable in a wide range of food product matrices (Abdou et al. 2007). Ovotransferrin is also found in the ESM and displays antimicrobial activity (Makkar et al. 2015). Like most of the transferrin family, ovotransferrin can deprive microbes of iron that is essential for their growth. In addition, a cationic peptide located within the 109–200 sequence of the N-lobe of hen ovotransferrin is known to permeate the cytoplasmic membrane of *Escherichia coli* leading to growth inhibition (Ibrahim et al. 2000). The extent of permeation was independent of the transmembrane potential. In the yolk, one of the most studied antimicrobial molecules is immunoglobulin Y (IgY), which is the major type of immunoglobulin present in high concentration in hen yolk. IgY is also stable in solutions of different pH and temperatures, making it ideal for food and biomedical applications (Berghman et al. 2005). Furthermore, *in vivo* treatment with specific IgY antibodies minimized *Pseudomonas aeruginosa* colonization in the lungs of cystic fibrosis patients (Hans et al. 2003), presenting a potential alternative to antibiotics treatment. In addition, yogurt fortified with anti-*Helicobacter pylori* IgY suppressed *H. pylori* infection in human subjects (Horie et al. 2004). *In vitro*, IgY inhibited the growth of *Piscirickettsia salmonis* in a liquid medium and in Atlantic salmon (Oliver et al. 2015), which shows a promising application in the treatment of epizootic diseases in fish. Egg phosvitins, an iron-binding phosphoprotein can disrupt bacterial cell wall and cause DNA leakage in gram-positive bacteria, *E. coli*, as a result of combined effect of its metal-chelating activity and high surface activity (Choi et al. 2004).

## 7.6 Commercial Application of Egg Byproducts

Egg yolk and eggshells are two byproducts of the egg industry, with eggshells being the most abundant. They play crucial roles in the development and safety of the egg embryo but also possess many applications in the food, feed, pharmaceutical, medical, and engineering industries. This section covers current and prospective applications of compounds and biomaterial derived from these egg byproducts.

### 7.6.1 Food Applications of Egg Yolks and Eggshells

Egg yolk granules contain proteins and peptides, which provide many applications in the food industry due to their foaming, coagulative, emulsifying, and coloring properties (Laca et al. 2014). Eggs contribute to both the texture and flavor of food items such as cakes, baked goods, batters, and mayonnaise. Egg yolk as a whole, as well as its granule and plasma fractions, are currently being used in the development and stabilization of different food and snacks (Valverde et al. 2016). Yolk lecithin is used in the pharmaceutical and cosmetic industry as an emulsifier (Palacios and Wang 2005). Lecithin is a triglyceride in yolk containing hydrophobic fatty acid residues and a hydrophilic head. These properties give it the ability to migrate between two immiscible phases and reduce interfacial tension, thereby allowing the formation of a stable emulsion (Rossi 2007). An edible application of lecithin is for the formulation of a mayonnaise-like item with reduced oil. In applications where there is a concern of allergic reactions soy lecithin is preferred, although at the moment the one from egg yolk is highly recommended for infant formulae due to its relatively high content of arachidonic acid and docosahexaenoic acid, which play a significant role in early infant nutrition (Gil et al. 2003). LDLs are another emulsifying compound in the egg yolk (Anton et al. 2003). Egg plasma is preferred for emulsification purpose because of its high content of LDL; however, a recent study showed increases in the emulsifying and foaming properties of granules after the removal of phosvitin (Chalamaiah et al. 2017).

Lysozymes mainly present in egg white, but also in ESMs and eggshell matrix (Hincke et al. 2000), are extensively used as preservatives due to their antimicrobial activity. Properties such as high heat stability in acidic conditions make them valuable to be applied in foods (Cunningham et al. 1991). In fact, their use as preservatives in the food and therapeutic industry has been approved by Food and Agriculture Organization (FAO)/World Health Organization (WHO) and in countries such as Japan, United Kingdom, Spain, Italy, and Germany (Mine et al. 2004). Fresh vegetables, fruits, fish and meat, have been preserved using lysozyme (Cunningham et al. 1991). They are used in cheese to prevent spoilage as there is no interference with the primary and secondary cultures required for the ripening of cheese (Anton et al. 2006), and in soya milk to preserve the curd (Mine et al. 2004). Additionally, lysozyme can be used to maintain the quality of kimchi, a Korean dish made from salted and fermented vegetables, Chinese noodles and custards (Yashitake and Shinichiro 1997), and also to control the bacterial activity of wine and shelf life of unpasteurized beers (Schneider et al. 2011; Daeschel et al. 1999). Biotechnological tools have enabled the incorporation of lysozyme into food packaging materials, mainly to preserve single food items that spoil at the surface (Conte et al. 2006; Rao et al. 2008).

There are numerous applications of the eggshell (Conte et al. 2006), which is composed mainly of minerals with calcium being by far the most abundant. Eggshell meal at a level of 0.4% (w/w) was incorporated into human food without any effect on cooking quality (Ockerman and Hansen 1999). Eggshell meal has also been approved for use in animal feed by the Association of American Feed Control Officials since 1982; however, limestone and oyster shells are frequently used as they are cheaper alternatives (Derere et al. 2017). The availability and digestibility of calcium in piglets are higher when fed eggshell compared to purified calcium carbonate (Schaafsma and Beelen 1999). Furthermore, eggshell powder can be used in the synthesis of calcium glutamate used as a calcium supplement, salt substitute, and flavoring agent (Wei and Wang 2009). In a study by Schaafsma et al. (2002), supplementation of eggshell powder in the meals of healthy post-menopausal women increased bone mineral density within 12 months.

### 7.6.2 Biomaterial and Biomedical Application of Eggshells

The high mineral content of eggshell coupled to its unique proteins make it a good bio-filler, as an inexpensive and renewable candidate for the preparation of polymer composites (Bootklad and Kaewtatip 2013). It is very useful in the synthesis of plastics such polyethylene, polypropylene, and poly(styrene-b-ethylene/butylene-b-styrene) (Kang et al. 2010; Iyer and Torkelson 2014; Supri et al. 2010). Toro et al. (2007) found that the benefit of eggshell was due to its higher dispersibility in the polypropylene matrix because of higher surface/volume ratio compared to commercial synthetic materials. A nanocomposite prepared with 2% eggshell powder-to-Bioplast GS 2189 polymer significantly increased the strength and modulus of the material (Hassan et al. 2014). In a related work, the use of eggshell power as a filler in the preparation of thermoplastic starch showed a stronger adhesion between components and better biodegradation of the resulting thermoplastic compared to the one made with commercial calcium carbonate (Bootklad and Kaewtatip 2013).

Eggshell powder is useful in the preparation of bioactive materials containing calcium and phosphate. One common example is hydroxyapatite that supports bone ingrowth, and can be produced from eggshell but also from coral, seashell, and body fluids (Gergely et al. 2010). Hydroxyapatite made from eggshell has been used in bio-inert implants, prosthetics, and bone fillers (Gergely et al. 2010; Rivera et al. 1999). At the nanoscale level, hydroxyapatite from eggshell has been applied in tissue engineering, drug delivery agent and as carriers for non-viral gene delivery (Prabakaran and Rajeswari 2009; Zhou and Lee 2011). In the medical industry, collagen from eggshells, but mainly from other sources (e.g. bovine, swine skins, and bones), are used to make dermal filler, wound dressing, artificial substitute for skin burns, and delivery systems (Parenteau-Bareil et al. 2010; Natarajan et al. 2010). Collagen can be used in combination with a mixture of fibroblasts, growth factors, silicone, and glycosaminoglycans for the design of implant materials (Harriger et al. 1997). ESM collagen has the advantage of a higher biosafety and lower autoimmune reactivity when compared to other mammalian collagens (King'ori 2011). The topical use of hydrolyzed ESM proteins in a formulated product over eight weeks significantly reduced facial skin wrinkle depth through an antioxidant mechanism and stimulation of dermal fibroblasts that secreted high levels of matrix components (Jensen et al. 2016). Lysozyme from eggs and other

sources have prospective application in the treatment of dental caries, protection of nasal tissue, and has been incorporated into creams for the treatment of burns and viral diseases (Abdou et al. 2013).

### 7.6.3 Absorbent Properties of Eggshells

The potential of ESM in the removal of metals from fluids dates back to 1994 when the shell membrane was proven to bind minerals, thereby promoting its use in purifying aqueous solutions (Suyama et al. 1994). Since then, ESM has been used to remove not only metals such as silver, gold, copper, or cobalt, but also organic molecules such as chromene dyes (Ishikawa et al. 2002; Arami et al. 2006). The adsorption properties of ESM are related to its large surface area and unique functional groups that enable it to form stable lattice networks with high homogeneity and adsorption capacity (Abdolmohammad-Zadeh and Talleb 2014; Park et al. 2016). Lead was adsorbed at  $24.94 \text{ mg g}^{-1}$  (Rao et al. 2010) on eggshell powder while up to  $161 \text{ mg g}^{-1}$  of chromium-VI, a known carcinogen and oxidative stress inducer, was adsorbed on modified ESM (Liu and Huang 2011).

The ESM has limited adsorption toward minerals like copper, zinc, nickel, and lead ions. Various structural modifications have then been performed to enhance their adsorption properties. Functionalized membranes prepared through crosslinking with polyethyleneimine were used to achieve high adsorption of cupric ions (Zou and Huang 2013) and chromium-VI (Liu and Huang 2011) while the functionalization with magnetite was used as a magnetic solid phase sorbent for the extraction of trivalent aluminum ions, a known neurological toxicant (Abdolmohammad-Zadeh and Talleb 2014). Moreover, chemical modification (i.e. methylation) of ESM carboxyl groups enhanced the adsorption capacity of pentavalent selenium suggesting that ionized carboxyl groups played an inhibitory role in arsenic (V) adsorption (Ishikawa et al. 2004). Thiol-functionalized ESM adsorption toward chromium (VI), mercury (II), copper (II), lead (II), cadmium (II), and silver (I) improved by 1.6–21.1 folds compared to that of the membrane control (Wang et al. 2013). Another reported use of the eggshell waste is for the removal of dyes and chemicals from aqueous solutions, such as the removal of cationic basic blue 9 and anionic acid orange 51 from aqueous solutions (Tsai et al. 2008) as well as fluoride from aqueous solutions and groundwater samples (Bhaumik et al. 2012). In other works, ESMs were used to adsorb dyes such as Direct Red 80 and Acid Blue 25, methylene blue, Brilliant Green (Arami et al. 2006; Kobiraj et al. 2012). This is important because elimination of high concentrations of these dyes in effluents before they are released into the surrounding environment will mitigate their negative ecological impact.

The adsorption of minerals by eggshells and ESMs is affected by the pH and concentrations of ions and the biomaterial. The pH of the solution is important because it directly affects the protonation of metal binding sites, the solubility of calcium carbonate, and metal speciation in the solution (Guru and Dash 2014). The uptake of chromium (III) by eggshells was reported to be dependent on pH of the solution with uptake rate at pH 5 being higher than those at pH 3 and 4 (Chojnacka 2005). Similarly, lead (II) uptake by eggshells decreased from pH 6 to pH 2 (Rao et al. 2010). In both cases, the decrease is related to the ion-exchange properties of carbonate groups and greater preponderance of hydrogen or hydronium ions at lower pH that might restrict the binding of the cationic

metal ions. In addition to the influence of pH, the uptake of lead ions by eggshell powder was enhanced by increasing the concentration of metal ions or the adsorbent, and decreased with higher particle size of the adsorbent (Rao et al. 2010).

#### 7.6.4 Applications in Chemical Processes

Eggshell is used in various chemical processes including the production of biodiesel (transesterification of oils), hydrogen/syngas (methane oxidation, wood gasification, coal gasification), bioactive compounds (chomenones, pyrans, lactulose), and nanocomposites (Guru and Dash 2014). The procedures to prepare eggshell catalysts differ, depending on the chemical reaction or process required for the formation of the end product. The process can include washing (water, acid, alkaline), drying (100–110 °C), calcination (200–1000 °C), treatment with metal oxide (e.g. TiO<sub>2</sub>), or a combination of some of these conditions. In the production of biodiesel, eggshell particles, as catalysts, are dried or calcined and then used to trans-esterify vegetable oils and animal fats in the presence methanol (Wei et al. 2009). The catalytic molecule derived from eggshell is calcium oxide (CaO), which can also be obtained from other waste materials such as ashes and bones (Laca et al. 2017). The use of eggshell as solid base catalysts would lower biodiesel production costs by eliminating the need for neutralization with acids and the removal of water (Guru and Dash 2014; Di Serio et al. 2008). Meanwhile, eggshell utilization is limited by the relatively low activity and, consequently, longer reaction time and high amount of the catalyst. The catalytic property of eggshell-derived materials is also applicable in the production of hydrogen gas. The process is environmentally friendly, because it lowers the generation of carbon dioxide, the major greenhouse gas emitted through anthropogenic activities. Materials such as wood and coals can be gasified for the production of hydrogen (Mostafavi et al. 2016). During the process, biomass is decomposed into gases (e.g. hydrogen, methane, carbon monoxide, carbon dioxide) and byproducts such as tar and char, and the addition of eggshell suppresses carbon dioxide production through absorption of the gas on its calcium monoxide, which promotes hydrogen gas generation (Taufiq-Yap et al. 2013). Eggshell catalysts have also been used the synthesis of chromene or benzopyran derivatives, which are mainly pigments and cosmetic dyes, but some also possess diuretic, antitumor, antioxidant, antihypertensive, and antimicrobial properties (Mosaddegh 2013). About 17–25% of lactose is converted to lactulose using eggshell powder (Nooshkam and Madadlou 2016; Montilla et al. 2005). Lactulose is of interest in the food and pharmaceutical industry because of its prebiotic activity and effects in the reduction of blood ammonia, serum lipids, blood glucose, and insulin (Nooshkam and Madadlou 2016; Panesar and Kumari 2011).

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# 8

## Egg Processing Discards

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### 8.1 Introduction

Eggs form a major source of protein in the diets of people all over the world. They are used in various forms in the food industry due to their nutritional value and their role in improving physical properties of other food products. Among the world's largest egg producers are China, the USA, and Japan (Zeidler 2002). The main components of eggs are the shell, albumen, and yolk. The commonest eggs used as food are hen eggs. The parts commonly used are the albumen and yolk mostly for nutritional purposes and their properties in food product development and processing. Over the years, as egg consumption and utilization has increased, egg product wastes have also increased. The major challenge with the generation of these waste products is the environmental impact. Eggshell wastes which form a bulk of the wastes generated from egg production and processing plants are transported often at a cost, to landfills where they are discarded (Yoo et al. 2009). Eggshell wastes in particular, have been found to be rich in organic and inorganic compounds, which when harnessed could be utilized in formation of useful products. Various works of literature have been carried out and have proven this fact. As a result of this, the further processing of egg production and processing wastes could both have economic and environmental benefits. This chapter seeks to provide information on the usefulness of egg processing wastes with much emphasis on eggshell wastes.

#### 8.1.1 Egg Formation

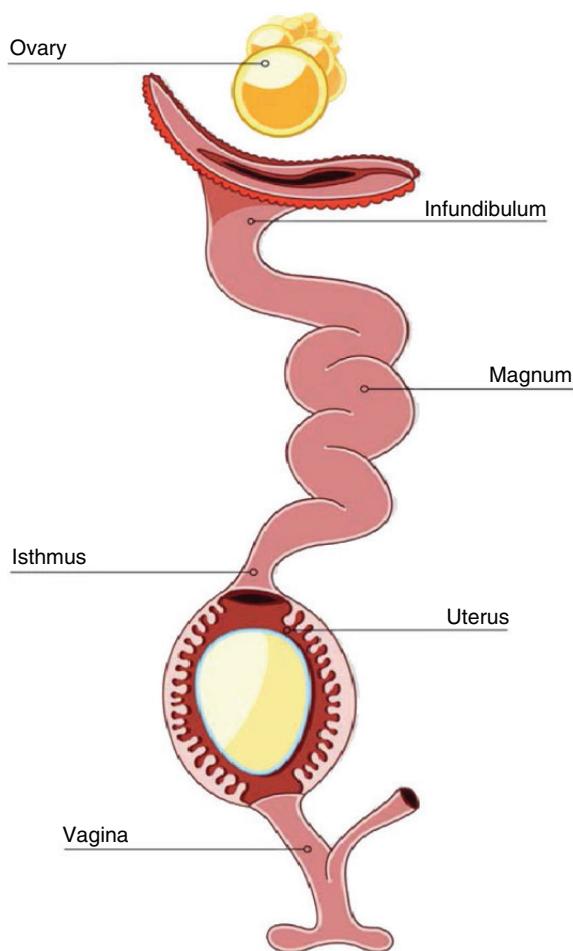
Young hens, referred to as pullets are raised for the purpose of egg production. In North America, the commonest breeds are the White Leghorn and Rhode Island Red (Wu 2014). The reproductive system of the pullet is the site for egg production, and is composed of two main parts: the ovary and the oviduct. When a pullet hatches, it has two ovaries and oviducts. However, as it matures only the left ovary and oviduct fully

develop to produce eggs (Wu 2014; Gill 2007). The right reproductive system on the other hand usually reverts to an inactive state, leaving the reproductive system with only one set of active ovary and oviduct.

(Figure 8.1).

The ovary is basically made up of a group of follicles which contain ova (yolks). A mature ovum (a yolk) is released from the ovary into the oviduct in a process called ovulation. It then travels through the oviduct for egg formation to be completed.

The oviduct has five parts: infundibulum, magnum, isthmus, uterus, and vagina. The infundibulum is the funnel shaped uppermost part of the oviduct that receives the mature ovum. Fertilization of the ovum can also occur if it encounters a sperm in this section. In the magnum, the albumen is secreted onto the yolk after which the albumen covered yolk moves on to the isthmus where the inner and outer shell membranes are formed. Calcification and shell pigmentation of the egg occurs in the uterus after which the shelled egg now moves to the vagina to be transported to the cloaca (Joyner et al. 1987; Wu 2014). See below the reproductive tract and a summary of the formation of an egg.



**Figure 8.1** The reproductive system of a hen with incompletely formed egg.  
Source: Adapted from Hincke M.T. et al. (2012).

### 8.1.2 Egg Structure, Shape, and Size

Eggs are mostly ovoid shaped and consist of three main parts: the shell, the albumen and the yolk (Wu 2014) (refer to Figure 8.2). It is however not uncommon for hens to produce eggs with slightly different shapes. These deviations have been mostly attributed to genetic factors while others have also been attributed to changes or abnormalities in the oviduct (Zeidler 2002). Egg size variation is observed among a flock bred for egg production. According to Zeidler (2002), factors accounting for this variation include the following:

*Feed.* The higher the protein and linoleic acid content, the larger the size of the eggs produced by the hen;

*Climate.* The hotter the weather, the less feed is consumed by the hen. This results in production of smaller sized eggs;

*Age of the hen.* Young hens lay many small and medium-sized eggs. However, as they grow and mature, they lay a larger percentage of large and extra-large eggs.

*Rate of egg production.* Smaller eggs are produced by hens that tend to lay a higher volume of eggs.

#### 8.1.2.1 The Shell

The egg is protected by its external shell. The shell has many pores which allow the exchange of air, moisture and other particles. It is composed of an outer layer referred to as the cuticle which serves as a protective barrier against the movement of microbes from the external (Gill 2007; Zeidler 2002). The inner layer of the shell is composed of the shell membrane, which serves as a barrier between the shell and the egg albumen; it further protects the egg against the movement of microbes which may have crossed the cuticle (Zeidler 2002; Stadelman 2000). The shell

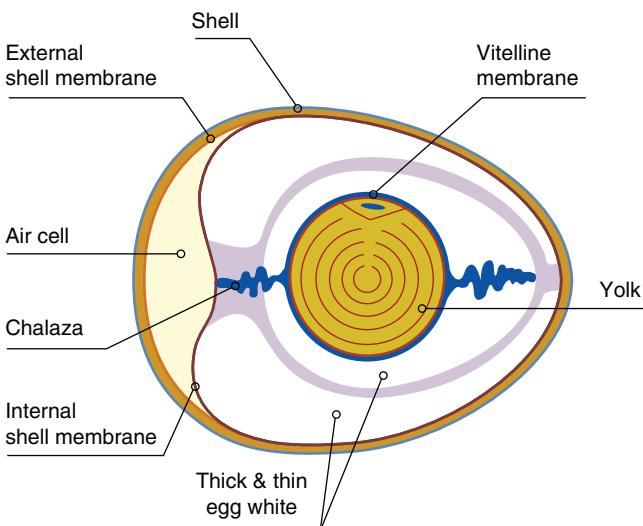


Figure 8.2 The structure of a shelled egg. Source: Adapted from Wu J. (2014).

membrane has two layers – the inner layer, which is in close contact with the albumen, and the outer layer, which is in close contact to the shell. Once an egg is laid, it adapts to its external temperature and loses water to its surroundings, this leads to the formation of an air sack between the inner and outer shell membrane at the base of the egg. The volume of air increases as the egg ages and as such is used as an indirect measure of the age of an egg in a process known as candling. This also explains why a freshly laid egg would sink to the bottom when placed in water as opposed to an older egg which would float when subjected to the same treatment (Zeidler 2002).

### 8.1.2.2 The Albumen

The albumen is made up of two thick and two thin layers. The thick layers are the inner-gel-like fibrous layer and the outer thick layer. The inner thick layer gets its gel-like characteristics as a result of the presence of the protein, ovomucin (Wu 2014; Zeidler 2002). A network of structures referred to as chalazae, which keep the egg yolk suspended in the egg, are located on opposite ends within the thick albumen layer. The two thin albumen layers have no ovomucin and are clearer and thinner in consistency as opposed to the thick inner albumen layers.

### 8.1.2.3 The Yolk

The yolk is located in the middle of the egg and is enclosed by a membrane called the vitelline membrane and yellow and white yolk layers (Gill 2007; Zeidler 2002). A germinal disc is also found on the yolk, and an embryo forms from this when the egg is fertilized (Zeidler 2002).

## 8.1.3 Byproducts from Egg Production

### 8.1.3.1 Egg Shells

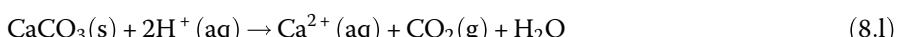
#### 8.1.3.1.1 Eggshell Powder ( $\text{CaCO}_3$ ) for pH Control and Heavy Metal Immobilization in Agricultural Soils

In the process of egg production, the waste generated could be also harnessed in order to serve other useful purposes. This process of value addition to waste products generated from the production process is the central focus of this chapter. Egg shells are known to form about 11% of the weight of an egg (Oliveira et al. 2013). Since the main part of eggs used in egg processing have to do with the albumen and yolk, the amount of waste generated after the industrial processing consists mostly of egg shells.

Egg shells, just like molluscs, are a rich source of  $\text{CaCO}_3$  (Walton et al. 1973; Zeidler 2002). This forms the basis for their use as substitutes for lime in soil pH treatments (Oliveira et al. 2013). The main reactive compound in lime, calcium carbonate, is responsible for neutralizing acids and also removing heavy metals in soils (Oliveira et al. 2013; Ok et al. 2010). There have been applications of shells as lead and cadmium immobilizers due to their calcium carbonate content and also the enhanced effectiveness as a result of the conversion of calcium carbonate to calcium oxide. Ok Y.S et al. (2010) in an attempt to evaluate the effectiveness of shell (oyster shells) calcium carbonate in removing heavy metals from soils proved that the metal removing capabilities of calcium carbonate in shells was improved upon high temperature treatments ( $770^\circ\text{C}$ ) as a result of the release of the more active form, calcium oxide ( $\text{CaO}$ ). The process of producing calcium oxide is

referred to as calcination. Lee et al. (2013) in a similar experiment, used egg shell wastes in combination with NPK (Nitrogen, phosphate and potash) fertilizer or rapeseed waste as Cd and Pb immobilizers. The experiment explored the effectiveness of  $\text{CaCO}_3$  from egg wastes in increasing soil pH and also in reducing the availability of the heavy metals Cd and Pb. The experiment confirmed that the presence of egg shell waste powder as an alternative to commercial lime or calcium carbonate, is not only an effective treatment for heavy metal immobilization, but also goes to curb or reduce the harmful effect of these metals in food consumed by both plants and animals. In addition, the treatment is effective in raising the soil pH of acidic soils in an attempt to improve crop yield. The increased alkaline phosphatase activity in the soil also confirms the rise in pH as the enzymes are less active in acidic soils (Lee et al. 2013).

When the soil pH is highly acidic (low pH), it affects the yield of crops. It is therefore important to control the soil pH in an effort to improve agricultural yield. Methods utilized in correcting acidic soil pH include the addition of lime or commercial calcium carbonate ( $\text{CaCO}_3$ ). The reaction between calcium carbonate and acids leads to the production of carbon dioxide, water and calcium ions. The carbon dioxide is required by plants in photosynthesis and the calcium ion serves as a mineral supplement for plants.



#### 8.1.3.1.2 Inkjet Paper Coating

Paper used for printing comes in either coated or uncoated forms. The coated paper usually provides higher quality printing and also sharper images once printing is completed. Coating material for inkjet paper provide quicker aqueous ink absorption and minimized smearing after printing hence, their advantage. In the production of coating paper, the coating material requires a binder in addition; this ensures that the ink is able to bond with the paper effectively (Hladnik and Muck 2002). The quantity of the binder to an extent depends on the coating material or pigment. "Silica oxide ( $\text{SiO}$ ) has been used as a synthetic source of coating agent for inkjet paper due to its high color density and resolution" (Yoo et al. 2009). The challenge however, is the high cost of silica which makes the manufacturing of the high color density paper relatively expensive when compared to uncoated printing papers (Hladnik and Muck 2002). Another challenge is the fact that it requires a higher amount of binding agent. Hladnik A. and Muck T. (2002), demonstrated that, silica required a much higher amount of binding material than precipitated calcium carbonate. In another demonstration, Yoo S. et al. (2009), extracted eggshell calcium carbonate as a substitute for silica coating and determined the effect on ink density and paper gloss. The experiment showed that eggshell calcium carbonate has a high affinity for dye-based dyes. It was then concluded that "eggshell calcium carbonate improved optical density for dye-based inks such as cyan, magenta, and yellow" (Yoo et al. 2009).

#### 8.1.3.1.3 Calcium Source or Supplement in Diet and Calcium Transport Enhancers

Due to the high content of calcium in egg shells, the idea of it being a nutrient supplement was thought a laudable one. Schaafsma et al. (2000) investigated the mineral, amino acid, and hormonal content of chicken egg shell powder. The experiment concluded that

the eggshell powder had calcium levels (mean  $\pm$  SD/g EPSs (Extracellular Polymeric Substances):  $401 \pm 7.2$  mg) comparable to the recommended daily intake levels for the elderly (50–70 years). Calcium is a mineral salt that plays a vital role in skeleton and teeth development. According to Lanham-New S. (2008), Calcium supplement intake seemed to reduce bone loss in postmenopausal women, especially those in their late menopausal stages. This was especially obvious in those who were in the habit of consuming less than 400 mg per day of calcium in their diet.

Eggshell is proposed as a potentially good supplement, not only for its calcium content, it is also known to contain proteins that potentially have nutraceutical properties. The protein content in eggshell is approximately 1.0% (w/w) as compared to the major component of calcium, which is 95% (w/w) (Daengprok et al. 2003). In experiments carried out by Daengprok W. et al. (2003), eggshell protein matrix was obtained and used to determine its role in enhancing the transport of calcium across Caco-2 cell monolayers – a form of human cell layers with properties of the small intestines; hence, its use in cell studies. It was observed that the eggshell protein matrix effectively enhanced the transport of calcium across the *in vitro* Caco-2 layers by about 64%. The amino acid sequence was determined by reverse-phase high-performance chromatography to be a sequence of Met-Ala-Val-Pro-Gln-Thr-Met-Val-Gln (Daengprok et al. 2003).

### 8.1.3.2 Egg Shell Membrane

#### 8.1.3.2.1 Collagen Production

Collagen is a useful protein material due to its wide application in various industries such as the pharmaceutical, cosmetic, and food industry. It has been commercially extracted from animal sources including bovine and porcine skin and bones. Due to religious reasons, the porcine sources are prohibited by Muslims and the only form, halal bovine collagen, is acceptable. Strict vegetarians do not consume collagen or collagen products for the obvious reason it is from animal sources. Another challenge with the use of bovine and porcine collagen is the issue of allergic and autoimmune reactions. It is estimated that 2–3% of the population experiences an allergic or autoimmune reaction upon exposure to animal collagen (Zhao and Chi 2009). Furthermore, due to the spread of diseases, such as foot and mouth disease and bovine spongiform encephalopathy, there have been restrictions with regards to the production of collagen; as such, alternate sources of the protein include fish tissues and surimi production waste (Hashim et al. 2015).

Zhao Y-H and Chi Y-J (2009) carried out experiments on eggshell membranes as they suspected the amino acid content of the egg shell membrane was a clear indication that the membrane proteins contained sequences similar to collagen. They extracted collagen from the eggshell membrane and compared its sequence to porcine collagen type 1. It was determined the eggshell membrane collagen has similar properties to that of porcine bone collagen type 1; hence, its suitability as a type 1 collagen substitute for its porcine counterpart in functional and cosmetic applications. This with the added advantages of its biosafety status and minimal religious restrictions, make it a very suitable collagen source for various commercial applications. Yi et al. (2004) in a similar experiment were also able to deduce that soluble eggshell membrane proteins could be used in the production of collagen type 1 protein.

### 8.1.3.2.2 Treatment for Burns

Over the years, methods used in medical science for treating burns have evolved. The use of animal proteins from porcine sources as well as human amniotic membranes has proven useful in the dressing of burn wounds (Maeda and Sasaki 1982). Eggshell membrane proteins have been found to be a rich source of collagen (Zhao and Chi 2009) which has the ability to heal tissue wounds and enable the development of proteins in connective tissues including bones, cartilage and tendons (Cheng et al. 2009; Hashim et al. 2015). This property of collagen is one of the basis for the application of collagen rich compounds and tissues in burn treatments.

Although eggshell protein membrane has been demonstrated to be useful in adhering to connective tissues and useful in managing burns, it has a limitation in the sense that it cannot be easily molded into different shapes, thickness, and sizes (Yi et al. 2004). This limitation however is remedied by the further treatment of eggshell membrane proteins in order to achieve a more soluble form, soluble eggshell proteins (Yi et al. 2004). Yi F. et al. (2004), prepared the soluble eggshell membrane proteins by treatment with aqueous 3-mercaptopropionic acid at 90 °C in the presence of 10% acetic acid. This was done in order to break the disulphide bonds in eggshell membrane proteins hence making it more soluble. The biocompatibility to NIH3T3 cells, a model which mimics the characteristics of human tissues, was also determined and compared to that of collagen type 1. It was observed that the cultured NIH3T3 cells readily attached to the soluble eggshell membrane proteins starting as early as one hour after culturing, whereas in the case of collagen type 1, none of the cells had attached to the protein.

### 8.1.3.3 Bioactive Compounds from Yolk and Albumen Processing Byproducts

Though many bioactive compounds have been identified from eggs, it has been observed that after processing or breaking the eggs, many of these compounds tend to lose their bioactive properties due to a less conducive environment (Zeidler 2002). It is, however, not a hopeless situation as some components of eggs under a carefully controlled environment are still able to be used in the production of useful bioactive compounds. In the production of egg products at processing plants, egg cracking units consisting of breakers and egg yolk–albumen separators are utilized, which separate the yolk from the albumen (refer to Figure 8.3) (Zeidler 2002).

The separated yolk and albumen then go through filtration and other processing steps prior to their being packaged as dried or frozen egg products (Zeidler 2002).

Byproducts obtained after this stage are then used as raw materials for bioactive compounds including lysozymes and Sialic acid or N-acetylneurameric acids (Zeidler 2002).

Lysozymes are enzymes, generally considered as safe and obtained from egg whites by cation exchange chromatography (Wu 2014). They are highly useful as anti-microbial agents in the food industry, especially in cheese production and in the preservation of foods such as tofu, meats, and sea food (Wu 2014). This is due to their ability to destroy gram-positive bacteria (Zeidler 2002). Mecitoğlu C. et al. (2006), used partially purified lysozyme from egg white in packaging material and observed their effectiveness against *Bacillus subtilis* and *Lactobacillus plantarum*. This experiment also confirms the high stability of lysozymes even after lyophilization making it a highly potent anti-microbial agent in food safety packaging material.



**Figure 8.3** Egg cracking units with breakers and egg-yolk separators.

The chalazae and vitelline membranes of egg yolks are also used as byproducts from egg processing plants in isolating Sialic acid (Zeidler 2002). *N*-Acetylneurameric acid, commonly referred to as Sialic acid is a “nine-carbon backbone” attached to the surface of most cells in humans and animals, and known to play a key role in the physiological reactions that occur in the bodies of organisms. Studies have shown a strong correlation in the intake of Sialic acid supplements and the cognitive development or improvement in the brain functionality of organisms (Wang et al. 2007). This is one of the bases for isolating Sialic acid from egg yolk after the egg yolk screening process.

## 8.2 Conclusion

The benefits derived from utilizing egg processing and production wastes are countless. Apart from the advantage of protecting the environment from tonnes of egg processing plant wastes, several medical, agricultural and health benefits have been attained. With the advancement in technology and the search for natural and safer alternatives into agriculture and health, it is a great possibility that more useful findings would arise over time.

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## 9

# Byproducts from Fish Harvesting and Processing

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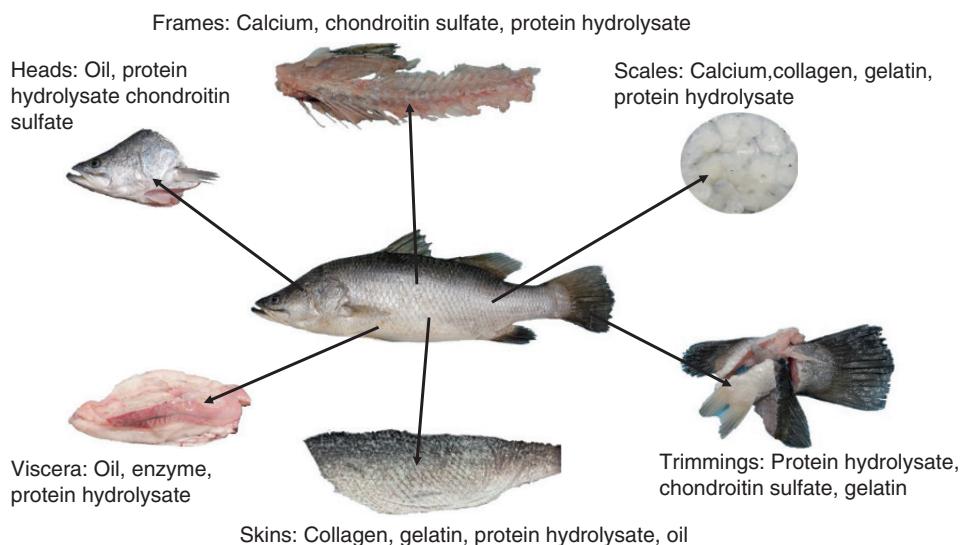
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## 9.1 Introduction

The current fish processing practically generates large amounts of byproducts, accounting for up to 75% of the total fish weight (Shahidi 1994). Despite the presence of several valuable components, fish processing byproducts are usually dumped in landfills or into the oceans, leading to potentially harmful environmental effects. Those byproducts are also used as raw materials for fish meal, silage, and fertilizer manufacturing (Rustad et al. 2011). Typically, fish processing byproducts consist of viscera, head, trimming, skin, scale, roe, and bone, as well as fish that are damaged or unsuitable for human consumption or bycatch (Rustad et al. 2011).

Fish processing byproducts contain a wide range of nutritional components, especially lipid and protein fractions as well as functional compounds or nutraceuticals. Fish oil is rich in n-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), phospholipids, squalene, fat-soluble vitamins, etc. Remaining proteins in fish processing leftovers are easily digestible and can be used for the production of hydrolysates containing different peptides and amino acids (Rustad and Hayes 2012). Furthermore, collagen, gelatin, as well as hydrolyzed collagen can be produced from collagenous materials such as bone, scale, or skin, etc. In addition to fish proteins and oil, other valuable components, including enzymes, nucleic acids, minerals, and other bioactive compounds such as chondroitin sulfate (CS), etc. can be recovered from those leftovers (Figure 9.1). By using the potential processing or technology, marketable new products with added value can be manufactured, in which the processors gain increased revenue. More importantly, the cost of disposal or discard can be reduced, while the pollution caused by those perishable leftovers is prevented. Further research to explore novel uses of byproducts is necessary for the benefit of both the environment and humans.



**Figure 9.1** Asian seabass byproducts as raw materials for production of biomolecules.

## 9.2 Fish Collagen

Collagen is the major structural protein of animal connective tissue, contributing to the strength and support of tissues in skins, tendons, bones, cartilages, etc. Collagen has been widely used in food, biomedical, pharmaceutical, and cosmetic applications (Regenstein and Zhou 2007). Commercial collagens are generally produced from bovine and porcine hides and bones. Currently, increasing attention has been paid to alternative sources for the replacement of mammalian collagens, especially from fish processing byproducts. Fish collagen can be used for Halal and Kosher products served for Muslim and Jewish communities, respectively. Because of the outbreak of bovine spongiform encephalopathy and bird flu, the increasing demand of fish collagen has been gained (Benjakul et al. 2012b; Regenstein and Zhou 2007).

Collagen is composed of tropocollagen, a rod-shaped protein consisting of three polypeptides units (called  $\alpha$ -chains) intertwined to form a triple-helical structure. Each  $\alpha$ -chain coils in a left-handed helix with three residues per turn and the three chains are twisted right-handed to form the triple helix. The three chains are held together primarily by hydrogen bonding between adjacent –CO and –NH group (Lee et al. 2001). The most common collagen is type I collagen. It contains two  $\alpha_1(I)$ -chains and one  $\alpha_2(I)$ -chain, which has different amino acid sequence and composition (Benjakul et al. 2012b). Glycine (Gly) represents nearly one-third of the total amino acid residues and it is distributed uniformly at every third position throughout  $\alpha$ -chain, except for the 10 amino acid residues from C-terminal and 14 amino acid residues from N-terminal (Foegeding et al. 1996). With the Gly-X-Y repeat, the frequent occurrence of proline (Pro) and hydroxyproline (Hyp) in the X and Y-positions is reported (Liu et al. 2015). The contents of proline and hydroxyproline, so called imino acids, vary with species and their living habitat (Foegeding et al. 1996). Pyrrolidine rings of proline and

hydroxyproline impose restrictions on the conformation of the polypeptide chain and help to strengthen the triple helix (Bae et al. 2008). Additionally, hydroxyl group of hydroxyproline plays an important role in stabilizing the helix by interchain hydrogen bonding via a bridging water molecule as well as direct hydrogen bonding to a carbonyl group (Wong 1989).

Nowadays, byproducts from fish processing have received considerable attention as potential sources for collagen production. There is a number of studies on extraction and characterization of collagen from fish processing byproducts, such as seabass scale (Chuaychan et al. 2015), Nile tilapia skin (Chen et al. 2016a), Amur sturgeon cartilage (Liang et al. 2014), bighead carp fin (Liu et al. 2012), silvertip shark skeletal and head bone (Jeevithan et al. 2014), carp bone (Duan et al. 2009), and seabass swim bladder (Sinthusamran et al. 2013). Different collagens from different raw materials have varying compositions and properties. Furthermore, the processes used for extraction not only affect the yield, but also the composition and chain length, etc. of resulting collagen.

### 9.2.1 Isolation of Collagen

Isolation of collagen is generally separated into three main steps, including raw material preparation, extraction, and recovery. Collagen can be extracted from fish skin, bone, scale, etc. Firstly, those raw materials are subjected to cleaning, size reduction, followed by appropriate pretreatment prior to extraction. The extraction is normally carried out with the aid of acid. However, due to the lower yield of collagen obtained from this process, a pepsin-aided process was developed to increase extraction yield (Nalinanon et al. 2007). All procedures for collagen production are performed at low temperature ( $4^{\circ}\text{C}$ ) to avoid thermal denaturation. All processes used for collagen extraction directly affect the extraction yield and properties of collagen obtained.

#### 9.2.1.1 Preparation of Raw Materials

In general, the raw materials such as skin still contain non-collagenous constituents, including lipids, pigments, etc. Calcium or other inorganic matters are found in fish scales and bones. Single or several pretreatments are employed to remove the undesirable matters prior to extraction in order to increase the purity of extracted collagen. The removal of residual meats and thorough cleaning are performed as the first step before further pretreatments. Size reduction of raw material is also beneficial to facilitate the removal of non-collagenous matters and increased the efficacy for collagen extraction. Alkaline pretreatment with diluted NaOH is commonly used to remove non-collagenous proteins and pigments. NaOH solution at concentration of 0.1 M has been used to remove non-collagenous proteins from raw material (Benjakul et al. 2012b). To remove lipids, the raw materials can be treated with 10% butyl alcohol and rinsed with water (Nagai et al. 2000). NaCl and  $\text{H}_2\text{O}_2$  have also been used to remove non-collagenous proteins and pigments, respectively (Benjakul et al. 2012b). For the certain raw materials such as fish bones and scales containing high amount of calcium, especially in the form of hydroxyapatite (HA), decalcification using ethylenediaminetetraacetic acid (EDTA) with its chelating ability is commonly implemented. The decalcification can be also achieved using inorganic acid, especially hydrochloric acid. However, harsh condition should be avoided, in which the native collagen can be retained (Benjakul et al. 2012b). EDTA in the range of 0.1–0.5 M has been employed for decalcification of fish

scale and bone (Moreira-Silva et al. 2016). Porous decalcified raw material with increased surface area can be readily extracted for collagen. The yield and properties of extracted collagen are influenced by the source and age of the raw material (Regenstein and Zhou 2007). The nature and concentration of acid or alkali used during pretreatment, the ratio of acid solution and raw materials and the temperature and time of pretreatment and extraction also determine yield and molecular properties of extracted collagen (Regenstein and Zhou 2007).

### 9.2.1.2 Extraction

#### 9.2.1.2.1 Acid Solubilization Process

Acid solubilization process has been widely used for collagen extraction. Collagen obtained is referred to as “acid-soluble collagen, ASC.” Extraction is conducted using acidic condition, in which the positive charge of collagen polypeptides becomes dominant. As a consequence, the enhanced repulsion among tropocollagen can be achieved, leading to increased solubilization (Benjakul et al. 2012b). Both organic acids (acetic, citric, lactic) and inorganic acid (hydrochloric) can be used for fish collagen extraction (Skierka and Sadowska 2007). Acetic acid is the most commonly used acid for collagen extraction (Schmidt et al. 2016). A concentration of 0.5 M is practically used (Pal and Suresh 2016). Schmidt et al. (2016) reported that acetic acid and lactic acid rendered collagen from Baltic cod skin with a higher yield than HCl and citric acid. The use of several consecutive extractions may get a better ASC yield, compared to simply prolonging the extraction time (Regenstein and Zhou 2007). However, extraction with a longer time mostly shows a higher yield than with a shorter time. Wang et al. (2009) found that the yield of grass carp skin collagen increased with increasing the extraction time from 12 to 24 hours.

#### 9.2.1.2.2 Pepsin Solubilization Process

Generally, the typical acid solubilization process renders a low yield of collagen. To tackle the problem, pepsin has been applied because it is able to cleave peptides specifically in the telopeptide region of collagen, leading to enhanced extraction efficiency (Nagai et al. 2002). Extraction of collagen with the aid of pepsin is a potential method for several reasons: (i) some of non-collagenous proteins are hydrolyzed and are easily removed by salt precipitation and dialysis, improving collagen purity; (ii) it is possible to hydrolyze those components and telopeptides of collagen to make the sample ready to solubilize in acid solution, resulting in improvement of extraction efficiency; and (iii) antigenicity is reduced caused by telopeptide in the collagen, which serves as the major problem in food and pharmaceutical applications (Lee et al. 2001; Werkmeister and Ramshaw 2000). Nalinanon et al. (2007) found that the use of fish pepsin such as bigeye snapper pepsin could increase the yield of collagen from skin of bigeye snapper. Pepsin is used simultaneously with acid solubilization process and the extracted collagen is further filtered, precipitated, and freeze-dried. The collagen is referred to as “pepsin soluble collagen, PSC.”

#### 9.2.1.2.3 Recovery of Collagen

After the extraction process, the collagen is typically recovered by salt precipitation, centrifugation, dialysis, and freeze-drying. The collagen solution is generally precipitated using NaCl in the presence of buffer such as tris(hydroxymethyl)aminomethane at

pH 7.5 (Nagai et al. 2000; Nalinanon et al. 2007). The concentrations of NaCl used for collagen precipitation can be varied from 0.9 to 2.6 M. This process aims to maximize the collagen recovery and removal of impurities. The resultant precipitate is then collected by centrifugation. The pellet is dissolved in 0.5 M acetic acid with minimal volume prior to dialysis against 0.1 M acetic acid and distilled water. The dialysate containing collagen is finally freeze-dried and the powder obtained is named as ASC or PSC (Benjakul et al. 2012b).

### 9.2.2 Emerging Technologies for Isolation/Extraction and Purification of Fish Collagen

The use of conventional methods for collagen extraction is restricted by several drawbacks. Recently, isolation, extraction, and purification methods using emerging technologies have been continuously developed. Those techniques can reduce solvent/chemical consumption, shorten processing time and increase extraction efficiency (Huang et al. 2013). Emerging technologies, including ultrasonic, microwave-assisted extraction and high-pressure processing, have been introduced for collagen extraction (Mustafa and Turner 2011).

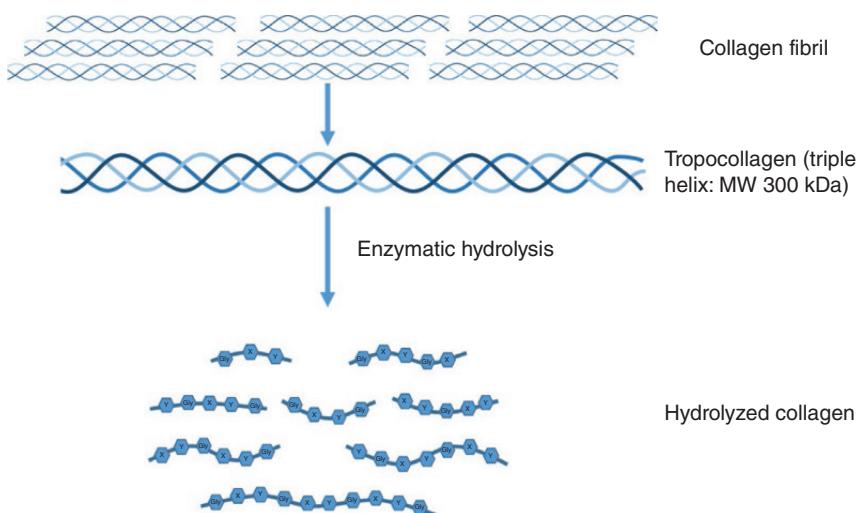
Ultrasounds are the high-frequency sound waves (20 kHz) that exceed the frequency hearing capacity of the human ear. Ultrasound principally acts by generating bubble cavitation in a biological medium and is considered as an efficient alternative to traditional methods (Kadam et al. 2015). It facilitates the extraction of heat sensitive compounds with minimal damage and higher yield. Kim et al. (2012a) revealed that the extraction yield of ASC from seabass skin increased rapidly with increasing amplitude (20–80%) of ultrasonic treatment. The ultrasonic treatment requires a lower amount of acid and a shorter time for collagen extraction. However, longer duration of ultrasonic treatment may lead to the structural damage of extracted collagen to some degree (Kim et al. 2013). Moreover, Zeng et al. (2012) reported that ultrasound-papain treatment of grass carp scales rendered a higher extraction yield of collagen with shorter processing time than the conventional method.

Recently, Huang et al. (2016) developed a novel extrusion–hydro-extraction process for extraction of collagen from tilapia scales. This technique utilized the extrusion process to decompose the strong linkage between collagen and hydroxyapatite, and facilitates the release of collagen from fish scale extrudates by water extraction. Moreover, the extrusion process may also alleviate the unpleasant smell of fish scale and yielded odorless collagen. The developed process rendered two to three times higher extraction yield than control sample. Hydrophilic ultrafiltration process was also used for isolation and purification of collagen from scales of red drum fish (Chen et al. 2016b). The membrane material for ultrafiltration was hydrophilic polyethersulfone, which is suitable for hydrophilic proteins, such as collagen, with high permeation and fouling resistance. Collagen isolated using this process showed higher extraction yield and purity than that isolated using conventional method. This process was more efficient, compared to conventional salt precipitation/dialysis. It therefore meets the requirements for large-scale production in the food and nutraceutical industries.

### 9.2.3 Hydrolyzed Fish Collagen

Dietary proteins are sources of biologically active peptides, which are inactive in the parent proteins but can be liberated during gastrointestinal digestion, food processing, or fermentation. Collagenous materials, including collagen and gelatin, have been served as the sources of biologically active peptides with promising health benefits. Hydrolyzed collagen which gains drastic increasing interest for consumers is generally obtained by enzymatic hydrolysis of collagenous proteins using commercial proteases, including trypsin, chymotrypsin, pepsin, alcalase, neutrase, protamex, properase E, pronase, collagenase, bromelain, and papain (Figure 9.2) (Kim et al. 2001; Mendis et al. 2005; Yang et al. 2008). Apart from commercial proteases, enzyme extracts from fish viscera or shrimp hepatopancreas have the potential to be used for production of bioactive hydrolyzed collagen (Phanturat et al. 2010; Senphan and Benjakul 2014). Protease specificity affects size of peptide, amount, free amino acid, amino acid sequences of resulting peptides, which in turn influence the biological activity of the hydrolysates. Enzymatically hydrolyzed fish skin collagen has shown better biological activities, compared to that of the peptides derived from fish muscle protein (Kim and Mendis 2006). Collagen peptides have unique Gly-Pro-Hyp sequence in their structures, and it is presumed that the observed bioactivities of collagen peptides are associated with their unique amino acid compositions and sequences (Kim et al. 2001). Hydrophobic amino acids such as proline play a role in inhibiting lipid oxidation and contribute to various bioactivities (Byun and Kim 2001; Mendis et al. 2005). Peptides with proline and/or hydroxyproline are generally resistant to degradation by digestive enzymes, and therefore have a better chance of reaching their target site *in vivo* in an intact form (Sarmadi and Ismail 2010).

Hydrolyzed collagens from fish processing byproducts of various species have been prepared and characterized. Different proteases and processes have been employed to produce hydrolyzed collagens from different sources, e.g. carp swim bladder (Pal and



**Figure 9.2** Enzymatic hydrolysis of collagen.

Suresh 2017), scales of *Lates calcarifer*, *Mugil cephalus*, *Chanos chanos*, and *Oreochromis* spp. (Huang et al. 2015), tuna skin (Lee et al. 2017), haddock skin (Qi Feng et al. 2015), Malabar grouper skin (Hema et al. 2017), grass carp skin (Wang et al. 2017), thornback ray skin (Lassoued et al. 2015), Alaska pollock skin (Guo et al. 2015), skin and bone of striped catfish (Baehaki et al. 2015), seabass skin (Sae-leaw et al. 2016e), basa skin (Zhang et al. 2016), etc. Those hydrolyzed collagens have different peptides, which are varied in sizes, amino acid sequences, as well as bioactivities (Table 9.1).

### 9.2.4 Bioactivities of Hydrolyzed Fish Collagen

Bioactive hydrolyzed fish collagen or peptides are known to possess various potential health benefits (Table 9.1). Many studies have reported that hydrolyzed fish collagen exhibit a wide range of bioactivities, including antihypertensive, antioxidant, antimicrobial, mineral binding, anticoagulant, anti-adipogenic, immunomodulatory, and antiproliferative activities, which can be applied as functional foods and pharmaceuticals for health promotion and disease risk reduction (Visessanguan and Benjakul 2012). The technological approaches in terms of recovery and production of these compounds from various fish processing byproducts have been investigated for potential commercial applications in the food, health, and allied industries (Pasupuleti and Braun 2010; Visessanguan and Benjakul 2012).

Due to the decrease of collagen synthesis in the body, its demand for the skin, hair, and bone tissues increases with aging (Iwai et al. 2005). Over recent years, various beneficial effects have been reported on the consumption of hydrolyzed collagen that includes improvements of joint pains (Clark et al. 2008), wound healing (Zhang et al. 2011), inhibition of intestinal glucose uptake (Iba et al. 2016), improvement of moisture and elasticity, and reduction of wrinkles of facial skin (Inoue et al. 2016) as well as suppression of UV-B-induced skin damage and photoaging (Tanaka et al. 2009).

### 9.2.5 Applications of Fish Collagen/Hydrolyzed Collagen

#### 9.2.5.1 Food Applications/Supplement

Collagen can be used to produce edible casing for sausages or other related products. Sausage casings were originally derived from the gastrointestinal tract of cattle, sheep, and pig. However, with the rapid growth in demand for sausage products, the collagen casings were developed. They have some advantages over the natural gut casings, due to the convenience, economic efficiency, and uniformity of the regenerated collagen casings (Hood 1987). The collagen can be used in processed meat products to improve protein functionality through the immobilization of free water, thereby increasing the stability of the finished product (Prabhu et al. 2004). The use of collagen as a binder and extender in emulsion sausages and hams enhances the absorption and binding of water (Prabhu et al. 2004).

Recently, hydrolyzed collagen or collagen peptides have attracted considerable attention due to their beneficial biological functions. Hydrolyzed collagens with molecular weights from 500 up to 20 000 Da are commercially available. As a food ingredient, usage of low molecular weight (2000–5000 Da) hydrolyzed collagen is preferred to lower precipitation and turbidity problem in foods and drinks (Bilek and Bayram 2015). It is widely known that the digestion of collagen can generate peptides that may be useful

**Table 9.1** Sources, active peptide sequence and molecular weight (MW) of bioactive peptides from some hydrolyzed fish collagens.

Sources	Enzymes used for hydrolysis	Bioactivity	Active peptide sequence	MW (Da)	References
Thornback ray skin	Neutrase or crude enzyme from <i>Bacillus subtilis</i> A26	Antihypertensive Antioxidant	Gly – Ile – Pro – Gly – Ala – Pro Ala – Val – Gly – Ala – Thr	510.58 417.46	Lassoued et al. (2015)
Alaska pollock skin	Trypsin	Mineral binding	Gly – Pro – Ala – Gly – Pro – His – Gly – Pro – Pro – Gly Gly – Pro – Ala – Gly – Pro	842.90 397.43	Guo et al. (2015)
<i>Lates alcarifer</i> , <i>Mugil cephalus</i> <i>Chanos chanos</i> <i>Oreochromis</i> spp.	Papain and flavourzyme	Iron-binding activity	–	1300.00	Huang et al. (2015)
Alaska pollock skins	Alcalase	Antioxidant	Tyr – Gly – Cys – Cys Asp – Ser – Ser – Cys – Ser – Gly Asn – Asn – Ala – Glu – Tyr – Tyr – Lys Pro – Ala – Gly – Asn – Val – Arg	444.11 554.16 900.39 612.33	Sun et al. (2016)
Seabass skin	Alcalase	Antioxidant	Gly-Leu-Phe-Gly-Pro-Arg	646.37	Sae-Leaw et al. (2016d)
Nile tilapia skin	Trypsin	Antihypertensive Antioxidant	Gly – Pro – Glu – Gly – Pro – Ala – Gly – Ala – Arg Gly – Glu – Thr – Gly – Pro – Ala – Gly – Pro – Ala – Gly – Ala – Ala – Gly – Pro – Ala – Gly – Pro – Arg	810.87 1490.61	Choopicharn et al. (2016)
Unicorn leatherjacket skin	Partially purified glycol endopeptidase from papaya latex	Antioxidant	Glu – Gly – Pro – Leu – Gly	472.24	Karnjanapratum et al. (2016)

for organic biosynthetic processes (Cúneo et al. 2010). The consumption of collagen-rich foods might be beneficial for bone health. Keratinocyte culture experiments demonstrated that collagen had significant effects on cell attachment and proliferation (Li et al. 2005). Ingestion of hydrolyzed collagen could increase type I and IV collagen in Wistar rats and inhibit native metalloproteinase 2 (MMP2). Thus, hydrolyzed collagen may reduce aging-related damages of extracellular matrix by stimulating anabolic processes in skin tissues (Zague et al. 2011). In food legislations, there is no restriction for the amount of collagen usage. However, the addition of 2–30% hydrolyzed collagen to liquid foods is proposed in view of its positive effects. Beverages can be fortified with hydrolyzed collagen to improve their functional and nutritional properties. Fruit juice drinks containing hydrolyzed collagen were formulated and new functional drinks from orange, apple, and white grape juice blends are produced (Bilek and Bayram 2015). Addition of 2.5% hydrolyzed collagen into fruit juice drinks was significantly preferred by panelists. Chuaychan et al. (2017) reported that the incorporation of hydrolyzed collagen from scale of potted golden goatfish at level of 1–5% had no effect on quality and sensory property of apple juice.

#### 9.2.5.2 Biomedical Applications

The primary reason for the usefulness of collagen in biomedical application is that collagen can form fibers with extra strength and stability through its self-aggregation and cross-linking. Collagen is the most desirable biopolymer for biomedical applications because of their high biocompatibility and low immunogenicity (Lee et al. 2001). Collagen can be used as surgical suture, hemostatic agents, and tissue engineering including basic matrices for cell systems and replacement/substitutes for artificial blood vessels and valves (Auger et al. 1998). As a commercial medical product, collagen can be part of natural, stabilized tissue that is used in the device such as in a bioprosthetic heart valve. It can be fabricated as a reconstituted, purified product from animal sources, for example, as in wound dressings (Ramshaw et al. 2008). The main applications of collagen as drug delivery systems are collagen shields in ophthalmology, sponges for burns/wounds, mini-pellets and tablets for protein delivery, gel formulation in combination with liposomes for sustained drug delivery. It can be used as controlling material for transdermal delivery, nanoparticles for gene delivery and basic matrices for cell culture systems. It is also utilized for tissue engineering including skin replacement, bone substitutes, and artificial blood vessels and valves (Lee et al. 2001).

#### 9.2.5.3 Cosmetic Applications

Collagen and collagen derivatives have been marketed for use in skin care products. These products have gained commercial popularity in the marketplace, at least partly due to the claimed beneficial biological effects on skin conditions (e.g. anti-aging effects) (Peres et al. 2017). Supplementation with collagen and collagen-derived products may potentially stimulate extracellular matrix anabolic metabolism. This leads to an increase of collagen biosynthesis in the skin using the corresponding absorbed degradation products as substrates and to a promotion of the repair processes in dermal wounds. These positive effects might result from the biochemical similarities of exogenous collagen, such as the unique amino acid and peptide profiles, to the endogenous collagen in connective tissue and in particular to the major type I collagen in the dermis (Wijesundara and Malaweera 2013).

### 9.3 Fish Gelatin

Gelatin is derived from collagen by thermal denaturation or partial hydrolysis. Gelatins are currently used in diverse fields including foods, cosmetics, and pharmaceuticals. Generally, major industrial sources of gelatin production are bovine and porcine skins and bones. Similar to collagen, due to religious constraint and some associated diseases, gelatin from fish processing byproducts gains increasing attention. Various byproducts from fish processing such as skin, bone, scale, or internal organs can be used as raw material for gelatin extraction. Thornback ray skin (Lassoued et al. 2014), spotted golden goatfish scale (Chuaychan et al. 2016), shark cartilage (Kwak et al. 2009), Baltic cod offal (Kołodziejska et al. 2008), lizardfish bone (Taheri et al. 2009), skipjack tuna fin (Aewsiri et al. 2008), yellowfin tuna swim bladder (Kaewdang and Benjakul 2015) were used for gelatin extraction. Type and freshness of raw material are the key factors determining the properties of fish gelatin. Additionally, the methods for preservation of the starting raw materials also affect certain properties of fish gelatin. Icing and freezing are important preservation methods to maintain the quality of raw materials during handling and storage. Fernández et al. (2003) reported that gelatin from flounder skins frozen at -12 °C had lower gel strength, compared to those from fresh skin and skin frozen at -20 °C. Liu et al. (2008) revealed that the rheological properties of gelatin from channel catfish skin were highly affected by preservation methods. Gelatin from dried channel catfish skin exhibited higher gel strength, compared to those from fresh and frozen skins. Icing of seabass skin for gelatin production had a marked influence on fishy odor and color of resulting gelatin due to lipid oxidation but it did not drastically affect gelling property (Sae-leaw and Benjakul 2015).

#### 9.3.1 Production of Gelatin

Gelatin manufacturing process generally consists of three major steps: (i) pretreatment of raw material; (ii) extraction of the gelatin; and (iii) purification and drying. All processes used for gelatin extraction have direct impact on the yield and properties of gelatin obtained (Benjakul et al. 2012a).

##### 9.3.1.1 Pretreatment

In general, a mild acid pretreatment of the fish skin is prerequisite for gelatin extraction. Pretreatment enhances the cleavage of non-covalent bonds of protein structure, thus affecting swelling and collagen solubilization (Stainsby 1987). Swelling must be conducted under the appropriate condition. Solubilization of collagen can occur during acid pretreatment if it is not operated properly. Excessive swelling may lead to the lower yield. Solubilization can be promoted and associated with the loss of mother collagen during pretreatment. Type of acid has been considered as the essential factor governing yield and properties of fish gelatin (Benjakul et al. 2009). Giménez et al. (2005) reported that gelatin from Dover sole skin treated using lactic acid had the similar properties to that treated with acetic acid and those treatments had no negative impact on organoleptic properties of gelatin.

An alternative approach to acid pretreatment is the use of particular proteolytic enzymes. Some raw materials with a high amount of molecular cross-links generally

provide the lower extraction yield (Galea et al. 2000). To increase the extraction yield, some proteases capable of solubilizing the collagen or cleaving the cross-links have been implemented during acid pretreatment process. Nalinanon et al. (2008a) demonstrated that the extraction of gelatin from bigeye snapper skin treated with pepsin during swelling process markedly increased the yield from 22.2% to 40.3% in comparison with that of the skin without pepsin treatment. Nevertheless, treatment with pepsin could enhance the degradation of  $\alpha$ - and  $\beta$ -chains of the gelatin from thornback ray skin, thereby decreasing particular properties such as gel strength (Lassoued et al. 2014).

Raw material with high fat content can be associated with the negative effect on gelatin properties such as off-odor and dark color due to the enhanced lipid oxidation during extraction. Pretreatment of seabass skin with citric acid, followed by defatting with 30% isopropanol prior to gelatin extraction could reduce lipids, particularly membrane phospholipids, thereby lowering lipid oxidation and fishy odor in the resulting gelatin (Sae-Leaw et al. 2016a). Khamtaphant and Benjakul (2008) prepared defatted skin from brownstripe red snapper using 10% butanol prior to gelatin extraction.

### 9.3.1.2 Extraction and Drying

Conversion of collagen into soluble gelatin can be achieved by heating. Heat treatment destabilizes triple-helix, resulting in helix-to-coil transition and conversion into soluble gelatin by breaking a number of intra- and intermolecular covalent crosslinks and hydrogen bond present in collagen (Giménez et al. 2005). The properties of gelatin are greatly influenced by the extraction process, which may depend on pH, temperature, and time during extraction process (Gómez-Guillén and Montero 2001). These extraction variables also influence the length of the polypeptide chains, thus affecting the functional properties of gelatin, particularly gelling property (Kołodziejska et al. 2008). Among those processing parameters, heating temperature plays the major role. Gelatin from skins of brownbanded bamboo shark and blacktip shark underwent the pronounced degradation when extracted at high temperature, around 75 °C (Kittiphattanabawon et al. 2010). Nevertheless, gelatin from unicorn leatherjacket skin had more retained  $\alpha$ - and  $\beta$ -chains when extracted at high temperature (75 °C) (Kaewruang et al. 2013). Proteolysis induced by heat-activated and heat-stable indigenous proteases associated with skin matrix can contribute to the destabilization as well as disintegration of collagen in skin of unicorn leatherjacket by disrupting the intra- and intermolecular cross-links (Ahmad et al. 2011). Those indigenous proteases hydrolyzed gelatin from skin during extraction at the optimal temperature (50–55 °C), thereby affecting the yield, proteinaceous components and properties of the resulting gelatin. Ahmad et al. (2011) reported that addition of soybean trypsin inhibitor during gelatin extraction could prevent the degradation during extraction and improve functional properties of gelatin from unicorn leatherjacket skin.

After extraction, the gelatin is filtered to remove suspended or insoluble matters including fat, unextracted tissues, and other residues. Diatomaceous earth or activated carbon can be used to clarify the gelatin solution. The final stage is evaporation, sterilization, and drying. These operations are performed as quickly as possible to minimize loss of properties (Johnston-Banks 1990). Gelatin from shark cartilage was dried using three different drying methods including freeze drying, hot-air drying, and spray drying (Kwak et al. 2009). Freeze-dried gelatin showed the highest gel strength and foam formation ability, but had the lowest foam stability. Nevertheless, spray-dried gelatin

exhibited the greatest emulsion ability. Sae-Leaw et al. (2016b) revealed that freeze-dried seabass skin gelatin had the higher gel strength, compared to the spray-dried counterpart. However, the lower lipid oxidation products, fishy odor as well as volatile compounds were detected when spray drying was used.

### 9.3.2 Emerging Technologies for Extraction of Fish Gelatin

Several emerging technologies such as ultrasound, microwave, and high-pressure processing have been shown to be promising for gelatin extraction. Ultrasound can increase the extraction yield of gelatin from fish processing byproducts. Gelatin from pollock skin extracted with the aid of ultrasound treatment with a frequency of 20 kHz had the increased extraction yield by 11.1%, compared to that from the conventional process (Olson et al. 2005). Moreover, ultrasound could significantly increase the yield and improve rheological properties, gel strength, and functional properties of gelatin from bighead carp scales (Tu et al. 2015). However, a longer period of ultrasound could decrease gel strength and melting points by weakening the gel network. Therefore, ultrasound-assisted extraction with optimal amplitude and time is a potential method to increase the yield and provide gelatin of high quality.

Microwave-assisted extraction process has also been suggested as one of the effective methods for improving the quality of gelatin, including gel strength, melting point, and viscosity of gelatin. This method considerably reduced extraction time in comparison to the conventional extraction processes (Park et al. 2013). Binsi et al. (2017) extracted gelatin from scales of rohu using microwave-assisted extraction.

High pressure is another useful technique to shorten the extraction time and enhance extraction efficiency of gelatin from fish skins. High pressure treatment of Dover sole skin at 250 and 400 MPa for 10 or 20 minutes, was applied during skin pretreatment in acid at 10 °C to facilitate destabilization of acid labile crosslinks, or during extraction in water at 45 °C to accelerate collagen hydrolysis. Pressure level and time induced noticeable changes in molecular weight distribution and consequently affected the viscoelastic properties of gelatin (Gómez-Guillén et al. 2005).

### 9.3.3 Improvement of Properties of Fish Gelatin

Fish gelatin generally exhibits inferior properties, especially gel-forming ability, to mammalian counterparts. This is due to the lower content of imino acids, proline, and hydroxyproline (Haug et al. 2004). However, chemical and physical treatments can be applied to modify the gelatin network through cross-linking of gelatin chains to improve gelling properties (Cao et al. 2007). Cross-linking agents including glutaraldehyde, genipin, carbodiimides, calcium salts, and transglutaminase have been used (Benjakul and Visessanguan 2003; Chiou et al. 2006). Phenolic compounds are used as protein cross-linkers, which strengthen the gel network of gelatin. Ethanolic extract from coconut husk rich in tannic acid was used as the potential cross-linker for strengthening the gel of gelatin from yellowfin tuna swim bladder (Kaewdang and Benjakul 2015). Phenolic compounds could also be used as an antioxidant to prevent lipid oxidation during gelatin extraction. Sae-leaw et al. (2016c) reported that incorporation of tannic acid during gelatin extraction from seabass skin could lower lipid oxidation, fishy odor, as well as the formation of volatile compounds, especially hexanal and heptanal, in gelatin from seabass skin.

Furthermore, physical treatments, such as UV (Otoni et al. 2012),  $\gamma$ -irradiation (Islam et al. 2014; Sung and Chen 2014) and high pressure technology (Montero et al. 2002) have also been applied to improve gelling and particular properties of gelatin.

### 9.3.4 Applications of Fish Gelatin

In the food industry, gelatin is an extremely versatile ingredient and can be used to improve the consistency, elasticity, and stability of foods, such as confectionery, fruit juices, dairy products, soups, and others (Gómez-Guillén et al. 2002; Jeya Shakila et al. 2012). Gelatin is used as the processing aid of fruit juices, beer, and wine, not only for clarification and precipitation of substances causing turbidity, but also for reducing the concentration of polyphenols such as tannins and anthocyanogens (Schrieber and Gareis 2007). Lassoued et al. (2014) used gelatin from the skin of thornback ray for clarification of apple juice. Thornback ray skin gelatin exhibited higher ability in clarifying the apple juice than bovine gelatin. Fish gelatin could also be used for microencapsulation of food flavors such as vegetable oil, lemon oil, garlic flavor, apple flavor, or black pepper (Soper 1997). Fish gelatin with lower melting temperatures had a better release of aroma and offered a stronger flavor (Choi and Regenstein 2000). It is usually recommended to be used in foodstuffs to improve the protein levels and is also used to reduce the carbohydrate levels in foods formulated for diabetic patients (Karim and Bhat 2009). Fish gelatin has been used as biomaterial for preparing edible films (Tongnuanchan et al. 2013). Such a film can be heat sealed, in which the bag or pouch could be made as the edible container to carry dry food or condiment (Tongnuanchan et al. 2016).

For the pharmaceutical industry, gelatin is often used in the manufacturing of various products such as capsules, cosmetics, tablet coatings, and emulsions. Another main application is the embedding of oil-based vitamins. Cold water fish gelatin was used for the microencapsulation of oil soluble substances such as vitamins A, D, E, and carotenoids (Schrieber and Gareis 2007).

## 9.4 Fish Oil

Fish oils are rich in PUFAs, especially n-3 PUFA, including linolenic acid (C18:3), eicosapentaenoic acid (EPA; C20:5n-3), docosapentaenoic acid (DPA; C22:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) (Harris et al. 2008). The n-3 PUFAs in fish oils play an important role in reducing the risk of a number of diseases, including atherosclerosis, coronary heart disease, hypertension, inflammation, hypotriglyceridemic, allergies, and diabetes (Leigh-Firbank et al. 2007). Long-chain PUFAs are essential for the growth and development of infants, and they have been fortified in food as supplements in the form of concentrated fish oil. Hence, there is a marked demand for marine fatty acids as a health food and for the use as a dietary supplement (Lee et al. 2014). Fish oils have benefits to reduce the risk of degenerative diseases such as cardiovascular disease (atherosclerosis, thrombosis, stroke), cancers, diabetes, depression, immune disorders, proper neural and brain development, and other diseases (Rizliya and Mendis 2014). Fish oils are widely used in feed and aquaculture.

### 9.4.1 Extraction of Fish Oil

Extraction process is an important procedure affecting quality and oxidative stability of fish oil. The head is the important fish leftover used for fish oil production. Additionally, other byproducts such as viscera, especially liver of lean fish, have been used for fish oil manufacture. Several methods can be used to extract fish oils from different raw materials. These methods vary widely in terms of yield and affect the quality of the extracted oils differently.

#### 9.4.1.1 Traditional Process

Wet pressing method, as described by the Food and Agriculture Organization of the United Nations (FAO 1986), is the most common process used for the production of crude oil. This process involves the cooking of the raw material, pressing of the cooked material and final filtration or centrifugation to recover the oil from the protein-rich meal. Different fish processing byproducts have been proposed as sources of fish oil. Chantachum et al. (2000) studied the separation of oil from non-precooked and precooked tuna heads by a wet reduction method at 85 °C for 30 minutes. Oil from non-precooked samples had superior yield and quality, compared to the oil from precooked samples. Crude oil from byproducts of Nile tilapia and hybrid sorubim processing was extracted by cooking at 40 °C for 3 hours (Menegazzo et al. 2014). Byproducts from Norwegian spring spawning herring, including heads, tails, belly flaps, backbones, and viscera were used for oil extraction by wet rendering at 70 °C or enzymatic hydrolysis with Alcalase or a mixture of papain and bromelain (Carvajal et al. 2015). The wet rendering process yielded the oil with lower lipid oxidation and higher stability, compared to those prepared by enzymatic hydrolysis. In general, this traditional process for fish oil production provides good results when using fish products or byproducts with high-oil content such as herring, tuna, sardine, salmon, etc. However, it is not feasible when the oil content is low (Rubio-Rodríguez et al. 2010). Recently, Sae-leaw and Benjakul (2017) produced fish oil from visceral depot fat from seabass by heating under vacuum condition. The obtained oil had higher yield than that produced using solvent extraction method.

#### 9.4.1.2 Supercritical Fluid Extraction (SFE)

The rendering process employs high temperature, which increases the susceptibility of the extracted oil to thermal degradation. As a consequence, non-heat treatment methods for fish oil extraction have gained considerable attention. Although solvent extraction with hexane and similar solvents is applicable, it is not a recommended method for extraction of food grade fish oils (Maqsood et al. 2012). Supercritical fluid extraction (SFE) is a promising process for the extraction and fractionation of fish oils containing labile PUFA, which can be carried out under mild operating conditions and oxygen free media, leading to the reduction of oxidation of n-3 fatty acids during the extraction process (Létisse et al. 2006). Moreover, SFE selectively extracts low polar lipid compounds, thus avoiding the co-extraction of polar impurities such as some inorganic derivatives with heavy metals. Oils from salmon byproducts (belly part, trimmed muscle, frame, and skin) were extracted using different methods, including hexane extraction, pressing, and SFE (Haq et al. 2016). Oil extracted by SFE had better physical characteristics (color

and viscosity), oxidative stability and radical scavenging activity. Oils from off cuts of hake, orange roughy and salmon, and liver of jumbo squid were extracted with different methods, including cold extraction, wet reduction, enzymatic extraction, and SFE (Rubio-Rodríguez et al. 2012). SFE yielded the oil with the lowest lipid oxidation products and certain pollutants such as some arsenic species (mainly polar derivatives). Fish oils from different parts of Indian mackerel were extracted using various techniques of supercritical CO<sub>2</sub> extraction, including continuous, co-solvent, soaking, and pressure swing (Sahena et al. 2010). The amounts of PUFAs recovered (as a percentage of total extracted fatty acids) were in the ranges of 73.24–74.68% in the skin, 68.36–69.37% in the flesh, 56.20–57.3% in the viscera and 61.21–62.09% in the head. The soaking and pressure swing techniques used with supercritical CO<sub>2</sub> are highly effective in extracting fish oil with the highest n-3 PUFAs. Different methods, including wet reduction, SFE, ammonia, and enzymatic extraction were used for oil extraction from common Kilka (Sayyad and Ghomi 2017). SFE showed the highest oil yield (96.94%), the best oxidative and color characteristics. Oil had the highest total unsaturated fatty acids. Furthermore, the oil obtained by SFE had the greatest oxidative stability throughout 90 days of storage at 4 °C. Nowadays, SFE is generally considered as a useful technology to replace traditional extraction processes such as wet rendering or solvent extraction. Therefore, SFE is a promising technology to be scaled up in order to produce high quality fish oil at industrial scale.

#### 9.4.1.3 Enzymatic Method

Other processes, such as enzymatic hydrolysis with proteases, have been implemented to extract crude oil from fish processing byproducts. Proteases can facilitate the release of oil from fish tissues without the use of solvent or high temperature. Approximately 80% of total lipids could be recovered from salmon frames after enzymatic hydrolysis with Protamex (Liaset et al. 2003). Commercial proteases (Alcalase, Neutrase, and Flavourzyme) were tested for their ability to release the oil from salmon heads (Linder et al. 2005). Alcalase rendered fish oil with the highest yield. Fish oil extraction with enzymatic hydrolysis generally yields the same or higher amount of oil, compared to conventional extraction method. With milder conditions, a high product quality can be achieved, while leaving protein fractions that can also be used as valuable ingredients (Rustad et al. 2011). de Oliveira et al. (2017) prepared oils from tuna heads using various methods including enzymatic hydrolysis using Alcalase, the physical method by cooking and pressing after fishmeal production and the solvent extraction method. The oil extracted by enzymatic hydrolysis had the lowest acidity and peroxide value and showed the highest levels of EPA and DHA. Hathwar et al. (2011) studied the enzymatic hydrolysis to extract oil from viscera of Indian major carps and rohu with various commercial proteases, including Protease-P-Amano, Alcalase, Protex 7L, and Neutrase. Hydrolysis by Protease-P-Amano resulted in the maximum lipid recovery (74.9%), followed by that hydrolyzed with Alcalase (61.7%). Qi-Yuan et al. (2016) optimized the fish oil extraction from mackerel viscera using Neutral protease by response surface methodology. The yield of oil was greatly influenced by pH, enzyme concentration, temperature, agitation, and incubation time. Using the optimized condition, the yield of 78.66% was obtained.

### 9.4.2 Fish Oil Refining

Crude fish oils obtained from any extraction processes are still inedible because they contain free fatty acids (FFAs), primary oxidation products, minerals, pigments, moisture, phospholipids, and other insoluble impurities, which reduce their quality. Therefore, it is necessary to remove these impurities from the crude oils, in which purified edible oils with desirable shelf-life and commercial value are produced (Huang and Sathivel 2010). Crude fish oils are normally subjected to further refining processes by chemical methods via several steps including degumming to remove phospholipids and other gummy materials, neutralization or deacidification with caustic soda for FFA removal, washing with water to remove residual alkali, bleaching to remove pigments, soap and trace metals, and deodorization by vacuum distillation to remove remaining FFA, aldehydes, ketones, alcohols, and other odor compounds (Huang and Sathivel 2010). These processes present several drawbacks, since it involves the use of chemicals such as alkali that can contaminate the environment and some neutral oil is lost, mainly in oils with FFA content (Rubio-Rodríguez et al. 2010). Physical refining processes such as the application of superheated steam under low pressure has been proposed as an alternative to remove FFA and volatile compounds. However, with high temperature used, it is not suitable for thermolabile oils such fish oil (Čmolík and Pokorný 2000). Physical adsorption on activated carbon has been used to remove contaminants. Deodorization is also an important process to remove undesirable odor, especially fishy odor. Fishy odor reduces sensory quality and limits the application of fish oil in the food industry. Traditional oil deodorization is based on the application of high temperature. Alternative methods using vacuum steam distillation at low temperature followed by a treatment in a silica gel column, adsorption with a resin, or treatment with activated carbon or diatomaceous earth was proposed for the removal of odorous compounds from fish oil (Čmolík and Pokorný 2000). Oil from carp viscera was refined using a mixture of adsorbents (activated carbon and Tonsil Supreme-110FF bleaching earth) (Monte et al. 2015). Recently, Charanyaa et al. (2017) developed a refining process for removal of phospholipids, FFA, and metal ions without affecting n-3 PUFA present in the crude oil from Indian sardine. Degumming with 5% (w/w) orthophosphoric acid, two stage solvent extraction with methanol at 1 : 1 (w/w) in each stage and bleaching with 3% (w/w) activated charcoal at 80 °C for 10 minutes resulted in the reduction of phospholipid content from 612.66 to 5.66 ppm, FFA from 5.64% to 0.56% with the complete removal of iron and mercury. The proposed refining process was able to produce sardine oil of superior quality by removing almost all the impurities without any loss of n-3 PUFA content.

Supercritical fluid technology is the alternative method for oil refining to replace the use of chemicals or high temperature. This technology is implemented for degumming, bleaching, and deacidification of fish oil (Bhosle and Subramanian 2005; Čmolík and Pokorný 2000). Catchpole et al. (2000) developed a method for the removal of impurities, including peroxides, fatty acids, or odorous compounds, from different crude fish oils (orange roughy oil, deep sea shark liver oil, spiny dogfish oil, and cod liver oil) using supercritical CO<sub>2</sub> and CO<sub>2</sub> – ethanol mixture. A mixture of CO<sub>2</sub> – ethanol yielded the oil with lower acidity and negligible fishy odor, compared to the oil obtained using pure supercritical CO<sub>2</sub> under the same experimental conditions.

Winterization is also used in fish oil refining in order to concentrate the valuable n-3 PUFAs, especially EPA and DHA (Crexi et al., 2010). Other methods that can be used to

concentrate valuable n-3 PUFAs include freezing, crystallization, urea complexation, molecular distillation, SFE, and lipase hydrolysis (Hamam and Shahidi 2008; Zuta et al. 2003). The concentration of n-3 PUFAs using urea complexation is more efficient than the other methods and more protective for the PUFAs against autoxidation (Liu et al. 2006). Separation of urea complexes from the non-urea complexing fraction effectively removes saturated and monounsaturated fatty acids and enriches the PUFAs in the liquid fraction.

#### 9.4.3 Applications of Fish Oil

Due to the beneficial effects of fish oil, it has been widely used in the food, feed, and pharmaceutical industries. Edible uses of fish oil can be divided into three major categories: as a pharmaceutical component, as a functional food component, and as a commodity for the food industry. With the emphasis on the importance of long chain n-3 PUFAs, there are various fish oils containing capsules commercially available in the market as dietary supplement (Sahena et al. 2009).

For food applications, fish oil can be used mainly for two purposes, i.e. to replace vegetable oil and animal fat, as well as to improve the nutritional value of food products (Irianto et al. 2014). Fish oils are mainly used in the production of margarines, salad oil, and salad dressing, mayonnaise, and several types of spreads and pastes in bakery products. The unsaturated nature of fish oil provides good creaming properties and the varied chain lengths in these oils contribute to the smoothness and plasticity of margarines and shortenings (Rizliya and Mendis 2014). Recently, there has been increasing interest among food manufacturers to start using liquid fish oil instead of hydrogenated counterpart. The microencapsulation technique seems to be a promising way of incorporating the oil into food products without affecting the organoleptic properties (Rizliya and Mendis 2014). Fish oils from cod liver and shark were micro-encapsulated using fish gelatin, chitosan, and maltodextrin as wall materials (Pourashouri et al. 2014). Fish gelatin-based micro-encapsules had the highest oxidative stability during storage at 20 °C for 60 days. Moreover, nano-encapsulated fish oil was prepared by liposomal method and it was used to fortify in yogurt (Ghorbanzade et al. 2017).

Fish oil is an important constituent in aquaculture feeds, contributing essential fatty acids needed by fish for normal growth, health, and reproduction. A small amount of fish oil is being also used in the feed of farm-raised animals, such as pigs, poultry, cattle, and sheep, and it is also used in pet foods. The incorporation of fish oil in animal feed has been shown to improve the immunity of the animals against diseases, increase feed appeal, reduce incidences of deformities, and enhance growth (Rizliya and Mendis 2014).

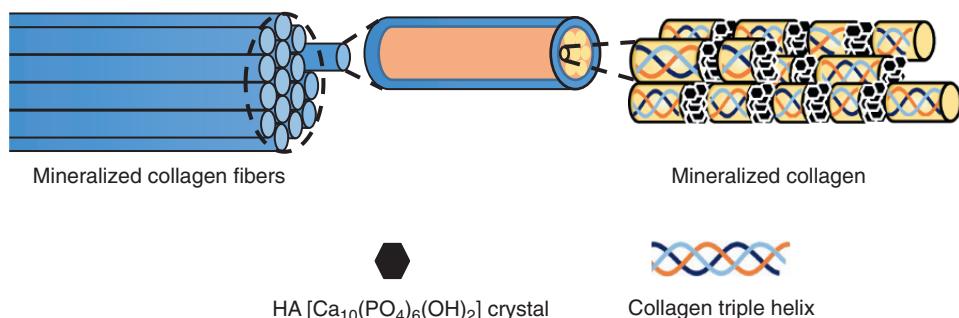
### 9.5 Fish Calcium

Calcium (Ca) is known to be an essential element required for numerous physiological functions in human body including the strengthening of teeth and bones, nerve function, blood coagulation, muscle contraction, and many enzymatic reactions that require calcium as a cofactor (Anderson and Garner 1996). Calcium can be obtained from dairy

products such as milk or cheese, dietary supplements, and calcium fortified products. Generally, dairy products are common food sources of calcium. However, due to lactose indigestion and intolerance in certain populations, especially Asian people, consumption of milk or dairy products may be limited. As a consequence, calcium fortified foods or calcium salts can be alternative calcium supplements. Small fish are often eaten whole, due to high calcium in bones. Calcium from such fish has been shown to have comparable absorption to that from skimmed milk both in rats (Larsen et al. 2000) and humans (Hansen et al. 1998).

Among fish processing byproducts, fish bone or skeleton serves as a potential source of minerals and calcium. Fish bones contain 60–70% minerals including calcium, phosphorous, and hydroxyapatite (Kim and Mendis 2006). HA with the following formula:  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  has Ca/P mole ratio of 1.67, while tricalcium phosphate (TCP)  $\text{Ca}_3(\text{PO}_4)_2$  has the ratio of 1.50 (Hamada et al. 1995). HA crystals are assembled in the gap zones between collagen fibril to form mineralized collagen fibril. A number of mineralized collagen fibrils align parallel to each other to form mineralized collagen fibers (Figure 9.3) (Cui et al. 2007). Calcium from fish can be easily absorbed by the body (Larsen et al. 2000). Biological utilization and clinical trial showed that the bone is a good source of bioavailable calcium, much better than common calcium powders, suggesting the potential of fish bone as a source of dietary calcium (Ishikawa et al. 1990). In order to incorporate fish bone into calcium-fortified food, it should be converted into an edible form by softening its structure. This can be achieved by different methods, including hot water treatment and the addition of hot acetic acid solution. Superheated steam has been used to reduce the loss of soluble components from fish tissue, which enabled the better recovery of bone within a shorter period. Furthermore, the treated bones need further saponification, degreasing, and degumming (Venugopal 2008a).

Ca-based powder was prepared from Alaska pollock bones by autoclaving in water to soften the bone prior to powdering. Autoclaving for an optimum period (40 minutes) resulted in a powder having appreciable soluble calcium content. Autoclaving for longer periods did not affect the mineral yield, and soluble calcium ratio (Choi et al. 1998). Apart from steaming, treatment with different acids has been performed to extract calcium from the Alaska pollock frame. Alaska pollock frame treated with hydrochloric and lactic acid had calcium content of 60% and 30%, respectively, whereas those treated with acetic acid had the lowest content. The obtained fish bone powder contained 38.3% Ca and



**Figure 9.3** The hierarchical structure of a self-assembled HA-collagen composite in bone tissues.

17.7% P, with a ratio of 2:1, which is similar to that of human bones (Changhu et al. 1995).

Recently, biocalcium from pre-cooked skipjack tuna bone was prepared using several treatments including alkaline treatment, defatting, and bleaching (Benjakul et al. 2017). Cleaning and defatting of pre-cooked skipjack tuna bone had a marked influence on the removal of lipid substrate and lipid oxidation products, associated with fishy odor. Bleaching using NaOCl and H<sub>2</sub>O<sub>2</sub>, respectively effectively improved the whiteness of biocalcium. The resulting biocalcium contained Ca and P at levels of 23.02% and 10.14%, respectively. It also consisted of collagen or peptides and had the negligible amount of volatiles associated with lipid oxidation. Nemati et al. (2016) reported that tuna bone powder (TBP) recovered by alkaline treatment contained calcium and phosphorus of 38.16% and 23.31%, respectively. The ratio of Ca:P was close to 2:1, which was comparable to that in human bones. The processing of bones and skeletal matter from sardine oil and ribbon fish using sodium hydroxide and ethanol yielded powder with calcium content ranging from 27.2% to 32.7% (Logesh et al. 2012). Calcium supplement was prepared from Nile tilapia bone by soaking in 0.8% sodium hydroxide for 90 minutes, heating at 121 °C for 90 minutes and drying at 90 °C for 60 minutes (Techochatchawal et al. 2009). The obtained calcium supplement in capsule contained 25.01% calcium and its quality met the requirements for calcium supplement standard. Malde et al. (2010b) prepared calcium powder from the backbones of salmon and cod with the aid of proteases. Salmon and cod backbones had calcium content of 20.8% and 26.1%, respectively. Baltic cod and Atlantic salmon backbones with remaining muscle tissue, devoid of heads and fins, were used for calcium powder preparation (Bubel et al. 2015). The obtained powders had calcium contents of 27.79–24.92%, with low protein and fat contents.

### 9.5.1 Bioavailability of Fish Calcium

Fish bone contains bioactive and nutraceutical molecules, but a few efforts have been made to utilize fish bones as functional materials due to the low solubility (Kim et al. 2012b). Calcium must be ionized and soluble to be absorbed in the body. Although a calcium salt is precipitated because of alkaline conditions in the ileum, some calcium ions are present in the solution. In general, calcium is poorly absorbed at the intestine. After calcium is dissolved in the stomach, the gastric contents move into the small intestine, where calcium is absorbed. Bicarbonate (HCO<sup>-</sup><sub>3</sub>) is secreted into the intestine to neutralize the contents. Therefore, the concentration of calcium available for absorption in the jejunum and ileum, which maintain a pH of approximately 7, is expected to be lower than that dissolved in the stomach (Goss et al. 2010). The total calcium absorption is a complicated function of local solubility, the rate of transepithelial movement (i.e. absorption), and the sojourn time in the particular intestinal segment (Duflos et al. 1995).

According to Goss et al. (2010), peptides act as calcium carrier, thereby enhancing calcium absorption at small intestine. Jung et al. (2007) reported that calcium binding fish bone peptides could inhibit the formation of insoluble calcium salts at neutral pH *in vitro* study. Supplementation of calcium binding peptide increased calcium retention and the loss of bone mineral was decreased in ovariectomized rats. Heterogeneous enzyme extracted from the intestine of bluefin tuna was able to digest the skeletal frames of hoki (*Johnius belengerii*) and oligophosphopeptides were generated. Those peptides

could solubilize more calcium, thus improving the calcium bioavailability (Jung et al. 2005).

Malde et al. (2010a) reported that calcium from salmon and cod bones were well absorbed in young healthy men. Calcium absorption from the bones of salmon and cod were 22.5 and 21.9, respectively. The availability of calcium from TBP was 53.93%, which was significantly higher than most calcium salts (Nemati et al. 2016). The study on SaOS-2 cells demonstrated that the mineral content of hake fish bone powder is bioavailable and did not exhibit a cytotoxic effect on the cell line (Flammini et al. 2016). *In vivo* data revealed that hake fish bone powder is a suitable calcium supplement with comparable efficacy to commercial calcium tablets on rat bone mineralization (Flammini et al. 2016).

### 9.5.2 Applications of Fish Calcium

Fish calcium has been used in pharmaceutical application as a dietary supplement and it is sold commercially as tablets. Additionally, fish calcium can be supplemented or fortified in many food products. TBP and TPC were formulated as a calcium supplement in bakery products, including cookies and bread (Nemati et al. 2016). The result showed that cookies fortified with TBP had higher *in vitro* bioavailability, compared to the fortified bread. The percentage of calcium bioavailability of TBP and TCP in cookies were 38.9% and 39.5%, respectively, while bioavailability of TBP and TCP for bread were 36.7% and 37.4%, respectively. Fortified bread and cookies had the comparable scores with control.

## 9.6 Fish Enzymes

Enzymes are biological catalysts capable of speeding up chemical reactions and are biological tools for improving food quality or food processing operations. Use of an enzyme as a processing aid has a number of advantages over the use of chemicals, including high specificity, efficiency of catalysis at moderate temperatures, and being environmentally friendly. Enzymes recovered from fish processing byproducts can be an alternative source of enzyme apart from those of plant or microbial origins (An and Visessanguan 2000). Fish processing leftovers, especially viscera, are potential sources of different enzymes, such as proteases, lipases, cellulase, and collagenase (Klomklao 2008).

### 9.6.1 Proteases

Fish viscera has a relatively large portion of the animal round weight with approximately 5% (Gildberg 1992). Fish viscera contains high levels of digestive enzymes, making it a suitable source for recovering proteases for applications. Fish proteases are multifunctional enzymes, which catalyze the hydrolytic degradation of proteins. Digestive enzymes found in fish viscera include pepsin, gastricsin, trypsin, chymotrypsin, collagenase, elastase, carboxypeptidase, and carboxyl esterase (An and Visessanguan 2000). Pepsin and trypsin are two main groups of proteinases found in fish viscera. Pepsin found in fish stomach is active in acidic conditions, while trypsin is concentrated in the pyloric cecum and active at neutral and alkaline conditions (An and Visessanguan 2000). Crude visceral

proteases from little tuna (*Euthynnus affinis*), catla (*Catla catla*) and tilapia (*Oreochromis mossambicus*) were isolated and characterized (Murthy et al. 2017). Trypsin was found to be the major protease. Trypsins from spleen of skipjack, yellowfin, and tongol were purified. They had MW of 24 kDa and showed the optimal pH around 8.5–9.0 using Na $\alpha$ -p-tosyl-L-arginine methyl ester hydrochloride (TAME) as a substrate (Klomklao et al. 2006a,b; Klomklao et al. 2009b). Purified trypsin from *Luphiosilurus alexandri* pyloric cecum with a molecular weight of 24 kDa showed an optimum at pH 9.0 and 50 °C when Na $\alpha$ -benzoyl-DL-arginine p-nitroanilide (BAPNA) was used as a substrate (dos Santos et al. 2016).

Commercial pepsin is normally extracted from the glandular layer of hog stomachs. Due to the high demand for pepsin and high cost associated with hog pepsin, considerable attention has been directed to find substitutes. Marine fish viscera-derived pepsin is a competitive option in the substitution of hog pepsin (Kim and Dewapriya 2014). Several pepsin and pepsin-like enzymes have been isolated from fish, including cold- and warm-water fish such as pepsin from rainbow trout stomach (Wald et al. 2016), skipjack tuna stomach (Nalinanon et al. 2011), mandarin fish stomach (Zhou et al. 2008), etc. Pepsin from the stomach of albacore tuna, skipjack tuna, and tongol tuna had the optimal pH and temperature of 2 and 50 °C, respectively (Nalinanon et al. 2008b).

### 9.6.2 Lipases

Commercially, lipases are extracted from the pancreas and serous glands of ruminants, especially from young calves and pigs. During the past decade, fish processing byproducts, especially digestive organs, could be used as a prospective source of lipases. Sae-leaw and Benjakul (2018) extracted lipases from Asian seabass digestive organs, including stomach, liver, pyloric caeca, and intestine. Lipase from liver had the highest activity, compared to other organs. Various lipases from fish digestive organs, such as annular seabream pyloric caeca (Smichi et al. 2017b), red seabream pyloric caeca (Smichi et al. 2017a), smooth-hound pancreas (Achouri et al. 2017), golden gray mullet viscera (Smichi et al. 2013), carp liver (Görgün and Akpinar 2012), and Chinook salmon and New Zealand hoki pyloric caeca (Kurtovic et al. 2010) have been isolated and characterized. Lipase from liver of Asian seabass had MW of 60 kDa and exhibited optimal pH and temperature of 8.0 and 50 °C, respectively when  $\rho$ -nitrophenyl palmitate ( $\rho$ -NPP) was used as a substrate (Sae-leaw and Benjakul 2018).

### 9.6.3 Applications of Fish Enzymes

Enzymes have become an essential component of food, pharmaceutical, and cosmeceutical production with their great catalytic power. Fish-derived enzymes could be used as a processing aid for many seafood products due to a low cost. Enzymatic peeling is a popular application of enzymes in the food as well as cosmetic industries (Kim and Dewapriya 2014). Some enzymes are capable of dissolving only the outer layer of muscles, without damaging the original muscle tissue, taking advantage of the biochemical differences between the skin and muscle cells. This type of enzyme is applied in the formulation of skin-peeling agents in the cosmetics industry (Kim and Dewapriya 2014). Moreover, peeling enzymes are commonly used in the removal of undesirable parts from many economically valuable marine fish species, such as tuna, skate, filefish, squid, and

many other shellfish (Suresh et al. 2015). Enzymes derived from fish have been applied for several processes of the dairy industry. Particularly, fish digestive enzymes could be used as a cheap and alternative source for cheese production. In addition, several industrial applications of fish-derived enzymes have been reported (Kim and Dewapriya 2014; Shahidi and Janak Kamil 2001). The ability of the fish enzymes to work at low temperature and high pressure are the main characteristic features that have brought about their great potential to be used as a competitive alternative for traditional enzymes (Kim and Dewapriya 2014).

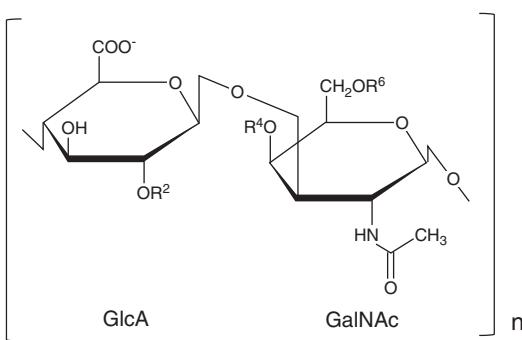
Fish proteases have been employed for the production of protein hydrolysates with bioactivity. Trypsins from pyloric caeca of brownstripe red snapper and bigeye snapper were used for production of protein hydrolysate from skin of brownstripe red snapper (Khantaphant and Benjakul 2008). Visceral peptidase from giant catfish was used for preparation of gelatin hydrolysate from the skin of giant catfish with antioxidant and angiotensin converting enzyme ACE-inhibitory activities (Ketnawa et al. 2017). Trypsin from unicorn leatherjacket pyloric caeca was used for preparation of protein hydrolysate from Indian mackerel protein isolate (Zamani and Benjakul 2016). Additionally, bluefish trypsin was employed for recovery of carotenoprotein from black tiger shrimp (Klomklao et al. 2009a).

There is a wide range of actual and potential applications of fish lipases ranging from cleaning products to modified foods, flavor development, biodiesel production, and synthesis of structured lipids (Kurtovic et al. 2010). Important applications of lipases in foods include modification of flavor and preferential hydrolysis of ethyl esters of PUFAs for enrichment of EPA and DHA in marine oils (Shahidi and Wanasyunda 1998). Lipases have been used intensively in the dairy industry for the hydrolysis of milk fat, contributing to flavor enhancement in cheeses, creams, and other milk products and accelerating cheese ripening. In addition, lipases can also be used to prevent the generation of trans-fats in margarines (Kurtovic et al. 2009). The attachment of a fatty acid to the bioactive compounds such as antioxidants using lipases provide the improved solubility in food and cosmetic applications (Kurtovic and Marshall 2013). For pharmaceutical application, lipases have been used to synthesize lovastatin, a drug that lowers serum cholesterol level (Choudhury and Bhunia 2015).

Due to the unique characteristics of fish-derived lipases, they show similar activity to commercially available lipases in terms of fatty acid release and flavor and odor development in dairy cream (Kurtovic et al. 2011). Recently, lipase from the liver of Asian seabass was used for defatting of fish skin prior to gelatin extraction (Sae-leaw and Benjakul 2018). Another interesting application of fish lipases is the production of n-3 PUFAs concentrate using marine oils (Rubio-Rodríguez et al. 2010). Due to the high substrate specificity of lipases, commercially available lipases are not suitable for the production of n-3 concentrates using long-chain PUFAs. However, it has been found that the fish lipase-immobilized system works well in concentrating PUFAs with long-chain PUFAs (Kim and Dewapriya 2014).

## 9.7 Fish Chondroitin Sulfate

Fish cartilaginous tissues contain glycosaminoglycans such as CS. CS is a complex heteropolysaccharide consisting of an alternating sequence of sulfated or unsulfated D-glucuronic acid (GlcA) and N-acetyl-D-galactosamine (GalNAc) residues linked



CS type	$\text{R}^2$	$\text{R}^4$	$\text{R}^6$
Nonsulfated chondroitin	H	H	H
Chondroitin-4-sulfate (CSA)	H	$\text{SO}_3^-$	H
Chondroitin-6-sulfate (CSC)	H	H	$\text{SO}_3^-$
Chondroitin-2,6-disulfate (CSD)	$\text{SO}_3^-$	H	$\text{SO}_3^-$
Chondroitin-4,6-disulfate (CSE)	H	$\text{SO}_3^-$	$\text{SO}_3^-$
Chondroitin-2,4-disulfate (CSB)	$\text{SO}_3^-$	$\text{SO}_3^-$	H
Trisulfated chondroitin	$\text{SO}_3^-$	$\text{SO}_3^-$	$\text{SO}_3^-$

**Figure 9.4** Structure of chondroitin with various sulfation positions.

through alternating  $\beta(1 \rightarrow 3)$  and  $\beta(1 \rightarrow 4)$  glycosidic bonds (Figure 9.4). CS is often modified by sulfate groups replacing one or more of the OH groups on C4 and C6 of GalNAc and C2 and C3 of GlcA (Venugopal 2008b). Sulfation in these different positions, which is mediated by specific sulfotransferases, confers specific biological activities to chondroitin. CS sulfation varies with sources, tissue, location within a tissue and age (Lauder 2009). CS is abundant and widely distributed in humans, mammals, and invertebrates, reflecting its important role in biological processes.

### 9.7.1 Production of Fish CS

Commercial CS is mainly derived from trachea of cow, nasal septa of pig, chicken keel, and shark cartilage (Lauder 2009; Venugopal 2008b). However, mammalian cartilage represents only 0.6%, compared to chondrichthyes, which represent 6–8% (Lee and Langer 1983). Apart from fish cartilage such as skate cartilage (Lignot et al. 2003), shark cartilage (Wang and Tang 2009), ray cartilage (Garnjanagoonchorn et al. 2007), fish processing byproducts such as bones of monkfish, cod, spiny dogfish, salmon, and tuna (Maccari et al. 2015), fin, head, and skeleton of *Scyliorhinus canicula*, head of *Prionace glauca*, and skeleton of *Raja clavata* (Novoa-Carballal et al. 2017) have been used for extraction of CSs.

In general, isolation of CS can be done by various steps, including: (i) chemical hydrolysis of cartilage; (ii) breakdown of proteoglycan core; (iii) elimination of proteins

and CS recovery; and (iv) purification of CS. The first two stages are mostly conducted by alkaline hydrolysis at high concentrations of NaOH, urea or guanidine HCl, subsequently combined with selective precipitation of CS using cationic quaternary ammonium chemicals (such as cetylpyridinium chloride), potassium thiocyanate, non-ionic detergents or alcoholic solutions, deproteinization by trichloroacetic acid and finally purification with gel filtration and/or ion-exchange and size-exclusion chromatography (Vázquez et al. 2013). Nevertheless, those economically operated processes lead to unsatisfactory purity for clinical uses of CS. The techniques that improve final product quality need larger amounts of reagents and are time-consuming. Therefore, customers and manufacturers try to develop more environmentally friendly and economical processes to obtain CS based on non-contaminant solvent strategies. Various alternative isolation methods have been recently developed to replace traditional methods for pursuing sustainability (Murado et al. 2010). The process can be performed by digestion of raw materials and proteins mediated using enzymes, selective precipitation of proteins with alcoholic solution, resuspension and neutralization with salt solution and separation by molecular-weight using ultrafiltration-diafiltration technologies (UF-DF). Murado et al. (2010) prepared CS from skate cartilage by enzymatic hydrolysis using papain and chemical hydrolysis with 0.2 M NaOH, selective precipitation using ethanol together with membranes technology. This quick and highly efficient process provided high yield (15%, w/w). CS from cartilaginous materials of small-spotted catshark byproducts including heads, fins, and skeletons were recovered using three sequential steps (Blanco et al. 2015). Those consisted of enzymatic hydrolysis with alcalase, alkaline proteolysis using NaOH and selective precipitation of CS with ethanol. Finally, ultrafiltration process and subsequent diafiltration were employed in order to achieve a high CS purity. The final yields of CS were 4.8, 3.3 and 1.5% of wet weight when heads, fins, and skeleton, were used as raw materials, respectively. A solvent-free mechanochemical extraction technology was applied to the extraction of CS from shark cartilage (Wang and Tang 2009). This process was carried out via mechanical pretreatment and mechanochemical treatment in AGO-2 high-intensity planetary activator at room temperature under solvent-free condition. The yield of CS was increased from 8.5% to 9.3% and the purity of product was improved from 86.4% to 95.1%. High intensity pulsed electric field (PEF), ultrasound-assisted method, alkaline method, and enzymatic method were used to extract CS from fish bone (He et al. 2014). PEF yielded the highest content of CS, followed by ultrasound-assisted method, alkaline method, and enzymatic method, respectively. CS content obtained by PEF was 2.02 times higher than enzyme method, 1.84 times higher than alkaline method, and 1.42 times higher than ultrasonic method.

### 9.7.2 Applications of Fish CS

CS has been reported to have a wide range of applications in the pharmaceutical, cosmetic, and food industries due to its anti-degenerative arthritis, anti-inflammation, anti-atherogenic, antitumor, and hypolipidemic activities (Campo et al. 2004). CS is an essential component of extracellular matrix of connective tissues, which plays a central role in various biological processes, such as the function and elasticity of the articular cartilage, hemostasis and inflammation, regulation of cell development, cell adhesion,

proliferation, and differentiation (Schiraldi et al. 2010). The main pharmaceutical application of CS is the treatment of osteoarthritis. CS is currently recommended by the European League Against Rheumatism (EULAR) as a SYSADOA (symptomatic slow acting drug for osteoarthritis) in Europe in the treatment of knee and hand osteoarthritis based on research evidence and meta-analysis of numerous clinical studies (Volpi 2009). Both *in vitro* and *in vivo* studies have shown chondroprotective properties of CS, resulting from an increase in the biosynthesis of connective tissue components (collagen, proteoglycans, and hyaluronan) and an increase of the viscosity of the synovial fluid at disease sites (Belcher et al. 1997). Low-molecular weight CS is applied for the treatment and prevention of osteoarthritis, because it competitively inhibits some cartilage degradative enzymes. Moreover, CS supplements may have an effect in relieving pain and stiffness caused by arthritis with fewer side effects compared to conventional drugs. The anti-inflammatory effect of CS was comparable to non-steroidal anti-inflammatory drugs such as indomethacin and ibuprofen (Ronca et al. 1998). Orally administered Condrosulf® (commercial CS from shark fins) was studied for its bioavailability and pharmacokinetics (Conte et al. 1995). It was persistent for more than 24 hours in humans when using a single dose of 0.8 or 0.4 g/day. Ronca et al. (1998) demonstrated the anti-inflammatory and chondroprotective effects of Condrosulf in rats and in healthy volunteers. The result suggested that CS reached synovial fluid and cartilage and modifies some pharmacologic and biochemical markers in experimental animals and osteoarthritic patients.

CS is also used in the engineering of biological tissues associated with the processes of bone repair, cartilage, and cutaneous wound (Wang et al. 2006). Moreover, it can be used in combination with other biopolymers (such as collagen, proteoglycans, and hyaluronic acid) to produce scaffolds with slow and controlled biodegradability that promote and accelerate the regeneration of damaged structures (Chang et al. 2003).

## 9.8 Concluding Remarks

Large amounts of fish processing byproducts are generated and currently wasted or used for production of low-value products. These byproducts are the potential sources for converting to marketable value-added products. To ensure better utilization of fish processing byproducts for applications in food, nutraceutical, cosmetic, or medical products, it is necessary to (i) use high quality byproducts; (ii) increase the yield of recovered products; (iii) develop the standardized and controlled processes accounting for variation in raw material, providing stable, healthy, and high-quality products; and (iv) measure and enhance the selected properties, especially bioactivities. Thus, the consistent prime quality products can be manufactured from underutilized leftovers.

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## 10

# Byproducts from Shellfish Harvesting and Processing

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## 10.1 Introduction

Production of shellfish byproducts from shellfish processing industries has undergone a dramatic increase in recent years. Due to the increased world production and consumption of shellfish, the shellfish industry is focused on an appropriate destination and/or reuse for these byproducts. The leftover produced each year by the shellfish processing industries represent a practical challenge. In general, the byproducts are generated at approximately 75% of the total weight of crustaceans (shrimp, crabs, prawns, lobster, and krill), with the current lack of acceptable waste management, there is a potentially large environmental hazard concern. Generally, seafood byproducts are thrown away at sea, burned, landfilled, or simply left out to spoil (Hamed et al. 2016). These byproducts are an excellent source of valuable compounds. The major marketable compounds isolated from shellfish byproducts are proteins, bioactive peptides, pigment, chitin, and chitosan. Also, protease and lipase which are the major enzymes can be found in shellfish wastes. Hence, the recovery and extraction of those compounds from shellfish processing byproducts and their uses may be a solution to minimize the byproducts and to generate valuable biomolecules for application in many fields.

## 10.2 Enzymes from Shellfish Processing Byproducts

Shellfish processing byproducts generally contain digestive enzymes, such as proteinases and lipases that have the potential for biotechnological utilization. As the marine animals adapt to different environmental conditions, their enzymes are associated with certain

unique properties, compared with enzymes from animals, plants, and microorganisms (Simpson 2000; Klomklao and Benjakul 2018). Recently, several enzymes from shellfish processing byproducts have been isolated and characterized. This has led to the emergence of new applications of these enzymes in industries. Moreover, the extraction and recovery of enzymes from shellfish processing byproducts may contribute significantly to reduce the local pollution problem and end up with valuable products.

### 10.2.1 Proteinases

Proteinases have been known to degrade proteins through hydrolysis of peptide bonds (Klomklao et al. 2006). For shellfish, digestive organs are rich in proteinases and play a role in digestion. Hepatopancreas can serve as an excellent source of proteinases in shellfish (Jeong et al. 2000). Trypsin is one of the main proteinases in the digestive tract of shellfish. Trypsin has a catalytic triad of three essential amino acid residues that include serine (Ser), histidine (His), and aspartate (Asp). Trypsin specifically cleaves the peptide bond on the carboxyl side of lysine or arginine residues (Poonsin et al. 2019). The high specificity of trypsin for lysine and arginine results from the negative charge of Asp at the S1 binding pocket of trypsin matching the positive charge of the P1 side chain of the substrate (Kishimura et al. 2010). In the shrimp hepatopancreas, trypsin not only functions as a digestive enzyme, but is also responsible for activating all the pancreatic enzymes by cleaving a short activation peptide from the amino-terminus of inactivate zymogens (Gates and Travis 1969).

Shellfish trypsin has low thermal stability but shows high activity at low temperature. Furthermore, it tends to be more stable at alkaline pH, and has a lower content of basic amino acid residues in the polypeptide chain in comparison with mammalian trypsins (Gates and Travis 1969). The molecular weight (MW) of shellfish trypsins can vary with species. Oh et al. (2000) reported that the purified protease from the hepatopancreas of shrimp (*Penaeus orientalis*) had high proteolytic activity in the pH range of 7.0–9.5. The optimum temperature for casein hydrolysis of protease was 70 °C. The protease was stable at neutral and alkaline pH but unstable at acidic pH. Major proteases isolated from the hepatopancreas of Northern shrimps (*Pandalus eous*) were classified as serine proteases (Aoki et al. 2003). Four trypsin-like enzymes ([collagen peptide] CP-I, II, III, and IV) from the hepatopancreas of crawfish (*Procambarus clarkii*) were purified using Diethylaminoethyl Sepharose (DEAE-Sepharose) chromatography. (Jeong et al. 2000). Trypsin CP-I, II, III, and IV had MW of 35.0, 41.2, 37.9, and 39.5 kDa, respectively. These enzymes had optimal activity at pH 8.0–8.5 and showed the highest activity at 60–70 °C. Trypsin from a king crab (*P. camtschaticus*) was isolated in a homogeneous state by successive ion-exchange chromatography on DEAE-Sephadex, affinity chromatography on Agarose modified with peptide ligands from trypsin hydrolysate of salmon, and ion-exchange chromatography on a MonoQ column. The trypsin contained 237 amino acids which correspond to its MW of 24.8 kDa (Kislitsyn et al. 2003). Wu et al. (2008) purified trypsin from North Pacific krill (*Euphausia pacifica*) by ammonium sulfate precipitation, ion-exchange, and gel-filtration chromatography. The MW of purified trypsin was 33 kDa. It was active over a wide pH (6.0–11.0) and temperature (10–70 °C) range. The optimum pH for activity was 9.0 and the maximal activity was found at 40–50 °C. Sriket et al. (2012) purified trypsin from the hepatopancreas of freshwater prawn (*Macrobrachium rosenbergii*) by Q-sepharose, Superdex 75, and MonoQ columns. The purified trypsin had a MW of

17 kDa. The optimal pH and temperature for Boc-Val-Pro-Arg-MCA hydrolysis were 8.0 and 55 °C, respectively. Trypsin was stable to heat treatment up to 40 °C, and over a pH range of 7.0–11.0.

Recently, Wu et al. (2014a) purified trypsin from the cephalothoraxes of Antarctic krill (*Euphausia superba*) by ammonium sulfate precipitation, ion-exchange, and gel-filtration chromatography. Antarctic krill cephalothoraxes contained three trypsins. The MW of three trypsins (I, II, and III) were estimated to be approximately 28.7, 28.8, and 29.2 kDa, respectively. Trypsins I, II, and III showed maximal activity at 40, 45, and 40 °C, respectively, using N $\alpha$ -benzoyl-DL-arginine p-nitroanilide (BAPNA) as a substrate. Trypsin from the hepatopancreas of Pacific white shrimp (*Litopenaeus vannamei*) was purified and characterized (Senphan et al. 2015). Purification was carried out by ammonium sulfate precipitation and a series of chromatographies including DEAE-Sepharose and soybean trypsin inhibitor-Sepharose 4B columns. Trypsin had a MW of 24 kDa as estimated by sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE). The enzyme exhibited the maximal activity at 60 °C and had the optimal pH at 8.0 using BAPNA as a substrate. Five trypsin-like enzymes were purified from lobster (*Panulirus argus*) by a combination of size exclusion (Sephadex G-75 column) and anion exchange (MonoQ anion exchange column) chromatographies. The MWs of enzymes were determined as 35–36 kDa. The optimum pH and temperature for BAPNA hydrolysis of most trypsin isoforms were found to be around 7.0 and 60 °C, respectively (Perera et al. 2015). Sriket and Sriket (2015) also purified trypsin from the hepatopancreas of royal red prawn (*Haliporoides sibogae*) by ammonium precipitation, Benzamidine affinity column, and MonoQ column chromatography. The purified trypsin had a MW of 27 kDa as estimated by SDS-PAGE. The optimal pH and temperature for Boc-Val-Pro-Arg-MCA hydrolysis were 9.0 and 50 °C, respectively, while the purified enzyme was stable to heat treatment up to 50 °C and over a pH range of 7.0–11.0. The biochemical properties of shellfish trypsins are summarized in Table 10.1.

Apart from trypsin, chymotrypsin is also found in shellfish. Chymotrypsins, which cleave the peptides on the carboxyl side of phenylalanine, tyrosine, and tryptophan residues, have been isolated and characterized from several shellfish, including shrimps Pacific white shrimp (*L. vannamei*) (Hernández-Cortés et al. 1997), crabs (*Callinectes bellicosus* and *Callinectes arcuatus*) (Díaz-Tenorio et al. 2006), and Caribbean spiny lobster (*P. argus*) (Perera et al. 2008). Although chymotrypsins from marine animals are basically similar to mammalian counterparts, differences in structural and functional properties were reported (Fong et al. 1998). Navarrete-del-Toro et al. (2015) purified chymotrypsin from the midgut gland of yellow leg shrimp (*Penaeus californiensis*) by affinity chromatography (trypsin inhibitor-agarose gel columns), followed by preparative electrophoresis. Chymotrypsin had a MW of 37.5 kDa. The optimal pH and temperature for N-succinyl-L-alanyl-L-alanyl-L-prolyl-L-phenylalanine-p-nitroanilide (SAAPFNA) hydrolysis were 7.0 and 50 °C, respectively. The enzyme was stable at 50 °C and sensible to low pH. Chymotrypsin was purified from the gastric juice of California spiny lobster (*Panulirus interruptus*) by preparative electrophoresis and affinity chromatography on agarose-p-aminobenzamidine (Bibo-Verdugo et al. 2015). The purified chymotrypsin had MW of approximately 28 kDa. The enzyme exhibited maximal activity at pH 7.0–8.0 and 55 °C, using SAAPFNA as a substrate.

**Table 10.1** Biochemical properties of various shellfish trypsins.

Identified species	MW (kDa)	Optimum pH	Optimum temperature (°C)	Substrate	References
Sand crab ( <i>Portunus pelagicus</i> )	34.8	8	60	TAME <sup>a</sup>	Dionysius et al. (1993)
Crawfish ( <i>Procambarus clarkia</i> )	35.0–41.2	8.0–8.5	60–70	TAME	Jeong et al. (2000)
White shrimp ( <i>Penaeus vannamei</i> )	30.2–32.9	8–10	60	BAPNA <sup>b</sup>	Sainz et al. (2004)
North Pacific krill ( <i>Euphausia pacifica</i> )	33	9	40–50	BAPNA	Wu et al. (2008)
Freshwater prawn ( <i>Macrobrachium rosenbergii</i> )	17	8	55	Boc-Val-Pro-Arg-MCA <sup>c</sup>	Sriket et al. (2012)
Antarctic krill ( <i>Euphausia superba</i> )	28.7–29.2	—	40–45	BAPNA	Wu et al. (2014a)
Pacific white shrimp ( <i>Litopenaeus vannamei</i> )	24	8	60	BAPNA	Senphan et al. (2015)
Lobster ( <i>Palinurus argus</i> )	35–36	7	60	BAPNA	Perera et al. (2015)
Royal red prawn ( <i>Haliporoides sibogae</i> )	27	9	50	Boc-Val-Pro-Arg-MCA	Sriket and Sriket (2015)

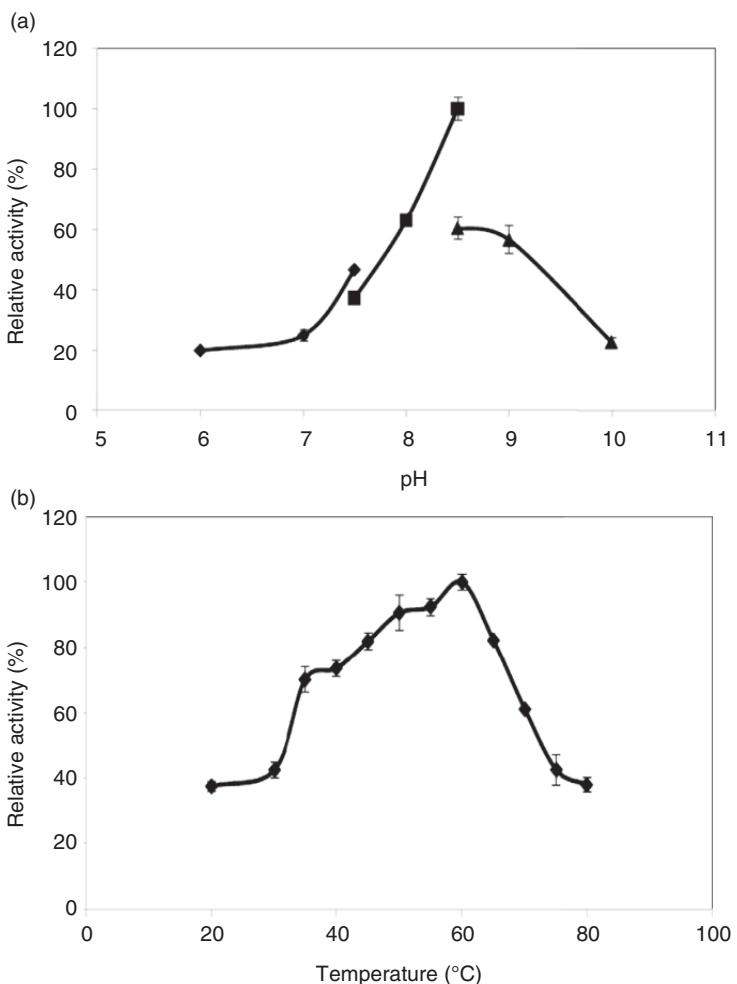
<sup>a</sup>α-N-p-toluene-sulfonyl-L-arginine methyl ester.

<sup>b</sup>α-N-benzoyl-DL-arginine-p-nitroanilide.

<sup>c</sup>t-Butyloxy-carbonyl-Val-Pro-Arg-4-methyl-coumaryl-7-amide.

### 10.2.2 Lipase

Lipase is an enzyme that catalyzes the hydrolysis of triacylglycerols at the oil–water interface to release glycerol and free fatty acids (Kuepethkaew et al. 2017). Shellfish byproducts are a potentially rich source of enzymes such as lipases (Kurtovic et al. 2009) that may have some unique properties for industrial applications. The hepatopancreas is generally assumed to be the major source of digestive lipase in shellfish as it is found in mammals (Tocher 2003). Cherif et al. (2007) reported that digestive lipase from the hepatopancreas of crab (*Carcinus mediterraneus*) had the MW of 65 kDa. The crab digestive lipase displayed its maximal activity on long and short-chain triacylglycerols at a temperature of 60 °C. The optimal pH of crab digestive lipase was found around 8.0. Lipase was purified from the midgut gland of whiteleg shrimp (*L. vannamei*) (Rivera-Pérez et al. 2011). The purified lipase was obtained after Superdex 200 gel filtration and Resource Q anionic exchange. The MW of purified lipase was about 48 kDa, as determined by SDS-PAGE. The lipase hydrolyzed short and long-chain triacylglycerols and naphthol derivatives at comparable rates. Specific activities of 1787 and 475  $\mu\text{mg}^{-1}$  were obtained when triolein and tributyrin were used as substrates, respectively, when tested at pH 8.0 and 30 °C. Recently, Kuepethkaew et al. (2017) isolated lipase from the hepatopancreas of Pacific white shrimp (*L. vannamei*). The optimal profiles of pH and



**Figure 10.1** pH (a) and temperature (b) profiles of lipase from hepatopancreas of Pacific white shrimp.  
Source: Kuepethkaew et al. 2017.

temperature of lipase were 8.5 and 60 °C, respectively, using *p*-nitrophenyl palmitate as a substrate (Figure 10.1). The enzyme was stable to heat treatment up to 40 °C and over a pH range of 7.0–10.0 for 30–120 minutes.

## 10.3 Protein and Bioactive Peptides from Shellfish Processing Byproducts

### 10.3.1 Protein

Shellfish proteins have bioactive properties that make them a very interesting alternative for food industry (Sila and Bougatet 2016). In shellfish processing leftover, protein levels can vary from 30% to 65% of its dry weight. A number of proteins depend on the process

used and species. Shellfish processing byproducts contain significant quantities of protein rich in essential amino acid (Senphan et al. 2014). The shellfish head, shell, and undersized shellfish, etc. had a high content of essential amino acids, indicating a high nutritional value and can be used for animal feed supplement and human nutrition (Mao et al. 2017; Poonsin et al. 2018). Lopez-Cervantes et al. (2006) found that free amino acid contents in the fermented shrimp waste ranged from 9.3 to 56.9 mg g<sup>-1</sup> dry mass. The sample contained arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, asparagines, serine, glutamine, glycine, alanine, and proline. Tyrosine was the most abundant amino acid (56.9%). The shells of tropical periwinkle (*Tymanonotus fuscatus*) were rich in glutamine, lysine, asparagines, and leucine, which accounted for 19.92%, 11.95%, 11.71%, and 11.55% of the total amino acids, respectively. The shells had an essential amino acid/non-essential amino acid ratio of 1.09 (Ehigitor and Oterai 2012). Klomklao et al. (2009) reported that black tiger shrimp (*Penaeus monodon*) shells contained 30.88% protein. The protein content in Brazilian red spotted shrimp (*Farfantepenaeus paulensis*) waste was 49.0% (Sánchez-Camargo et al. 2011). Sila et al. (2012a) found that pink shrimp (*Parapenaeus longirostris*) processing wastes contained 31.3% protein. Senphan et al. (2014) also reported that Pacific white shrimp (*L. vannamei*) shells consisted of 43.89% protein and had total amino acid contents of 169.47 mg g<sup>-1</sup> sample. Pacific white shrimp shells were rich in glutamic acid/glutamine (67.17 mg g<sup>-1</sup> sample) and aspartic acid/asparagines (47.38 mg g<sup>-1</sup> sample). The protein existing in shellfish discards are closely associated with chitin and minerals (protein-chitin-minerals complex).

#### 10.3.1.1 Protein Extraction

Proteins from shellfish processing byproducts can be isolated by solubilization process. Then the isolated protein can be processed into powder, paste, and liquid, and further used as a supplement in food (Rustad 2003). The extraction efficiency of protein from shellfish processing byproducts varies depending on the processing methods. Chemical and biological methods are used for isolation of proteins from shellfish discards. However, the chemical process generally yields products with reduced nutritional qualities, poor functionality and restricted use as flavor enhancers (Kristinsson and Rasco 2000). Furthermore, the utilization of protein isolated by chemical process is diminished because of the presence of sodium hydroxide (Oh et al. 2007). To overcome the hazards from chemical treatments, alternative methods including biocatalysis (Manni et al. 2010) and fermentation (Mao et al. 2017) have gained attention for the recovery of crustacean wastes.

#### 10.3.1.2 Enzymatic Hydrolysis

Recovery of protein from the shellfish processing byproducts by enzymatic hydrolysis has several advantages since hydrolysis can be controlled, thus minimizing undesirable reactions (Klomklao and Benjakul 2017). Hydrolysis of proteins from shellfish processing byproducts can be achieved by both digestive enzymes of the shellfish itself, as well as by addition of external sources of enzymes (Rustad 2003). Cao et al. (2009) recovered protein from white shrimp (*Penaeus vannamei*) head by autolysis. The protein content of shrimp head was found to be 84.8/100 g sample. Cahu et al. (2012) reported that endogenous enzymes from white shrimp (*Pe. vannamei*) head had a strong autolysis capacity for recovering protein from shrimp processing waste. The content of protein hydrolysate was 12/100 g sample. Mukhin and Novikov (2001) reported that production of protein

hydrolysates from deep-water prawn (*Pandalus borealis*) and king crab (*P. camtschaticus*) processing byproducts could be achieved with the aid of protease from king crab hepatopancreas. Limam et al. (2008) reported that shrimp head of *Penaeus kerathurus* were hydrolyzed with commercial trypsin (0.1%). The hydrolysate was rich in protein (79.2/100 g sample) and had improved functional properties. Nguyen et al. (2016) also reported that protein hydrolysate from Australian rock lobster shells (*Jasus edwardsii*) was prepared with Alcalase. The hydrolysate had excellent functionality and high nutritional quality with potential applications for the food industry. Protein hydrolysates are the excellent nitrogen source in the growth media for microorganisms (Mao et al. 2017; Sila and Bougatef 2016)

#### 10.3.1.3 Fermentation

One interesting technology for the extraction of protein from shellfish discards is fermentation by using microorganisms. Fermentation is an eco-friendly and cost-effective alternative process for protein hydrolysis (Mao et al. 2017). Proteolytic enzymes from microorganisms such as *Pediococcus acidolactici* (Sachindra and Bhaskar 2008), *Bacillus subtilis* (Yang et al. 2000), and *Bacillus licheniformis* (Mao et al. 2013) have been used to form protein hydrolysis. The shrimp (*Penaeus japonicus*) waste fermented by *Pediococcus acidolactici* CFR2182 had low MW proteins or peptides. The formation of peptides and amino acids during hydrolysis of shrimp waste protein during fermentation is expected to be responsible for the antioxidant activity (Sachindra and Bhaskar 2008). Bueno-Solano et al. (2009) reported that protein hydrolysates were prepared through lactic acid fermentation of shrimp byproducts. The protein contents of the three hydrolysates have been compared, ranging from 8.43 to 46.73/100 g of wet mass. Sun and Mao (2016) also reported that the polypeptide content from fermentation broth of Antarctic krill (*E. superba*) with *B. subtilis* OKF04 was 9.5 g l<sup>-1</sup>.

#### 10.3.2 Bioactive Peptides

Bioactive peptides can be produced from shellfish processing byproducts. Enzymatic hydrolysis of proteins is an efficient way to recover potent bioactive peptides (Klomklao et al. 2013). Bioactive peptides from shellfish processing byproducts have been shown to possess many physiological functions, including antihypertensive or angiotensin converting enzyme (ACE) inhibition, antioxidant, and antimicrobial activities (Harnedy and FitzGerald 2012). Moreover, some of these bioactive peptides possess nutraceutical potentials that are beneficial in human health promotion (Wijesekara and Kim 2010). Stensvaga et al. (2008) reported that bioactive peptide, known as arasin 1, derived from spider crab (*Hyas araneus*), inhibited the growth of *Corynebacterium glutamicum*. The peptide from American lobster (*Homarus americanus*) exhibited bacteriostatic activity against some gram-negative bacteria and both protozoastatic and protozoacidal activity against two scuticociliate parasites *Mesanophrys chesapeakensis* and *Anophryoides haemophila* (Battison et al. 2008). Furthermore, antimicrobial activity and growth inhibition of bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *B. subtilis*, and fungi such as *Botrytis cinerea* and *Penicillium expansum* by peptide, CgPep33, derived from oyster (*Crassostrea gigas*) have been also reported (Liu et al. 2008).

A number of studies have demonstrated that peptides derived from shellfish processing byproducts act as potential antioxidants such as oyster (*C. gigas*) (Qian et al. 2008), shrimp (Binsan et al. 2008; Benjakul et al. 2009; Manni et al. 2010; Dey and Dor 2014; Sila

**Table 10.2** Biological activity of peptides derived from shellfish processing byproducts.

Identified species	Origin	Biological activity	References
American lobster ( <i>Homarus americanus</i> )	Hemocytes	Antimicrobial	Battison et al. (2008)
Oyster ( <i>Crassostrea gigas</i> )	Muscles	Antimicrobial	Liu et al. (2008)
Spider crab ( <i>Hyas araneus</i> )	Hemocytes	Antimicrobial	Stensvaga et al. (2008)
Brown shrimp ( <i>Penaeus aztecus</i> )	Head	Appetite suppressant	Cudennec et al. (2008)
Shrimp ( <i>Acetes chinensis</i> )	Shell	ACE inhibitory	He et al. (2006)
Oyster ( <i>Crassostrea talienwhanensis Crosse</i> )	Muscle	ACE inhibitory	Wang et al. (2008)
White shrimp ( <i>Litopenaeus vannamei</i> )	Cephalothorax	ACE inhibitory	Benjakul et al. (2009)
Oyster ( <i>Crassostrea gigas</i> )	Muscle	ACE inhibitory	Shiozaki et al. (2010)
White shrimp ( <i>Litopenaeus vannamei</i> )	Cephalothorax	Antioxidant	Binsan et al. (2008)
Shrimp ( <i>Metapenaeus monoceros</i> )	Shell	Antioxidant	Manni et al. (2010)
Shrimp ( <i>Penaeus monodon</i> and <i>Penaeus indicus</i> )	Cephalothorax, shell, and tail	Antioxidant	Dey and Dor (2014)
Deep-water pink shrimp ( <i>Parapenaeus longirostris</i> )	Head, shell cephalothorax and appendix	Antioxidant	Sila et al. (2014)

et al. 2014). The beneficial effects of bioactive peptides from shellfish byproducts are well known in scavenging free radicals and reactive oxygen species (ROS) or in preventing oxidative damage by interrupting the radical chain reaction of lipid peroxidation (Kim and Wijesekara 2010). In addition, the bioactive antioxidant peptide LeuLys-Gln-Glu-Leu-Glu-Asp-Leu-Leu-Glu-Lys-Gln-Glu, isolated from oyster (*C. gigas*), exhibited a higher activity against polyunsaturated fatty acid peroxidation than  $\alpha$ -tocopherol (Qian et al. 2008). Peptides derived from shellfish processing byproducts displaying biological activity are presented in Table 10.2.

## 10.4 Carotenoid and Carotenoprotein from Shellfish Processing Byproducts

### 10.4.1 Carotenoid

Shellfish are one of the important natural sources of carotenoid. The carotenoids are the group of pigments that contribute to the yellow, orange, and red colors of the skin, shell, or exoskeleton of aquatic animals. Astaxanthin, a red-orange carotenoid, is found to be

the major carotenoid in several shellfish such as Indian shrimp (*P. monodon*, *Penaeus indicus*, *Metapenaeus dobsonii* and *Parapenaeopsis stylifera*) (Sachindra et al. 2005a), marine crab (*Charybdis cruciata*) (Sachindra et al. 2005b), tropical spiny crayfish (*Panulirus ornatus*) (Barclay et al. 2006), giant freshwater prawn (*M. rosenbergii*) (Kumar et al. 2009), Brazilian red spotted shrimp (*F. paulensis*) (Sánchez-Camargo et al. 2011), red king crab (*P. camtschaticus*) (Daly et al. 2013), and Pacific white shrimp (*L. vannamei*) (Senphan et al. 2014). Guillou et al. (1995) reported that astaxanthin in shrimp (*P. borealis*) was presented as diester, monoester, and free form (76%, 20%, and 4%, respectively, relative to total astaxanthin). Three forms of astaxanthin, namely monoester, diester, and free, were found in black tiger prawns (*Penaeus monodon*) (Okada and Yamaguchi 1994). Senphan et al. (2014) also reported that the major carotenoids from Pacific white shrimp (*L. vannamei*) shells were astaxanthin monoester and astaxanthin diester. The amounts of carotenoid from shellfish vary with species, life-history stage, developmental stage, molt stage and the organ or tissue of the animals. Carotenoid contents in various sources of shellfish wastes are presented in Table 10.3.

Generally, shellfish cannot synthesize carotenoids and must obtain them from their diets (Wade et al. 2017). However, various Decapod crustaceans can convert different dietary carotenoids (including canthaxanthin, lutein, or zeaxanthin) into the predominant astaxanthin (Maoka et al. 2011). Katayama et al. (1973) proposed that shellfish fall into two broad classes based on their biosynthetic capacity:

- 1) Shellfish can convert  $\beta$ -carotene to astaxanthin in their internal organs, such as Penaeid shrimp.
- 2) Shellfish can convert  $\beta$ -carotene to astaxanthin in their internal organs but also convert metabolic intermediates in other tissues of their body, such as lobsters and crabs.

#### 10.4.1.1 Carotenoid Extraction

Several studies have been carried out to recover the carotenoid from shellfish processing byproducts using various methods such as fermentation process, supercritical fluid extraction, extraction using organic solvents and vegetable oils. Guillou et al. (1995) reported that the silage of northern shrimp (*P. borealis*) waste has the higher yield of carotenoid recovered by solvent extraction. Sachindra et al. (2007) recovered carotenoid from fermented shrimp (*P. indicus*) waste. The optimized conditions for preparation of fermented silage from the shrimp waste were 20.5% of glucose,  $19.5 \times 10^4$  cells  $\text{g}^{-1}$  of starter culture and fermentation time of 70 hours.

A mixture of polar and non-polar solvents for improved recovery of carotenoids from shellfish has been demonstrated. Carotenoids in marine crab (*C. cruciata*) and fresh water crab (*Potamon potamon*) waste were extracted using acetone and subsequently partitioned using petroleum ether (Sachindra et al. 2005b). Sachindra et al. (2006) extracted carotenoids from shrimp (*P. indicus*) processing byproducts using different organic solvents and solvent mixtures. A 50 : 50 mixture of isopropyl alcohol and hexane gave the highest carotenoid extraction yield, compared to acetone, methanol, ethanol, isopropyl alcohol, ethyl acetate, ethyl methyl ketone, petroleum ether, and hexane individually, and to a mixture of acetone and hexane. Astaxanthin from crawfish (*Procambarus clarkii*) shells were extracted using alcohol as a cosolvent in supercritical carbon dioxide extraction (Charest et al. 2001). Radzali et al. (2016) also isolated astaxanthin

**Table 10.3** Carotenoid contents from different shellfish processing byproducts.

Identified species	Content (mg kg <sup>-1</sup> )	Carotenoid	References
Fairy shrimp ( <i>Streptocephalus dichotomus</i> )	52.3	Canthaxanthin	Velu et al. (2003)
	34.5	Astaxanthin	
Indian shrimp ( <i>Parapenaeopsis stylifera</i> )	104.7–153.1	Astaxanthin	Sachindra et al. (2005a)
Marine crab ( <i>Charybdis cruciata</i> )	152.0–267.0	Astaxanthin	Sachindra et al. (2005b)
Fresh water crab ( <i>Potamon potamon</i> )	420.0–748.0	Zeaxanthin	Sachindra et al. (2005b)
American clawed lobster ( <i>Homarus americanus</i> )	220.0	Astaxanthin	Tlusty and Hyland (2005)
Tropical spiny crayfish ( <i>Panulirus ornatus</i> )	30.0–120.0	Astaxanthin	Barclay et al. (2006)
Marine shrimp ( <i>Xiphopenaeus kroyeri</i> )	91.7–121.0	Astaxanthin	De Holanda and Netto (2006)
Rock lobsters ( <i>Jasus lalandii</i> )	54.0	Astaxanthin	Auerswald and Gade (2008)
Black tiger shrimp ( <i>Penaeus monodon</i> )	87.9	Astaxanthin	Klomklao et al. (2009)
Brazilian redspotted shrimp ( <i>Farfantepenaeus paulensis</i> )	87.0–148.0	Astaxanthin	Sánchez-Camargo et al. (2011)
Pacific white shrimp ( <i>Litopenaeus vannamei</i> )	25.0–150.0	Astaxanthin	Ju et al. (2011)
Pink shrimp ( <i>Parapenaeus longirostris</i> )	51.85–67.42	Astaxanthin	Sila et al. (2012a)
	93.2	Xanthophylls	
Red king crab ( <i>Paralithodes camtschaticus</i> )	380.0	Astaxanthin	Daly et al. (2013)
Giant tiger prawn ( <i>Penaeus monodon</i> )	100.0–250.0	Astaxanthin/β-carotene	Niu et al. (2014)
Mud crab ( <i>Scylla paramamosain</i> )	44.5–106.1	β-carotene	Tantikitti et al. (2015)
	14.7–229.1	Zeaxanthin	

from tiger shrimp (*P. monodon*) processing byproducts by supercritical carbon dioxide with 15% (v/v) ethanol.

Since carotenoid in shellfish processing byproducts is an oil soluble pigment, several vegetables such as sunflower oil, ground nut oil, ginger oil, palm oil, mustard oil, soybean oil, coconut oil, rice bran oil, and cod liver oil have been used to extract carotenoid (Kandra et al. 2012). Sachindra and Mahendrakar (2005) extracted and determined carotenoids from shrimp (*P. indicus*) waste using different vegetable oils. The highest carotenoid yield was obtained when sunflower oil was used, compared to groundnut oil, gingelly oil, mustard oil, soy oil, coconut oil, and rice bran oil. Handayani et al. (2008)

used palm oil to extract astaxanthin from giant tiger shrimp (*P. monodon*) waste. Parjikolaei et al. (2015) also reported that the extraction of astaxanthin from northern shrimp (*P. borealis*) processing byproducts by sunflower oil. The highest astaxanthin content was achieved at a temperature of 70 °C, using an oil to waste ratio of 9 and a stirring speed of 400 rpm. Sunflower oil could extract astaxanthin with a yield of 80%, relative to total astaxanthin extracted by organic solvent. Other attempts to concentrate carotenoid by drying resulted in significant losses of carotenoids due to high temperatures and oxidation (Charest et al. 2001). Supercritical fluid extraction operated at ambient temperatures can eliminate heat damage to the carotenoids. Nevertheless, the carotenoid content is low (Babu et al. 2008). Those techniques are not effective in extracting total carotenoids. Therefore, to increase the extraction efficiency, the enzymatic process has been explored as an alternative method to extract carotenoids from shellfish byproducts. De Holanda and Netto (2006) recovered astaxanthin from industrial shrimp (*Xiphopenaeus kroyeri*) processing byproducts using enzymatic treatment with Alcalase and pancreatin. Alcalase was more efficient than pancreatin, increasing the recovery of astaxanthin from 4.7 to 5.7 mg astaxanthin/100 g of dry waste. The use of a mixture of two enzymes, Tunisian barbel (*Barbus callensis*) and bovine trypsin, to recover carotenoid yielded the high levels of xanthophylls (93.2 µg g<sup>-1</sup> of shrimp waste) (Sila et al. 2012a). Sadighara et al. (2015) reported that the extractability of carotenoids from shrimp (*Penaeus semisulcatus*) processing byproducts with trypsin gave the improved extraction efficacy of carotenoids over acid and alkaline extraction.

#### 10.4.2 Carotenoprotein

Generally, carotenoid presents in shellfish as protein-pigment complex. In nature, protein-pigment interaction increases carotenoid stability. (Armenta-López et al. 2002). When the molecule includes a carotenoid with a protein in stoichiometric proportion, it is called a carotenoprotein (Klomklao et al. 2009). They may be present in a variety of tissue such as eggs, ovaries, blood, and the integument (Garate et al. 1984). The pigments in the crustacean exoskeleton are located in the pigmented layer of the endocuticle and the various color appearances of crustacean are originally due to the carotenoids (Goodwin 1960). The color appearances of carotenoprotein range from red to purple and various colored carotenoproteins seem to co-exist in the same animal over different parts of the exoskeleton in different proportion (Nakagawa et al. 1971).

According to Klomklao et al. (2009), carotenoprotein can be classified into two groups: true carotenoproteins, those complexes containing no lipids and showing carotenoid-protein stoichiometry; and carotenolipoproteins, which contain lipids and are not necessarily in stoichiometric combination. Chessman et al. (1967) defined the carotenoprotein as proteins, in which carotenoids are present in stoichiometric proportions as prosthetic groups. The main carotenoid in the carotenoprotein was free astaxanthin, and no significant difference was found between astaxanthin diester and astaxanthin monoester (Okada and Yamaguchi 1994). This complex can be green, purple, or blue in the living animal, acquiring a red color when subjected to heat treatment (Armenta-López et al. 2002). The red color of cooked crustaceans appears by the release of the individual astaxanthin prosthetic groups from the carotenoproteins when denatured by heat (Lorenz 1998). Several theories, such as polarization, etc. have been proposed concerning the nature of the interaction between carotenoids and proteins (Salares et al. 1979). Chessman et al. (1967) indicated

that the linkage between astaxanthin and apoprotein in the visual pigments is of the nature of a protonated Schiff's base. It has been noted that the reactive 4 and 4'-keto groups in the  $\beta$ -ionone rings of astaxanthin are prerequisites for interaction between carotenoid carbonyl groups and amino acid residues to form carotenoprotein (Zagalsky and Herring 1972). Generally, carotenoproteins are readily split into carotenoids and apoprotein by treatment of an aqueous solution with acetone or ethanol; thus, the carotenoids are bound non-covalently to the proteins (Young and Williams 1983). Several studies indicated that the union of carotenoid with apoprotein stabilizes the carotenoid against photo-oxidation and the protein against denaturative changes of tertiary structure.

#### 10.4.2.1 Carotenoprotein Extraction

A number of studies have been carried out on the recovery of several carotenoprotein shellfish species, e.g. tropical brown shrimp (*Metapenaeus monoceros*) (Chakrabarti 2002), crayfish (*P. clarkii*) (Cremades et al. 2003), shrimp (*P. monodon*, *P. indicus*, *M. monoceros* and *P. monodon*) (Babu et al. 2008), black tiger shrimp (*P. monodon*) (Klomklao et al. 2009), lobster (*Jasus lalandii*) (Timme et al. 2009), deep-water pink shrimp (*P. longirostris*) (Sila et al. 2012a), and Pacific white shrimp (*L. vannamei*) (Senphan et al. 2014). Various techniques have been employed to recover carotenoprotein from shellfish processing byproducts. Carotenoproteins of shellfish can be extracted with water or dilute salt solutions, fractionally precipitated with ammonium sulfate and purified by chromatography on ion exchange celluloses or by selective absorption on calcium phosphate or aluminum hydroxide or a combination of these processes. The extraction of carotenoprotein from shellfish necessitates a preliminary decalcification of the finely ground material. Many decalcification agents have been used to solubilize carotenoprotein, such as boric acid with Tris-HCl, pH 6.8 solution (Timme et al. 2009) and ammonium sulfate (Czeczuga et al. 2005). Carotenoprotein is best preserved in solution of high ionic strength or as precipitates in strong ammonium sulfate solution. Velu et al. (2003) isolated carotenoprotein of freshwater fairy shrimp (*Streptocephalus dichotomus*) with acetone and suspended in ethylene diamine tetra-acetic acid (EDTA) solution (40 mM EDTA, 10% NaCl, pH 7.0), fractionally precipitated with ammonium sulfate and purified by ion exchange chromatography on DEAE-cellulose. Based on thin layer chromatography and mass spectral analysis, a variety of carotenoprotein complexes such as astaxanthin, canthaxanthin, antheraxanthin, lutein,  $\beta$ -cryptoxanthin, and violaxanthin were found. Freshwater fairy shrimp had astaxanthin and canthaxanthin as predominant carotenoids.

The procedures for recovery of pigment and protein content described in the previous section are tedious and/or time consuming. In particular, low recovery of carotenoprotein was obtained. Since, about one-third of the dry matter in shellfish waste is protein. Therefore, an enzymatic process has been developed to increase the extraction efficiency. Cano-lopez et al. (1987) reported that using proteolytic enzyme (e.g. trypsin) conjunction with a chelating agent (EDTA) in the extraction medium increased the efficacy in recovering both protein and pigment from shrimp processing byproducts. Simpson et al. (1993) showed the effect of trypsin from bovine pancreas and Atlantic cod offal on the recovery of carotenoproteins from lobster (*H. americanus*) waste. The use of the enzyme preparations resulted in more than a twofold increase in the yield of the product as compared with the extraction process carried out without enzymes. Chakrabarti (2002) isolated carotenoprotein from tropical brown shrimp (*M. monoceros*) shell by

enzymatic process including trypsin, papain, and pepsin. Trypsin yielded the maximum recovery of carotenoprotein pigment while pepsin and papain showed lower recovery during the same period of hydrolysis.

Klomklao et al. (2009) isolated carotenoprotein from black tiger shrimp (*P. monodon*) shells using bluefish (*Pomatomus saltatrix*) trypsin. The product obtained was found to have higher protein and pigment content than those of untreated black tiger shrimp waste and had low contents of chitin and ash. Bluefish trypsin showed similar recovery efficacy of protein and carotenoids, compared with bovine trypsin. Sila et al. (2012a) also reported that the addition of Tunisian barbel (*B. callensis*) trypsin to the deep-water pink shrimp (*P. longirostris*) shells was effective in improving the recovery of carotenoproteins. The results indicated that higher protein and carotenoid recovery were obtained in shrimp shells treated with Tunisian barbel trypsin. Senphan et al. (2014) also recovered carotenoprotein from Pacific white shrimp (*L. vannamei*) shells with the aid of proteases from hepatopancreas of the same species. Carotenoprotein extracted with the enzyme had higher protein, fat, and pigment contents than the shrimp shells. It contained astaxanthin and astaxanthin diester as major carotenoids (Senphan et al. 2014).

#### 10.4.2.2 Physical and Chemical Properties of Carotenoprotein

##### 10.4.2.2.1 Molecular Size

The protein patterns of carotenoprotein from different shellfish species have been extensively studied. The carotenoprotein contained a variety of proteins with different MWs. Buchwald and Jencks (1968) reported that MW of carotenoproteins from yellow lobster (*Homarus gammarus*) shells ranged from 48 to 90 kDa. Carotenoprotein isolated from the carapace of crayfish (*P. clarkii*) had proteins with MW of 19.2 and 22.4 as the major protein (Garate et al. 1986). Carotenoproteins recovered from lobster (*H. americanus*) waste with the aid of bovine trypsin and semi-purified trypsin from Atlantic cod offal had an apparent MW of 43.9 and 89.5 kDa (Simpson et al. 1993). Klomklao et al. (2009) reported that the carotenoprotein from black tiger shrimp (*P. monodon*) shells recovered by autolysis process contained two major proteins. The MWs of the major proteins were estimated to be 211 and 45 kDa when analyzed using SDS-PAGE. After treatment of shrimp shells with bluefish trypsin, the protein band with high MW, especially 211 kDa, was totally removed. However, the band with MW of 45 kDa still remained and appeared as the major protein. Protein with MW of 45 kDa, most likely actin, has been reported to resistant to hydrolysis by proteinase. Senphan et al. (2014) also reported that the MWs of the major proteins of carotenoprotein from Pacific white shrimp (*L. vannamei*) shells recovered with and without proteinase were estimated to be 93 and 45 kDa. The differences in the MW of the carotenoproteins may probably be due to the differences in shellfish species. Those proteins might be associated with carotenoid, particularly astaxanthin, which is the predominant pigment in crustacean (Klomklao et al. 2009).

##### 10.4.2.2.2 Amino Acid Compositions

The amino acid compositions of various carotenoprotein from shellfish processing discards have been analyzed. Carotenoprotein derived from several shellfish species (e.g. crayfish, black tiger shrimp, Pacific white shrimp) had high essential amino acids (Cremades et al. 2003; Klomklao et al. 2009; Sila et al. 2012a; Senphan et al. 2014).

Cremades et al. (2003) reported that carotenoprotein derived from crayfish (*P. clarkii*) contained all the essential amino acids, accounting for 46.6% of the total amino acid content. Armenta and Guerrero-Legarreta (2009) analyzed amino acid profiles of carotenoproteins extracted from fermented and non-fermented shrimp waste. Essential amino acids in fermented and non-fermented carotenoproteins were 49% and 47%, respectively, with respect to total amino acids. Both carotenoproteins contained high amounts of aspartic acid, glutamic acid, leucine, and lysine. Klomklao et al. (2009) reported that carotenoproteins isolated from black tiger shrimp (*P. monodon*) shells with and without the aid of bluefish (*P. saltatrix*) trypsin were rich in glutamate (13.00% and 13.25%) and aspartate (11.18% and 10.43%). Furthermore, glycine, alanine, leucine and lysine were found at high amounts in both carotenoprotein. Sila et al. (2012a) reported that carotenoprotein extracted from pink shrimp (*P. longirostris*) with the aid of Tunisian barbel (*B. callensis*) trypsin was also rich in glutamate and aspartate. Glycine, alanine, leucine, and lysine were present in relatively large percentages in carotenoprotein. Senphan et al. (2014) also found that glutamic acid and aspartic acid were the dominant amino acids in carotenoproteins isolated from Pacific white shrimp (*L. vannamei*) shells with and without the aid of proteinases. Carotenoproteins recovered by both processes had higher essential amino acid contents than those found in Pacific white shrimp (*L. vannamei*) shells. However, low contents of cysteine and hydroxyproline were found in carotenoproteins.

## 10.5 Chitin and Chitosan from Shellfish Processing Byproducts

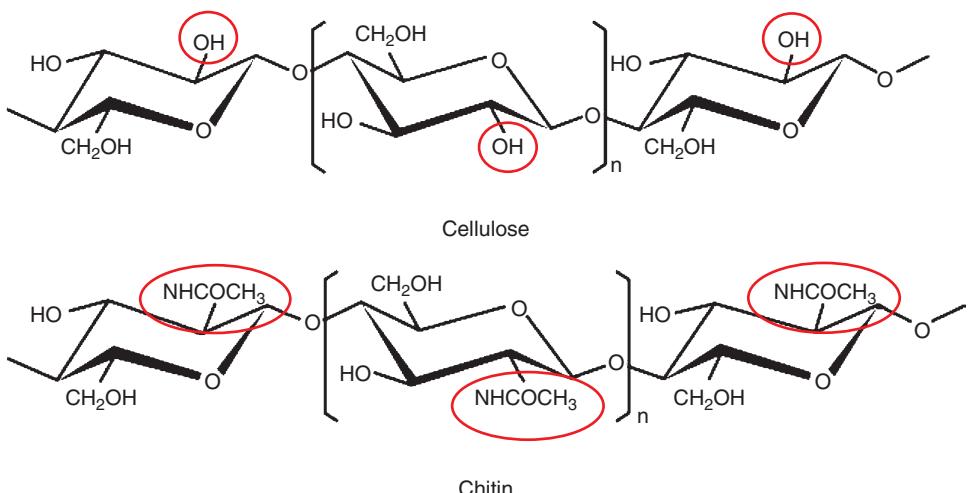
### 10.5.1 Chitin

In general, chitin is mainly found in the exoskeletons of shellfish. The amounts of chitin from shellfish discards vary with species and seasons. Much attention has been paid to chitin and its derivatives as natural bioactive materials owing to their non-toxicity, biocompatibility and biodegradable nature (Kim and Mendis 2006). These materials have important structural and functional properties that make them attractive for a wide variety of applications in many fields such as food and nutrition, microbiology, agriculture, environmental protection, biomedicine, tissue engineering, and biotechnology (Mao et al. 2017).

#### 10.5.1.1 Chitin Structure and Physicochemical Properties

Chitin is a polysaccharide constituted substantially of  $\beta$ -(1 → 4) linked *N*-acetyl-D-glucosamine units (Younes and Rinaudo 2015). The structure of chitin resembles cellulose, except for the carbon residue at position 2 which has an acetamide group (-NHCOCH<sub>3</sub>) attached to chitin instead of the hydroxyl group of cellulose (Ghorbel-Bellaaj et al. 2012) (Figure 10.2).

Chitin occurs in nature as ordered crystalline microfibrils. It is found in three polymorphic forms:  $\alpha$ -,  $\beta$ -, and  $\gamma$ -chitin. The  $\alpha$ -form is the most stable form of the three crystalline variations (Hamed et al. 2016). The  $\alpha$ -chitin chains are arranged in anti-parallel stands. The anti-parallel arrangement in  $\alpha$ -chitin gives rise to strong hydrogen bonding and consequently makes it more stable (Rinaudo 2006). The  $\alpha$  configuration is mainly obtained



**Figure 10.2** Comparison between the chemical structures of cellulose and chitin.

from crab and shrimp shells (Kandra et al. 2012).  $\beta$ -chitin is less stable than the  $\alpha$ -chitin. The  $\beta$  form is arranged in parallel chains.  $\gamma$ -chitin is the least common form. It is a mixed form, i.e. a combination of  $\alpha$  and  $\beta$  structures. The structures of  $\alpha$ - and  $\beta$ -chitin are shown in Figure 10.3.

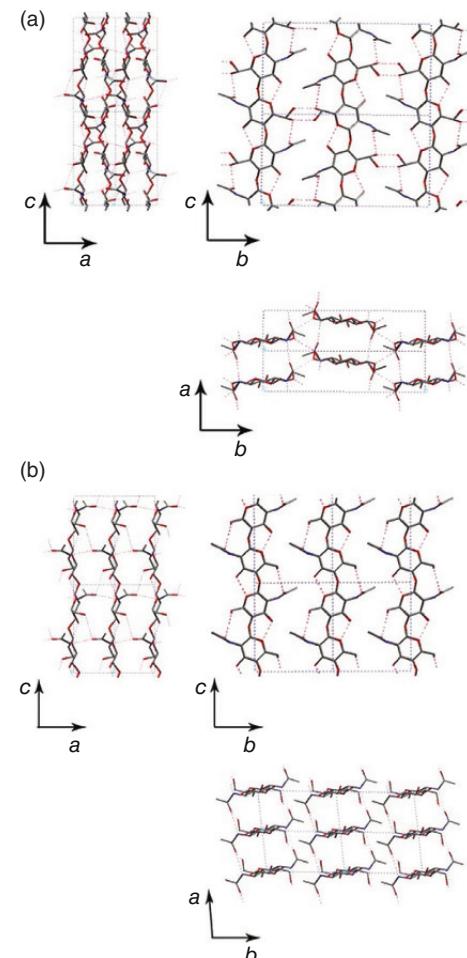
Chitin is a nitrogenous polysaccharide which in its pure state is white or yellowish. It is also odorless and tasteless (Hamed et al. 2016). Chitin is highly hydrophobic. Therefore, it is insoluble in water and even most organic solvents. The solubility of chitin is limited due to the strong intermolecular hydrogen bonding (Wilson and Omokanwaye 2013).

#### 10.5.1.2 Chitin Extraction

In general, chitin in shellfish found as a constituent of a complex network with proteins onto which calcium carbonate deposits to form the rigid shell. The interaction between chitin and protein is very intimate and there is also a small fraction of protein involved in a polysaccharide-protein complex (Kjartansson et al. 2006). Thus, chitin isolation from shellfish requires the removal of the two major constituents of the shell. Proteins are removed by deproteinization and inorganic calcium carbonate is eliminated by demineralization. Simultaneously, small amounts of pigments and lipids are generally removed during the two previous steps. In some cases, an additional step of decolorization is applied to remove residual pigments (Younes and Rinaudo 2015). A traditional method for preparation of chitin and its derivatives from shellfish is presented in Figure 10.4.

##### 10.5.1.2.1 Chemical Extraction

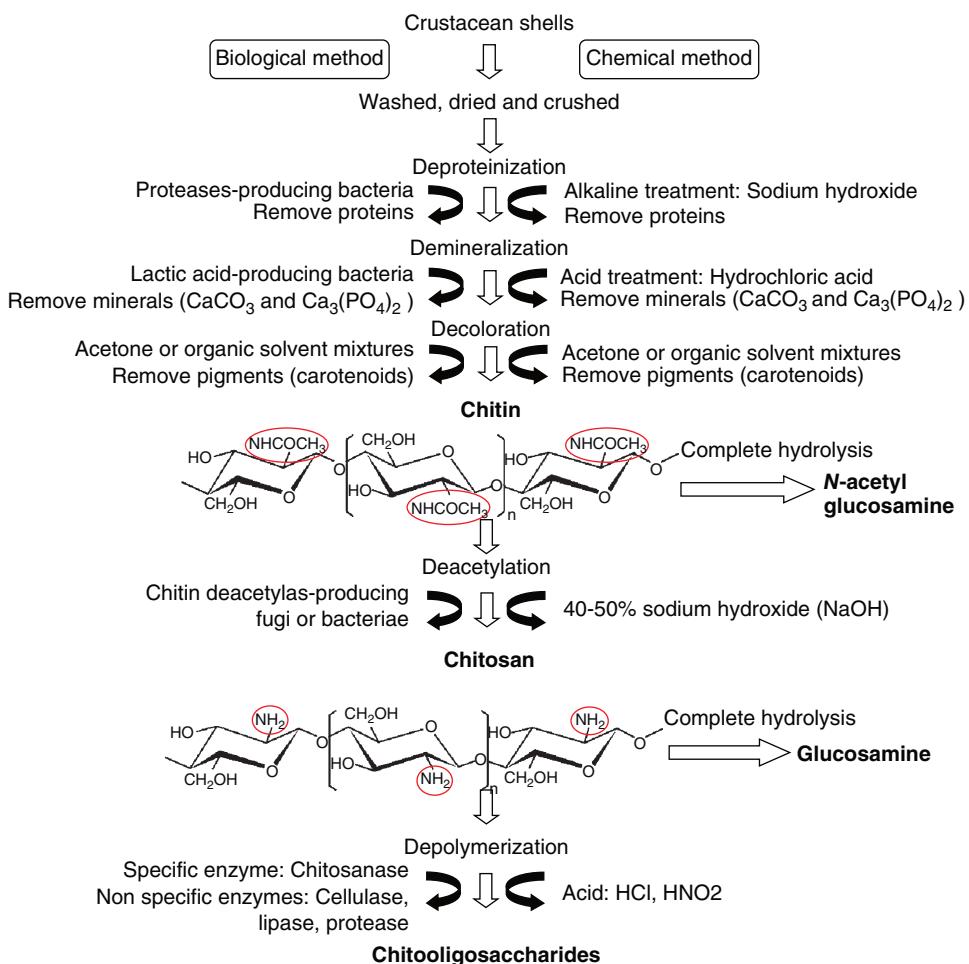
Chemical methods have been widely employed for deproteinization and demineralization by treatment with alkali and acid to remove protein and minerals (calcium carbonate and calcium phosphate), respectively (Percot et al. 2003). Chemical methods were the first approach used in deproteinization. The deproteinization step aims to induce the disruption of chemical bonds between chitin and proteins. However, chemicals used also



**Figure 10.3** The molecular structure of  $\alpha$ -chitin (a) and  $\beta$ -chitin (b) viewed from three different directions. Source: Naleway et al. 2016.

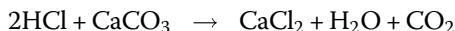
depolymerize the biopolymer. A wide range of chemicals have been tested as deproteinization reagents including NaOH,  $\text{Na}_2\text{CO}_3$ ,  $\text{NaHCO}_3$ , KOH,  $\text{K}_2\text{CO}_3$ ,  $\text{Ca}(\text{OH})_2$ ,  $\text{Na}_2\text{SO}_3$ ,  $\text{NaHSO}_3$ ,  $\text{CaHSO}_3$ ,  $\text{Na}_3\text{PO}_4$ , and  $\text{Na}_2\text{S}$  (Younes and Rinaudo 2015). NaOH is the preferential reagent and it is applied at concentration ranging from 0.125 to 5.0 M, at varying temperature (up to 160 °C) and treatment duration (from a few minutes up to a few days) (Cahu et al. 2012). Under these conditions, the protein becomes detached from the solid component of shellfish discards. After completion of the deproteinization step, the solids are separated from the protein slurry by filtration. The solid fraction consists mainly of chitin and calcium carbonate. It also contains most of pigments.

In the next step, the solid fraction is treated with acid solution. Demineralization is generally performed by acid treatment using HCl,  $\text{HNO}_3$ ,  $\text{H}_2\text{SO}_4$ ,  $\text{CH}_3\text{COOH}$ , and  $\text{HCOOH}$  (Arbia et al. 2013). Among these acids, the preferential reagent is dilute hydrochloric acid (Abdulkarim et al. 2013). Demineralization is easily achieved via the



**Figure 10.4** Chitin chitosan chitooligosaccharides N-acetyl glucosamine and glucosamine extractions by chemical and biological methods.

decomposition of calcium carbonate into the water-soluble calcium salts along with the release of carbon dioxide as shown in the following equation:



Most of the other minerals present in the shellfish cuticle react similarly and give soluble salts in the presence of acid. Then, filtration of the chitin solid phase is performed, followed by washing using deionized water (Younes and Rinaudo 2015).

A decoloration step is added if a colorless product is required. Acetone or an organic solvent mixture is used to remove pigments such as carotenoids (Kaur and Dhillon 2015). With appropriate deproteinization and demineralization, the remaining product consists mainly of chitin. The major concern in chitin production is the quality of the final product, which is a function of the molecular mass (average and polydispersity) and the

degree of acetylation (Arbia et al. 2013). Harsh treatment may cause hydrolysis with, inconsistent physical properties of chitin. Percot et al. (2003) reported that the use of strong acid for the demineralization of chitin results in detrimental effects on MW and the degree of acetylation that negatively affect the intrinsic properties of the purified chitin. Arbia et al. (2013) reported that the chemical method allows almost complete removal of organic salts, but at the same time, reactions of deacetylation and depolymerization may occur. Moreover, high sodium hydroxide concentrations and high proteinization temperatures can cause undesirable deacetylation and depolymerization of chitin. In addition, these chemical methods are hazardous and energy consuming and lead to environmental pollution (Gortari and Hours 2013). The chemical treatment also creates a disposal problem for the wastes, since neutralization and detoxification of the discharged wastewater may be necessary. Another disadvantage of chemical chitin purification is that the valuable protein components can no longer be used as animal feed (Valdez-Pea et al. 2010). Therefore, the interest in biological extraction is increasing since it is safer and cheaper treatment for chitin recovery.

#### 10.5.1.2.2 Biological Extraction

Instead of using the chemical method that could be a source of environmental problems, the biological method offers an alternative to extract chitin from shellfish discards. The development of the biological treatment techniques is gaining greater attention, by the application of enzymes and microorganisms for chitin extraction. A comparative study was carried out by Khanafari et al. (2008) for extraction chitin from shrimp wastes by chemical and biological methods. The results indicated that the biological method (using microorganisms) was more effective for the recovery of chitin from shrimp wastes, compared to the chemical method. Bustos and Healy (1994) also reported that chitin obtained by the deproteinization of shrimp shells with various proteolytic microorganisms has higher MWs in comparison with chemically prepared shellfish chitin. The biological extraction of chitin offers high reproducibility in shorter time, simpler manipulation, smaller solvent consumption and lower energy input. However, the biological method is still limited to laboratory scale studies (Younes and Rinaudo 2015).

##### 10.5.1.2.2.1 Enzymatic Deproteinization

Deproteinization processes have been reported for chitin production from shellfish discards using proteases. Many proteases such as Alcalase, pepsin, papain, pancreatin, devolvase, and trypsin have been used to remove proteins from shellfish discards and minimize the deacetylation and depolymerization during chitin isolation (Kandra et al. 2012). This treatment may be performed either after or before the demineralization step of the solid material, which modifies the accessibility for the reactants (Younes and Rinaudo 2015).

These methods allow obtaining a liquid fraction rich in proteins and a solid chitin fraction. The liquid fraction can be used either as a protein supplement for human consumption or as an animal feed (Rao et al. 2000).

Many reports have demonstrated the application of proteolytic microorganism for deproteinization of shellfish discards to produce chitin. Synowiecki and Al-Khateeb (2000) applied the enzymatic deproteinization on previously demineralized shrimp (*Crangon crangon*) shell processing wastes. Alcalase 2.4 L, a serine endopeptidase obtained from *B. licheniformis*, was used. Gildberg and Stenberg (2001) also used Alcalase 2.4 L for chitin recovery from the processing wastes of Northern shrimp (*P. borealis*).

Jo et al. (2008) reported that deproteinization of the snow crab (*Chionoecetes opilio*) shell using protease-producing *Serratia marcescens* FS-3 could be applicable to chitin production. Oh et al. (2007) used protease producing strain *P. aeruginosa* F722 for the extraction of chitin from crab shells. Manni et al. (2010) used protease from *Bacillus cereus* SV1 for chitin extraction from shrimp (*M. monoceros*) waste. High enzymatic deproteinization of shrimp wastes was achieved and reached  $88.8 \pm 0.4\%$  with enzyme/substrate of 20.  $^{13}\text{C}$  CP/MAS-NMR (crosspolarization,magic-angle-spinning, nuclear magnetic resonance) spectral analysis of chitin prepared by the enzymatic deproteinization of shrimp wastes was found to be similar to that obtained by alkaline treatment and to the commercial  $\alpha$ -chitin. Giyose et al. (2010) evaluated the potential use of protease produced by *Erwinia chrysanthemi* for the deproteinization of lobster (*Palinurus* sp.) heads in a chitin production process. Protease producing *E. chrysanthemi* was successfully used for deproteinization of lobster wastes. Enzymatic deproteinization was optimized by Younes et al. (2012) before demineralization. In this study, many microbial proteases were compared on the basis of their efficiency in deproteinization of shrimp (*M. monoceros*) shells. Six alkaline crude microbial proteases from *Bacillus mojavensis* A21, *B. subtilis* A26, *B. licheniformis* NH1, *B. licheniformis* MP1, *Vibrio metschnikovii* J1, and *Aspergillus clavatus* ES1 were used. The highest deproteinization degree was obtained with *B. mojavensis* A21 proteases, being at about 76%. Then, the effect of reaction conditions, i.e. mainly enzyme/substrate ratio, temperature, and incubation time, on the deproteinization degree were optimized using response surface methodology. *B. mojavensis* A21 proteases were found to remove up to  $88 \pm 5\%$  of the shell proteins under the optimized conditions.

Furthermore, proteases from marine animals have been applied for deproteinization of shellfish discards. El-Hadj Ali et al. (2011) used alkaline proteases from viscera of striped seabream (*Lithognathus mormyrus*) for deproteinization of shrimp wastes up to 79% using an enzyme/substrate ratio of 10 after incubation for three hours at  $40^\circ\text{C}$ . Proteases from zebra blenny (*Salarias basilisca*) viscera were found to be effective in the deproteinization of shrimp wastes. The protein removal after three hours at  $40^\circ\text{C}$  with an enzyme/substrate ratio of  $5 \mu\text{mg}^{-1}$  was about 77% (Ktari et al. 2014). Sila et al. (2012b) reported that the enzymatic deproteinization of the shrimp shell wastes, using proteases from goby (*Zosterisessor ophiocephalus*) viscera, could be applicable to the chitin production process. Younes et al. (2015) also reported that alkaline proteases from red scorpionfish (*Scorpaena scrofa*) viscera were applied for deproteinization of shrimp shell wastes to produce chitin. The high level of deproteinization (83%) was obtained with an enzyme/substrate ratio of  $10 \mu\text{mg}^{-1}$  after incubation at  $50^\circ\text{C}$  for three hours. NMR analysis of prepared chitin gave similar peak pattern to that of commercial chitin. Moreover, prepared chitin using *S. scrofa* proteases was still completely acetylated presenting a degree of acetylation equal to that of commercial chitin.

**10.5.1.2.2.2 Microbial Fermentation** One interesting new technology for the extraction of chitin that offers an alternative to the more harsh chemical methods is fermentation by using microorganisms. The cost of using enzymes can be decreased by performing deproteinization by the fermentation process, which can be achieved by endogenous microorganisms (so called auto-fermentation) or by adding selected strains of microorganisms (Younes and Rinaudo 2015). This latter can be achieved by single-stage fermentation, two-stage fermentation, co-fermentation, or successive fermentation.

Many microorganism species were proposed for the shells of shellfish fermentation (Arbia et al. 2013). Fermentation methods could be separated into two major categories: lactic acid fermentation and non-lactic acid fermentation.

**Lactic Acid Fermentation** Fermentation of crustacean shells can be performed by selected *Lactobacillus* sp. strain as inoculum which produces lactic acid and proteases. Lactic acid is obtained by conversion of glucose resulting in lower pH condition of silage and suppressing the growth of spoilage microorganisms. Lactic acid produced by bacteria reacts with the calcium carbonate component in the shellfish waste resulting in the formation of calcium lactate, which can be precipitated and removed by washing (Arbia et al. 2013). Thus, deproteinization of the shellfish wastes can occur by the action of the proteases produced by added strains, or by gut bacteria present in the intestinal system of the treated shellfish, or by proteases present in the shellfish itself (Oh et al. 2007). The efficiency of lactic acid fermentation depends on many factors such as the species and quantity of inoculums, carbon source and its concentration, initial pH and pH evolution during fermentation, temperature, and duration of fermentation (Gortari and Hours 2013).

A number of studies on the use of lactic acid fermentation for the recovery of chitin from shellfish discards have been carried out. Rao et al. (2000) studied the effect of different fermentation parameters (initial pH, initial glucose concentration and inoculation with different quantities of *Lactobacillus plantarum* 541) on deproteinization and demineralization degrees of shrimp wastes. Combined treatment with *Lactobacillus* and the reduction of initial waste pH by the addition of acetic acid produced lower deproteinization but higher demineralization degrees than treatment with *L. plantarum* 541 or acid individually. In addition, inoculation with *Lactobacillus* resulted in a high-quality protein liquor output, whereas autofermented waste (due to the presence of shrimp microflora) gave a strong stinky protein fraction. In the fermentation with lactic acid bacteria, the demineralization efficiency and the quality of the derived product are high, and the addition of commercial proteases may even increase deproteinization. Cremades et al. (2001) isolated chitin from crawfish (*P. clarkii*) byproducts using semi-solid-state fermentation with *Lactobacillus paracasei*. The chemical and spectrometric (fourier-transform infrared spectroscopy ((FTIR) and  $^{13}\text{C}$  NMR) characterization of the obtained chitin was high quality and similar to that of commercial chitin. Successive two-step fermentation was carried out from red crab (*Chionoecetes japonicus*) shells for the extraction of chitin in combination of the first step with a lactic acid bacterium *L. paracasei* subsp. *tolerans* KCTC-3074 and the second step with a protease producing bacterium *S. marcescens* FS-3, and vice versa. The successive fermentation in the combination of FS-3 and KCTC-3074 gave the best result in co-removal of  $\text{CaCO}_3$  and proteins from crab shells. In this combination, the rates of demineralization and deproteinization were 94.3% and 68.9%, respectively, at the end of fermentation (Jung et al. 2007). Choorit et al. (2008) used response surface methodology to optimize demineralization efficiency in fermented shrimp (*L. vannamei*) head. The following variables were tested: sucrose concentration, initial pH value and soaking time, using *Pediococcus* sp. L1/2. An increase in demineralization degree was caused by higher sucrose concentration and soaking time. An important effect of the initial pH was noticeable. Demineralization degree reached about 83% using the following condition: at pH 7,  $50\text{ g l}^{-1}$  sucrose concentration and 36 hours soaking time. Aytekin and Elibol (2010) extracted chitin from prawn wastes using lactic acid-producing bacterium, *Lactococcus lactis*, and a protease-producing marine

bacterium, *Teredinibacter turnerae*. Both bacteria were cultivated individually and co-fermented. The results showed that *L. lactis* removed the inorganic materials efficiently (66.5% of deproteinization, 78.8% of demineralization, and 52.2% process yield), while *T. turnerae* was more efficient (77.8% deproteinization, 23.3% demineralization, and 49.2% process yield). The highest process yield (95.5%) was obtained during co-fermentation of both bacteria.

**Non-lactic Acid Fermentation** In non-lactic acid fermentation, both bacteria and fungi were also used for shellfish discards fermentation, such as *Bacillus* sp. (Ghorbel-Bellaaj et al. 2012), *Exiguobacterium* sp. (Sorokulova et al. 2009), *Gluconobacter* sp. (Liu et al. 2014), and *Aspergillus* sp. (Teng et al. 2001). Sini et al. (2007) reported that *B. subtilis* was found to be an efficient starter culture for fermentation of shrimp shells to produce chitin. About 84% of the protein and 72% of the minerals were removed from the fermented residue at the end of fermentation. The FTIR spectrum of the prepared chitin was similar to that of commercial chitin. Sorokulova et al. (2009) investigated *B. cereus* and *Exiguobacterium acetyllicum* on the fermentation of shrimp shell wastes. Fermentation of 3% shell wastes with *B. cereus* resulted in 97.1% deproteinization and 95% demineralization. For *E. acetyllicum*, the level of deproteinization and demineralization was 92.8% and 92%, respectively. High activity of isolated cultures in decomposition of shrimp shell waste suggests broad potential for application of these bacteria in environmentally friendly approaches to extract chitin from shellfish processing wastes. Ghorbel-Bellaaj et al. (2012) evaluated the ability of six protease-producing *Bacillus* species (*Bacillus pumilus* A1, *Bacillus mojavensis* A21, *B. licheniformis* RP1, *B. cereus* SV1, *Bacillus amyloliquefaciens* An6, and *B. subtilis* A26) to ferment shrimp (*M. monoceros*) shells waste for chitin extraction. All the *Bacillus* strains were found to be able to deproteinize shrimp waste. The highest deproteinization degree was obtained using *B. cereus* SV1. Liu et al. (2014) isolated chitin from shrimp (*L. vannamei*) head wastes using successive co-fermentation with protease-producing bacterium, *B. licheniformis* 21886, and an acid-producing bacterium, *Gluconobacter oxydans* DSM-2003 (dried skim milk). When shrimp shell wastes were fermented with *B. licheniformis* 21886 followed by *G. oxydans* DSM-2003, the chitin content was up to 90.8%, with the deproteinization and demineralization efficiencies as 87% and 93.5%, respectively. Crab shells waste were fermented using six protease-producing *Bacillus* species (*B. subtilis* A26, *B. mojavensis* A21, *B. pumilus* A1, *B. amyloliquefaciens* An6, *B. licheniformis* NH1, and *B. cereus* BG1) for the production of chitin. The highest deproteinization and demineralization were obtained with *B. licheniformis* NH1 and *B. pumilus* A1. Furthermore, glucose supplementation was found remarkably to promote demineralization efficiency, and enhance slightly deproteinization rates. The FTIR spectra of the prepared chitins after fermentation showed the characteristics bands of  $\alpha$ -chitin (Hajji et al. 2015).

Proteolytic enzymes released from fungus *Aspergillus niger* were also used for deproteinization and demineralization of shellfish discards. Teng et al. (2001) evaluated concurrent production of chitin from shrimp shells in a one-pot fermentation process where proteases from the fungi hydrolyze proteins into amino acids that in turn act as a nitrogen source for fungal growth. The results showed that residual proteins in the isolated shrimp chitin were below 5%. All samples displayed characteristic profiles for chitin in their FTIR and solid-state NMR spectra. All chitin samples evaluated with

3-[4,5-dimethylthiazolyl-2]-2,5-diphenyl tetrazolium bromide (MTT) and Neutral Red assays with cell lines did not display cytotoxic effects.

The biological extractions of chitin are simple, more productive and environmentally friendly when compared to chemical processes. However, these methods have their drawbacks such as longer processing time compared to chemical methods and limited to laboratory scale studies (Younes and Rinaudo 2015; Hamed et al. 2016).

## 10.5.2 Chitosan

The term chitosan usually refers to a family of polymers obtained after chitin deacetylation to varying degrees. When the degree of deacetylation of chitin is more than 50%, the product is called chitosan and becomes soluble in acidic solution (Rinaudo 2006). During deacetylation, acetyl groups are removed but also depolymerization reaction occurs, indicated by changes in the MW of chitosan.

Chitosan has been of interest in the past few decades due to its potential broad range of application. Chitosan has great properties, such as polycationic, non-toxic, biodegradable, and biocompatible. As a consequence, chitosan has been applied in different fields such as medical biotechnology, agriculture, cosmetic, and food (Kumar 2000).

### 10.5.2.1 Chitosan Structure and Physiochemical Properties

Chitosan is a linear polysaccharide that contains copolymers of D-glucosamine (deacetylated units) and *N*-acetyl-D-glucosamine (acetylated units) linked by  $\beta$ -1,4-glycosidic bonds. This biopolymer is obtained by the partial deacetylation of chitin (Figure 10.4).

The degree of deacetylation is generally defined as the glucosamine/*N*-acetyl-D-glucosamine ratio, which goes up as chitin is converted to chitosan. Therefore, when the percentage of *N*-acetyl-D-glucosamine is higher than glucosamine, the biopolymer is called chitin and when the percentage of glucosamine exceeds *N*-acetyl-D-glucosamine the compound is called chitosan (Hamed et al. 2016). The degree of deacetylation has often been cited as an important parameter that determines many physiochemical and biological properties of chitosans such as crystallinity, hydrophilicity, degradation, and cell response (Freier et al. 2005). Nunthanid et al. (2001) reported that tensile strength and moisture adsorption of chitosans with high MW (600–1000 kDa) were significantly greater than those with lower MW (50–60 kDa). Xu and Du (2003) also reported that increasing chitosan MW increased protein encapsulation efficiencies and reduced release kinetics.

Chitosan has three types of reactive groups including an amino group, and both primary and secondary hydroxyl group at the C-2, C-3, and C-6 positions, respectively. Chemical modification of these groups has provided numerous useful materials in the different fields of application (Kumar 2000). Chitosan is the only commercial cationic polymer due to the positive charges on its amino groups. It is soluble in dilute acids ( $\text{pH} < 6$ –6.5) such as acetic acid, formic acid, succinic acid, lactic acid, and malic acid along with dilute HCl (Tungtong et al. 2012). The solubilization of chitosan occurs by protonation of the amino group on the C-2 position of the D-glucosamine repeat unit, whereby the polysaccharide is converted to a polyelectrolyte in acidic media (Rinaudo 2006). The physical properties of chitosan in solution depend on the degree of deacetylation and the acetyl group distribution along the chains (Philippova et al. 2012).

### 10.5.2.2 Chitosan Preparation

Chitosan can be prepared by deacetylation of chitin through chemical or enzymatic methods. Chemical methods are used extensively for commercial purpose of chitosan preparation due to their low cost and suitability for mass production (No and Meyers 1995).

#### 10.5.2.2.1 Chemical Deacetylation

Chitin can be converted to chitosan by the chemical process. From a chemical point of view, either acids or alkaline can be used to deacetylate chitin. However, glycosidic bonds are very susceptible to acid; therefore, alkaline deacetylation is used more frequently (Hajji et al. 2014). Chitosan is commonly prepared by deacetylation of chitin using 40–50% alkaline solution at 100–160 °C for a few hours. Chitosan is produced as an insoluble residue deacetylated up to 95% (Honarkar and Barikani 2009). The alkaline treatments hydrolyze the acetyl groups and transform the *N*-acetyl-D-glucosamine units into D-glucosamine units with free amino groups (Kumar 2000).

Many parameters in the deacetylation reaction can impact the characteristics of chitosan. Rege and Block (1999) reported that the temperature and processing time had a significant effect on degree of deacetylation and MW of chitosan. Tsaih and Chen (2003) used 50% NaOH solution to deacetylate chitin at 99 °C or 140 °C for one to nine hours. The degree of deacetylation of chitosans increased with increasing reaction time and temperature. The MWs of chitosan deacetylated at 140 °C were smaller than those at 99 °C. Yuan et al. (2011) reported that the degree of deacetylation of chitosan increased with increasing reaction time and temperature. Hamed et al. (2016) reported that chitosans with a different degree of deacetylation and MW were generated depending on the reaction time, temperature, alkaline concentration and repetition of alkaline steps. Moura et al. (2015) also reported that the MW of chitosan increased with decreasing NaOH concentration, reaction time and NaOH/chitin ratio. The MWs of obtained chitosan were in the range of 101–201 kDa.

Chemical deacetylation can cause environmental pollution, requires significant energy and produces poor quality of chitosan. Therefore, enzymatic methods could replace it to overcome those limitations (Raval et al. 2013).

#### 10.5.2.2.2 Enzymatic Deacetylation

In recent years, the use of chitin deacetylases has become an alternative method to obtain chitosan from chitin because of the maintenance of the compound's properties and environment friendly processing (Mao et al. 2017).

Chitin deacetylase (EC 3.5.1.41) catalyzes the hydrolysis of *N*-acetamido bonds in chitin to produce chitosan. Chitin deacetylase was found in several fungi such as *Mucor rouxii* (Martinou et al. 1993), *Absidia coerulea* (Gao et al. 1995), and *Aspergillus nidulans* (Alfonso et al. 1995). The presence of this enzyme activity has also been reported in bacteria and insects (Mao et al. 2017). Chitin deacetylase is secreted either into the periplasmic region or into the culture medium (Younes and Rinaudo 2015). The enzyme is an acidic glycoprotein of about 75 kDa with 30% (w/w) carbohydrates. Biochemical characterization indicates a very narrow specificity for  $\beta$ -(1,4)-linked *N*-acetyl-D-glucosamine polymers (Zhao et al. 2010). It is interesting to notice that chitin deacetylases produced by *C. lindemuthianum* and *A. nidulans* are not inhibited by acetate (a product of the deacetylation), which make them suitable for chitin deacetylation (Alfonso et al.

1995). However, there are also some problems with the use of enzyme-producing fungi such as a low yield of deacetylase producing strains, low enzyme activity, and fermentation requirements that are complicated. Therefore, chitin deacetylase producing bacteria may replace the current fungal strains (Hamed et al. 2016). Bacteria are easier and faster than fungi to grow in large-scale fermentation systems (Kaur et al. 2012). Zhou et al. (2010) reported that chitin deacetylase producing bacteria isolated from soil was identified as *B. amyloliquefaciens*. The optimization of culture medium components for chitin deacetylase production was 2% starch at pH 7 and 30 °C. Kaur et al. (2012) isolated chitin deacetylase producing bacteria from beaches of Chennai, India. The isolated bacteria were identified as *Bacillus* sp. and *Serratia* sp., respectively. The yield of chitosan by *Bacillus* sp. and *Serratia* sp. was 0.16 and 0.1 g l<sup>-1</sup>, respectively, using chitin as a carbon source. These bacteria can be exploited for biotransformation of chitin to chitosan at industrial scale and proved to be a promising candidate for an economical and environmentally friendly process.

### 10.5.3 Chitooligosaccharides

Chitooligosaccharides are the depolymerized products of chitosan. Chitosans with degrees of polymerization less than 20 and an average MW less than 3900 Da are called chitooligosaccharides (Hamed et al. 2016). Crab and shrimp shell processing byproducts are currently utilized as the major industrial source of biomass for the large-scale production of chitooligosaccharides. Basically, the very high MW and high viscosity of chitosan make it difficult to use in many applications. Chitooligosaccharides are therefore much easier to manipulate in large-scale commercial applications due to their low MWs, low viscosity, and short chain lengths.

#### 10.5.3.1 Chemical Method

Chitooligosaccharides are generated by depolymerization of chitosan using acid hydrolysis. Acidic depolymerization is done using different reagents such as hydrochloric acid, hydrofluoric acid, nitrous acids, acetic acid and sulfuric acid (Lodhi et al. 2014). However, the use of chemical solutions has been shown to have several disadvantages such as lower yields of chitooligosaccharides and a large amount of monomeric D-glucosamine units. Furthermore, strong acids must be disposed, thereby causing an environmental pollution. In addition, the chitooligosaccharides prepared by the acidic hydrolysis might be toxic because of chemical changes during conversion (Kim and Rajapakse 2005).

#### 10.5.3.2 Enzymatic Method

Chitooligosaccharides can be produced by specific enzymes such as chitosanases and non-specific enzymes such as cellulases, lipases, glycanases, and proteases (Hamed et al. 2016). Enzymatic hydrolysis for chitooligosaccharides production is preferable, since this method renders the higher yields of oligomers with a higher degree of polymerization. Jeon et al. (2000) reported that optimum conditions for hydrolyzing 1% chitosan (89% deacetylation) using chitosanase from *B. pumilus* BN-262 were 45 °C at 5.5 pH for one hour. Ilyina et al. (2000) hydrolyzed commercial crab chitosan using chitosanase from *Streptomyces kurssanovii*. The chitosan hydrolysis carried out by lipase at 37 °C produced a larger quantity of chitooligosaccharides than that at 55 °C when the reaction

time was longer than 6 hours, and chitooligosaccharides yield of 24 hours hydrolysis at 37 °C was 93.8% (Lee et al. 2008). Laokuldilok et al. (2017) hydrolyzed chitosan using lysozyme, papain, or cellulase (0.003% w/w) for 0–16 hours. Papain showed the highest MW reduction of chitosan. After 16 hours of chitosan hydrolysis with papain, the average MW was 4.3 kDa. The chitooligosaccharides produced with papain had high antioxidant and antimicrobial activities, especially against gram-negative pathogens (*E. coli*).

#### 10.5.3.3 Modes of Action and Applications

Chitooligosaccharides are also reported to have excellent biological properties such as cholesterol lowering, antioxidant activity, antimicrobial activity, antitumor, and immuno-enhancing effects, etc. (Lodhi et al. 2014). Chitooligosaccharides are easily absorbed through the intestine, quickly get into the blood flow and have systemic biological effects in the organism. In the food industry, chitooligosaccharides attract a greater interest as antimicrobial agents, antioxidants, and nutritional enhancers of food (Kim and Rajapakse 2005).

Chitooligosaccharides possess primary amino groups in their structures. The number of these amino groups has proven to play a major role in antimicrobial activity. Chitooligosaccharides can alter permeability characteristics of microbial cell membrane and further prevent the entry of materials or cause leakage of cell constituents that finally leads to death of bacteria, yeasts, and fungi (Kim and Rajapakse 2005). Jeon and Kim (2000) reported that chitooligosaccharides with degrees of polymerization of 3–6 showed a higher inhibitory effect on *E. coli* with increasing concentration and 0.5% chitooligosaccharides solution completely inhibited the growth of *E. coli*. Choi et al. (2001) evaluated the *in vitro* antimicrobial activity of chitooligosaccharides (derived from the exoskeletons of marine crustaceans) against two representative oral pathogens, *Actinobacillus actinomycetemcomitans* and *Streptococcus mutans*. Their morphology from spherical shape to irregular condensed masses with bleb-like structures was observed indicating a separation of cytoplasmic membrane from cell wall and coagulation of cytosolic components. The site of chitooligosaccharides action is probably the bacterial envelope and the killing of the organism could be due to membrane disruption. Benhabiles et al. (2012) also reported that chitooligosaccharides from shrimp shell byproducts completely inhibited the growth of *Staphylococcus aureus* and *P. aeruginosa* ATCC 27853 after 2 and 2.5 hours of incubation, respectively.

Currently, the antioxidant activity of chitooligochitosans has attracted a greater attention. Antioxidant activities of chitooligochitosans depend on their MW. Laokuldilok et al. (2017) studied the antioxidant activity of chitooligochitosans with three different MWs of 5.1, 14.3, and 41.1 kDa. The lower MW chitooligochitosans showed the higher 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, reducing power, and metal chelating activity. Park et al. (2004) studied antioxidant activity of chitooligosaccharides with different MW and degree of deacetylation. Chitooligosaccharides with MW range of 1–3 kDa and highly deacetylated (90%) chitooligosaccharides have a higher potential to scavenge different radicals such as DPPH, hydroxyl, superoxide, and alkyl radicals. Fernandes et al. (2010) reported that chitooligosaccharides can be used as antioxidants in biological systems. They reduced either the hemolytic and DNA damage, by inhibiting  $H_2O_2^-$  and 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH)-radicals.

### 10.5.4 Glucosamine

Glucosamine (2-amino-2-deoxyglucose, chitosamine) is an amino sugar that occurs in acetylated and polymerized forms in chitin. Glucosamine can be produced from shellfish processing byproducts using acid hydrolysis or using enzymes to digest molecular chain of chitin into *N*-acetyl glucosamine monomers (Benavente et al. 2015). D-glucosamine can be produced by hydrolysis of chitosan into D-glucosamine monomers as showed in Figure 10.4. There are three forms of glucosamine supplements in the market, i.e. *N*-acetyl glucosamine, glucosamine hydrochloride, and glucosamine sulfate (Wanichpongpan and Attasat 2016).

#### 10.5.4.1 Chemical Method

Generally, the chemical degradation of chitin or chitosan can be performed through hydrolysis using a strong acid, such as HCl. The processing temperature and concentration of the acid should be carefully selected. These parameters must be high enough to sufficiently degrade chitin and chitosan, in which the *N*-acetyl glucosamine and glucosamine product are not destroyed (Chen et al. 2010). Mojarrad et al. (2007) isolated glucosamine from exoskeleton of Persian Gulf shrimp (*M. monoceros*) by hydrolysis of chitosan with hydrochloric acid. The optimum conditions for glucosamine preparation were 37% hydrochloric acid, chitin/acid solution ratio of 1 : 9 at 100 °C for four hours. Wanichpongpan and Attasat (2016) prepared glucosamine from chitosan extracted from shrimp shell by treating chitosan with 14.4% hydrochloric acid at 80 °C for six hours. Benavente et al. (2015) produced glucosamine from crustacean shell in four main stages: (i) acid hydrolysis of chitin with 12 M hydrochloric acid using the reflux technique; (ii) filtration of the solution to discard solid impurities; (iii) recrystallization of the product using 95% ethyl alcohol as solvent; and (iv) filtration, washing and drying of final product at 50 °C. The yield of glucosamine hydrochloride with respect to chitin was 58%. Although the production of glucosamine by chemical methods is estimated to be sufficiently economic, the product is not considered a natural material due to its chemical modification. The large quantities of chemical waste resulting from chemical processes are not environmentally friendly. However, the enzymatic methods can produce glucosamine under mild conditions. Thus, biological methods especially enzymatic hydrolysis has received considerable attention, in order to apply mild reaction conditions as well as product consistency (Nidheesh et al. 2015).

#### 10.5.4.2 Enzymatic Method

Several enzymatic processes can be implemented to produce glucosamine. Pan et al. (2011) reported that glucosamine can be produced with commercial  $\alpha$ -amylase and glucoamylase. It takes two steps ( $\alpha$ -amylase 6 hours, glucoamylase 12 hours) to hydrolyze chitosan to glucosamine. Sun et al. (2013) obtained glucosamine by degrading chitosan using crude enzymes including chitosanase and  $\beta$ -D-glucosaminidase. Chitosanase and  $\beta$ -D-glucosaminidase are specific hydrolases for chitosan and it takes less time to hydrolyze chitosan to glucosamine. In addition, glucosamine is of high purity when the crude enzymes are added into chitosan. The crude exo- $\beta$ -D-glucosaminidase obtained from *P. decumbens* CFRNT15 was used for the hydrolysis of chitosan from shrimp processing byproducts to produce D-glucosamine. The crude exo- $\beta$ -D-glucosaminidase was used

to produce D-glucosamine with the maximum yield of 546 µM at pH 5.6 and 42 °C for 30 hours (Nidheesh et al. 2015).

#### 10.5.4.3 Biomedical Applications

There are numerous evidences from *in vitro* and preclinical studies, indicating the anti-inflammatory and immunosuppressive properties of glucosamine. Osteoarthritis, especially in the knee, is the most common type of arthritis or degenerative joint disease in the elderly. Glucosamine and its derivatives are the most commonly used over-the-counter supplements in the treatment of osteoarthritis. The disease-modifying ability of glucosamine in osteoarthritis is mainly attributable to both anti-inflammatory and chondroprotective effects (Dalirfardouei et al. 2016). The mechanism for anti-inflammatory effects of glucosamine is inhibition of NF-κB signaling activation. Jeong et al. (2010) found that glucosamine can bind to transglutaminase 2 (TGase 2) directly and stops IκB polymerization, therefore prevents canonical NF-κB activation. Moreover, Largo et al. (2003) glucosamine inhibits IL-1β-induced NF-κB activation, IκBα degradation in the cell cytoplasm and the NF-κB subunits nuclear translocation in human osteoarthritic chondrocytes. Glucosamine also prevents cytokine-induced demethylation of a specific CpG site in the IL-1β promoter and so decreased IL-1β expression (Dalirfardouei et al. 2016).

A number of studies have shown the antioxidant activities of glucosamine. Xing et al. (2006) reported that 0.8 mg ml<sup>-1</sup> glucosamine hydrochloride showed 84% superoxide radical scavenging activity. The hydroxyl scavenging activity of glucosamine hydrochloride was about 54% inhibition at 3.2 mg ml<sup>-1</sup>. The results also showed that glucosamine can protect macromolecules such as protein, lipid, and deoxyribose from oxidative damage induced by hydroxyl radicals. Jamialahmadi et al. (2014) reported that glucosamine could be a suitable agent to prevent H<sub>2</sub>O<sub>2</sub>-induced DNA damage in human peripheral lymphocytes. Glucosamine at concentrations of 2.5–40 mM showed a potent antigenotoxic effect, while its N-acetylated analog only indicated a slight DNA protection at concentration of 40 mM. The protection activity of glucosamine may be related to free amine or positive NH<sub>2</sub> group on glucosamine backbone. This amino sugar has protective antioxidant and cytoprotective properties and so can be considered as a promising agent in preventing and/or treating ROS-related diseases. Oral administration of glucosamine to pulmonary inflammation murine models can directly inhibit nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase activity, decrease reactive oxygen species production and prevent P47<sup>phox</sup> translocation from cytoplasm to membrane, which is crucial for the activation of NADPH oxidase (Wu et al. 2014b).

Several studies indicate that glucosamine can inhibit growth of microorganisms and controls bacterial infections. Appelt et al. (2013) reported that glucosamine possesses antimicrobial properties against gram-positive bacteria (*S. aureus* [ATCC 25923], *Listeria monocytogenes* [ATCC 7644] and *Enterococcus faecalis* [ATCC 29212]) and gram-negative bacteria (*E. coli* [ATCC 25922], *P. aeruginosa* [ATCC 27853], *Salmonella choleraesuis* [ATCC 10708], and *Klebsiella pneumoniae* [ATCC 700603]). The parenteral form of N-acetyl glucosamine is also capable of controlling systematic toxic symptoms caused by viral and bacterial infections (Dalirfardouei et al. 2016). The mechanism of antibacterial activity of glucosamine seems to probably disrupt bacterial cell wall via free amino group like para-aminobenzoic acid and sulfonamide (Rozin 2009).

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# 11

## Microbial Enzymes from Fish Processing Discards

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### 11.1 Introduction

Enzymes have been used for many years as industrial processing aids to make products such as food, textiles, leather, detergents, pharmaceuticals, and as diagnostic tools. Their use as processing aids derives from their superior attributes over chemicals including faster reaction rates, greater specificity leading to more consistent products, effectiveness in small amounts and under milder reaction conditions, low capital investments costs, and ease of their control and ready inactivation once the desired transformation in the food is attained. Their use is also more environmentally friendly and results in less waste and/or undesirable co-products. Traditionally, enzymes have been produced from plant, animal, and microbial sources. Of the three, microorganisms are by far the most commonly used as an enzyme source. The reasons for this include the fact that they grow and produce enzymes in higher yields faster; they take less space and require fewer and inexpensive substrates and resources to grow and proliferate. They are also not subject to the same restrictions due to agricultural and environmental regulations. Another major issue surrounding their use is the medium or substrate(s) required to make microorganisms grow and proliferate. Currently, they are grown using substrates such as peptones which are relatively costly and increase the costs of the enzymes produced. Research is now ongoing using agricultural and fishery harvesting and processing discards as substrates for enzyme production. These discards are rich in all the essential nutrients and biochemicals (e.g. C, N, and minerals such as Ca) necessary to sustain the growth of the microorganisms for enzymes production. While many papers exist on the utilization of agricultural waste for industrial enzyme production (Martins et al. 2011), fish waste has yet to be given the same attention. The use of this waste could greatly benefit the economies of fish producing countries such as Thailand, India, Vietnam, the Scandinavian countries, and Canada. In most countries, waste disposal often represents a large problem.

Fish and shellfish harvesting and processing is accompanied by generation of large quantities of discards in various forms, either as bycatch (considered as "trash" fish by

commercial fishers) and damaged products that cannot be sold, or as “inedible” processing byproducts (e.g. heads, bones, fins, tails, viscera, waste water). Fish processing involves steps such as stunning, sorting, grading, slime removal, de-heading, scaling, gutting, trimming of fins, washing, meat bone separation, and sectioning into steaks and fillets (Suresh et al. 2018; Ghaly et al. 2013). For example, in cod processing plants, almost 60% of the fish is considered discards. Love et al. (2015), intimated that around 20 million tonnes of seafood discards are produced annually. Based on these descriptions, it is estimated that around 20–80% of the whole animal ends up as discards which are put to low value-added uses such as fish silage, fishmeal, and fish sauce. Some of the discards are also transformed into byproducts such as proteins, fish oil, amino acids, minerals, enzymes, bioactive peptides, collagen and gelatin, chitin and chitosan (Klomklao et al. 2012; Benjakul et al. 2012a, b; Ghaly et al. 2013; Simpson et al. 2000). The benefits derived from this use include: (i) serving as important nutrient source for animals; (ii) adding value to a hitherto abundantly available underutilized resource material could serve as valuable substrates to produce enzymes for the global enzyme market; and (iii) as a means of reducing dumping to protect environment health.

There is currently increased consumer awareness about global issues as they pertain to the environment they inhabit. There is now consumer agitation for better greener processes with greener outcomes. These aspirations span several industries including the food industry, where there is now a steady shift toward putting harvesting and processing discards to better economic use, as well as movement away from synthetic compounds for their more natural alternatives (Rohrig 2015). For example, Kraft Heinz is now using natural and organic food colorings in its macaroni and cheese packaged dinners in response to a public outcry. Other companies (e.g. Mondelez International, ConAgra Foods, General Mills, Kellogg's, Nestle, Mars, Pizza Hut, and Taco Bell) have all curtailed use of synthetics in their products such as non-alcoholic beverages, cream cheese spreads, M&Ms, skittles, etc.). This trend of replacing synthetic compounds in the food industry has been spurred by their being perceived as having cosmetic effect only, and by research connecting artificial coloring with hyperactivity in children. With the use of chemicals in foods currently under intense scrutiny, the agricultural and fishery processing sectors must find novel solutions to keep up with the changes in consumer attitudes and demands.

This chapter deals with discards produced from fish and shellfish harvesting and processing, their composition, and use as substrates for microbial enzyme production. It also discusses current and other potential uses of these enzymes.

## 11.2 Seafood Harvesting and Processing Discards

### 11.2.1 Global Production of Fish and Shellfish

Global fish production has been booming in recent years, and was forecasted to top \$150 billion in year 2017 (FT 2018). This trend has been attributed to economic recovery in key European importing countries, as well as steady demands from traditional fish importing countries like the U.S., Japan, China, Spain, Vietnam, France, Italy, and Germany, and its greater consumption (ITC 2018; FAO 2013). This remarkable growth in

the fish and shellfish sector has been largely helped by increased production from aquaculture. In the developed countries, fish is highly regarded for its high-quality protein, omega-3 polyunsaturated fatty acids (n-3 PUFAs), mineral, and vitamin contents. Fish also have relatively lower saturated fat content. In developing countries, fish is mostly consumed as a cheap protein source. According to Suresh et al. (2018), fish and seafood represent approximately 17% of all animal protein consumed. The Worldatlas report of 2017, listed the 10-leading seafood producing countries as China, Indonesia, India, Vietnam, United States, Myanmar, Japan, Philippines, the Russian Federation, and Chile (Worldatlas 2017). China and Indonesia in particular, steadily increased their catch by more than one million metric ton from 2011–2014. Globally, the seafood harvest was estimated at approximately 174 million metric tons in 2017 (FAO 2018). Of this volume, the major portion was used as food for human consumption, with most of it derived from aquaculture. China's aquaculture industry, for example, quadrupled over the past 20 years to produce over 40 million tonnes of fish. The FAO has described global aquaculture as the fastest growing food-producing sector, now providing more than 50% of the world's fish used as food (FAO 2017).

## 11.3 Fish and Shellfish Processing Discards

Fish and shellfish harvesting and processing is accompanied by the generation of large quantities of discards in various forms, either as by-catch (considered as “trash” fish by commercial fishers) and damaged products that cannot be sold, or as “inedible” processing byproducts (e.g. heads, bones, fins, tails, viscera, waste water). As alluded to above, the discards are put to low value-added uses such as fish silage, fishmeal, and fish sauce; or used to a lesser extent to produce value-added products such as proteins, fish oil, amino acids, minerals, enzymes, bioactive peptides, collagen and gelatin, chitin and chitosan.

### 11.3.1 Types of Fish and Shellfish Discards

Fish and shellfish harvesting and processing discards come in two forms; the by-catch being fish species with undesirable characteristics and of little or no commercial value, and the inedible waste generated from the various processing operations mentioned above. Guerard (2007) described by-catch as those that cannot be sold, but can be further transformed into low value products such as fish meal, fish sauce, fish paste, and imitation seafood analogs; whereas the inedible waste were forms that were mostly composted or transformed into fish silage.

#### 11.3.1.1 Harvesting By-Catch

These are either by-catch or species with undesirable characteristics and little appeal to consumers. Several factors contribute to their non-preferred status by consumers, such as their high oil content, high bone content, peculiar odors, flesh color, or size. When caught, they are often used to produce bait, fish oil, fish meal, fish sauce, fish paste, and similar low-value products. For example, anchovies caught off the coasts of Peru

and Chile represent up to 10% of the total marine catch, but they are used mainly to produce fish sauce, fish meal, or fish oil. However, it must be noted that the uses to which this category of discards are put vary greatly among countries. Certain species that may not be desired by inhabitants of some countries are much desired by certain populations. For example, mackerel is better perceived in Japan than it is in Canada where it is often transformed into fish oil. In Canada, by-catch from the fish and seafood harvest include capelin, cunner, herring, mackerel, and green crab.

#### 11.3.1.2 Processing Discards

Various reports have estimated fish and shellfish processing discards to range between 35% and 80% of the whole animal. This category of discards is generated by processing operations, and includes two main sub-categories, i.e. solid waste generated from de-heading, deskinning, deboning, evisceration, trimming, and filleting operations, and process wastewater (also known as stickwater or fish solubles). The trend toward more ready-to-eat fish and shellfish products makes production of this kind of waste quite abundant and steady. For finfish, the solid waste includes heads, bone and cartilage, skin, scales, fins and tails, blood, viscera. The process wastewater or stickwater includes water from washing steps and pressing exudates from the manufacture of fish meal. Shellfish processing waste depends greatly on the species. Crustaceans are mostly exploited for their meat leaving behind the hepatopancreas, heads, shells, carapace, legs, and tails. These parts constitute between 70% and 80% of the whole animal and are mostly discarded as waste in Canada and the US, although they are eaten with the meat in some cultures including China and Ghana. Other shellfish species like bivalves (e.g. oysters, mussel, and scallops), gastropods (e.g. snails and slugs), some echinoderms (e.g. sea urchins) generate shells as waste, while cephalopods (e.g. squids and cuttlefish) produce “pens”.

#### 11.3.1.3 Composition of Fish Discards and Some Current Uses

Fish and shellfish processing generate large quantities of several byproducts that include backbones (~14%), fins and lungs (~10%), guts (~7%), heads (~21%), livers (~5%), roe (~4%), and skins (~3%) (Ghaly et al. 2013). These components are all rich in useful nutrients and biochemicals such as digestive enzymes, proteins, fat, pigments, chitinous polymers, carotenoid pigments, flavorants, and anti-freeze proteins (Simpson 2000). According to Ramalingam et al. (2014), the discards also contain high amounts of nitrogen (6%), phosphorus (5%), and potassium (4%). These components can be used to sustain the growth of living organisms, i.e. plants, animals, and microorganisms (Ramalingam et al. 2014), or to produce value-added products of commercial relevance (Simpson 2007).

Fish guts or viscera are obtained from degutting by cutting fish longitudinally to remove blood and internal organs (i.e. stomach, intestine, pyloric ceca, gall bladder, liver, kidney, and gonads). Blood is removed by water washing to get rid of endogenous enzymes, pigments, and gut microorganisms that could all accelerate deterioration of fish meat. The stomachs, pyloric ceca, pancreas, and intestines, are rich in proteolytic enzymes such as pepsins, trypsins, chymotrypsins, collagenases, gastricsins, etc. (Poonsin et al. 2017; de Melo Oliveira et al. 2017; Simpson 2000), and lipases (Aryee et al. 2007; Noriega-Rodríguez et al. 2009).

Fish heads represent a significant portion of fish and shellfish processing waste. However, it is mostly dumped or used to produce fish protein hydrolysates. Due to the relatively high protein content, the head are often a good source of N that can also be used as feed supplement or fertilizer.

Fish filleting operations also generate large quantities of fish skins with the skins constituting about 3% of the waste. Fish skins have high fat and high collagen contents, but are low in ash. Gelatin is derived from collagen by partial hydrolysis, and the gelling properties of gelatin is exploited extensively in the food, pharmaceutical, and cosmetic industries. While the primary production of gelatin has been from pork skins and bovine hides, consumer concerns and varied diet restrictions has pushed the industry toward researching other sources of gelatin such as fish skins. In this connection, fish discards from species such as cod, pollock, and salmon have been shown to be good sources of gelatin.

After fish are filleted, a significant part of the meat is left attached to the fish bones. The meat present on these frames often have higher content of minerals than the filets. The meat scraps are often transformed into products such as fish burgers, fish sticks, fish dumplings. The bones are also rich in minerals, collagen (for gelatin) and hydroxyapatite (Sunil and Jagannatham 2016).

With regards to shellfish waste, there is a large body of research on shrimp heads which consist of about 74% of the whole animal. Shrimp represent one of the most commercially important shellfish species, with several shrimp fishing fleet very active worldwide (Gillet 2008). The composition of shrimp heads is like that of the shells with high protein, chitin, and mineral contents. Shells and pens form the backbone, support, and protection for the animal. The shells and pens constitute a major fraction of the waste generated in shellfish processing, amounting to about 80% of the shellfish waste. Chitin is a structural polysaccharide made up of several N-acetyl glucosamine units linked together by beta 1-4 glycosidic bonds. It is the second most abundant organic compound after cellulose and is quite ubiquitous in nature. In the realm of shellfish processing discards, shrimp species such as pink shrimp (*Pandalus borealis*) (also known as North Atlantic cold-water prawn), brown shrimp (*Crangon crangon*), and the Asian tiger shrimp giant, aka tiger prawn (*Penaeus monodon*), as well as crab species snow crab (*Chionoecetes opilio*) and the blue crab (*Callinectes sapidus*) are the main sources of the wastes. The discards from these crustacean species and lobster shells, as well as ink pens from cephalopods are all important sources of chitin and calcium carbonate. The chitin is commonly converted to its more versatile derivative chitosan, and these biopolymers have several commercial uses for making cosmetics, textiles, and as an antimicrobial agent. Crustacean shells also have bright red pigments known as carotenoids. These pigments impart the colors we associate with salmonid flesh and cooked crustacea. Wild fish have easy access to these pigments through the consumption of other small crustacea like krill and many other microorganisms. However, farmed animal species like salmonids and shrimp, must have the pigments incorporated in their diets as they are incapable of de novo synthesis of the pigments. Discards from crustacea in various forms can be included in the diets of farmed animals to impart color to their skins (Simpson et al. 2000). Among crustacea discards, shrimp waste is the commonly used for this purpose because of its abundance and popularity, as well as ease of handling.

Multiple other byproducts can be created from wastes fish and shellfish harvesting and processing waste. A variety of minces and hydrolysates can be extracted from the carcass

of filleted fish using enzymatic approaches. These minces have a high mineral and collagen content. Despite being esthetically unappealing, they can nevertheless be added in canned fish, surimi, and a variety of other foodstuff such as soups, pizzas, and pasta.

### 11.3.2 Non-Edible Uses of Fish and Shellfish Discards

Fish waste has also been used as fertilizers. Coastal regions of Canada such as Prince Edward Island traditionally use the fish wastes as a fertilizing material (MacLeod et al. 2006). The protein of fish and shellfish waste make them excellent N sources for the decomposers present in the soil. The shells of shellfish can also add calcium carbonate as an important mineral supplement for the soil. Another interesting byproduct that is the so-called “mussel mud” which is basically soil containing large quantities of broken mussel, oyster, quahog, and clam shells that is used as fertilizer.

Commercial fish and shellfish processing also co-produce large quantities of effluents or wastewater, whose treatment is tedious due to the high content in organic matter and salt (Cristóvão et al. 2015). Wastewaters contains significant amounts of N and P, and can also be used as eco-friendly fertilizer for certain agricultural practices (Rahman 2014).

Finfish skins have similar compositions to those of mammals, e.g. bovine and porcine (Koliada et al. 2014). The skins of certain eel species have long been used to produce leather for similar applications as bovine leather. This industry easily recuperates the skins of commercially important fish such as cod, salmon, carp, and tilapia to create a product that can be applied in bags, shoes, and belts.

Fish and shellfish by-catch and fish harvesting rejects may be transformed into fish meal for animal feed. Such discards and rejects are often homogenized then pressed to expel liquid. The byproduct thus obtained is then milled and dried to produce a dark brown powder. The benefit derived from this use is twofold: (i) as important nutrient source for animals, and (ii) as a means of reducing dumping to pollute the environment. Fish discards such as viscera, heads, and trimmings are high in protein, and in countries such as Norway and Sweden, discards from cod, haddock, flatfish, plaice, saithe, mackerel, sprat, and sardine are commonly used in aquaculture feeds for species such as trout and gilthead sea bream to provide them with important nutrients for growth (Ween et al. 2017). Incorporating fish and shellfish discards also adds omega-3 fatty acids in the diets fed to the animals. Although made up of proteins predominantly, fish meal also contains several essential oils, vitamins and minerals for livestock and aquaculture animals’ health and welfare (Oliva-Teles 2012). The main drawback of this source material is its high content of heavy metals such as Pb and Hg that could accumulate in fish tissue and pose health risks to consumers.

Fish oil is a byproduct of fishmeal production from fish harvesting and processing discards. After the solids have been pressed and milled, the liquid homogenate is centrifuged to separate the oil from the aqueous phase (NSF 2010). Fish oil represents an important commodity in the fish processing sector. Produced first in the Scandinavian countries and North America in the early 1800 (FAO 1986), production is now widespread globally with Peru and Chile as major producers. In 2016, non-official estimates of global fish oil and fishmeal production suggested that about 25–35% of the products were derived from fish and shellfish byproducts and processing discards (Seafish 2018).

Demand for fuel worldwide has grown steadily in tandem with the growing global population. The variability in the supply of fossil fuels since the mid-1970s, coupled with their non-renewable nature, and undesirable environmental effects, have all given impetus to the search for alternative fuels such as biodiesel (Aryee et al. 2011; Canakci 2007). Biodiesel is alkyl esters of fatty acids, and has been portrayed as a suitable, greener, biodegradable, and sustainable alternative to fossil fuel (Meher et al. 2006). Biodiesel is produced from animal (livestock and fish) and vegetable (e.g. jatropha, palm oil, soybean, and canola) oils, as well as used frying oils, and oils produced from microorganisms (Demirbas 2009; Silva et al. 2010). As alluded to above, the fish and shellfish harvesting and processing sectors generate large quantities of discards that are either put to low value use or discarded as waste. It is estimated that global fishery processing practices generate between 25% and 60% of the whole animal as waste (Falch et al. 2006). Biodiesel may be produced via transesterification with chemicals (either acids or bases), or with lipase enzymes. As well, wastewaters contain large amount of solid organic wastes which can be fermented into biomethane for use as a household heating fuel (Achinis et al. 2017).

## 11.4 Seafood Discards as Substrates for Microbial Enzyme Production

Enzymes are biological molecules that accelerate biological reactions. They catalyze synthesis of other biomolecules, or their break down into products. Enzymes do this by binding with substrates to form transient complexes that may continue to form the products or decay to regenerate the substrates. Enzymes are traditionally derived from animals, plants, and microorganisms; however, commercial enzymes are mostly produced from microorganisms.

Animal and plant sources account for only about 8% and 4%, respectively, of commercial enzymes. Animal enzymes are commonly obtained from cow and pig pancreas, liver and stomach. Nonetheless, homologous enzymes from other animals, e.g. fish, are also of interest for commercial uses because the broader range of environmental conditions in their habitat have endowed them with enzymes that are better suited to different conditions (e.g. pH, temperature, salinity) versus homologous enzymes from mammals. Some common plant enzymes include papain, a protease from papaya for use in: the clarification of beverages and the tenderization of meats, as a digestive aid, in bathing, and as debriding agent for the cleaning /treating of wounds; ficin, a protease derived from the fig tree widely used as a meat tenderizer; and bromelain, also a protease derived from pineapple stems used as meat tenderizer.

The greater use of microorganisms for enzyme production is due to their ready availability and less restrictions from agricultural, environmental, and regulatory policies that influence large scale cultivation and harvesting of animals and plants as source material for enzymes. Microorganisms are also easier and cheaper to produce in predictable and sustainable quantities. Fungi form the predominant source of commercial enzymes (~50%); examples are *Aspergillus*, *Candida*, *Mucor*, *Penicillium*, and *Saccharomyces*. Bacteria follow fungi as the second major commercial enzymes sources (~30%), with examples such as *Bacillus*, *Klebsiella*, *Lactobacilli*, and *Streptomyces*.

The global industrial enzymes market was valued US \$6.1 billion in 2017 and is expected to reach US \$8.5 billion by 2022. Several factors influence cost-effective production and use of enzymes for industrial processes. There has been an upsurge of interest and extensive research on microbial enzymes for industrial applications. In addition to the relative advantages of using microorganisms for commercial enzyme production, there is also the important consideration that microorganisms can now be readily manipulated via genetic engineering or other approaches to produce commercial enzymes more abundantly and consistently; or tweaked to adjust their performance characteristics to suit certain applications. In terms of microbial enzyme production, the formulation and cost of the culture media are also crucial because they can affect enzyme concentration and yield, as well as cost of the products formulated with the enzymes. Fish and shellfish byproducts, such as heads, guts, frames, trimmings, skins, exoskeletons, and, wastewater, etc. are rich in nutrients (C, N, and minerals such as Ca), that can be used in formulating excellent and inexpensive media to sustain growth of microorganisms for enzyme production. These materials are generally used after various pretreatments, e.g. boiling followed by filtration to recover peptones in the soluble supernatant; or by cooking, mincing, pressing, and drying into powdered forms. In other cases, the raw materials are first defatted with solvent, then subjected to hydrolysis with acid, alkali, or proteolytic enzymes (Rebah and Miled 2013; Triki-Ellouz et al. 2003). The various products obtained from the pre-treatments are then incorporated in microbial culture media to grow bacteria such as *Bacillus cereus* and *Bacillus subtilis* for protease production (Esakkiraj et al. 2009; Ellouz et al. 2001), or the fungus *Rhizopus oryzae* for lipase production (Ghorbel et al. 2005).

#### 11.4.1 Microbial Proteases

Proteases (also known as proteolytic enzymes) are hydrolases and they catalyzes the breakdown of peptide bonds in proteins and polypeptides to produce low molecular weight peptides and amino acids. Proteases represent over 60% of the global enzyme market (Banik and Prakash 2004), and they have been produced from several different microorganisms such as *B. cereus*, *Bacillus licheniformis*, *B. subtilis*, *Pseudomonas aeruginosa*, *R. oryzae*, *Serratia marcescens*, *Streptomyces* sp., *Vibrio parahaemolyticus*, etc. (Joo and Chang 2005; Vazquez et al. 2006). Proteases have a plethora of applications in the biomedical, cosmetic, detergent, food, leather, and pharmaceutical industries, etc. (Banik and Prakash 2004). Various studies have shown that processing byproducts and wastewater from fish and shellfish are good and inexpensive nutrient sources for microbial growth to produce proteases. For example, fish and shellfish heads, viscera and peptones from various marine species, e.g. rainbow trout, squid, sardine, and tuna were incorporated in media for successful protease production by various microorganisms such as *P. aeruginosa*, *B. subtilis*, *Bacillus mojavensis*, and *Vibrio* sp., (Ellouz et al. 2001; Triki-Ellouz et al. 2003).

Furthermore, supplementation of microbial culture media with shellfish material rich in chitin and minerals (e.g.  $\text{CaCO}_3$ ), e.g. shrimp and crab shells, cuttlefish and squid pens, has also been shown to be effective in enhancing microbial growth and protease production by several microorganisms including *Bacillus* sp., *Chryseobacterium* sp., *Lactobacillus paracasei*, *P. aeruginosa*, and *Serratia ureilytica* (Souissi et al. 2008; Bhadra et al. 2005). Microbial proteases have wide ranging applications in industry. In the food

industry, they are used in cheesemaking, baking, brewing, protein hydrolysates, and the clarification of xanthan gum. The enzymes are also used in various other industries such as the detergents, leather, cosmetic, and biomedical industries (Aguilar and Sato 2018).

Microbial proteases have several uses as industrial biocatalysts for the food (e.g. baked goods, cheeses, fermented products, etc.), and in the cleaning (detergents), textiles (desizing), and leather (bating) industries (Contesini et al. 2018; Paul et al. 2014; Ward et al. 2009). Microbial proteases are also used to clarify xanthan gum for use as a viscosity control agent in the food, pharmaceutical, cosmetic, and related industries (Contesini et al. 2018).

#### 11.4.2 Microbial Lipases

Lipases like proteases, are hydrolytic in action. However, unlike proteases, lipases catalyze the breakdown fats into simpler molecules, primarily the hydrolysis of triglycerides into glycerol and fatty acids. Lipases also catalyze transesterification reactions. Various animal (e.g. mammals, insects, fish), plant, and microbial lipases have been described. Animal lipases include mammalian, fish and avian gastric and pancreatic lipases (López-Amaya and Marangoni 2000); and insect lipases that are found in their eggs, plasma, salivary glands and muscles (Pahoja and Sethar 2002). Plant lipases occur in several plant tissues such as seeds and bran. Microbial lipases have been reported in microorganisms including several species of fungi, e.g. *Aspergillus carneus*, *Absidia corymbifera*, *Aspergillus fumigatus*, *Rhizopus chinensis*, *R. homothallicus*, *Penicillium* sp., *Geotrichum* sp., and *Rhizomucor* sp., *Mucor pusillus*, *S. thermophile*; yeasts, e.g. *Candida antarctica*, *Caulerpa cylindracea*, *Candida rugosa*, *Rhodolultal glutinis*, *Pichia xylosa*, *Yarrowia lipolytica*, *Saccharomyces crataegensis*, *Torulaspora globose*; and bacteria, e.g., *Bacillus alcalophilus*, *Bacillus coagulans*, *B. licheniformis*, *B. subtilis*, *P. aeruginosa*, *Staphylococcus caseolyticus* (Thakur 2012). The increasing utilization of lipases in industrial operations has given a fillip to the search for novel lipases. In this regard, microorganisms are the obvious and preferred candidates. Hitherto, supplementation of microbial culture media with material derived from fish and shellfish processing discards has proven beneficial in enhancing production of lipase enzymes by microorganisms (Rebah and Miled 2013). Thus, peptones from both fish (e.g. sardine and tuna) and shellfish (e.g. cuttlefish and shrimp) species were incorporated in microbial growth media to increase lipase production by *Staphylococcus epidermidis* and *Staphylococcus xylosus* (Rebah and Miled 2013). Microbial lipases are considered as the most widely used biocatalyst for applications in industries such as food, oil and fat, detergent, leather, textile, pulp and paper. Lipases are also used for organic synthesis, and production of cosmetics and perfumery, and biodiesel (Andualema and Gessesse 2012; Pahoja and Sethar 2002; López-Amaya and Marangoni 2000).

#### 11.4.3 Chitinolytic Enzymes

Living organisms, including animals, insects, higher plants, bacteria, and fungi, are all known to produce chitin degrading enzymes that breakdown chitinous polymers into various low molecular weight forms. Examples of chitinolytic enzymes include chitinases, chitosanases, chitin deacetylases, and N-acetylglucosaminidase. Chitinolytic enzymes are of interest for biotechnological applications for reasons such as their use

in agriculture to control plant pathogens. Chitinases also break down chitins into chitin oligomers that are used in human health care as immune-enhancers, and as antimicrobial and antitumor agents.

Commercial use chitinolytic enzymes depends on their availability as highly active preparations at reasonable cost. Again, microorganisms are the obvious candidates to achieve these effects. Thus, use of fish and shellfish waste as inexpensive source of C and N to support the growth of microorganisms for production of chitinolytic enzymes is beneficial. Chitinous materials from marine sources are excellent sources of C (as chitin) and N (proteins, amino acids, peptones), and can serve as good inducers for chitinolytic enzyme production by microorganisms. In this connection, shellfish waste materials were incorporated in media for chitinolytic enzyme production by bacteria such as *B. cereus* YQ308, *B. subtilis* W-118, *Monascus purpureus* CCRC31499, and *P. aeruginosa* K-187.

Chitinolytic enzymes from microorganisms have applications in waste management, biocontrol in agriculture, and in biomedicine (Stoykov et al. 2015).

#### 11.4.4 Ligninolytic Enzymes

Lignins are complex organic polymers that occur abundantly in nature as important structural materials in plant tissues and in some algae (Kirk and Farrell 1987). Lignins are highly recalcitrant and resistant to hydrolysis by both acids and alkalis. However, they are degraded by a complex of enzymes collectively known as ligninolytic enzymes. Ligninolytic enzymes include various peroxidases (e.g. lignin peroxidase, manganese peroxidase, heme peroxidase), oxidases, and copper-dependent laccases (Martinez et al. 2009). Ligninolytic enzymes oxidize lignins, thus can act on and degrade lignins and harmful xenobiotics such as pesticides, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs). Thus, ligninolytic enzymes are versatile and can find several important applications in several sectors including the agricultural, fuel, paper, and textile industries (Rodríguez and Toca Herrera 2006). Although the enzymes are produced by various living organisms, their production from microorganisms is much more cost effective. And the use of inexpensive nutrients to promote of the appropriate microorganisms to produce the enzymes is desirable. Hitherto, fishery processing waste have been used to promote growth and production of various ligninolytic enzymes, viz., lignin peroxidase, manganese peroxidase and laccase, by the bacteria *Phanerochaete chrysosporium* BKM-F-1767 (Gassara et al. 2010).

Ligninolytic enzymes from microorganisms have been used in the manufacture of paper, animal feed, and bioethanol (Plácido and Capareda 2015).

#### 11.4.5 Microbial Transglutaminases (TGases)

Transglutaminases (or TGases), EC number EC 2.3.2.13, have the systematic name, protein-glutamine  $\gamma$ -glutamyltransferase. They catalyze acyl transfer reactions between the  $\gamma$ -carboxamide groups of glutamine residues in peptides, and primary amines or water molecules (Zhang et al. 2017). TGases are widely distributed in tissues of living organisms such as vertebrate (e.g. guinea pig and several fish species) and invertebrate animals (e.g. Antarctic krill), plants, and microorganisms (Zhang et al. 2017; Binsi and Shamasundar 2012; Serafini-Fracassini and Del Duca 2008; Della Mea et al. 2004;

Yokoyama et al. 2004). Although TGases are widespread in nature, commercial TGases are derived from limited sources, mainly from guinea pig liver and microorganisms such as *Streptomyces* sp. and *Streptomyces* sp. (Zhu and Tramper 2008). Wastewater generated from surimi preparation is a rich and inexpensive C and N source for microbial growth and proliferation. H-Kittikun et al. (2012) incorporated threadfin bream processing waste in microbial media for the cultivation of *Enterobacter* sp. C2361, *Streptomyces moharaense*, and *Providencia* sp. C1112, and successfully demonstrated varying degrees of production of MTGase by these microorganisms.

MTGases are used in food industry to restructure small meat and fish pieces into larger chunks to improve their texture and shelf-life, for improving the functional characteristics of milk and other dairy products, and for the manufacture of tofu, among others. It is also used in the pharmaceutical industry such as the generation of site-specific antibody drug conjugates (Rickert et al. 2016), as well as in the textile industry to soften wool, smoothen leather and silk and increase the wettability of fabrics. The enzyme also enhances the shrink resistance of the wool and it improves the tensile strength of the wool fibers (Asmamaw and Assefa 2014).

#### 11.4.6 Microbial Alpha-Amylases (or $\alpha$ -Amylases)

Alpha-amylases (or  $\alpha$ -amylase) are hydrolytic enzymes that randomly cleave  $\alpha$ -1,4 glycosidic bonds within large polysaccharide molecules such as starch and glycogen, to produce low molecular weight byproducts, i.e. glucose and maltose.  $\alpha$ -Amylase is ubiquitous in nature and has been isolated from animals, plants, and microorganisms. Plant  $\alpha$ -amylases have been produced from barley, cassava, rice and malt (Sundaram and Murthy 2014). Animal  $\alpha$ -amylases include those from bovine and swine pancreas.

Then,  $\alpha$ -Amylase is produced by several bacteria, fungi, and genetically modified species of microbes. The most widely used microbial source for commercial production of the enzyme is *Bacillus amyloliquefaciens* and *B. licheniformis*. Others bacteria sources of the enzyme include *B. cereus* and *B. subtilis*. Only very few fungi are used as sources of  $\alpha$ -amylase. These fungi include *Aspergillus* sp (e.g. *Aspergillus oryzae*, *Aspergillus niger*, and *Aspergillus awamori*) and *Penicillium* sp (e.g. *Penicillium chrysogenum*, *Penicillium fellutanum*, *Penicillium expansum*).

Al-Asady (2016) used microbial medium comprised of potato peels (as C source) fish waste (as N source) to cultivate *B. licheniformis* for production of extracellular  $\alpha$ -amylase.

Microbial  $\alpha$ -amylases find widespread use as digestive aids, for desizing of textiles, in baked goods, in detergents, and in animal feed, as well as for starch liquefaction to produce starch syrups, glucose, and maltose. It is also used in paper making to coat paper, and in fuel alcohol production (de Souza and de Oliveira 2010).

### 11.5 Conclusions

Fishery waste are abundantly available, and their disposal poses challenges to environmental health. Nonetheless, these discards are rich in nutrients and various other useful components that may be recovered and put to profitable use. One such use is as source

material for the nutrients and energy that are needed to sustain the growth and proliferation of microorganisms for commercial enzymes production. Various microorganisms including bacteria, fungi, and yeasts, have been grown in submerged and semi-submerged systems to produce a wide range of microbial enzymes including various hydrolases (e.g. proteases, lipases, amylases, chitinases, and pectinases), oxidoreductases (e.g. peroxidases, laccases, oxidases), and transferases (e.g. transglutaminases), etc. Conditions and media for growth of microorganisms selected for the production microbial enzymes must first be optimized with respect to pH, temperature, aeration, agitation, as well as C and N contents, etc. to ensure the enzymes can be produced cost-effectively. Nonetheless, microbial enzymes are still generally easier, cheaper, and faster to produce versus their homologs from plant and animal sources. Once produced, microbial enzymes can be put to similar uses as their counterparts derived from plant and animal.

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## 12

### Animal Discards in Livestock Feed Manufacture

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#### 12.1 Introduction

Animal discards or animal byproducts are defined as all or any animal parts that are unfit for human consumption (Shirley and Parsons 2001). However, after processing (usually heat processing), these discards could be good sources of energy and nutrients for livestock. Depending on the nature of the products, they could be relatively cheap and readily available alternative sources of nutrient and energy for livestock. In addition to savings on feed cost, it also provides a good avenue through which livestock discards or byproducts, which could result in environmental pollution, are judiciously recycled, resulting in economic and environmental sustainability. It has been estimated (Rodehutscord et al. 2002) that for every kilogram of animal discards, about 1.4 kg of CO<sub>2</sub> and 0.2 kg of NO<sub>x</sub> are released into the atmosphere through incineration of meat and bone meal (MBM) and other livestock discards. Hence, subjecting animal discards to incineration is not a sustainable approach, both from the economic and environmental perspectives (Rodehutscord et al. 2002). Animal discards have been included in the diets of livestock as a source of energy and nutrients for several years. However, the interest and demand for animal byproduct declined significantly after the link between mad cow disease (*Bovine spongiform encephalopathy*, BSE) in cattle and MBM was established.

The livestock sector is highly dynamic, and it is evolving with the industrialization of animal agriculture. Feeding strategies, and the animal feed industry as a whole, have evolved in tandem with this growth. According to FAO (2010), livestock production has grown substantially since the 1960s, resulting in increased demand for animal products (FAO 2010). Global beef and milk production have more than doubled, egg production has increased by nearly four times, and chicken meat production has grown 10 times over (Speedy 2003). Combining the effects arising from breeding techniques and health of the animal with optimal nutrition and environmental sustainability, this upward trend is likely to continue. The increase in the consumption of animal-based food products conferred by population growth, is likely to increase the competition between humans and farm animals for food resources (CAST 2013; De Boer and Bickel 1988).

The current increase in global demand for cereal grains and protein sources for both humans and animals inadvertently reduces availability and increases cost. Data from FAO-STAT (2001) showed that 40% of cereals are fed to livestock in the United States, and globally, one-third of total world cereal is used in the livestock industry (Speedy 2003).

To compensate for the high feed cost associated with livestock feeding operation, producers are having to source for alternative feed ingredients. The quantity of animal byproducts available is estimated to be in excess of 30–40% after processing of animal food products (Ockerman and Hansen 1998; Denton et al. 2005) hence, the obligatory need to recycle safe, yet otherwise valueless discards from livestock enterprise to eliminate waste is essential. These products are further utilized as byproducts for both agriculture and industrial use (Jha and Prasad 2013, 2014). The United States of America's meat industry describes every waste generated during processing as a byproduct and these animal byproducts fall into two categories; edible and inedible (Ockerman and Hansen 1998). As of 2013, the US rendering industry generated more than 8 million metric tons of rendered animal products, including MBM, poultry byproduct meal (PBM), blood meal, and feather meal (National Renderers Association Inc. 2006; Sapkota et al. 2007). By effectively using these livestock discards, the livestock industry benefits from a potential revenue source, eliminates the need to incur increasing cost of disposing these products (Ockerman and Hansen 1998), and contributes to environmental sustainability.

Animal discards or animal byproducts are defined as all or any animal parts that are unfit for human consumption (Shirley and Parsons 2001). However, after processing (usually heat processing), these discards could be good sources of energy and nutrients for livestock. Depending on the nature of the products, they could be relatively cheap and readily available alternative sources of nutrient and energy for livestock. In addition to savings on feed cost, it also provides a good avenue through which livestock discards or byproducts, which could result in environmental pollution, are judiciously recycled, resulting in economic and environmental sustainability. It has been estimated (Rodehutscord et al. 2002) that for every kilogram of animal discards, about 1.4 kg of CO<sub>2</sub> and 0.2 kg of NO<sub>2</sub> are released into the atmosphere through incineration of meat and bone meal (MBM) and other livestock discards. Hence, subjecting animal discards to incineration is not a sustainable approach, from both an economic and environmental perspectives (Rodehutscord et al. 2002). Animal discards have been included in the diets of livestock as a source of energy and nutrients for several years. However, the interest and demand for animal byproduct declined significantly after the link between mad cow disease (*Bovine spongiform encephalopathy*, BSE) in cattle and MBM was established.

Furthermore, the inclusion of animal byproducts into livestock feeds has played an important role in animal feeding operations for many years, with the poultry industry being a major user of animal protein (Denton et al. 2005). Though plant-based protein sources supply most of the livestock's dietary requirement, they are considered nutritionally imbalanced, because they do not meet certain essential amino acids and vitamin B<sub>12</sub> requirements of some animals, reducing their biological value. Thus, supplementary sources of protein such as crystalline or synthetic amino acids or animal protein to augment amino acids from plant-based feed ingredients are added to the feed. In nursery pigs for example, reduced protein digestibility has been associated with poor growth performance and digestive disorders in pigs fed raw soybeans and soybean products (e.g. soybean meal, SBM) without proper heat treatment (Maxwell and Carter 2001). The antinutritional factor contents of plant protein sources, as well as transient

hypersensitivity to some antigens in the case of SBM, have been implicated in the decreased digestibility in pigs (Maxwell and Carter 2001). Animal protein sources, however, are excellent sources for calcium and phosphorus, B-complex vitamins (particularly riboflavin and B<sub>12</sub>) as well as amino acids, lysine, and methionine (Denton et al. 2005; Ravindran and Blair 1993), which makes them great supplements to cereal-based diets. The adequacy and usefulness of a protein feedstuff for poultry and livestock, will depend on its ability to provide high quality amino required for maintenance growth, and productive functions (Beski et al. 2015; NRC 2012). The objective of this chapter is to present information on the different classes of animal discards that are available as well as factors that could influence their use in the diets of livestock.

## 12.2 Animal Discards and Byproducts

Based on human population growth projection by WHO/FAO, animal protein consumption will continue to increase which will eventually lead to an increase in the quantity of animal discards that are generated annually. There are only two ways through which these discards could be handled, complete destruction or through recycling of the discards. The first approach may include incineration or composting. Although relatively straightforward, this procedure comes with some troubling consequences on the environment as well as the wellbeing of livestock and the human population. The second approach includes industrial use or incorporation into livestock feed. The later approach was highly embraced for several years until the BSE incident after which the interest in the use of the animal discards in livestock feed steadily declined. In addition to discards from the livestock industry, fish discards (e.g. fish meal), play an important role in livestock feed. The Food and Agriculture Organization (FAO 2017) defined fish discards or discarded catch (dead or alive) as the portion of the total organic material of animal origin in the catch, which is thrown away, or dumped at sea for whatever reason. It does not include plant materials and post-harvest waste such as offal (Kelleher 2005). Hence, animal or livestock discards could be defined as any portion of the total organic materials of livestock, including poultry that does not enter into the human food chain.

## 12.3 Regional Importance of Livestock Discards

Livestock and poultry are raised primarily to supply the much-needed animal protein (meat, egg, and milk) for human consumption. Meat and bone meal (MBM) is one of the most important animal discards that are important in swine and poultry feed both in terms of availability, cost, and nutrient and energy composition. One of the challenges with this discards is its energy and nutrient variability (Table 12.1). Moreover, in the 1980s and 1990s, its use in livestock feed hit a major setback as a result of BSE outbreak which was believed to have arisen as a result of animal products in the feed given to cattle (Wilesmith et al. 1991; Ducrot et al. 2013). In some European countries such as Germany, the use of discards from terrestrial animals in the feedstuffs for ruminants was banned in 1994, but a complete ban was placed on these products in 2002 (Rodehutscord et al. 2002) after the first case of BSE was discovered in Germany.

**Table 12.1** Nutrient and energy composition of 12 meat and bone meal samples sourced across United States of America.<sup>a</sup>

MBM ID	Source	Dry matter (g kg <sup>-1</sup> )	Crude protein (g kg <sup>-1</sup> )	Crude fat (g kg <sup>-1</sup> )	Phosphorus (g kg <sup>-1</sup> )	Calcium (g kg <sup>-1</sup> )	Ash, (g kg <sup>-1</sup> )	Gross energy, (kcal kg <sup>-1</sup> )
1	Beef parker slaughter material	921.2	496.7	91.1	61.7	145.8	381.9	3493
2	Beef parker slaughter material + some offal	963.7	512.4	97.7	46.5	106.4	317.4	3881
3	30% from bovine whole carcass + 65% from swine whole carcass	945.1	564.2	110.8	28.3	61.6	232.3	4469
4	Whole cattle carcass + small amount of mixed species	939.9	538.0	140.8	25.6	54.3	200.3	4661
5	Mixed species from processing of trims and bones	962.3	549.1	110.3	40.8	93.5	279.6	4107
6	About 70% beef, 30% pork + small quantity of poultry processing and whole bird carcass	982.1	619.1	96.9	26.7	61.6	202.7	4732
7	Mixed raw materials of about 50% beef and 50% pork. No whole carcass	979.3	542.5	63.3	43.4	102.5	291.2	4155
8	Exclusively from swine slaughter	990.7	537.5	115.5	39.4	88.0	261.4	4342
9	About 80% beef slaughter, 10% whole beef and swine carcass, and small quantity of poultry parts	989.4	535.7	106.5	36.1	84.3	248.2	4320
10	60% beef parker, 25% poultry, 15% grocery store treamings, outdated muscle meats and processed meat	971.9	525.4	120.5	36.8	85.1	250.3	4377
11	About 45% each of beef and pork slaughter materials with about 10% turkey slaughter of higher bone content	973.4	604.8	113.4	27.4	66.3	213.0	4671
12	Nearly all pork from parker processing sows for sausage	969.2	537.2	151.2	37.6	87.2	261.2	4490
Average		965.7	546.9	109.8	37.5	86.4	261.6	4308
Min		921.2	496.7	91.1	25.6	54.3	200.3	3493
Max		990.7	619.1	151.2	61.7	145.8	381.9	4732

<sup>a</sup> Adedokun and Adeola (2005).

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Phosphorus is the third most expensive component of feed given to swine and poultry (after energy and protein) and the requirements for this nutrient is typically met from the inorganic sources mostly from mining of rock phosphate which is not renewable (Rodehutscord et al. 2002). The uniqueness of MBM is that it is able to significantly contribute to energy, protein, and minerals (especially Ca and P) in the diet. Prior to the complete ban of MBM in Germany, it was estimated that about 57% of the phosphorus need of swine and poultry was met through MBM alone (Rodehutscord et al. 2002).

The quantity and quality of animal discards would vary with the source as well as the geographical location from which they are derived, hence the inclusion of these discards in the diets of livestock should be done with caution. Because the proportion of meat that is left on the bone would vary by the region of the world, MBM from the developing countries may be relatively low in crude protein and high in minerals (calcium and phosphorus) compared to MBM from the developed countries. The proportion of discards for meat processing facilities in the United Kingdom was estimated to be about 32% (Foxcroft 1984). With an estimated total meat production of 321 288 000 tonnes of carcass weight of meat produced worldwide in 2016, more than 102 812 000 tonnes would be designated as discards. This discard was projected to increase by more than 1 000 000 tonnes of carcass weight in year 2017 (FAO 2017).

## 12.4 Factors Influencing the Use of Animal Discards in Livestock Feed

Despite the preponderance of animal discards from several sources, their use in livestock feed is rather strictly controlled. A good example of this is the inclusion of MBM in the diets of livestock, in general but ruminants in particular. Since the outbreak of BSE, the European Union passed laws prohibiting the use of MBM in the diets of livestock. While Canada allows MBM to be included in the feed of ruminants, these are restricted to the MBM from swine and horses (Forge 2005). This, to a great extent, has resulted in a situation in which the inclusion of some of the animal discards in the diets of livestock could be said to be regional. Despite the abundance of animal discards that are rich in energy and nutrients, it is important to note that its use in livestock feed is driven primarily by its cost which is influenced by the prevailing market price of competitive feed ingredients such as SBM (soybean meal), inorganic sources of minerals (such as mono- and di-calcium phosphate, limestone), plant based lipid sources, and the nature of the discards. A high level of MBM, as well as other discards in poultry and swine diets, could result in a decrease in feed intake and performance as a result of a decrease in the palatability of the diet. Furthermore, the relative level of a certain class of nutrient or individual nutrient could play a role in whether or not an animal discard would be used in livestock feed. A good example is the level of Ca and P in MBM which could limit the quantity that could be included in a typical diet (Table 12.2). Some of the products from animal discards such as MBM, meat meal, and fish meal have excellent amino acid profile which in most cases rivals that of SBM and canola meal (Table 12.3). In the case of some animal discards such as feather meal, the poor digestibility and amino acid profiles would limit the level of inclusion in the diets of nonruminant (Miller and De Boer 1988).

**Table 12.2** Energy and nutrient composition of feed ingredients of animal origin.<sup>a</sup>

Ingredient	Gross energy (kcal kg <sup>-1</sup> )	Crude protein (%)	Crude fat (%)	Calcium (%)	Phosphorus (%)	Magnesium (%)	Copper (ppm)	Zinc (ppm)	Manganese (ppm)	Iron (ppm)	Selenium (ppm)
Meat meal	4497	56.40	11.09	6.37	3.16	0.35	10.00	94.0	10.00	440	0.37
Meat and bone meal	3806	50.10	9.21	10.90	5.30	0.41	11.00	96.0	17.00	606	0.31
Milk, lactose	4143										
Milk, whey permeate, 85% lactose	3657	3.00	0.20	0.27	0.34						
Milk, whey powder	3647	11.60	0.83	0.62	0.69	0.13	6.60	9.90	3.00	57.0	0.12
Poultry byproduct	5300	64.03	12.02	4.54	2.51	0.18	10.00	94.00	9.00	442	0.88
Poultry meal	NA	64.72		2.82	1.94		35.70	99.40	5.20	230	
Salmon protein hydrolysate	4713	90.79	2.12	0.09	0.84	0.07		54.13			6.29
Blood cells	5216	92.83	1.50	0.02	0.34	0.02	2.55	15.75	0.40	2675	1.00
Blood meal	5330	88.65	1.45	0.05	0.21	0.11	7.60	49.10	0.00	1494	
Blood plasma	4733	77.84	2.00	0.13	1.28	0.03	14.75	13.45	2.50	81.00	1.60
Egg, whole, spray dried	6283	50.97	34.26	0.29	0.69		1.80	43.70	0.00		61.00
Feather meal	5467	80.90	5.97	0.41	0.28	0.20	10.00	111.00	10.00		76.00
Fish meal, combined <sup>b</sup>	4496	63.28	9.71	4.28	2.93	0.13	8.00	88.98	38.90		411
Gelatin	5645	100		0.00							

<sup>a</sup>Nutrient Requirements of Swine (NRC 2012).<sup>b</sup>All species.

**Table 12.3** Dry matter, crude protein, and amino acid contents of some animal discards, soybean and canola meals, %.<sup>a</sup>

	MBM <sup>b</sup>	Meat meal	Blood meal	Feather meal	Fish meal <sup>c</sup>	Soybean meal <sup>d</sup>	Canola meal <sup>e</sup>
Dry matter	95.16	96.12	93.23	94.24	93.70	89.98	91.33
Crude protein	50.05	56.40	88.65	80.90	63.28	47.73	37.50
<i>Essential amino acid</i>							
Arginine	3.53	3.65	3.83	5.63	3.84	3.45	2.28
Histidine	0.91	1.24	5.39	0.82	1.44	1.28	1.07
Isoleucine	1.47	1.82	0.97	3.63	2.56	2.14	1.42
Leucine	3.06	3.70	11.45	6.59	4.47	3.62	2.45
Lysine	2.59	3.20	8.60	2.00	4.56	2.96	2.07
Methionine	0.69	0.83	1.18	0.59	1.73	0.66	0.71
Phenylalanine	1.65	1.98	6.15	3.95	2.47	2.40	1.48
Threonine	1.63	1.89	4.36	3.72	2.58	1.86	1.55
Tryptophan	0.30	0.40	1.34	0.60	0.63	0.66	0.43
Valine	2.19	2.61	7.96	5.75	3.06	2.23	1.78
<i>None essential amino acid</i>							
Alanine	3.87	3.82	7.29	3.90	3.93	2.06	1.61
Aspartic acid	3.74	4.28	7.78	4.95	5.41	5.41	2.56
Cysteine	0.46	0.56	1.26	4.32	0.61	0.70	0.86
Glutamic acid	6.09	7.03	7.18	8.40	7.88	8.54	6.35
Glycine	7.06	5.98	3.69	7.08	4.71	1.99	1.80
Proline	4.38	3.92	5.03	10.16	2.89	2.53	2.02
Serine	1.89	1.99	4.64	8.18	2.43	2.36	1.49
Tyrosine	1.08	1.35	2.66	2.12	1.88	1.69	1.06

<sup>a</sup> Nutrient requirements of swine (NRC 2012).<sup>b</sup> Meat and bone meal, >4% phosphorus.<sup>c</sup> Combined for all fish species.<sup>d</sup> Dehulled, solvent extracted.<sup>e</sup> Solvent extracted.

## 12.5 Production and Composition of the Animal Discard or By Product

About 3 and 1.5 million tons of MBM and animal fat, respectively, were produced in the European Union, EU-15 (EURA 1999). The chemical composition of MBM is variable and its composition and quality will largely be dependent on the raw materials, geographical location as well as the processing conditions (Rodehutscord et al. 2002). The nutrient composition of most of these products is dependent on the nutrient contents of the animal discards from which they are derived. For example, the level of Ca and P (usually

<10% and 4%, respectively) places an upper limit on how much of this discard can be included in the diets of swine and poultry. High levels a level of Ca and P in a diet, especially if their ratio is outside of the 1:1 to 2:1, could create problems with the digestion and absorption of several minerals in the gastrointestinal tract. Another factor is the level of fat in the discards. Animal discards such as MBM, meat meal, and eggs have a relatively higher level of crude fat (10–35%) compared to other discards. This high level of fat would also minimize the level of inclusion in livestock feeds as a result of a potentially high energy value of the discards. Fat, especially from MBM, could easily be oxidized with increasing storage time of feeds in the absence of or an insufficient level of antioxidants in the diet (Chanadang et al. 2016). Furthermore, oxidative peroxidation could result in intestinal damage and a decrease in nutrient and energy utilization.

## 12.6 Economic Importance of Animal Discards

In our quest to meet the demand for animal protein, livestock production has steadily been on the rise. With an increase in the competition for grains and cereals, the cost of livestock feed is also on the rise. This could potentially lead to a situation in which animal protein gets too expensive and is prized out of the reach of an average consumer. In addition to the fact that one of the easiest ways to get rid of these discards is to recycle them by including them in the diets of livestock, the use of animal discards also influences the economics of animal production. When animal discards are included in livestock feeds, it can bring down the cost of feed. For example, when MBM is used to supply a portion of the crude protein (and to some extent Ca and P) in the diets of swine and poultry, a subsequent decrease in the level of SBM (as well as inorganic sources of Ca and P) that would be required, hence, this could lead to a reduction in feed cost because SBM is usually more expensive than MBM. Additionally, if included in the diet at recommended levels (Hill et al., 1998; Miller and De Boer, 1988; Reese et al. 1995), it could improve the palatability of the diets (Lessire et al. 1985).

## 12.7 Livestock Discards and Feed Formulation

In general, livestock discards and byproducts of animal origin are mostly organic with high moisture content. Hence, they are subject to deterioration within a short period of time if not properly processed and stored. By virtue of the nature of the discards, they can only be incorporated into livestock fees after proper processing. Processing helps in decontaminating the raw materials as well as enhancing the value of the discard by concentrating the nutrient, as well as increasing the shelf value of the final product. The lack of sufficient resident intestinal microbes in the fore- and mid-gut of monogastric animals compared to ruminants, makes it essential to have adequate levels of all the six classes of nutrients in the feed given to poultry and swine with ad libitum access to water. This is important for three reasons: (i) to meet nutrients and energy requirements; (ii) to reduce feed cost and maximize net return on investment without negatively impacting growth, performance, and reproductive efficiency; and (iii) to minimize the amount of nutrients that is excreted into the environment. In order to formulate diets that meet these

conditions, it is important to have access to information on nutrient and energy requirements. This information is available in different Nutrient Requirements Tables as well as from the animal different breeding companies.

The main challenge is the predictability of the quality of nutrients in most of the animal discards. Depending on the major components of a diet, especially when it is corn-SBM-based, lysine and methionine are the top two amino acids that could be limiting if such diet is fed to poultry or swine. Because of the nature of these discards (organic with high moisture level), heat processing is an important procedure that these discards must be subjected to. However, considerable high temperature could negatively influence the quality of some of the nutrients, most importantly, lysine (Johnson et al. 1998). Although MBM is high in lysine (2.59%, NRC 2012), the availability or usefulness of this lysine to swine and poultry is a function of the level of the Maillard reaction that may have taken place during heat processing. If a diet is formulated on the assumption that the lysine content is highly available, then the requirements for lysine will not be met. If this is the case, the utilization of the other amino acids for protein synthesis will be negatively affected because the requirements for the first and subsequently second (methionine or lysine) limiting amino acids are not met (Waibel 1959; Kanbe 1996; Uzu 1982). This process is based on the ideal protein concept, which compares the abundance of amino acids relative to the lysine level of the diet (Fuller and Chamberlain 1985). When the ideal amino acid ratio is different from what is should be, performance of an animal is negatively impacted and a relatively higher level of nitrogen may be excreted into the environment. In addition to lysine and methionine, close attention should also be paid to threonine, tryptophan, and the sum of the sulfur containing amino acids (methionine + cysteine; Kanbe 1996). These are generally the next limiting amino acids in a typical corn-SBM-based diets, hence their levels in any animal discard that would be included in the diet of nonruminants should be evaluated. The species of livestock, age of the animal, and the nature of the animal discards (Miller and De Boer 1988; Reese et al. 1995; Hill et al. 1998) will influence the level of inclusion of different discards in livestock feed.

It is a generally accepted that not all the nutrients in a particular feed ingredient is available to the animal. This has led to a situation in which diets are formulated on digestible rather than on total amino acids (Adedokun et al. 2007; NRC 2012). In order to optimally use animal discards in the diets of nonruminants, digestible rather than total values should be used. This allows diets to be formulated based on the nutrients that is actually available to the animal, which enhances our ability to formulate diets that closely meet the animal's requirements which may lead to a reduction in nutrient excretion. In addition to its use in livestock feed, 25% of rendered products produced in the United States is used in commercial pet foods (Watson 2006).

## 12.8 Meat and Bone Meal and Meat Meal

Meat and bone meal (MBM) tankage, as well as meat meal and meat meal tankage are used to describe different types of animal discards that are composed primarily of meat and bone or meat alone. Tankage contains "blood", while the "meal" does not (Chiba 2001). On the other hand meat meal is similar to MBM except that, in general, the level of phosphorus (>4%) and calcium are higher in MBM, while the levels of crude protein

and crude fat are usually higher in meat meal compared to MBM (Chiba 2001; NRC 2012).

Meat and bone meal (MBM) is defined as rendered animal offal, which may include restaurant grease, plate waste, trimmings and bones, viscera and digesta, blood, heads, hooves, hides, and dead livestock that are considered unfit for human consumption (Kirstein 1999; Shirley and Parsons 2001; Narodoslawsky 2003). In general, the crude protein contents of MBM is relatively high, with a range of between 42% and 65% has been reported by Batterham et al. (1970) and Evans and Leibholz (1979). This huge range (23%-point) has been attributed to variations in the proportion of bones in a particular MBM meal sample. Bone could account for between 21 and 61% of MBM (Batterham et al. 1970). This variation can be attributed to the source of the MBM as well as the proportion of dead animals in the MBM. MBM is one of the most popular and abundant animal discards. The importance of the quality and profile of amino acids in MBM to animal performance cannot be over-emphasized, because it closely resembles the amino acid profile of the expected product (meat and to a lesser extent egg; Table 12.3). In addition to this, animal proteins, in general, are good sources of Ca and P (Table 12.2) as well as vitamin B<sub>12</sub>. Despite this, however, the quality of MBM varies widely depending on the source and the processing technique, especially heat treatment, which the original material has been subjected (Cromwell 1998). The vast majority of the poultry discards in MBM come predominantly from broiler (>60%) and turkeys (>30%) with minimal overall contributions from water fowls (Rodehutscord et al. 2002).

## 12.9 Blood Meal

The total volume of blood in different species of livestock varies with the size of the animal and it is proportional to the body weight but not to the surface area (Courtice 1943). Blood is the red colored liquid that is comprised of all the six classes of nutrients. This component of an animal is completely discarded except in some parts of the world where blood meal is consumed by humans. In order to efficiently use this discard, it is subjected to extensive heat treatment to get rid of the moisture and to eliminate any potential biological contamination (Campbell 1998), as well as to increase its shelf life. Unlike meat meal and MBM, dried blood meal is usually not included in swine diets, because it is low in lysine. It also has the potential to reduce feed palatability (Miller 1990). In order to improve the level of lysine and the palatability of diet containing blood meal, spray-drying, and flash drying methods as against vat cooking method are employed (Miller 1990; Cromwell 1998). In addition to the effect of processing temperature on lysine availability and the issue with palatability, the relatively high level of leucine in blood meal (Chiba 2001; Johnson et al. 1998) is another factor that limits the inclusion level of blood meal in livestock feed. The inclusion of 2.5% of spray-dried blood meal in the diets of weanling pigs have been shown to have resulted in superior weight gain compared to other protein sources (Kats et al. 1994a). Better nitrogen retention in pigs fed diets with blood meal supplementation compared to pigs fed a SBM-based diet has also been reported (Parsons et al. 1985). Miller and De Boer (1988) and Reese et al. (1995) provided information on the recommended level of inclusion of some animal discards in livestock feeds.

## 12.10 Poultry-Byproduct Meal

For a successful livestock production, improving the efficiency of feed utilization is important to maximize animal performance and profit. To achieve this, supplying essential nutrients without exceeding the requirements is important. This is especially true for energy and protein sources which are classified as the major components of feed in livestock production. Currently, SBM (CP 40–48%) is the major source of high-quality vegetable protein for feeds in the poultry industry. With its excellent amino acid profile and high level of digestibility, it has become a standard against which other protein sources are compared (Chiba 2001). However, due to the high demands of soybean brought about by increased competition and exports, SBM has become an expensive commodity. As an economically conservative industry, the primary force that drives changes in livestock feeding strategies is the cost of production, especially feed costs. Thus, the economic production of cost-effective nutritionally balanced diets would justify the inclusion of alternative feed ingredients in the diets, provided animal performance is not compromised.

To make the most efficient use of resources to maximize sustainability in animal agriculture, the postharvest processing stage becomes a vital part. The poultry-processing industry generates enormous quantities of offal that can be effectively converted into a useable commodity. Poultry byproducts such as poultry by-product meal (PBM) are a derivative of this process and often used as an alternative source of protein from animal origin. PBM by definition, is a dry rendered product from carcass of slaughtered poultry such as condemned or discarded chickens, necks, feet, undeveloped eggs and organs, except for feathers (Ockerman and Hansen 1998; Cruz-Suárez et al. 2007; Najafabadi et al. 2007). The rendering processes involves the use of a high-pressure processing equipment that thoroughly cooks the offal at high temperatures ranging from 120 to 140 °C, to extract the moisture and excess fat (Cruz-Suárez et al. 2007; Denton et al. 2005). The remaining product often referred to as “crackling” back in the early 1900s, is dehydrated and ground into a fine powder and sold as PBM and has been shown to enhance growth rate compared to animals fed diets containing only plant proteins (Denton et al. 2005). Variation in the composition of PBM and the quality of the finished product has largely been attributed to variability in raw material composition and their quality, as well as the rendering process (Najafabadi et al. 2007). Over the years, improvements have been made in the rendering process, all of which has been aimed at creating a consistent chemical composition and quality nutrient profile (Cruz-Suárez et al. 2007; Cortes Cuevas et al. 2011). To be considered high quality, the calculated chemical composition of PBM is usually in the range of 58% to 63% crude protein, 12% to 23% ether extract, and 18% to 23% ash (Cortes Cuevas et al. 2011; Senkoylu et al. 2005), and these levels vary depending on the manufacturer (Dong et al. 1993). The calcium to phosphorus level cannot be more than 2.2 : 1.0, and not more than 4% acid-insoluble ash (Pesti 1987; Senkoylu et al. 2005; Cruz-Suárez et al. 2007).

PBM has long been utilized by the poultry (Escalona and Pesti 1987; Senkoylu et al. 2005; Hosseinzadeh et al. 2010), swine (Keegan et al. 2004; Zier et al. 2004), pet (Yamka et al. 2003), and aquaculture (Turker et al. 2005) industries as a nutrient substitute, or, in the case of ruminants, as a ruminally undegradable protein source, due to their high-quality protein, palatability, calcium, vitamin B<sub>12</sub>, and phosphorus contents. Several authors have documented the successful use of PBM in both the broiler and laying hen diets as far back as the 1950s. Wisman et al. (1958) reported that by incorporating 20% of the crude protein

in a typical broiler or layer diet to provide 3% of the crude protein needed, PBM increased growth and improved feed efficiency, where no additional protein supplement was added. Furthermore, previous work observed no differences in body weight gain or feed efficiency when PBM was incorporated at 5% of the crude protein needed in an isocaloric, isonitrogenous corn-SBM-based practical diets, compared to those fed the animal protein-free control diet. However, in excess of the 5% level, PBM causes a slight depression in performance (Escalano and Pesti 1987; Pesti 1987; Hassanabadi et al. 2008). Inclusion levels up to 7.5% of PBM in laying hen diets had no effect on feed intake, egg production, egg mass, egg weight, feed conversion ratio, egg shape index, egg shell thickness, and Haugh unit (Hosseinzadeh et al. 2010). Similarly, no significant difference on egg production, egg mass and feed conversion ratio using different levels of PBM as substitution for SBM in Japanese quail breeders' diet (Ertürk and Celik 2004). Methionine and lysine are reported to be the first and second limiting amino acid in PBM respectively (Wang and Parsons 1998; Senkoju et al. 2005; Hosseinzadeh et al. 2010), while lysine bioavailability, according to Cortes Cuevas et al. (2011), is high. They recorded values greater than 90% (95.2–97.8%) and ash content of 12.1% (Cortes Cuevas et al. 2011). As the sole lysine source in the diet, PBM significantly improved final weight, weight gain, and feed efficiency. In a swine research evaluating PBM, Keegan et al. (2004) reported that in a 15-day study, nursery pigs fed diets containing either fish meal or PBM as the protein source presented no differences in daily gain or feed intake however, within the grade of PBM (low ash vs. high ash content), pigs fed diets containing low-ash PBM had greater gain-to-feed ratio compared with pigs fed diets containing high-ash PBM. Another study comparing different animal protein sources (spray-dried porcine plasma, blood meal, fishmeal, PBM, and SBM) at different nursery phases, observed that replacing PBM with blood meal or fish meal in nursery pig ration had no effect on performance however, might not be adequate to replace spray dried porcine meal (Zier et al. 2004).

Due to the refinements made in the processing of PBM resulting in a meal containing a higher protein content (68–72%) and a lower ash content (8–11%), associated with decreased levels of inorganic materials, it is thought that this would improve the nutritive value of the meal with the hope that PBM could replace some portion of fish meal in fish diets without compromising growth. By replacing fishmeal with up to 20% PBM, in chinook salmon diets, weight gain and feed efficiencies were not compromised but comparable with those whose protein source was mainly from fish meal (Fowler 1991). A 30% replacement however, impaired growth and feed efficiency. In a pacific white shrimp study on the other hand, replacement levels up to 80% of a pet feed grade PBM (CP 66%) as a substitute for a fish meal blend (CP 65%), did not affect the survival rate and feed conversion ratio. Protein efficiency ratio, nitrogen retention efficiency, as well as digestibility coefficients (protein, dry matter, and energy), were comparable to the fish meal diet (Cortes Cuevas et al. 2011). Research on the digestibility of two different sources of PBM (pet-grade or food grade) compared to menhaden fishmeal in a hybrid striped bass diet showed that the dry matter of fish meal, pet-grade, and food-grade PBM (79.2%, 73.5%, and 64.4%, respectively) were not different (Thompson et al. 2008). Protein digestibility was comparable for both the fish meal and pet-grade PBM (86.4% and 78.4%) but different from the feed grade PBM (75.1%). Similarly, apparent digestibility coefficients for protein, lipid, and dry matter were 91%, 92%, and 81%, respectively, in PBM fed to cobia (Zhou et al. 2004), and Tibbetts et al. (2006) reported protein digestibility of 80% in PBM fed in test diets to cod.

## 12.11 Fish Meal

Fish meal is in popular demand in every sector of animal agriculture as a good source of protein. An annual figure of 7 million tons of fishmeal (Roger 2004) was estimated to be used by the animal agriculture industry, with the bulk of this being used in aquaculture. This demand puts other groups, such as swine and poultry, at a disadvantage as they cannot compete with farmed fish in terms of return on investment. Despite this however, the aquaculture industry is in search of alternative protein sources because of the high cost of fish meal, which is expected to continue to be on the increase (Dong et al. 1993; Turker et al. 2005). Fish meal is often described as a composition of clean, dried, ground carcasses of fish either caught for this purpose or a byproduct from fish processing for human consumption (Ravindran and Blair 1993). Often times, menhaden and anchovy are the main fish species used for fish meal with lesser quantities of herring, ocean perch, and whitefish used (Leeson and Summers 2009). It is an exceptionally good source of high quality protein (50–75%) with relatively high energy, calcium, phosphorus, vitamins (A, E, choline, biotin, and B<sub>12</sub>), and essential fatty acids levels (Chiba 2001; Ravindran and Blair 1993), which makes it an excellent source of protein for poultry. Therefore, diverse range of raw materials are processed into fishmeal. The quality of fish meal varies and depends greatly on the quality and type of the fish material used as well as the processing methods. Based on this, the proximate analysis of the fish meal varies from one species to another, and often dependent on the geographical area, season, age, and sex of the fish used (Stoner et al. 1986). Inclusion of fishmeal in livestock, aquaculture, and companion animal diets, have been shown to improve performance, feed efficiency, and disease resistance due to its growth promoting effects and its anti-inflammatory properties (Allen et al. 1998; Klasing 1998).

Depending on the processing method, the residual oil in fish meal differs, thus metabolizable energy (ME) is calculated based on this knowledge (Leeson and Summers 2009). According to Leeson and Summers (2009), at 4% fat and 63% CP, a fish meal sample is said to have ME of about 3289 kcal kg<sup>-1</sup>. However, ME value drops with a reduced fat content of 1% and 58% CP to 2836 kcal kg<sup>-1</sup>. Furthermore, the chances of oxidation are high even with only 2% residual oil therefore fish meals must be stabilized with antioxidants to preserve its quality (Chiba 2001; Leeson and Summers 2009). The value of fish meal as a protein source for livestock is improved with the addition of ethoxyquin, an antioxidant, which protects nutritionally important polyunsaturated fatty acids from rapid oxidation during the drying and storage phases. Lecithin, a chelator that helps to prevent the formation of free radicals, has also been used to stabilize fish meals.

Nutrient and energy digestibility information of a feed ingredient is particularly important when intended for animal use, to improve the accuracy of diet formulations and to allow for appropriate substitution of feedstuffs in least-cost diet formulation. Sullivan and Reigh (1995) reported the digestibility coefficients for dry matter, crude protein, and energy, when fish meal and other protein sources were used as test ingredients in a hybrid striped bass study. Fish meal had 83.7%, 88.2%, and 95.6%, respectively, for dry matter, crude protein, and energy, which was higher than coefficients for SBM (44.5%, 80%, and 55%). Similarly, apparent digestibility coefficients from a sunshine bass study where test diets consisted of 70 : 30 mixture of reference and test ingredient, in this case fish meal, were 86.4%, 92.1%, and 89.4% for protein, lipid, and organic matter digestibilities (Thompson et al. 2008). The inclusion of 8% fish meal in growing pig's diet

elicited a quadratic response in average daily gain (ADG) and feed intake (Stoner et al. 1986). Additionally, substituting about 8% fish meal of the total 20% crude protein provided by dried whey in early weaned pigs did not alter growth or performance (Stoner et al. 1990). According to Green (1989), essential amino acids' digestibility values were higher in milk and fish meal than meat meal. The calculated apparent digestibility values derived for fish meal were lysine 94%, threonine 91%, and methionine 94% in castrated pigs.

Despite the desirable characteristics of fish meal in poultry diets, few investigators have considered the effects of feeding fish meals or fish oils on egg flavor. Egg and carcass palatability tests revealed a fishy taint in eggs compared to chicken meat when birds were fed high levels (10–25%) of fish meal. This was more evident in the low-grade fish meal (Cruickshank 1939). Koehler and Bearse (1975) reported that inclusion of certain fish meals at 10% or fish oil at 1.5% in laying hen's diet caused a "musty," "fishy" flavor in eggs, which intensified when the eggs were stored for four weeks at 10 °C. However, the addition of 5% fish meal to the diet had no effect on the flavor of chicken carcass. Other potential problems with feeding fish meal in broilers is gizzard erosion. Gizzerosine, a compound formed by a reaction between free histidine or histamine and lysine during fish meal production (overheating process), has been implicated in this condition (Masumura et al. 1985; Leeson and Summers 2009; Gjevre et al. 2013). Ten times more potent than histamine, gizzerosine stimulates increased gastric acid production resulting in localized cracks in the gizzard, gizzard erosions, hemorrhagic lesions, and subsequently affecting growth rate. This condition is, however, dose dependent (Leeson and Summers 2009; Gjevre et al. 2013). Several methods have been attempted to mitigate this problem including the addition of sodium bicarbonate to the diet to serve as a buffer against reduced pH, thereby reducing the severity of the erosion (Leeson and Summers 2009). Others include adopting low rendering temperature and a careful control of drying process to reduce microbial decarboxylation of histidine and subsequent formation of high levels of gizzerosine (Gjevre et al. 2013).

## 12.12 Feather Meal

About 5–7% of the total weight of a matured chicken is feathers and are considered a poultry processing byproduct (Williams et al. 1991). In the United States alone, 2–4 billion pounds of feathers are produced each year. This enormous output generated by the poultry processing industry necessitated the need for further processing in order to make it into a useful alternative byproduct (feather meal) rather than dealing with the problems associated with their disposal. Although feathers, like other keratinous products, are not digestible in their natural state, they contribute virtually no nutritional value to monogastric animals (Chiba 2001; Morris and Balloun 1973). Feather meal contains approximately 85–99% crude protein and if made available, could supply cheap source of animal protein for livestock feeding. The indigestible state of feather meal is attributed to the  $\beta$ -keratin molecule in feathers, which are composed of hydrogen bonds and hydrophobic interactions of protein strands folded into  $\beta$ -pleated sheets and further crosslinked in cysteine disulfide bonds (Williams et al. 1991). In its natural state, analysis of feather protein showed that it contains large amounts of glycine, cysteine, arginine,

and phenylalanine (Block et al. 1939) and after steam pressure processing, only cysteine showed a considerable loss (Gregory et al. 1956).

This challenge can partially be overcome by hydrolyzing the feathers. This procedure destroys the disulfide bonds within the keratin molecule making it highly digestible. Various methods of processing raw feathers have been adopted, ranging from the use of sodium sulfate and sodium hydroxide, as well as autoclaving (Draper 1944). Other methods include dry-rendering type cooker (Binkley and Vasak 1950), steam pressurized vessel to induce hydrolysis, and application of thermal drying to reduce moisture content (Morris and Balloun 1973; Williams et al. 1991). This ultimately results in a free-flowing friable meal. Other methods involve enzymatic treatments using microorganisms such as *Bacillus licheniformis* (Strain PWD-1) (Williams et al. 1990), *Vibrio sp. strain kr2* (Sangali and Brandelli 2000), *Streptomyces fradiae* (Elmayergi and Smith 1971) that have keratinase activity. Variability in quality is often associated with the processing conditions such as the time, temperature, pressure, and moisture, which can affect its digestibility and biological value (Johnson et al. 1998). According to Gregory et al. (1956), processed feathers using steam and pressure resulted in a relatively stable amino acid content, with the exception of cysteine content which decreased from 8.8% to 3.6%. The loss of cysteine is often associated with the cleavage of the disulfide bonds which apparently weakens the keratin molecule. This limits its resistance to enzymatic hydrolysis, thereby making it more susceptible to proteolytic enzymes (Gregory et al. 1956; Papadopoulos et al. 1986; Morris and Balloun 1973). Furthermore, unstable residues of dehydroalanine formed as a result of the desulfurization reaction, can condense with cysteine to form lanthionine (Papadopoulos 1984; El Boushy et al. 1990). Research has shown that the lower the processing time and pressure, the lower the level of cystine digestibility, and subsequently increasing processing temperatures increases lanthionine content, which is inversely proportional to cysteine content (Papadopoulos et al. 1986; Leeson and Summers 2009). It is suggested that lanthionine levels should not be more than 20–30% of total cysteine levels in hydrolyzed feather meals (Leeson and Summers 2009). Similarly, raw feather meal with 16% pepsin digestibility depressed growth rate when compared to treated feather meals with 64–83% pepsin digestibility (Naber et al. 1961). In addition to this, the effect of processing factors on different individual amino acid varies.

In addition to challenges associated with feather digestibility, feather meal is deficient in methionine, lysine, histidine, and tryptophan (Morris and Balloun 1973; Williams et al. 1991; Chiba 2001). These amino acids, being the common limiting amino acids in a corn-SBM-based diet, makes feather meal less attractive as a source of protein in poultry and swine diets. Assessing the amino acid limitations using a chick bioassay, revealed that methionine and lysine were first and second limiting respectively, and tryptophan and histidine equally third limiting (Papadopoulos 1984). This means, crystalline form of these amino acids has to be added to a diet containing feather meal in order to meet their (methionine, lysine, histidine, and tryptophan) requirements leading to an increase in feed cost. Naber et al. (1961) observed that growth rate improved with corn-feather meal-based diet when supplemented with lysine (0.7%), methionine (0.4%), and tryptophan and histidine (0.1%), but it was not comparable to the corn-soybean oil meal-based diet. Moran et al. (1966) reported that corn-feather meal diet including unprocessed feather, feathers autoclaved for 30 minutes at 121 °C, feathers treated with sodium sulfide, and feathers autoclaved for 18 hours at 121 °C failed to support growth

regardless of amino acid supplementation. However, feather meal hydrolyzed at 142 °C for 30 minutes, supplemented at 5% of the total protein, with appropriate amino acids, supported chick growth equivalent to the corn-SBM-based diet which was supported by Papadopoulos (1984). Morris and Balloun (1973), observed that by increasing processing time from 30 to 60 minutes at steam pressure of 54 psi, methionine, lysine, and histidine levels decreased. However, an increase in pressure to 65 psi for 30 minutes with intermittent agitation, increased net protein values of all the feather meals tested. Also, at 2.5% of the total protein, supplemented feather meal supported growth comparable to the control diet without supplementation with crystalline amino acids. However, increasing the inclusion level of feather meal to 5%, methionine and lysine should be supplemented to achieve considerable results (Morris and Balloun 1973).

In terms of reducing abdominal fat deposition in broilers, studies have shown that dietary manipulation, especially energy and protein levels, can alter carcass composition. By increasing dietary protein and decreasing energy levels to achieve a reduction of carcass fat, less expensive protein sources offer a possible alternative. Feather meal has been replaced with other protein sources in limited quantities (Naber et al. 1961; Moran et al. 1966; Morris and Balloun 1973; Papadopoulos 1984). Thus, based on this strategy, Cabel et al. (1987) added feather meal to the broiler finishers diet assuming a 100%, 50%, or 0% amino acid availability. They observed that regardless of the amino acid availability level, feather meal reduced abdominal fat. Likewise, body weight, feed efficiency, and dressing percentage were comparable, suggesting that excessive abdominal fat deposition can be reduced by supplementing broiler finisher diets with feather meal 7–14 days prior to slaughter. Further investigation stated that increasing the dietary protein level, regardless of the source, significantly reduced abdominal fat accumulation. However, feather meal can be used effectively as a nitrogen source (Cabel et al. 1988).h

## 12.13 Grease in Livestock Feeds

Grease is an animal byproduct commonly supplemented in livestock feeds as a dietary lipid. Dietary lipids are digested, absorbed, and either oxidized to yield energy in the form of ATP or incorporated into body lipids (Birkett and de Lange 2001). Demand for animal fats is on the rise because soybean oil prices tend to be unstable (Liang et al. 2010). Choice white grease (CWG) or yellow grease (YG) are typically supplemented in swine, poultry, cattle, and sheep diets as an added fat to supply energy. According to American Fats and Oils Association (AFOA 1999), CWG is defined as the fat rendered from pork tissue. According to Zinn (1988), YG is a commercial feed fat composed of waste grease. Yellow grease has been given several names in the literature such as frying fats, frying oil, and recycled restaurant grease (Awawdeh et al. 2009a). The U.S. Census Bureau Current Industrial Report M311K (2005) reported 1.472 billion pounds of YG was being produced annually by the U.S. rendering industry. White grease, included in "other grease" reportedly produced 1.209 billion pounds annually. Titer value is important in distinguishing between tallow and grease. It is determined by melting the fatty acids after a fat has been hydrolyzed. Then, the fatty acids are cooled and the congealing temperature in degrees centigrade is the titer. If the titer value is below 40, it is considered grease (Meeker 2009). Benz et al. (2011) stated that supplementing swine diets with fat is a

practical method in achieving greater rate and efficiency of body weight gain. The ME needs of the animal (especially swine and poultry) is easily met with the addition of fat to the diets (Clapperton and Steele 1983).

In broiler chickens, up to 8% of white grease can be supplemented into the diet without affecting the growth rate (Siedler and Schweigert 1953). This study also reported that the calories from white grease were utilized efficiently at 2 and 4% fat, but the calories were not completely utilized when 8% fat was added to the ration. The addition of fat to poultry feeds ultimately reduces the amount of dust and improves the texture and color the feed (Sunde 1954).

Mateos and Sell (1981) evaluated the ME of YG in practical diets for laying hens by using actual ME determination and lipid digestibility data. Yellow grease was supplemented into the diet at 0%, 5%, 10%, 15%, 20%, 25%, or 30%. With each increment of supplemental YG, the ME of the diets increased. Regardless of the method of estimating energy contribution of fat to the diet, supplemental YG exerted an extra-metabolic effect on dietary ME. Cullen et al. (1962) conducted a study to evaluate 16 different types and grades of fat and their effect on growth performance. It was observed that when fed semi-practical diets, the absorbability of CWG was higher than bleachable fancy tallow (95.6% vs. 86.7%). Wu et al. (2011) investigated whether free fatty acid (FFA) content of YG influenced the performance and carcass characteristics of broiler chickens. Three FFA of YG were incorporated at low, medium, and high levels (2.74%, 12.59%, and 19.05%). The results show that the performance of broilers fed diets supplemented with YG with low level FFA was similar to that of birds on SBM. Additionally, when high levels of FFA were in the diet with YG, body weight gain and feed intake decreased significantly. Dressing percentage and percentage of breast muscle did not vary across the three YG treatments, but in comparison to soybean oil treatment, the YG diets were significantly lower.

According to Stahly (1984), lipids are essential ingredients in swine diets because of their high energy value and palatability. In growing-finishing pigs, Benz et al. (2011) evaluated the influence of feeding duration, between 0 and 82 days, of white grease on growth performance, carcass characteristics, and carcass fat quality. It was reported that increasing the feeding duration of white grease increased the amount of unsaturated fatty acids, which led to softer carcass fat. Additionally, Rentfrow et al. (2003) also reported an increase in total unsaturated fatty acids with the addition of white grease to the diet, while decreasing the total saturated fatty acid content. Engel et al. (2001) reported that up to 6% of white grease can be added to swine diets as an energy source without any detrimental effect to the quality of the longissimus muscle, belly, or bacon. Rosero et al. (2012) conducted a study evaluating the response of lactating sows and progeny to the increasing level (2%, 4%, and 6%) of supplemental CWG. The results showed that the total BW gain in sows and litter improved with the addition of CWG in comparison to an animal-vegetable blend fat source. Feed efficiency also tended to improve with increasing levels of CWG. Although there was a significant difference with the addition of CWG in sows, no difference was observed on litter performance. Additionally, piglet mortality showed a tendency to increase with the addition of dietary CWG.

Two sources of YG, griddle grease (GG) or conventional yellow grease (CYG) are commonly added to cattle diets. GG has a threefold greater concentration of FFA in comparison to CYG (42% vs. 15%) (Plascencia et al. 1999). This article also reported that fat supplementation of either GG or CYG increased average daily gain (ADG), feed

efficiency, diet net energy (NE), carcass weight, dressing percentage, and kidney, pelvic, and heart fat. Although there was a significant difference in FFA concentration, no difference was observed in growth performance of feedlot dairy cattle. In feedlot steers, Zinn (1988) observed increases in ADG when YG was supplemented at 4%. In these growing-finishing diets, the average net energy value for maintenance and gain ( $NE_m$  and  $NE_g$ ) were 6.20 and 4.53 Mcal kg<sup>-1</sup>, respectively. These values are considered higher than the feed standards listed in the NRC (1984).

In growing-finishing diets for feedlot steers, Zinn and Plascencia (1996) fed a low-forage diet (10% alfalfa hay) and high forage diet (30% alfalfa hay) with the supplementation of 0% or 6% YG. Results show that the high-forage diet with 6% YG increased ADG and longissimus muscle area. The authors (Zinn and Plascencia (1996) concluded that supplementation of 30% forage finishing diet with 6% YG will allow growth similar to that of steers fed a 10% forage diet without any supplemental fat. The improved performance can be related to the increase in energy density of the diet, a positive association on protein flow to the small intestine, and decreased ruminal methane production. There is also a relationship between fat supplementation and magnesium. Data reported by Ramirez and Zin (2000) showed that supplemental fats (YG or GG) may depress magnesium absorption.

Livestock producers are always exploring alternative sources of lipids to incorporate into the diet. Yellow grease was evaluated, at 6%, as an alternative ingredient in a study conducted by van Cleef et al. (2016). The article indicated that soybean oil or YG supplementation did not change feeding behavior or carcass characteristics. In addition to this, the lambs fed diets with added YG presented a lesser ADG than those fed diets with soybean oil. The use of YG increased the lambs' days on feed. Awawdeh et al. (2009a) evaluated YG as an alternative energy source compared to soybean oil. No difference in performance was observed in both the control group, YG-based diet, and soybean-oil-based diet. However, in regards to price, the cost of feeding 10 ewes for 55 days was \$612, \$544, and \$601 for the control, YG and soybean oil group, respectively. Also, the cost of producing 1 kg of milk was \$1.84, \$1.37, and \$1.52 for the control, YG, and soybean oil group, respectively. They concluded that YG supplementation was economically feasible and would keep the cost of production lower than that of soybean oil and a barley-wheat-based control diet.

In another barley-grain-based diet, Awawdeh et al. (2009b) conducted another study evaluating the effect of YG to that of soybean oil. The results were similar to what was reported earlier (Awawdeh et al. 2009a) – no difference between YG and soybean oil. In this study the lambs fed YG or soybean oil grew faster and were heavier than the barley-grain based control group. These lambs also had heavier carcass weights than those offered the control diet.

## 12.14 Whey

Whey is the liquid portion remaining from casein precipitation during cheese manufacturing (Lievore et al. 2015). According to Guirguis et al. (1993) whey is a byproduct of the cheese and casein manufacturing industry and contains over 50% of the initial milk solids and over 85% of initial milk volume. Liquid whey has been used in swine diets for many

decades. It was reported in 1949 that 4%, 8%, and 20% whey in the diet caused diarrhea in weanling pigs (Krider et al. 1949). Dried whey on the other hand, contains about 80% of lactose (Samli et al. 2007). Whey is composed of predominately lactose, protein, and minerals. The world production of whey is over 165 million tons and cheese whey contributes about 95% (Macwan et al. 2016). In Europe, the utilization of whey is 75%, while other countries are much less. In 1987, the United States reportedly used about 47% of whey solids in human and animal diets (Clark 1987).

## 12.15 Types of Whey

There are two categories of whey, including sweet whey and acid whey. Sweet whey is produced from enzymatic coagulation of milk proteins during manufacture of cheddar or Swiss cheese (Chandra 1980). Acid whey is composed from cottage cheese manufacturing. It is produced during acid coagulation of milk proteins and has a low pH, which helps retain its storage life in comparison to sweet whey (Chandra 1980). The main differences between the two whey types are in the mineral content, acidity, and composition of the whey protein fraction. The United States produces 2–6 million tons of acid whey and 20 million tons of sweet whey (Clark 1987). The pH of whey varies depending on the type with sweet whey having a pH range of 6.02–6.58, while acid whey has a pH range of 3.57–4.34 (Alsaed et al. 2013).

## 12.16 Production and Processing of Whey

Whey is produced from dairy industries all over the world. Around 50% of whey produced is used directly in liquid animal feeds, as fertilizer, or discarded as waste. In order to preserve the nutritional quality of the final product (whey), the first three steps during processing are important. The first step of processing is clarification. This is the process of removing the curds using centrifugation or a clarifier. Next, the fat from the curds are recovered by separation (Zadow 1992). This process is typically achieved with a self-discharging separator. Then, pasteurization must immediately be completed in order to preserve shelf life and microbiological quality. The final step of whey processing is determined by the nature of the final product desired such as whey powder, deproteinized whey, and whey protein concentrate (WPC). Grinstead et al. (2000) explained how the drying process occurs from liquid whey. The process begins with water removal using evaporation and drying, which can remove upwards to 90% of water from the liquid whey. Then, the whey is crystallized in agitation tanks where the temperature is set depending on desired viscosity. Drying the crystallized whey can be completed using spray drying or roller drying. Spray drying is used to dry at a low temperature quickly, to prevent denaturing. Roller drying uses a heated cylinder where the whey comes in contact with the roller to produce a thin sheet, which is then ground to a fine powder. Using a roller might diminish the nutritional value of the now dried whey. Spray drying has been reported to be superior to roller drying (Sohn et al. 1993).

## 12.17 Whey in Animal Feed

An essential use of whey is supplementation into livestock feeds. Whey can be supplemented into calf and pig starter and booster diets, dairy cow grain mixes, dry, pelleted, or block poultry feed, horse feeds, sheep pellets, and whey containing pet-foods. However, it is most commonly used in cattle and swine feeds. The amount of solid whey supplemented in the feed varies from 1.5% to 5% (Gillies 1974). According to Zadow (1987), 28% of the 1.65 million tons of whey produced in Australia is used for pig feed. Additionally, 5% is used as calf milk replacer. In the Netherlands, 15 000 tons of liquid whey and 120 000 tons of delactosed whey powder is manufactured for calf milk replacer. According to Lynch and McDonough (1979) large ruminants like cattle offer the best potential for the use of large quantities of liquid whey or whey products for the production of high-quality meat and milk. The study used Holstein calves fed supplemental liquid acid or sweet whey, and reported no differences in final body weight or daily gain. Ruminants can consume up to 30% of their dry-matter intake as liquid whey without impaired performance.

Landblom and Nelson (1980) experimented with the use of liquid and dried sweet whey in growing and finishing pigs. The results showed pigs fed diets supplemented with liquid whey were efficient in body weight gain. In comparison to the SBM or lysine diets in this experiment, the liquid whey resulted in feed saving of about 48.6 kg less feed per 45.5 kg gain. In a separate experiment from the same article, dried sweet whey was supplemented at 0%, 15%, 30%, and 45% in an attempt to replace barley in growing-finishing rations. The results from this study show that pigs whose diets were supplemented with dried sweet whey had a higher body weight gain than the pigs on the control diet which contained no whey. The whey diets were lower in fiber content than the barley-oat-based control diet and this ultimately resulted in faster gains and an improved feed efficiency.

## 12.18 Whey Disposal

There are many methods of whey disposal including disposal as sewage, returned to farmers, or paying processors for disposing the whey. In 1962, over half of the cheese plants surveyed reported they disposed all or part of their whey as waste and sewage, because they had no other way to dispose of the whey (Gillies 1974). Whey directly disposed into the environment has become a major cause of pollution. Because of the acidity of whey, it can cause problems if dumped into streams with low water discharge. A recent study by Utama et al. (2017) reported that cheese whey could be safely disposed of as fertilizer after it undergoes ethanol fermentation.

## 12.19 Whey in Non-Ruminants Feed

Dried whey has been fed to non-ruminants for decades. The addition of whey to poultry diets proved most effective when whey was supplemented at 3–4% (Schingoethe 1976). If more than 20% of lactose is in the diet of poultry, weight gain is negatively impacted

(Riggs and Beaty 1947). Dried whey utilization in several pig phases has been extensively researched (Krider et al. 1949; Hanrahan 1971; Ekstrom et al. 1975). Weight gain in pigs has been shown to increase with dried whey supplementation, however, the important question of how much lactose from the whey could a pig tolerate has not been adequately addressed. Studies show that too much dried whey (40%) will cause diarrhea in finishing pigs. However, suckling pigs are able to tolerate 60% dried whey with no obvious signs of negative effect (Becker et al. 1957). Furthermore, the inclusion of lactose (from whey) in diets significantly increased calcium and phosphorus absorption (Bergeim 1926).

## 12.20 Whey in Ruminant Feed

In ruminants, dried whey is often fed as an additive to prevent milk fat depression on high concentrate rations. This is seen as advantageous because whey is palatable (Schingoethe 1976). A 1970 study by Bishop and Bath reported a slight increase in milk fat percentages when dried whey of less than 5% of concentrate was fed. Other studies report increased weight gain of between 2% and 13% compared to the control group when 1–4% dried whey was added to the ration of growing-finishing beef cattle (Woods and Burroughs 1962; Hendrix and Klopfenstein 1972).

Forages have small amounts of fermentable carbohydrates. Therefore, whey can be added to grass and legume silages to improve the quality of the ration. Whey is an excellent fermentable carbohydrate that allows for faster and more complete fermentation, better quality of silage, higher acid content, and higher digestibility (Schingoethe 1976). Between 1% and 5% of dried whey was most effective in increasing the acidity of alfalfa silage prepared in lab silos (Nevens and Kuhlman 1936). Positive effect of whey supplementation urea-treated corn silage has been reported. Whey supplementation resulted in reduced nitrogen losses and improved animal performance (Schingoethe and Beardsley 1975).

## 12.21 Milk Replacer in Calves

The primary source of protein in milk replacers is whey. Studies show that whey is a rapidly hydrolysable protein source that can be included at high levels in milk replacer diets in calves (van den Borne et al. 2006). In early studies the primary ingredient in milk replacers was dried skim milk (DSM). The DSM was replaced with WPC because it was 40% cheaper and consisted of the same chemical composition. When whey undergoes ultrafiltration it produces WPC (Lammers et al. 1998). Additionally, because WPC has a better amino acid profile for growing calves than DSM, WPC has a higher bioavailability. Dried whey has often been added with skim milk to be used as a milk replacer to raise baby calves. The performance of whey protein milk replacers was equal to commercial milk replacers, in term of nitrogen digestion and retention. Furthermore, the addition of fat to whey protein milk replacers increases nitrogen digestibility to the level of whole milk (Lynch and McDonough 1979). The best level at which whey could be included in milk replacers without negatively impacting performance has not been unequivocally

established. Overall, more research is needed to determine how much whey will consistently improve animal performance.

## 12.22 Spray-Dried Plasma Protein

Plasma protein is a protein-rich liquid animal byproduct typically from a bovine or porcine source (Van der Peet-Schwingering and Binnendijk 1997; Torrallardona 2010; Álvarez, et al. 2018). The plasma is acquired by adding an anticoagulant, like sodium citrate, to the blood and centrifuging to separate out the cell fraction (Ockerman and Hansen 1998; Coffey and Cromwell 2001; Almeida et al. 2013). The collected plasma is concentrated, occasionally filtered, then dried in a manner to preserve biological activity (Ockerman and Hansen 2000). The bioactive compounds are retained using two methods for drying the plasma, which are spray drying and fluidized bed drying. The more common method of spray drying consists of atomizing the plasma into small particles and spraying into a stream of hot air. The hot air evaporates the water from the plasma, while staying cool enough to reduce any Maillard reactions occurring in the proteins (Ockerman and Hansen 1998). Fluidized bed drying is completed by spraying the plasma on a bed of small poly-carbonate balls that are suspended by a warm stream of air. After the plasma dries on the balls, they are forced against a screen to remove the dried plasma (Ockerman and Hansen 1998). In addition to the drying method, the quality of blood plasma protein can also be influenced by the processing time and volume, centrifugation force, and the concentration of phosphate saline buffer in the plasma (Álvarez et al. 2018).

The animals that are typically fed plasma protein are young pigs (Borg et al. 2002), with some instances of use in calves (Quigley and Wolfe 2003; Jones et al. 2004), poultry diets (Campbell et al. 2004; Bregendahl et al. 2005; King et al. 2005), and companion animal diets (Quigley et al. 2004; Rodriguez et al. 2016). The inclusion of spray-dried blood meal in livestock diets may give rise to some palatability issues, which has been shown to be improved by using spray-dried protein plasma. (Kats et al. 1994a, b). Plasma protein provides a good source for the amino acids: lysine, tryptophan, and threonine in high concentrations, however it is low in methionine and isoleucine (Coffey and Cromwell 2001; Torrallardona 2010). There is evidence that plasma protein can provide immune support, which has been shown by using blood products in calf and weanling pig diets (Jones et al. 2004; Pierce et al. 2005; Campbell et al. 2016). Immunoglobulins are present in the plasma (Torrallardona 2010) and have been shown to be beneficial for improving the health of different species challenged with various stress factors (Quigley and Wolfe 2003; Campbell et al. 2006; Campbell et al. 2016). In addition to being a good source for protein supplementation in animal diets, it may also enhance nutrient digestibility as well as improving health and performance when challenged with various stressors.

## 12.23 Sources of Plasma Protein

The two main sources of blood used in producing plasma protein are pigs (porcine) and cattle (bovine). Little differences have been seen in the amino acid composition of plasma proteins from both sources (Torrallardona 2010). Regardless of animal source, weanling

pigs fed spray-dried plasma protein had significantly better performance (weight gain and feed intake) with only the porcine-sourced plasma having a higher feed intake in 0–14 day old pigs (Torrallardona 2010). When the WPC in calf milk replacer was substituted with porcine or bovine protein plasma, either source of plasma reduced morbidity and mortality (Quigley and Wolfe 2003). The differences that were seen in the two sources were speculated to come from minor differences to which the plasma was subjected.

## 12.24 Amino Acid Concentration of Protein Plasma

The protein content of plasma is between 70% and 80% (Torrallardona 2010), and is highly-digestible as a feedstuff (Almeida et al. 2013). It has been shown to improve overall nutrient digestibility in companion animal diets (Quigley et al. 2004; Rodriguez et al. 2016). Plasma protein can rival the nutritive quality of other common protein sources. However, it is low in some essential amino acids, particularly methionine (Torrallardona 2010). To successfully use plasma protein as a component of swine and poultry feed, other sources of protein with sufficient levels of methionine would be required to meet the animal's nutrient requirements. The 10 essential amino acids, and their concentrations, as found in plasma from porcine and bovine sources are listed in Table 12.4.

## 12.25 Immune Support of Spray-Dried Protein Plasma

Spray-dried plasma has been studied in several species for its use in immune support. In commercial swine production, pigs are weaned early and may not have the ability to

**Table 12.4** Amino acid concentrations of plasma protein (%), as-is basis.<sup>a</sup>

	Porcine	Bovine
Arginine <sup>bcd</sup>	4.19	4.26
Histidine <sup>bcd</sup>	2.31	2.36
Isoleucine <sup>bcd</sup>	2.56	2.19
Leucine <sup>bcd</sup>	6.78	7.10
Lysine <sup>bcd</sup>	6.17	6.67
Methionine <sup>bcd</sup>	0.55	0.86
Phenylalanine <sup>bcd</sup>	3.99	3.92
Threonine <sup>bcd</sup>	4.12	4.84
Tryptophan <sup>bd</sup>	1.37	1.42
Valine <sup>bcd</sup>	4.69	5.16

<sup>a</sup> Superscripts are averages of numbers from the corresponding sources.

<sup>b</sup> Van der Peet-Schwering and Binnendijk (1997).

<sup>c</sup> Ravindran and Morel (2006).

<sup>d</sup> Hansen et al. (1993).

produce sufficient IgA (Perez-Bosque et al. 2016). Spray-dried plasma has been used in studies to evaluate the effect biologically active fractions of the plasma have on performance and health of the animal. In some instances, the animals sourced for the plasma are vaccinated for specific diseases to supplement specific immunoglobulins. This method of obtaining plasma protein is referred to as spray-dried immune porcine plasma (Torraldardona 2010).

The usefulness of spray-dried plasma in weanling pigs have been studied over the years. The rise in resistant-strain bacteria has forced meat producers to consider alternative methods of growth promoter. Spray-dried plasma has been a suggested alternative to antibiotics (Perez-Bosque et al. 2016), which were previously used as a growth promoter. When fed to early weaned pigs, spray-dried plasma (bovine or porcine) can decrease the postweaning growth lag seen, due to IgG fraction (Pierce et al. 2005). Reduced mortality and morbidity, and increased growth, in dairy calves has also been reported when fed a diet containing spray-dried plasma compared to diet containing WPC in milk replacer (Quigley and Wolfe 2003). The biologically active component of plasma protein may be responsible for the positive effects observed.

Additionally, spray-dried plasma has been shown to reduce the negative effects of different stressors impacting various animals. The health and performance of weanling pigs were improved when challenged with different respiratory diseases, after being orally fed spray-dried plasma (Campbell et al. 2016). While the effects of spray-dried plasma on stress is well-studied in swine, there are fewer studies in other species. Positive effects of spray-dried plasma in poultry under stress have been reported. In broilers, improved feed intake and growth were observed in birds challenged with necrotic enteritis (Campbell et al. 2006). Furthermore, it was shown that the form (particle size) of the plasma protein affected the response, resulting in higher improvements in feed intake and growth in broilers fed the granular form compared to those fed the powdered form (Campbell et al. 2006). Turkeys challenged with *Pasteurella multocida*, had increased performance in pouls, and reduced mortality in adult turkeys, when given spray-dried bovine serum through drinking water (Campbell et al. 2004).

## 12.26 Summary

As long as the demand for animal protein source exists, animal discards and byproducts will be available. The use of these discards in livestock feed will mostly be influenced by cost rather than competition for these products with humans. Unlike plant sources of nutrients, nutrients from animal sources have relatively higher crude protein (amino acid) and energy contents. An added advantage of animal discards is that they are highly digestible, because they are mostly devoid of crude fiber which nonruminant animals cannot optimally digest and utilize for growth and production. The main challenge of using animal discards in livestock feed is that the nutrient and energy contents of these products vary widely between and within discards depending on the source, processing techniques, and geographical location. Furthermore, there is the need for prompt processing and stabilization of animal discards in order to prevent contamination and to increase their feeding value as well as shelf life. The solution to these challenges is to always procure animal discards intended for livestock feed from a single and consistent

location, if possible. Furthermore, it is important to analyze the nutrient and energy contents of these products periodically to access the quality of the product being supplied. Efforts should also be made to ensure that the level of inclusion is optimal. Finally, the use of animal discards in livestock feeds could make livestock feeding operation more cost efficient and environmentally sustainable.

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## 13

# Bioactive Peptides from Fish Collagen Byproducts: A Review

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## 13.1 Introduction

In recent years, there is a growing interest regarding food protein derived bioactive peptides due to their numerous biological activities and their beneficial health effects (Hartmann and Meisel 2007; Nasri 2017). Indeed, numerous activities have been reported including antihypertensive (He et al. 2013a,b), anticoagulant (Nasri et al. 2012), antioxidant (Sarmadi and Ismail 2010), antimicrobial (Jemil et al. 2016), hypoglycemic (Nasri et al. 2015), antitumor (Xue et al. 2012), and cholesterol-lowering ability (Udenigwe and Rouvinen-Watt 2015). These biological activities are associated to peptides, which are inactive within the sequence of the original proteins and can be released by enzymatic hydrolysis either during food processing, through the gastrointestinal digestion by digestive proteases or by hydrolysis using exogenous proteases (Clare and Swaisgood 2000). Numerous proteins of plant or animal origin, have been used to generate biologically active peptides (Bhat et al. 2015; McCarthy et al. 2013). Recently, an increasing interest has been focused on marine proteins as a valuable source of bio-peptides, including collagen and gelatin. Collagen and gelatin have a wide range of industrial applications such as, pharmacology, food industry, cosmetics, tissue engineering, and biomedicine (Parenteau-Bareil et al. 2010; Jeevithan et al. 2013; Alfaro et al. 2015). Generally, most of the commercial collagens and gelatins are extracted from bovine and porcine skin or bone. However, due to the risk of bovine spongiform encephalopathy, and religious restrictions regarding the non-consumption of pork by Muslims and Jews, there has been increased interest in finding new collagen rich sources (Karim and Bhat 2009). To this end, fish collagens and gelatins are an alternative to their mammalian counterparts, as they are accepted by various religious groups (Silva et al. 2014).

Fish processing industries generated a large amount of byproducts, including skin, viscera, bones, scales, heads, and fins, which are generally either discarded or processed into low market-value products, such as fish meal, fish oil, animal feeds, or fertilizers (Ordonez-Del Pazo et al. 2014). These wastes, which constitute 30–50% of the total weight of the raw material, are promising sources of collagen, where up to 30% of the total fish byproducts are found to be collagen-containing products.

Moreover, these products can serve as a protein substrate to produce by enzymatic hydrolysis biologically active collagen hydrolysates, called also as gelatin hydrolysates. Due to their bioactivity, biocompatibility, high bioavailability, and safety (Gómez-Guillén et al. 2011; Xu et al. 2015; Venkatesan et al. 2017), the development of marine fish-derived biologically active peptides from collagen or gelatin, for human therapeutic, nutraceutical, and cosmeceutical uses is growing rapidly. Collagen peptides (CPs) used in foods and pharmaceuticals are generally recognized as safe food products by regulatory agencies (Moskowitz 2000). In addition, low molecular weight (LMW) peptides have been known to be less allergenic than their parent proteins, which may explain their wide use in the formulation of hypoallergenic infant foods (Host and Halken 2004).

This review highlights the biological properties and potential applications of fish collagen-derived active peptides.

## 13.2 Collagen Molecules

Collagen, a fibrous protein which is dominant in all the connective tissues, account for approximately 30% of the total protein content in the human body. Collagen plays an essential role in the structure of several tissues, including skin and bones, providing elasticity and strength (Myllyharju and Kivirikko 2001).

To date, up to 29 different types of collagen have been identified. Collagen molecules are composed of three helical polypeptide chains, called alpha chains, that assemble together to form a triple-helix structure stabilized by hydrogen bonds. There are approximately 25 different  $\alpha_1$  chains. Type I collagen, composed of two alpha 1 chains and one alpha 2 chain, is the most abundant collagen in connective tissues, such as skin, bone, and tendons, being responsible for skin tensile strength.

Collagens have very specific amino acid compositions, with Gly the most abundant amino acid may represent 35% of the total amino acid residues (Sotelo et al. 2015). Collagens also have a high content of amino acids (Pro and hydroxyproline [Hyp]) and Ala. Hyp is unique to collagen. Gly-X-Y is the most common amino acid sequence in collagen molecules, where X and Y are frequently Pro and Hyp, respectively (Nelson and Cox 2004).

## 13.3 Collagen and Gelatin Extraction Processes

Fish skins are widely used for the extraction of type I collagen. Generally, fish collagen and gelatin are extracted with acid solution process (Slade and Levine 1987). In the first step, washed fresh or frozen skins were cut into small pieces, homogenized, and then subjected to alkali treatment, typically NaOH, for at least six hours to remove non-collagenous substances (proteins, pigments, fats, etc.). To extract acid-soluble collagen, the pre-treated material was neutralized by washing with cold distilled water and then subjected to acidic treatment (usually 0.5 M acetic acid) for 24–72 hours under constant stirring at 4 °C or room temperature, depending on the raw material, followed by ultrafiltration (UF). Acidic extraction generally yields products with reduced functional and

biological properties and gives a low yield of collagen (Schmidt et al. 2016). An alternative approach to acidic treatment is the use of proteolytic enzymes (Nalinanom et al. 2008; Jridi et al. 2013). Regarding the enzymatic process, enzyme such as pepsin was added to acidic solution, and the pre-treated material was continuously stirred for about 24 hours at 4 °C or at room temperature.

Gelatin, the partially hydrolyzed form of collagen, is generally extracted from fish skins with acidic or enzymatic treatment followed by thermal treatment.

Compared to acidic process, pepsin treatment increases the yield of collagen extraction, as it is able to cleave specifically telopeptide region of collagen (Nalinanom et al. 2008; Benjakul et al. 2010). In addition, several pre-treatment steps could be used to increase the yield of collagen or gelatin extraction. In this respect, collagen yield was increased by 33.6% from turtle calipash (*Pelodiscus sinensis*) by ultrasound treatment (Zou et al. 2017).

The process and extraction conditions (nature of the acid, extraction temperature, enzyme level, incubation time, etc.) greatly influence the length of polypeptide chains and thereby their functional and biological properties. Collagens or gelatins extracted by enzymatic treatment contained more peptides with LMW, and their content increased with the increase of enzyme concentration applied in the enzymatic process. The functional properties and bioactivities of the obtained gelatins were improved compared to those obtained with the chemical approach.

To date, collagens and gelatins have been extracted and characterized from several fish species including: zebra blenny (*Salaria basilisca*) (Ktari et al. 2014), Black-barred half-beak skin (Abdelhedi et al. 2017), bigeye snapper (Benjakul et al. 2010), unicorn leatherjacket (Ahmad and Benjakul 2011). Further, they were extracted from other marine sources, such as mollusks (scallop (*Patinopecten yessoensis*) (Shen et al. 2007), cuttlefish (Jridi et al. 2013), octopus (Jridi et al. 2015)) and sponges (Tziveleka et al. 2017).

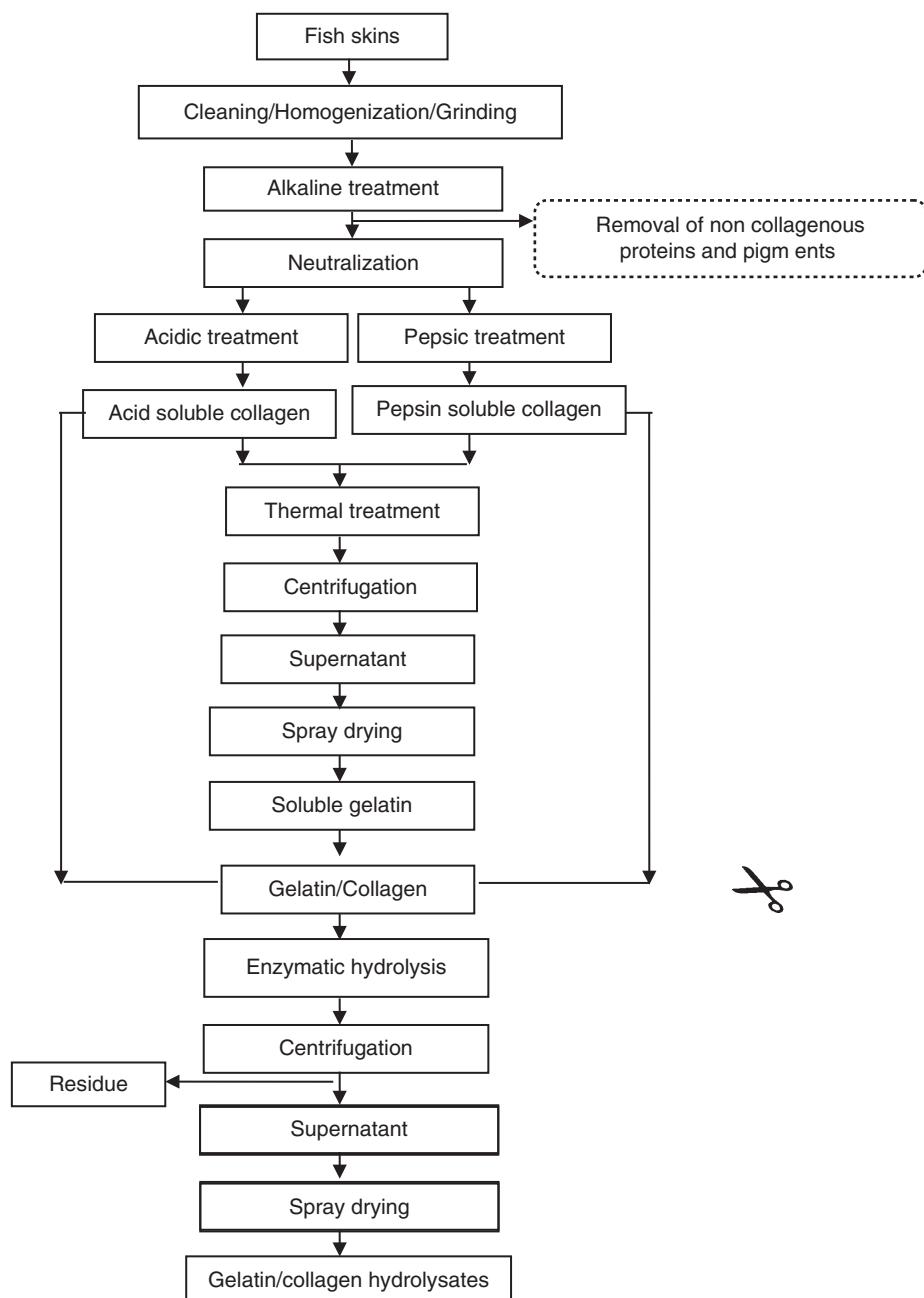
The general procedure (acidic and pepsin treatments) for the extraction of collagen and gelatin from the marine fish skins is shown in Figure 13.1.

### 13.4 Enzymatic Hydrolysis Process for the Production of Collagen Hydrolysates

Collagen is an excellent and inexpensive source for the production of peptides with biological activities as they can be extracted from fish processing byproducts. Various processes including chemical hydrolysis, enzymatic treatment with appropriate proteolytic enzymes, and fermentation with proteolytic bacteria, can be applied to produce biologically active collagen peptides (Saadi et al. 2015; Nasri 2017; Jemil et al. 2014). Enzymatic process is considered the most effective approach for the generation of bioactive peptides with defined characteristics. Indeed, compared to chemical and fermentation processes, enzymatic hydrolysis of protein substrates by specific proteases can be achieved under controlled conditions to produce reproducible functional collagen hydrolysates (Nasri 2017).

Collagen bioactive peptides could be generated by treatment with purified enzyme as well as crude digestive enzyme extract or bacterial enzyme preparation, often containing a mixture of several proteases. In addition, sequential enzymatic hydrolysis using enzymes with different specificities is recommended to enhance protein hydrolysis

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**Figure 13.1** Schematic representation of fish collagen and gelatin extraction, and collagen-based peptides production.

and obtain hydrolysate enriched with LMW peptides with desired biological activities. After enzymatic treatment, peptides in protein hydrolysates are usually fractionated using ultrafiltration and different chromatographic techniques (Nasri 2017). Most of the biopeptides are between 2 and 20 amino acid residues in length.

Up to now, several fish collagen and gelatin sources have been used for the production by enzymatic hydrolysis process of biologically active hydrolysates (Li et al. 2013; Blanco et al. 2017). The molecular weight, the amino acid composition and sequences, as well as the type of amino acid residues at N- and C-terminal of biopeptides, which are crucial for their biological activities, are governed by the nature of the collagen substrate sequence, the specificity of proteolytic enzymes and the processing conditions used in the hydrolysate preparation (Nasri 2017). In addition, incubation time and enzyme/substrate ratio greatly influenced the average molecular weight of released peptides and therefore their biological activities (Li et al. 2013; Chi et al. 2014). In general, high degree of enzymatic hydrolysis, leading to the release of small peptides, was appropriate to obtain hydrolysate with noticeable biological activities.

Due to their LMW, within the range of 500–2500 Da, collagen peptides are soluble in cold water and have no bitter taste owing to their high content in Gly. In addition, they are highly digestible and easily absorbed (Sibilla et al. 2015).

The schematic representation for the production of fish-derived peptides through enzymatic hydrolysis is illustrated in Figure 13.1.

## 13.5 Biological Activities of Fish Collagen-Based Peptides

### 13.5.1 Antioxidant Peptides

Lipid oxidation during food processing and storage (particularly those containing polyunsaturated fatty acids) by reactive oxygen species (ROS) is of great concern to food-industries and consumers, due to the loss of the food quality as well as the formation of undesirable off-flavors and potentially toxic reaction products (Lin and Liang 2002). Further, increased production of ROS, that may attack macromolecules such as proteins, DNA, and lipids, may lead to several degenerative diseases including diabetes, hypertension, cancer, atherosclerosis, etc. (Prior 1982). Synthetic antioxidants, including butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are widely used in food and pharmaceutical industries to prevent or delay lipid oxidation. However, these compounds have some undesirable side effects. Therefore, a great deal of interest has been expressed regarding the development of new and safe antioxidants from different natural sources, as alternatives to the synthetic ones (Becker 1993).

For instance, collagen and gelatin hydrolysates from several fish species, including thornback ray (Lassoued et al. 2015a), lizardfish (*Saurida* spp.) (Wangtueai et al. 2016), cartilage of *Sphyrna lewini*, *Dasyatis akjei* and *Raja porosa* (Li et al. 2013), and Black-barred halfbeak skin (Abdelhedi et al. 2017) have been found to display antioxidant activities. Moreover, numerous collagen-derived peptides are reported to possess antioxidant activity, and their amino acid sequences have been identified (Table 13.1).

Mendis et al. (2005) evaluated the antioxidant activity of Hoki (*Johnius belengerii*) skin gelatin hydrolysates generated with three commercial enzymes: trypsin,  $\alpha$ -chymotrypsin, and pepsin. Tryptic hydrolysate, exhibiting the highest antioxidant activity, was further

**Table 13.1** Examples of some bioactive peptides derived from collagenous sources.

Effects	Amino acid sequences	Protein substrates	References
Antioxidant activity	His-Gly-Pro-Leu-Gly-Pro-Leu Asn-His-Arg-Tyr-Asp-Arg Gly-Asn-Arg-Gly-Phe-Ala-Cys- Arg-His-Ala Gly-Phe-Arg-Gly-Thr-Ile-Gly-Leu- Val-Gly, Gly-Pro-Ala-Gly-Pro-Aal-Gly Gly-Phe-Pro-Ser-Gly Gly-Leu-Phe-Gly-Pro-Arg Gly-Ala-Thr-Gly-Pro-Gln-Pro-Leu- Gly-Pro-Arg Val-Leu-Gly-Pro-Phe Gln-Leu-Gly-Pro-Leu-Gly-Pro-Val Tyr-Gly-Cys-Cys, Asp-Ser-Ser-Cys-Ser-Gly, Asn-Asn-Ala-Glu-Tyr-Tyr-Lys, Pro-Ala-Gly-Asn-Val-Arg Glu-Pro-Gly-Pro-Val-Gly Leu-Pro-Gly-Pro-Ala-Gly Leu-Asp-Gly-Pro-Val-Gly Glu-Gly- Pro-Leu-Gly	Hoki skin gelatin Skin of horse mackerel Skin of croaker Scales of croceine croaker Gelatin of seabass skin	Mendis et al. (2005) Sampath Kumar et al. (2012) Wang et al. (2013) Sae-Leaw et al. (2016a)
	Gly-Pro-Glu, Gly-Ala-Arg-Gly-Pro-Gln, Gly-Phe-Thr-Gly-Pro-Pro-Gly- Phe-Asn-Gly,	Alaska Pollock skin collagen	Sun et al. (2016)
	Gly-Pro-Leu Gly-Pro-Met	Unicorn leatherjacket skin gelatin	Karnjanapratum et al. (2017)
Antihypertensive activity	Ala-Pro-Gly-Ala-Pro Gly-Ile-Pro-Gly-Ala-Pro GPEGPAGAR GETGPAGPAGAAGPAGPR Pro-Gly-Pro-Leu-Gly-Leu-Thr-Gly- Pro Gln-Leu-Gly-Phe-Leu-Gly-Pro-Arg Gly-Pro-Leu-Gly-Leu-Leu-Gly-Phe- Leu-Gly-Pro-Leu-Gly-Leu-Ser DPALATEPDPMFF Gly-Ala-Ser-Ser-Gly-Met-Pro-Gly Leu-Ala-Tyr-Ala	Scalloped hammerhead cartilage Alaska Pollack skin gelatin Thornback ray gelatin <i>Oreochromis niloticus</i> skin gelatin Skate ( <i>Raja kenojei</i> ) skin Squid tunic gelatin Nile tilapia gelatin Pacific cod skin gelatin	Li et al. (2017) Byun and Kim (2001) Lassoued et al. (2015a) Choonpicharn et al. (2016) Lee et al. (2011) Alemán et al. (2011) Vo et al. (2011) Ngo et al. (2016)
Antimicrobial activity	Gly-Leu-Pro-Gly-Pro-Leu-Gly-Pro- Ala-Gly-Pro-Lys	Atlantic mackerel collagen	Ennaas et al. (2015)
Hypoglycemic activity	Leu-Leu-Met-Leu-Asp-Asn-Asp- Leu-Pro-Pro	Pacific cod skin gelatin	Himaya et al. (2012)

successively fractionated by SP-Sephadex C-25 cationic exchange chromatography, Sephadex G-25 gel filtration and reversed-phase high-performance liquid chromatography (RP-HPLC). A strong radical scavenger peptide was purified and identified as His-Gly-Pro-Leu-Gly-Pro-Leu. In the same study, Mendis et al. (2005) investigated the effect of the purified peptide on antioxidative enzyme levels in cultured human hepatoma cells. The peptide was found to increase the activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx).

In their study, Sampath Kumar et al. (2012) isolated, from hydrolysates of skin horse mackerel (*Magalaspis cordyla*) and croaker (*Otolithes ruber*), two antioxidant peptides Asn-His-Arg-Tyr-Asp-Arg and Gly-Asn-Arg-Gly-Phe-Ala-Cys-Arg-His-Ala, respectively, which displayed excellent activities against polyunsaturated fatty acids peroxidation.

Furthermore, Wang et al. (2013) identified three antioxidant peptides with the following sequences, Gly-Phe-Arg-Gly-Thr-Iso-Gly-Leu-Val-Gly, Gly-Pro-Ala-Gly-Pro-Ala-Gly, and Gly-Phe-Pro-Ser-Gly, isolated from scales of croceine croaker (*Pseudosciaena crocea*) collagen.

More recently, gelatin of seabass skin was hydrolyzed with Alcalase and then fractionated by Sephadex G-25 size exclusion chromatography followed by RP-HPLC. Antioxidant peptides were identified in the most active RP-HPLC fractions (Sae-Leaw et al. 2016a). Among the identified sequences, four peptides were synthesized (Gly-Leu-Phe-Gly-Pro-Arg, Gly-Ala-Thr-Gly-Pro-Gln-Pro-Leu-Gly-Pro-Arg, Val-Leu-Gly-Pro-Phe, and Gln-Leu-Gly-Pro-Leu-Gly-Pro-Val), and their antioxidant activities were evaluated through 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity. The peptide Gly-Leu-Phe-Gly-Pro-Arg was found to exhibit the highest antioxidant activity.

Sun et al. (2016) evaluated the stability of antioxidative activity of Alcalase pollock skin collagen hydrolysate, with an hydrolysis degree (HD) of 13.17%, after two successive simulated gastro-intestinal digestion (SGID) steps. After twice SGID, the HD reached 26.17% and the antioxidant activity significantly increased compared to that of the initial Alcalase hydrolysate. Four peptides, with the following sequences Tyr-Gly-Cys-Cys, Asp-Ser-Ser-Cys-Ser-Gly, Asn-Asn-Ala-Glu-Tyr-Tyr-Lys, and Pro-Ala-Gly-Asn-Val-Arg, were purified from the hydrolysate obtained after the second simulated SGID. The enhancement of the antioxidant activity could be explained by the release of more potent peptides from Alcalase hydrolysate during gastro-intestinal digestion.

In a recent study of Karnjanapratum et al. (2017), antioxidant activity has been reported in enzymatic hydrolysate of unicorn leatherjacket skin gelatin produced with partially purified glycyl endopeptidase, isolated from papaya latex. A total of 56 antioxidant peptide sequences were identified. Four selected peptides, Glu-Pro-Gly-Pro-Val-Gly, Leu-Pro-Gly-Pro-Ala-Gly, Leu-Asp-Gly-Pro-Val-Gly, and Glu-Gly-Pro-Leu-Gly, were synthesized and their antioxidant activities were assayed. Glu-Gly-Pro-Leu-Gly showed the highest ABTS radical scavenging activity (4.95 µmol Trolox equivalent /g solid), while the other peptides displayed similar activity. However, the four synthesized peptides showed lower ABTS radical scavenging activity than gelatin hydrolysate, suggesting the synergistic effect of peptides present in the hydrolysate.

Other antioxidant peptides have been reported in Scalloped hammerhead (*S. lewini*) cartilage hydrolyzed with trypsin (Li et al. 2017). Three peptides were identified as Gly-Pro-Glu, Gly-Ala-Arg-Gly-Pro-Gln, and Gly-Phe-Thr-Gly-Pro-Pro-Gly-Phe-Asn-Gly.

The high amounts of hydrophobic amino acids within the peptide sequences, especially Gly and Pro may contribute significantly to their antioxidant activities due to the easy accessibility to hydrophobic targets.

Therefore, collagen and gelatin hydrolysates generated from fish skins contain electron donor peptides that could react with free radicals to convert them to more stable products, and hence, they can potentially be used as ingredients in functional foods and pharmaceuticals as an alternative source of natural antioxidants.

### 13.5.2 Antihypertensive Peptides

Angiotensin I-converting enzyme (ACE) plays an important physiological role in the renin-angiotensin system, which regulates human blood pressure. Renin converts angiotensinogen to inactive decapeptide angiotensin I, that will be converted by ACE to the potent vasoconstrictor octapeptide angiotensin II (Lavoie and Sigmund 2003). Moreover, ACE catalyzes the degradation of bradykinin, a known vasodilator peptide (Ondetti et al. 1977). Therefore, inhibitors of ACE are commonly used as therapeutic agents in the treatment of hypertension.

Specific inhibitors of ACE (such as captopril, lisinopril, and enalapril), used to treat and prevent hypertension, have demonstrated their usefulness. However, some of them are responsible for adverse side effects, including dry cough, lost of taste, renal failure and angioedema (Atkinson and Robertson 1979). Therefore, the development of new, natural, and specific ACE inhibitors, as alternative to the synthetic ones, is necessary for the prevention and remedy of hypertension. In this regard, various studies have been focused on the investigation of antihypertensive effects of protein hydrolysates from various protein sources (FitzGerald et al. 2004; He et al. 2013a,b).

ACE-inhibitory peptides are the most extensively studied biopeptides. To date, numerous peptides with anti-ACE activity have been identified from various collagen and gelatin hydrolysates. Byun and Kim (2001) reported two ACE-inhibitory peptides, Gly-Pro-Leu and Gly-Pro-Met, with  $IC_{50}$  values of 2.6 and 17.13  $\mu\text{M}$ , respectively, isolated from Alaska Pollack (*Theragra chalcogramma*) skin gelatin hydrolysate. Both peptides, and particularly Gly-Pro-Leu could be useful as a new hypotensive agent.

Zhao et al. (2007) evaluated the anti-ACE activity of sea cucumber gelatin hydrolyzed sequentially with bromelain and Alcalase. The resulting hydrolysate was fractionated by ultrafiltration into three fractions I, II, and III, that were composed of peptides with molecular weight <10, <5, and <1 kDa, respectively, displaying different degrees of activity. Thereafter, the antihypertensive effect of fraction III, which exhibited the strongest ACE-inhibitory activity, was studied *in vivo* after its oral administration to renal hypertensive rats (RHRs). The *in vivo* study revealed that administration of gelatin hydrolysate for one month decreased significantly both systolic and diastolic blood pressure of the RHR.

Lee et al. (2011) studied the ACE-inhibitory activity of several skate (*Raja kenojei*) skin hydrolysates obtained by treatment with  $\alpha$ -chymotrypsin, Alcalase, Neutrase, papain, pepsin, and trypsin. Two potent ACE-inhibitors were purified from the  $\alpha$ -chymotrypsin hydrolysate, which displayed the highest activity compared to the other hydrolysates. The amino acid sequences of the peptides were identified as Pro-Gly-Pro-Leu-Gly-Leu-The-Gly-Pro and Gln-Leu-Gly-Phe-Leu-Gly-Pro-Arg, with  $IC_{50}$  values of 95 and 148  $\mu\text{M}$ , respectively.

Similarly, Alemán et al. (2011) reported the anti-ACE activity of squid gelatin hydrolysates prepared with different proteases. Alcalase hydrolysate showed the most potent ACE inhibitory activity *in vitro* with IC<sub>50</sub> value of 0.34 mg ml<sup>-1</sup>, followed by the Neutrerase hydrolysate (IC<sub>50</sub> = 0.63 mg ml<sup>-1</sup>). The peptide Gly-Pro-Leu-Gly-Leu-Leu-Gly-Phe-Leu-Gly-Pro-Leu-Gly-Leu-Ser, identified from squid tunic gelatin, exhibited high ACE-inhibitory activity as well as interesting antioxidant activity.

In the same context, Vo et al. (2011) evaluated the ACE-inhibitory activity of gelatin hydrolysates from Nile tilapia (*Oreochromis niloticus*) generated with Alcalase, Pronase E, pepsin, and trypsin. A potent ACE-inhibitory peptide DPALATEPDPMMPF, which exhibited the strongest activity (IC<sub>50</sub> = 62.2 μM), was purified from the Alcalase hydrolysate.

In their study, Guo et al. (2015) reported the generation of bioactive peptides from Alaska pollock skin collagen through SGID. Moderate ACE inhibitory activity (IC<sub>50</sub> value of 2.92 ± 0.22 mg ml<sup>-1</sup>) was obtained using pepsin in the gastric stage, which significantly increased after intestinal digestion (IC<sub>50</sub> = 0.49 ± 0.02 mg ml<sup>-1</sup>).

In another study, Lassoued et al. (2015a) studied the ACE-inhibitory activity of thorn-back ray gelatin hydrolysates (TRGHs) obtained by enzymatic treatment with proteases from *Bacillus subtilis* A26 (TRGH-A26) and Neutrerase (TRGH-N). TRGH-A26 and TRGH-N displayed ACE-inhibitory activity with IC<sub>50</sub> values of 0.94 and 2.07 μg μl<sup>-1</sup>, respectively. Peptides from both hydrolysates were fractionated by Sephadex G-25 gel filtration, and the most active fractions were further separated by RP-HPLC and then analyzed using nanoESI-LC-MS/MS, and a total of 260 peptides were identified. By using Biopep data base, several potential active peptides, showing sequences with previously identified anti-ACE peptides, were synthesized and their activities were assessed. Among synthesized peptides Gly-Ile-Pro-Gly-Ala-Pro from TRGH-N and Ala-Pro-Gly-Ala-Pro from TRGH-A26 showed the highest activity, with IC<sub>50</sub> values of 27.9 and 170.23 μM, respectively. Both peptides had Pro residue at their C-terminal.

ACE-inhibitory potential of trypsin *O. niloticus* skin gelatin hydrolysate and its fractions, obtained by Sephadex G-50 gel filtration, was recently reported by Choopicharn et al. (2016). GPEGPAGAR and GETGPAGPAGAAGPAGPR, two main peptide sequences, were identified from fraction B, which exhibited high anti-ACE activity. Both synthesized peptides displayed similar levels of ACE-inhibitory activity.

Recently, Ngo et al. (2016) evaluated the ACE-inhibitory activity of Pacific cod skin gelatin hydrolysates produced using papain, α-chymotrypsin, pepsin, trypsin, Neutrerase, and Alcalase. Pepsin hydrolysate, the most potent ACE-inhibitor, was further fractionated into four hydrolysate fractions with molecular weight range of >10, 5–10, 1–5, and <1 kDa by sequential ultrafiltration (UF) using different kinds of UF membranes. Two potent ACE-inhibitor peptides, Gly-Ala-Ser-Ser-Gly-Met-Pro-Gly and Leu-Ala-Tyr-Ala, which showed IC<sub>50</sub> values of 6.9 and 14.5 μM, respectively, were purified from small-size peptides (below 1 kDa).

Although the structure-activity relationship of ACE inhibitory peptides has not been entirely elucidated, several works reported the importance of some specific amino acids at both C-terminal and N-terminal positions (Gobbetti et al. 2000). In fact, peptides with Trp, Tyr, Phe, Pro, or other hydrophobic amino acids at the C-terminal or at the three positions from the C-terminal are effective ACE-inhibitors. Nevertheless, peptide sequences that contained Pro at the carboxyl terminal were reported to be the most active. Further, peptides with Ile and Val at the N-terminal side were also potent ACE

inhibitors. Thus, the high content in Pro residue, may explain the potent anti-ACE activity of collagen substrates. As most of the highly active peptides reported in the literature contained Pro at C-terminal, enzyme which cleaves peptide bonds preferentially at the carboxyl side of Pro, could be used to generate highly active ACE-inhibitory peptides.

### 13.5.3 Antimicrobial Peptides

In recent years, there is an increasing interest in the discovery of novel and natural antimicrobial agents, with broad range activity against a wide spectrum of pathogenic microorganisms. Antimicrobials are used in foods to inhibit the growth of food spoilage microorganisms. Collagen-derived antimicrobial peptides could be an excellent alternative to conventional antibiotics. To date, few antimicrobial peptides have been identified from collagen hydrolysates. Most of them are composed of less than 50 amino acids, with a net positive charge of 2–8 (Rydlo et al. 2006).

Ennaas et al. (2015) reported the isolation and the identification of four antibacterial peptides from Protamex hydrolysate of Atlantic mackerel (*Scomber scombus*) byproducts, with the following sequences: SIFIQRFTT, RKSGDPLGR, AKPGDGAGSGPR, and GLPGPLGPAGPK. The last peptide, called collagencin, was identified as fragment of fish collagen. Collagencin was found to exhibit antibacterial activity against numerous Gram-positive and Gram-negative bacteria. In a more recent study, Ennaas et al. (2016) demonstrated that synthetic collagencin at 1.88 mM totally inhibited the growth of *Staphylococcus aureus*. Collagencin is a proline rich peptide, a common characteristic of numerous antimicrobial peptides reported in the literature. It contains as well Lys, a positively charged amino acid at the C-terminal side.

### 13.5.4 Hypoglycemic Peptides

Type 2 diabetes mellitus (T2DM) is a metabolic disorder, characterized by a high level of glucose, due to several fundamental defects, including impaired insulin secretion and insulin resistance (Lacroix and Li-Chan 2016). For the treatment of T2DM, insulin, and several oral hypoglycemic agents (such as dipeptidyl peptidase IV (DPP-IV) inhibitors) are used. DPP-IV plays an important role in serum glucose regulation in humans. Indeed, during the post-prandial phase, the enzyme catalyzes the degradation of incretins hormones, including glucagon-like peptide-1 and glucose inhibitory polypeptide. Both incretins are known to stimulate 50–60% of the total postprandial insulin secretion from  $\beta$ -cells (Creutzfeldt 2001). Their degradation results in the loss of their ability to enhance insulin secretion (Juillerat-Jeanneret 2014). Therefore, specific inhibitors of DPP-IV can be used as therapeutic agents in the treatment of T2DM (Jao et al. 2015).

Interestingly, several works reported the ability of peptides including those derived from collagen to inhibit DPP-IV. Li-Chan et al. (2012) investigated the anti-DPP-IV activity of Atlantic salmon skin gelatin hydrolysates obtained by treatment with Alcalase, bromelain, and Flavourzyme. Two peptides identified as Gly-Pro-Ala-Glu and Gly-Pro-Gly-Ala were synthesized and their  $IC_{50}$  values were estimated to be 49.6 and 41.9  $\mu$ M, respectively.

In their study, Wang et al. (2015) reported the anti-DPP-IV activity of halibut skin gelatin hydrolysate (HSGH) and tilapia skin gelatin hydrolysate (TSGH). At a concentration

of 1 mg ml<sup>-1</sup>, HSGH and TSGH displayed an inhibitory activity of 38.2% and 51.9%, respectively. Further, the *in vivo* study demonstrated that daily administration of TSGH for 30 days to streptozotocin-induced diabetic rats improved glucose tolerance.

In a more recent study, Zhang et al. (2016) demonstrated that Alcalase tilapia collagen peptides (TCPs), showed hypoglycemic effects in alloxan-induced diabetic mice. In fact, administration of TCP at 1.7 g kg<sup>-1</sup> body weight (bw) was found to reduce by 31.8% the blood glucose level after 25 days. In addition, TCPs improved antioxidant activity revealed by a significant increase in SOD and CAT activities by 23% and 59.2%, respectively, and a decrease of malondialdehyde (MDA, the product of lipid peroxidation) level by 39.1%.

### 13.5.5 Anticancer Peptides

Anticancer activity of few collagen hydrolysates has been reported in recent years. Alemán et al. (2011) studied the anticancer activity of giant squid (*Dosidicus gigas*) gelatin hydrolyzed with seven commercial proteases. They reported that Esperase hydrolysate displayed the highest cytotoxic and anti-proliferative effects on two cancer cells (MCF-7 human breast carcinoma and U87 glioma), while intact squid gelatin did not exhibit any cytotoxic or antiproliferative effects on cancer cells.

Recently, Baehaki et al. (2016) investigated the anti-cancer activity of Milk fish (*Chanos chanos*) skin collagen hydrolysate produced, after different incubation times, using collagenase from *Bacillus licheniformis* F11.4. Collagen peptides (CPs) showed the highest activities at 60 and 30 minutes toward HeLa and HCT-166 cells, respectively.

Phe-Ile-Met-Gly-Pro-Tyr, isolated from skate (*R. porosa*) cartilage protein hydrolysate was shown to exhibit, significant and dose-dependent antiproliferative activity in HeLa cells, with an IC<sub>50</sub> value of 4.81 mg ml<sup>-1</sup> (Pan et al. 2016). The identified hexapeptide induced apoptosis by upregulating the apoptotic protein (Bax)/the anti-apoptotic protein (Bcl-2) ratio and caspae-3 activation.

### 13.5.6 Other Biological Activities

In addition to bioactivities mentioned earlier, there are other activities which have been identified in various collagen hydrolysates, including neuroprotective effects of Chum Salmon skin collagen peptides on male rats with perinatal asphyxia (Xu et al. 2015). Sae-Leaw et al. (2016b) have revealed that sea-bass hydrolysates displayed immunomodulatory activity manifested by a significant decrease in interleukin-6 (IL-6) and IL-1 $\beta$  production in lipopolysaccharide stimulated RAW 264.7 macrophage cells.

Some examples of bioactive peptides derived from collagenous sources are listed in Table 13.1.

### 13.5.7 Multifunctional Activities

Numerous studies have shown that collagen hydrolysates are multifunctional and therefore, they can exert a wide variety of bioactivities. This is could be explained by the

presence in the same hydrolysate of a complex mixture of biopeptides, with different chain length and amino acid sequences, which may exhibit different biological activities.

In this context, Squid gelatin hydrolysates prepared by seven commercial proteases were reported to exert anti-ACE, anticancer, and antioxidant activities (Alemán et al. 2011). In another study, Popov et al. (2013) demonstrated that collagen peptides derived from Far-Eastern holothurians displayed numerous activities including antitumor, anticoagulant, anti-inflammatory, and wound healing effects. In a more recent study, Lassoued et al. (2015b) demonstrated that TRGHs may constitute a new source of antioxidant and anti-ACE peptides.

Similarly, Jumbo squid fins and arms collagen hydrolysates obtained by treatment with trypsin and protease type XIV were found to display multifunctional activities. They exhibit low antiproliferative activity of murine transformed cells, as well as antioxidative and antimutagenic activities (Suarez-Jiménez et al. 2015). Likewise, Guo et al. (2015) demonstrated the liberation of multifunctional peptides from Alaska pollock skin collagen following SGID, including antioxidant, DPP-IV-inhibitor, ACE-inhibitor, and metal binding peptides. Seabass gelatin hydrolysates were similarly found to exhibit antioxidant, immunomodulatory, and antiproliferative activities (Sae-Leaw et al. 2016b).

Recently, Abdelhedi et al. (2017) reported that black-barred halfbeak gelatin hydrolysate (BGH), obtained by treatment with Purafect® (HD of 12.5%), showed high antioxidant potential investigated by different antioxidant assays. Further, BGH sample exhibited antibacterial activity against different Gram<sup>+</sup> and Gram<sup>-</sup> bacteria, and displayed an ACE-inhibitory activity of 80.76% at 1 mg ml<sup>-1</sup>. Thus, black-barred halfbeak gelatin represents a promising source of antioxidant, ACE-inhibitory, and antimicrobial peptides that might prevent humans from several diseases.

Besides the multifunctional properties of collagen hydrolysates, some specific collagen peptide sequences have revealed multifunctional activities, which may therefore increase their potential biotechnological applications (Meisel and FitzGerald 2003). In this respect, the peptide Leu-Leu-Met-Leu-Asp-Asn-Asp-Leu-Pro-Pro, purified from Pacific cod skin gelatin hydrolyzed with gastrointestinal enzymes (pepsin, trypsin, and α-chymotrypsin) was found to inhibit ACE and to attenuate cellular oxidative stress (Himaya et al. 2012).

## 13.6 Potential Applications in Functional Foods or Pharmaceuticals

### 13.6.1 Bioavailability

Marine collagen peptides can be used in a wide range of applications. However, to reach their target site in an intact form after oral administration and to exert their biological activities, collagen peptides must be resistant to degradation by digestive enzymes (pepsin, trypsin, chymotrypsin) and then by plasma peptidases. In addition, they must be absorbed easily through the intestinal epithelial cells to reach the blood stream.

Several scientific studies reported the bioavailability of collagen peptides (particularly di- and tri-peptides) after oral administration to humans and animals. Bioavailability is defined as the relative amount of biopeptides that reach their target site. In general, small peptides are more bioavailable than free amino acids and proteins (Hajirostamloo 2010).

Collagen hydrolysates are a mixture of peptides with different lengths and containing mainly Gly, Pro, and Hyp. Small peptides are generally resistant to endogenous digestive proteases. The stability of collagen peptides could be attributed to the presence of Pro and Hyp in their sequence. In this respect, Gardner (1988) reported that Hyp- and Pro-containing peptides (di- or tri-peptides) are generally resistant to degradation by digestive proteases, and thereby have a high chance to reach their target site. In another study, Liu et al. (2009) evaluated the absorption, in the rat small intestine, of several Hyp-containing di-peptides. They demonstrated that Hyp-peptides are not completely hydrolyzed, and therefore are transported across the intestinal wall.

Regarding the absorption of peptides, several studies reported that small peptides are transported across the intestinal mucosa by PEPT-1 transporter (Leibach and Ganapathy 1996; Aito-Inoue et al. 2007). This was supported by the study of Iwai et al. (2005) who detected the presence of several food-derived collagen peptides in blood of healthy human volunteers after oral ingestion of gelatin hydrolysate. While Pro-Hyp was found to be the most abundant peptide, low levels of Ala-Hyp, Ala-Hyp-Gly, Pro-Hyp-Gly, Leu-Hyp, Ile-Hyp, and Phe-Hyp were also detected in human serum and plasma. Similarly, Shigemura et al. (2011) identified in human plasma, after fish scale collagen hydrolysate ingestion, in addition to Pro-Hyp, another major collagen peptide: Hyp-Gly.

### 13.6.2 Food Applications

As collagen-based peptides have been reported to possess antioxidant activity, they can therefore be used as functional ingredients to preserve food products against oxidation. In this context, Jridi et al. (2014) demonstrated that the addition of Alcalase-cuttlefish skin gelatin hydrolysate, which exhibited high antioxidant activity, into turkey meat sausage (at 0.5 mg g<sup>-1</sup>), delayed lipid oxidation monitored by Thiobarbituric acid reactive substances (TBARS) and conjugated diene up to 10 days compared to vitamin C. In the same context, Kittiphantanabawon et al. (2012) reported that gelatin hydrolysates from blacktip shark skin with different HD (10–40%) were found to inhibit lipid oxidation in linoleate and cooked comminuted pork model systems. Therefore, they could be used as alternative source of natural antioxidants.

Moreover, due to their antifreeze activity, collagen peptides could be added to frozen foods to retard or inhibit the rate of ice growth during storage and therefore to preserve their textural quality (Cornwell-Shuman 1960; Damodaran 2007; Wang et al. 2014). The antifreeze activity could be due to the reduction of water mobility. Wang et al. (2014) reported the purification of antifreeze peptide from shark skin collagen hydrolyzed with acid protease, followed by successive fractionation on SP-Sephadex cation exchange chromatography, Sephadex G-50 gel-filtration and RP-HPLC. Interestingly, the peptide identified as Gly-Ala-Ile-Gly-Pro-Ala-Gly-Pro-Leu-Gly-Pro showed hypothermia protection activity on *Lactobacillus bulgaricus*, commonly used in the manufacture of cheeses and fermented milks. In a more recent study, Damodaran and Wang (2017) demonstrated that Alcalase fish gelatin hydrolysate is able to inhibit the growth of ice crystal in an ice cream mix. Cationic peptides fraction obtained after fractionation using gel-filtration and ion exchange chromatography were found more effective than anionic and neutral peptide fractions.

According to Damodaran (2007), the antifreeze activity could be due to the tripeptide repeating structure of Gly-Pro-X or Gly-Z-Hyp in collagen peptides. In addition, the

cryoprotective effect could be attributed to the interaction of hydrophilic peptides with water, lowering thereby the migration of water to form crystals (Hossain et al. 2004).

### 13.6.3 Cosmetic Applications

Skin, the main barrier to the external environment, is known to be affected by intrinsic aging process as well as extrinsic factors. Intrinsic aging is due to genetic and hormonal processes, while external factors include ultraviolet (UV) radiation, smoking, stress, alcohol, lack of sleep, pollution, nutrition, etc. Intrinsic and extrinsic aging stimulate the production of free radicals, which affect skin elasticity and uniformity (Baumann 2007).

Several scientific studies reported the beneficial effects of fish collagen and collagen-based peptides on skin properties, due to their multifunctional bioactivities including antimicrobial, anti-aging, and anti-wrinkling activities. Moreover, as collagen peptides were found to exhibit antioxidant activities, they also can protect against free radicals, limiting their aging effects (Sibilla et al. 2015).

In this regard, Asserin et al. (2015) studied the effect of oral intake of fish collagen peptide (Peptan<sup>®</sup>) on skin hydration and the dermal collagen network in a clinical setting. Oral supplementation with Peptan increased the skin moisture level by 12% after eight weeks of intake compared with control group. Furthermore, Peptan prevented and reduced the fragmentation of dermal collagen network and increased significantly collagen synthesis. Similarly, Borumand and Sibilla (2015) demonstrated that oral drink containing hydrolyzed collagen improved the depth of facial wrinkles as well as elasticity and hydration of skin.

Among external factors, UV light exposure (photo-aging) is the major environmental factor that affects the structure and function of the skin. Indeed, UV exposure of skin leads to various damages such as inflammation, deep wrinkles, edema, irregular pigmentation, poor elasticity, increase in epidermal thickness and decrease in dermal collagen (Rittié and Fisher 2002; Kammeyer and Luiten 2015). In addition, UV radiation is the main cause of photocarcinogenesis (Agar et al. 2004).

Numerous studies have been focused on anti-photoaging effect of collagen peptides (CPs) (Venkatesan et al. 2017). In this context, Hou et al. (2012) evaluated the protective effects of CPs from cod skin, CP1 ( $2 \text{ kDa} < \text{MW} < 6 \text{ kDa}$ ) and CP2 ( $\text{MW} < 2 \text{ kDa}$ ) against UV radiation induced damage to mouse skin. CP1 and CP2 fractions were obtained by co-hydrolysis of collagen using pepsin and alkaline protease followed by ultrafiltration with 6 and 2 kDa cut-off membranes, respectively. Interestingly, in addition to their high moisture absorption and retention properties, CPs had the ability to protect skin from photoaging. The protective effect of CPs could mainly be attributed to the enhancement of immunity, reduction loss of moisture and enhancement of antioxidant activities.

In the same context, Fan et al. (2013) reported the protective effects of jellyfish collagen (JFC) and its jellyfish collagen hydrolysate (JFCH) on UV radiation-induced skin damage of mice. JFCH was more efficient than the native collagen.

Similarly, Chen et al. (2016) reported that the administration of salmon skin gelatin and its hydrolysate could alleviate the oxidative damage of mice skin induced by UV radiation mainly by increasing SOD, CAT, and GPx activities, as well as glutathione (GSH) content and decreasing MDA level. In addition, when administered orally, salmon gelatin and its hydrolysate enhanced the immune system.

**Table 13.2** Examples of commercially available collagen peptides.

Product name	Collagen source	Activities/Applications	Manufacturer	References
Fish collagen peptides	Collagen from fish scales hydrolyzed the average MW <3 kDa	<ul style="list-style-type: none"> <li>Anti-aging</li> <li>Skin moisturizing or antiwrinkle treatments</li> <li>Diet food products and natural food products</li> </ul>	Marine Biotech Jilidng	<a href="http://www.jilidng.com/fish-collagen.html">http://www.jilidng.com/fish-collagen.html</a>
Vinh wellness collagen peptides	<i>Pangasius hypophthalmus</i>	<ul style="list-style-type: none"> <li>Maintain connective tissues</li> <li>Build muscle</li> <li>Enhance joint structure</li> <li>Amino acid supplements</li> <li>Improves skin elasticity</li> <li>Enhances water absorption capacity</li> </ul>	Vinh Hoan	<a href="http://vinhwellness.com/premium-hydrolyzed-single-source-marine-collagen-peptides/">http://vinhwellness.com/premium-hydrolyzed-single-source-marine-collagen-peptides/</a>
Vital proteins marine collagen	Wild-caught snapper	<ul style="list-style-type: none"> <li>Promotes youthful skin, healthier hair, stronger nails, joint health, and bone health</li> </ul>	Vital Proteins	<a href="https://www.vitalproteins.com/products/marine-collagen-peptides">https://www.vitalproteins.com/products/marine-collagen-peptides</a>
Solu-Mar EN-30	Marine hydrolyzed collagen	<ul style="list-style-type: none"> <li>Used in shampoo, treatment (hair), leave-in, styling, bodywash, body lotion, facial moisturizer, mascara. Possesses moisturizing and film forming properties. Aids in the healing and structuring of connective tissue.</li> </ul>	SpecialChem	<a href="http://cosmetics.specialchem.com/product/i-lonza-solu-mar-en-30">http://cosmetics.specialchem.com/product/i-lonza-solu-mar-en-30</a>

(Continued)

**Table 13.2 (Continued)**

Product name	Collagen source	Activities/Applications	Manufacturer	References
Marine collagen capsules	Hydrolyzed marine collagen	<ul style="list-style-type: none"> <li>• Development and maintenance of skin tissue and elasticity</li> </ul>	Just vitamins	<a href="https://www.justvitamins.co.uk/Amino-Acids/Collagen-Marine-400mg.aspx#.WYdvQ7pFzIU">https://www.justvitamins.co.uk/Amino-Acids/Collagen-Marine-400mg.aspx#.WYdvQ7pFzIU</a>
Fish collagen powder	<ul style="list-style-type: none"> <li>• Salmon</li> <li>• Cod</li> </ul>	<ul style="list-style-type: none"> <li>• Supporting skin's elasticity.</li> </ul>	Interpharm Trading CO. Ltd	<a href="http://www.collagenhydrolysatesuppliers.com/index.html">http://www.collagenhydrolysatesuppliers.com/index.html</a>
SeaSource™ hydrolyzed marine collagen	Wild-caught, deep sea fish	<ul style="list-style-type: none"> <li>• Reduce wrinkles;</li> <li>• Create firmer, smoother more radiant skin, etc.</li> </ul>	Norland Products Inc.	<a href="https://www.seasourcecollagen.com/">https://www.seasourcecollagen.com/</a>
Hydrolyzed fish collagen	—	<ul style="list-style-type: none"> <li>• Promote joint and bone health;</li> <li>• Weight control;</li> <li>• Boost the immunity;</li> <li>• Improve sleeping;</li> <li>• Blood pressure;</li> <li>• Protect the gastric mucosa;</li> <li>• Protect the cornea.</li> </ul>	Jiangxi Cosen biology Co., Ltd.	<a href="http://www.chinacollagen.com/en/index.php/hydrolyzed-fish-collagen/">http://www.chinacollagen.com/en/index.php/hydrolyzed-fish-collagen/</a>
Fish collagen peptide	<ul style="list-style-type: none"> <li>• <i>Terapia</i></li> <li>• Salmon</li> </ul>	<ul style="list-style-type: none"> <li>• Suitable for health food, as tablet, capsule, sachet, etc.;</li> <li>• Skin beauty.</li> </ul>	Maruha Nichiro Corporation	<a href="http://www.maruha-nichiro.co.jp/food/english/fecd/product/pro00700.html">http://www.maruha-nichiro.co.jp/food/english/fecd/product/pro00700.html</a>

Norland hydrolyzed fish collagen	Deep water, ocean fish: Cod, Haddock and Pollock	<ul style="list-style-type: none"> <li>• Protein additive in nutraceutical, cosmetic, or food applications.</li> </ul>	Norland Products	<a href="https://www.norlandprod.com/Fishdefault.html">https://www.norlandprod.com/Fishdefault.html</a>
MAXENTA fish collagen peptide	Wild caught Alaska Pollock	<ul style="list-style-type: none"> <li>• Whitening and antioxidant properties</li> <li>• Increases skin elasticity and reduces sagging</li> <li>• Hydrates skin and helps to reduce fine lines</li> </ul>	Einfach Holding Sdn. Bhd.	<a href="http://www.maxentaplus.com/product_collagen.php">http://www.maxentaplus.com/product_collagen.php</a>
Marine collagen peptide	Deep ocean fish	<ul style="list-style-type: none"> <li>• Reduce moisture reduction in skin and muscle</li> <li>• Make skin remain young</li> </ul>	Green Stone Swiss Co., Ltd.	<a href="http://www.godowell.net/Nutrition-function-Add/Marine-Collagen-Peptide/">http://www.godowell.net/Nutrition-function-Add/Marine-Collagen-Peptide/</a>
Marine fish collagen hydrolysate peptide powder	Grass carp <i>Ctenopharyngodon idella</i>	<ul style="list-style-type: none"> <li>• Food ingredient, additive and nutraceutical.</li> </ul>	Shanghai Sunflower Food Ingredients Co., Ltd.	<a href="https://sunflower2016.en.ec21.com/Marine_Fish_Collagen_Hydrolysate_Peptide--10050912_10051770.html">https://sunflower2016.en.ec21.com/Marine_Fish_Collagen_Hydrolysate_Peptide--10050912_10051770.html</a>
Marine collagen Peptides	Wild-caught crimson snapper	<ul style="list-style-type: none"> <li>• Anti-aging</li> <li>• Decrease wrinkles</li> <li>• Beautify hair, skin, and nails</li> <li>• Strengthen joints</li> </ul>	Natural Force	<a href="https://naturalforce.com/product/marine-collagen-peptides/">https://naturalforce.com/product/marine-collagen-peptides/</a>

In a more recent study, Zhang et al. (2017) evaluated the effects of three different antioxidant collagen peptides (ACPs) as well as casein peptides and tea polyphenols on the photoaged skin of mice. The three ACPs, with high, medium, and low antioxidant activities, were obtained through enzymatic hydrolysis of silver carp skin collagen, using Alcalase, papain, and trypsin, respectively. Oral administration of ACPs alleviated UV radiation-induced abnormal alterations of skin components in both serum and skin. Further, ACPs were found to attenuate UV-induced oxidative stress attributed to the generation of several ROS by increasing the CAT and SOD activities and decreasing the MDA content compared with control group rats.

The overall studies demonstrated that collagen peptides could potentially be used as antioxidant and antiphotoaging agent in cosmetic industry.

#### 13.6.4 Potential Therapeutic Applications

Collagen hydrolysates could be used in therapeutic purposes especially in the prevention and/or treatment of cardiovascular diseases. For example, the ACE-inhibitory activity of collagen peptides reported *in vitro*, in SGID model system and *in vivo* (as mentioned above) demonstrated that collagen-derived antihypertensive peptides could be used as ingredients in functional foods and pharmaceuticals as an alternative for the prevention or the treatment of hypertension.

In another application, Liang et al. (2014) studied the healthful potential of Chum Salmon (*Oncorhynchus keta*) CPs against ethanol intoxication in female Sprague-Dawley rats. Orally administration of CPs at 4.9 and 9.0 g kg<sup>-1</sup> bw prior to the oral administration of ethanol could attenuate ethanol-induced loss of motor coordination, suggesting that CPs have protective effects against the toxicity induced by ethanol.

The *in vivo* wound-healing potential of collagen peptides, with antioxidant activities, has been recently studied. Zhang et al. (2011) demonstrated that oral administration of Chum Salmon skin collagen hydrolysate (at 2 g kg<sup>-1</sup>) to rats enhanced cutaneous wound healing. Indeed collagen peptide-treated rats showed faster closure and improved tissue regeneration at the wound site as well as angiogenesis. Similarly, in a recent study, Hu et al. (2017) evaluated the *in vitro* and *in vivo* wound healing potential of collagen peptides obtained through co-hydrolysis of Nile tilapia skin using neutral protease and papain. Collagen peptides were found to enhance the process of wound healing.

Today, numerous collagen peptides from various fish collagen sources are already commercialized by food and cosmetic industries (Table 13.2).

## 13.7 Conclusion

In recent years, there is an increasing interest in finding new and safe bioactive substances from natural sources. Fish processing collagen-containing byproducts are a potential source to provide by enzymatic hydrolysis novel biologically active peptides. As mentioned in this review, collagen peptides are endowed with several biological activities and numerous scientific studies have demonstrated their beneficial effects. Therefore, based on their plentiful and multifunctional biological activities, collagen

hydrolysates can be used as promising source of bioactive food, cosmetic, and pharmaceuticals ingredients.

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## 14

# Bioactive Compounds from Animal Meat Byproducts

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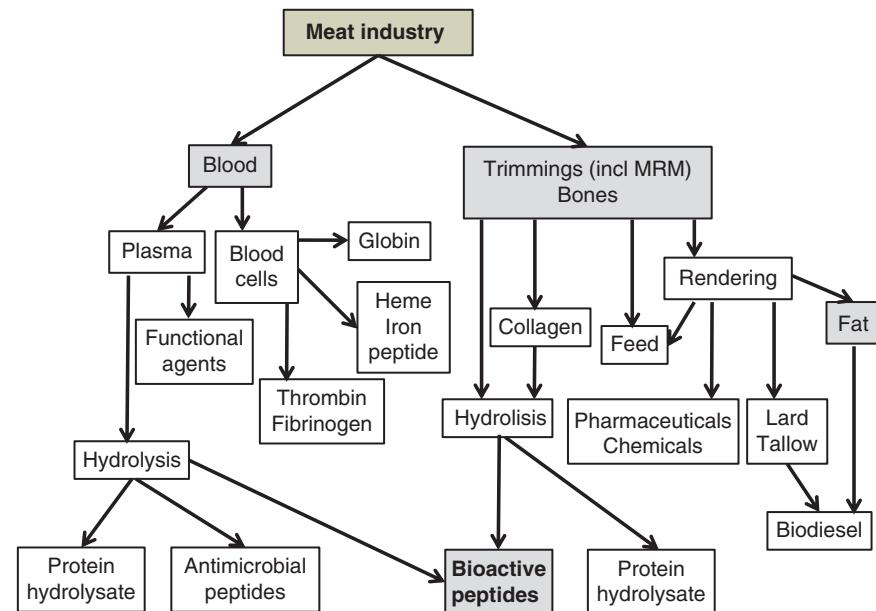
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## 14.1 Introduction

One of the main concerns nowadays in the modern world is about waste disposal and byproduct management of the food processing industry. This is an important problem in terms of environmental protection and sustainability (Russ and Pittroff 2004; Nollet and Toldrá 2011). In the meat industry, the highest amount of waste is produced during slaughtering, including bones, tendons, skin, contents of the gastro-intestinal tract, blood, and internal organs. However, the type of animal, tradition, culture, and religion are key factors to consider when assigning byproducts as edible or non-edible as these aspects will influence which portions of the slaughtered animal can be used for food or as food ingredients.

Despite meat byproducts being used for biodiesel production, fertilizers, feeds, and pet food, or for the manufacture of drugs by pharmaceutical industry, and plastics and leather by chemical industry, the use of compounds derived from meat byproducts as food ingredients such as flavor enhancers, emulsifiers, or nutrients, and as functional ingredients such as bioactive peptides is the main and most studied purpose during the last decade (Toldrá et al. 2016). In this sense, Figure 14.1 shows a diagram with main byproducts obtained from meat and meat products industry and different applications and uses of these wastes.

Certain molecules such as carnosine, anserine, conjugated linoleic acid, glutathione, creatine, creatinine, or taurine are naturally present in certain amounts and are thus characteristic in meat and meat products and have been described as biologically active in the human body. These molecules are also expected to be present in meat derived byproducts and probably exert their activity when used for food or food ingredients. In this sense, both carnosine (*b*-alanyl-L-histidine) and anserine (*N*-*b*-alanyl-1-methyl-L-histidine) are antioxidative histidyl dipeptides and the most abundant



**Figure 14.1** Flow diagram of main routes for value-addition to meat byproducts. Source: Reproduced from Mora et al. (2014b) with permission from Elsevier.

antioxidants in meats (Arihara 2006), whereas conjugated linoleic acid has been described as anticarcinogenic, antioxidant, and immunomodulative (Azain 2003).

## 14.2 Enzymatic Hydrolysis for Peptides Production

Despite peptides being able to be generated from protein matrices through different ways such as the natural gastrointestinal digestion or during food processing in fermentation and curing treatments, the most used methodology for the production of protein hydrolysates with the aim to generate potential bioactive peptides is enzymatic digestion with commercial enzymes or microbial fermentation. In fact, the generation of bioactive peptides through natural processes such as gastrointestinal digestion or food processing presents the challenge of limited control of hydrolysis conditions, as many endo- and exopeptidases are acting at the same time and a wide profile of peptides showing different sizes and characteristics is generated (Mora et al. 2015). Thus, the digestion of proteins from meat byproduct extracts under controlled hydrolysis conditions by known enzymes is the method of choice in industries. This allows the control of the generated bioactive peptides as well as the generation of more homogeneous batches.

The proteins obtained from meat industry considered as edible byproducts are usually subjected to hydrolysis with a wide range of commercial enzymes derived from animals such as pepsin, trypsin, chymotrypsin, corolase PP, or pancreatin, plants including the enzymes ficin, bromelain, and papain and microorganisms such as Neutrase®, a metallo-protease from *Bacillus amyloliquefaciens*, or Alcalase®, a serineprotease from *Bacillus licheniformis*. Some of these enzymes show specific cleavage sites, especially

those participating in the gastrointestinal digestion of animals, whereas others cover a broad range of specificities acting as endo- and exopeptidases as the microorganisms-derived enzymes. Despite the fact that this could cause poor reproducibility when unspecific enzymes are used, these are the best options in industry, as the highest costs are associated with enzymatic reactions, so cheap proteinases sources such as microorganisms are preferred (Agyei and Danquah 2011; Lafarga and Hayes 2014). Table 14.1 describes the characteristics and optimal conditions of the main enzymes used in the hydrolysis of meat and meat-derived byproducts, and the flow diagram for the enzymatic hydrolysis and generation of such bioactive peptides is shown in Figure 14.2.

The hydrolysis takes place in enzyme reactors that consist of a vessel or a series of vessels depending on reaction parameters such as the form of the enzyme (free or immobilized), the desirable scale of the reactor, and the need for controlling pH and temperature, as well as supplying or removing gases. In batch reactors, the product is removed after a fixed time. The main disadvantages of batch reactors are that the operating costs are higher than for continuous processes due to the necessity for the reactors to be emptied and regularly refilled. On the other hand, the variations from batch-to-batch are higher in batch reactors. Despite this, batch reactors show important advantages when compared with continuous reactors: their simplicity in use and easier reaction optimization. In fact, they are preferred for small-scale production and offer a closely controllable environment that is useful for slow reactions, where the composition may be accurately monitored, and conditions (e.g. temperature, pH, coenzyme concentrations) vary throughout the reaction. Once the desired degree of hydrolysis is reached, the product is then submitted to fractionation and partial purification either by filtration or chromatographic techniques, and then characterized for its bioactivity (Arihara 2006).

### 14.3 Bioactivities of the Obtained Peptides

Bioactive peptides is used for short protein sequences between 2 and 20 amino acids, which length permits them to access the blood stream easily through the gastrointestinal system and reach the different organs to exert their activity. The bioactive sequences are encrypted in the protein and have no effect until they are liberated. A very important aspect of bioactive peptides is that they must overcome enzyme degradation in the gastrointestinal tract after consumption in order to exert their biological activity once transported through the blood stream.

Bioactive peptides may exert different biological functions depending on the length and amino acids composition of their sequence, including ACE-inhibition, antioxidant, or antimicrobial activity.

#### 14.3.1 ACE-inhibitory Activity

The inhibition of the angiotensin I-converting enzyme (ACE) is the most studied bioactivity especially due to its relation with cardiovascular diseases. It participates in the renin–angiotensin system where angiotensin I is converted into the potent vasoconstrictor angiotensin II and, as a consequence, increases the blood pressure. It also degrades the vasodilative bradykinin, so the inhibition of ACE results in an effective way to reduce blood pressure (Ahmed and Mugurama 2010).

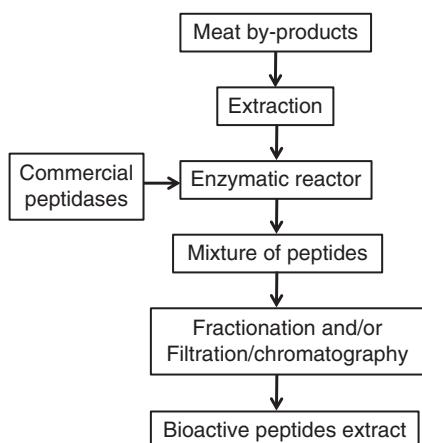
**Table 14.1** Characteristics and optimal conditions of main enzymes used in the hydrolysis of meat and meat-derived byproducts.

Enzyme name	EC number	Temperature <sup>a</sup>	pH <sup>a</sup>	Type	Origin
Papain	3.4.22.2	60–70 °C	pH 6–7	Cysteine protease	Exo- and endo-peptidase Papaya fruit
Bromelain	3.4.22.32	35–45 °C	pH 7	Sulfhydryl protease	Exo- and endo-peptidase Pineapple fruit
Thermolysin	3.4.24.27	65–85 °C	pH 5–8.5	Metalloproteinase	Exopeptidase <i>Bacillus thermoproteolyticus</i>
Pronase	3.4.24.4	40–60 °C	pH 7.5	Non-specific protease	Exo- and endo-peptidase <i>Streptomyces griseus</i>
Proteinase K	3.4.21.64	37 to 50–60 °C	pH 4–12	Serine protease	Exo- and endo-peptidase <i>Engyodontium album</i>
Neutrase	3.4.24.28	50 °C	pH 7	Metalloproteinase	Endopeptidase <i>Bacillus amyloliquefaciens</i>
Alcalase	3.4.21.62	50 °C	pH 8	Serine protease	Endopeptidase <i>Bacillus licheniformis</i>
Crude enzyme extract	—	40 °C	pH 8	Non-specific protease	Exo- and endo-peptidase <i>Raja clavata</i>

<sup>a</sup>Optimum conditions for activity.

Source: Reproduced from Mora et al. (2014b) with permission from Elsevier.

**Figure 14.2** Flow diagram for the generation of bioactive peptides through the enzymatic hydrolysis of edible meat byproducts. *Source:* Reproduced from Mora et al. (2014b) with permission from Elsevier.



Currently, there is an increasing interest in the study of natural ACE-inhibitory peptides as potential candidates to replace the already existing synthetic compounds such as Captopril or Enalapril which have been described to produce important side effects after consumption.

ACE-inhibitory peptides have been described to show short sequences between 2 and 12 amino. Several peptides showing hydrophobic amino acid residues in their sequences such as leucine, valine, alanine, tyrosine, phenylalanine, or tryptophan, have been described to exert ACE-inhibitory activity, especially when phenylalanine or other aromatic amino acids or proline are located at any of the three positions closest to the C-terminal site (Ambigaipalan et al. 2015). Moreover, it has been reported that the most potent antihypertensive peptides contain positively charged amino acids such as lysine and arginine at the C-terminal position.

### 14.3.2 Antioxidant Activity

Antioxidant peptides can be classified in two main groups depending on the type of reaction in which they are involved: (i) reactions based on hydrogen atom transfer that reduce free radicals and can be measured using oxygen radical absorbance capacity assay (ORAC), total radical trapping antioxidant parameter (TRAP) and  $\beta$ -carotene bleaching assay; and (ii) reactions based on electron transfer to reduce an oxidant which can be measured using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging assay, ferric-reducing antioxidant power, and 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity.

Synthetic antioxidant compounds such as t-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate are very common in food products. However, as occurs in ACE-inhibitory compounds, there is an increasing interest in the discovery of novel natural antioxidant peptides derived from the hydrolysis of proteins due to the recent safety concerns with synthetic antioxidants.

The antioxidant activity of the peptides generated from hydrolysis depends to a great extent on the presence of hydrophobic amino acids and at least one residue of histidine,

phenylalanine, tryptophan, or tyrosine in their structure (Mora et al. 2014a). So, amino acid residues with aromatic ring structure (phenylalanine, tyrosine, and tryptophan) can donate electrons to an electron deficient compound, and by this mechanism contribute to antioxidant activity (Sarmadi and Ismail 2010; Toldrá et al. 2017). Zhang et al. (2008) previously described that peptide fractions with higher concentrations of hydrophobic amino acids showed better power-reducing activity.

### 14.3.3 Antimicrobial Activity

Antimicrobial peptides have been described as being longer sequences than those of anti-hypertensive and antioxidant peptides. Their mode of action against the microorganisms is based on their capacity to form channels or pores within the microbial membrane (Castellano et al. 2016), so they are mostly hydrophobic as higher hydrophobicity is necessary in the affinity with the outer membrane of microorganisms. Peptides containing basic amino acids such as Tyr, Arg, and Lys have been described as having the ability to interact with the phospholipids of this membrane (Lopes et al. 2005).

## 14.4 Use of Byproducts as a Source of Bioactive Peptides

The use of byproducts as bioactive peptides has been extensively studied during recent years as an economically viable way for a waste obtained from one of the most contaminant industries nowadays. In this sense, blood, and collagen, very important byproducts from slaughterhouses and meat industry, have been the most assayed (Ryder et al. 2016).

Different reviews postulate slaughterhouse byproducts as an emerging source for the generation of bioactive peptides (Bah et al. 2013; Lafarga and Hayes 2014; Toldrá et al. 2016; Mora et al. 2014b) such as ACE-inhibition (Bhat et al. 2017), antimicrobial (Nedjar-Arroume et al. 2008; Etxabide et al. 2017), or antioxidant (Liu et al. 2016; Etxabide et al. 2017).

Blood constitutes an abundant source of proteins and can represent up to 4% of total animal weight. Its use depends to a great extent on the culture and religion of the country, so it could become a considerable environmental problem when it is not used for direct consumption or to increase the final value of certain food products by increasing their nutritional value, water-binding capacity, or emulsifying characteristics. Hemoglobin is the most abundant protein in blood (Ofori and Hsieh 2014) and most of the bioactive peptides described to date have been studied in these blood cells and the plasma fraction (Chang et al. 2007).

Despite peptides exerting different bioactivities such as ACE-inhibitory, antimicrobial or opioid have been described, studies showing ACE-inhibitory sequences are the most abundant. In this respect, some of the most recent bioactive sequences obtained from blood-derived byproducts are described in Table 14.2.

The generation of bioactive peptides from blood is an opportunity to add economic value and create novel applications for this product by overcoming the difficulties derived from differences in culture and religion that complicate both the direct consumption and use as an ingredient of this abundant byproduct.

**Table 14.2** Bioactive peptide sequences isolated and identified after enzyme hydrolysis of hemoglobin or plasma.

Source	Enzyme	Peptide sequences	$IC_{50}$	Activity	References
Hemoglobin $\alpha$ -chain	Pepsin	LDDLPGALSELSDLHAHKLRVDPVNFKLLSHSL	518.29 $\mu M$	ACE inhibitor	Adje et al. (2011)
Hemoglobin $\alpha$ -chain	Pepsin	KLLSHSL	42.51 $\mu M$	ACE inhibitor	Adje et al. (2011)
Hemoglobin $\alpha$ -chain	Pepsin	LLSHSL	1095.5 $\mu M$	ACE inhibitor	Adje et al. (2011)
Plasma	Trypsin	VYNEGGLPAP	3.1 $\mu M$	ACE inhibitor	Liu et al. (2010)
Porcine hemoglobin	Pepsin	LGFPTTKTYFPHF	4.92 $\mu M$	ACE inhibitor	Yu et al. (2006)
Porcine hemoglobin	Pepsin	VVYPWT	6.02 $\mu M$	ACE inhibitor	Yu et al. (2006)
Porcine hemoglobin	Pepsin	WVPSV	0.37 mg ml <sup>-1</sup>	ACE inhibitor	Ren et al. (2011)
Porcine hemoglobin	Pepsin	YTVF	0.23 mg ml <sup>-1</sup>	ACE inhibitor	Ren et al. (2011)
Porcine hemoglobin	Pepsin	VVYPWT	0.254 mg ml <sup>-1</sup>	ACE inhibitor	Ren et al. (2011)
Porcine hemoglobin	Pepsin	QELPG	0.02 mg ml <sup>-1</sup>	ACE inhibitor	Deng et al. (2014)
Hemoglobin	Alcalase	FQKVVA	5.8 $\mu M$	ACE inhibitor	Mito et al. (1996)
Hemoglobin	Alcalase	FQKVVAG	7.4 $\mu M$	ACE inhibitor	Mito et al. (1996)
Hemoglobin	Alcalase	FQKVVAK	2.1 $\mu M$	ACE inhibitor	Mito et al. (1996)
Hemoglobin	Alcalase	GKKVLQ	1.9 $\mu M$	ACE inhibitor	Mito et al. (1996)
Plasma	TCA	GVHVV	2 $\mu M$	ACE inhibitor	Park et al. (1996)

(Continued)

**Table 14.2** (Continued)

Source	Enzyme	Peptide sequences	$\text{IC}_{50}$	Activity	References
Plasma	n.d.	LVL	$4.2 \mu\text{g ml}^{-1}$	ACE inhibitor	Hazato and Kase (1986)
Hemoglobin $\alpha$ -chain	Pepsin	TKAVEHLDLPGALSELSDLHAHKLRVDPVNFKLLSHSLL	$366 \mu\text{M}$	ACE inhibitor	Adje et al. (2011)
Hemoglobin	Pepsin	KYR	—	Antimicrobial	Catiau et al. (2011)
Hemoglobin	Pepsin	RYH	—	Antimicrobial	Catiau et al. (2011)
Hemoglobin	Pepsin	$\alpha$ 107–136	—	Antibacterial	Daoud et al. (2005)
Hemoglobin $\alpha$ -chain	None	VNFKLLSHSLLVTLASHL	—	Antimicrobial	Hu et al. (2011)
Hemoglobin	Pepsin	QADFQKVAVGAVANALAHRYH	—	Antimicrobial	Nedjar-Arroume et al. (2006)
Hemoglobin	Pepsin	YPWT	$45.2 \mu\text{M}$	Opioid	Zhao et al. (1997)
Hemoglobin	Pepsin	TYPWTQ	$46.3 \mu\text{M}$	Opioid	Zhao et al. (1997)
Hemoglobin	Pepsin	YPWTQR	$4.3 \mu\text{M}$	Opioid	Zhao et al. (1997)
Hemoglobin	Pepsin	YPWTQRF	$2.9 \mu\text{M}$	Opioid	Zhao et al. (1997)
Hemoglobin	Pepsin	YPWTQRFF	$4.6 \mu\text{M}$	Opioid	Zhao et al. (1997)
Hemoglobin	Pepsin	LVVYPWTQ	$80.5 \mu\text{M}$	Opioid	Zhao et al. (1997)
Hemoglobin	n.d.	LVVYPWTQRF	$29.1 \mu\text{M}$	Opioid	Glamsta et al. (1992)
Hemoglobin	Pepsin	VVYPWTQ	$78.2 \mu\text{M}$	Opioid	Zhao et al. (1994)
Hemoglobin	Pepsin	VVYPWTQRF	$34.3 \mu\text{M}$	Opioid	Zhao et al. (1994)
Plasma	Flavourzyme	VSGVEDVN	$0.025 \mu\text{M}$	Calcium-binding	Lee and Song (2009)
Plasma	Flavourzyme	DLGEQYFKG	—	Ion-binding	Lee and Song (2009)

n.d.: non-determined.

Source: Reproduced with permission from Toldrá et al. (2016).

Collagen is the most abundant protein in vertebrates and can be easily and cheaply obtained from the meat industry. It is the main constituent of skin, hide, bones, and cartilage, and its nutritional value is very low, because it lacks essential amino acids. However, it has been described as a very rich source of bioactive peptides (Saiga et al. 2008, Herregods et al. 2011), especially antioxidant peptides (Li et al. 2007; Liu et al. 2010; Di Bernardini et al. 2012; Alvarez et al. 2012) due to the abundance of hydrophobic amino acids in collagen sequence. These bioactive peptides used to be obtained by enzymatic hydrolysis using alcalase, trypsin, chymotrypsin, neutrase, flavourzyme, pepsin, bromelain, and papain. However, the cheapest way to generate bioactive peptides from collagen is through hydrolysis by microorganisms. In fact, a recent study tested five commercially available microbial protease enzymes in connective tissue and myofibrillar proteins, and a *Bacillus* derived protease produced the most active antioxidant and ACE-inhibitory peptides (Ryder et al. 2016).

## 14.5 Future Trends

There are several critical steps to consider when evaluating the use of the generated bioactive peptides as they have to reach the target organ without degradation being bioavailable and, on the other hand, prove that do not result in toxins in the human body. The main difficulties in the industry for their manufacture are the scale of processing and the high prices for production due to the frequently low yields obtained, which might question the economic feasibility of the manufacturing. On the other hand, European legislation is very strict in terms of consumers' protection and there is a specific regulation for food of animal origin intended for human consumption (Mullen et al. 2017).

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## Part II

### On Plant Byproducts

## 15

# High-Value Products from Cereal, Nuts, Fruits, and Vegetables Wastes

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## 15.1 Introduction

Food is essential for human health and existence, however, a chunk of what we eat is disposed of as waste. In 2012, the Food and Agriculture Organization (FAO) reported that about 1.3 billion tonnes of foods including fresh vegetables, fruits, cereals, bakery, milk, and meat are lost along the food supply chain (FAO 2012). The amount of food waste will continue to rise, especially in the developing countries, because of population and economic growth (Kiran et al. 2014). Asian countries are the largest consumers of cereals and vegetables as such significant wastes are reported there. For instance, Asia's cereal wastes were 55% of the world's cereal wastes. Asia's vegetable wastes also constituted 74% of the world's cereal wastes (Gustafsson et al. 2011; Kiran et al. 2014; Melikoglu et al. 2013). Interestingly, some of these wastes are inevitable. For instance, the inedible parts of cereals such as husks and the hard shells of nuts must be discarded as wastes. The same is true for some fruits and vegetables. In any event, there is a sense that both the edible and inedible parts of cereal, nuts, and vegetables that constitute wastes can be converted to food and feed or used for other purposes beyond biofuels. Therefore, this chapter will focus on the conversion of these waste to valuable products.

## 15.2 Food Wastes by Categories

### 15.2.1 Cereals and Cereal Wastes

Cereal crops are cultivated for their edible seeds. Cereal grains give higher energy than any other crops, as such they are grown in greater quantities than most crops (Arvanitoyannis and Tserkezou 2008). Although there are many cereal crops, rice, wheat, and maize are the most widely consumed. These account for 94% of all cereal consumption (Ranum et al. 2014). Rice and wheat account for over 50% of the world's cereal

production (Arvanitoyannis and Tserkezou 2008). Rice is the staple food for half of the world population.

The global rice production as at 2016 was 753 million tonnes and 91% of this was produced in Asia alone (Sultan et al. 2018). Global rice consumption rose from 417 million tonnes in 2006 to 424 million tonnes in 2007, this growth is expected to reach 556 million tonnes by 2040 (Foo and Hameed 2009). On the other hand, it has been reported that wheat alone meets 21% of the world's food demand and is grown on about 494 million acres of farmland globally (Tsvetanov et al. 2016). Because wheat is used to produce a broad range of foods including bread, pasta, cakes, breakfast meals, among others, it is estimated that about 85% of the global population depend on wheat for basic calories and proteins (Chaves et al. 2013; Jasrotia et al. 2018). Another important cereal crop is maize or corn. It is preferred in Southern and Eastern Africa, North and Central America, and Mexico. It is of high economic importance to agriculture in the United States. The US ranks first in the world production of corn, with 35.5% of global corn production and approximate farm gate value of 51 billion US dollars (Soltani et al. 2016). The two most popular products of maize are maize flour and meal (Ranum et al. 2014).

Rice husk is the tough protective cover of the rice grain. It is one of the major byproducts of the rice milling industry. It is an unavoidable waste and constitutes 20% of the rice (Mansaray and Ghaly 1998). In the developing countries where an estimated 500 million tonnes of rice is produced annually, up to 100 million tonnes of these are rice husks (Daifullah et al. 2003). Rice husk has an annual global capacity of 700.7 million tonnes (Liu et al. 2016; Weldekidan et al. 2018). In some countries, rice husk ends up as leftover in the open air, they are sometimes burned or left to decay and these are of serious health and environmental concerns (Foo and Hameed 2009; Weldekidan et al. 2018). Rice bran is another byproduct from rice milling. It is obtained during the conversion of brown rice to white rice. Rice bran constitutes 10% of the rough rice and is an underutilized byproduct (Shi et al. 2017). Global production of rice bran has been reported to be between 50 and 60 million metric tonnes per year (Lee et al. 2005). In Japan, about 30% of the rice bran produced goes to waste (Pourali et al. 2009), although it is a rich source of dietary fiber, carbohydrate, protein, and lipids (Peng et al. 2017). Rice straw is the vegetative part of the rice plant. In China, rice straw waste in 2007 was estimated to be between 180 and 270 million tonnes based on dry content (Yanli et al. 2010). India, the second largest producer of rice generates around 112 million metric tonnes of rice straw annually (Sharma and Garg 2018; Singh et al. 2016). In Indonesia, a total annual production of 100 million tonnes of rice straw was reported (Manurung et al. 2016). More than 80% of these straws remain underutilized (Sharma and Garg 2018).

Significant amount of wastes has also been reported from other cereals. For instance, wheat husks constitute 15–20% of the grains (Bledzki et al. 2010). The non-food part of rye was reported to be as high as 20% (Bledzki et al. 2010). In some places, these husks are burned in open air fires, producing ashes that has negative impact on land fertility and cause environmental pollution (Terzioglu et al. 2013). A 2009 study found that the production rate of wheat straw in India was 110 million metric tonnes. Although some of these were used as cattle feed, majority of them were agro-wastes (Sukumaran et al. 2010). An average of 15.6 million metric tonnes of sorghum wastes are produced annually in India (Sukumaran et al. 2010). In Nigeria, 2–3 million metric tonnes of sorghum crop wastes is generated annually with less than 40% used for livestock feed and fencing (Nasidi et al. 2015). Maize or corn produces a lot of wastes. For instance, the average corn

**Table 15.1** Cereal waste estimates with respect to geographical locations.

Waste origin	Volume (million tonnes)	Region	References
Rice bran	50–60	Worldwide	J. W. Lee et al. (2005)
Rice husk	700.7	Worldwide	Weldekidan et al. (2018)
Rice straw	180–250	China	Yanli et al. (2010)
	112	India	R. Singh et al. (2016)
	100	Indonesia	Manurung et al. (2016)
Wheat bran	90	Worldwide	Onipe et al. (2015)
	1.6	Italy	Di Gioia et al. (2007)
Wheat straw	110	India	Sukumaran et al. (2010)
	82.4	United States	Kadam and McMillan (2003)
	26.4	Turkey	Sürmen 2003
Corn stover or stalk	153	United States	Kadam and McMillan (2003)
	9.6	Nigeria	Jekayinfa and Scholz (2009)
	4.2	Turkey	Balat (2005)
Corn cob	1.3	Nigeria	Jekayinfa and Scholz (2009)
Corn husk	1.0	Nigeria	Jekayinfa and Scholz (2009)
Rye straw	40–50	Worldwide	Ghaly and Ergüdenler (1994)
	0.4	Turkey	Balat (2005)
Sorghum waste	15.6	India	Sukumaran et al. (2010)
	2–3	Nigeria	Nasidi et al. (2015)
Sorghum straw	6.8	Nigeria	Jekayinfa and Scholz (2009)
Barley straw	13.5	Turkey	Balat (2005)
Oat straw	0.5	Turkey	Balat (2005)
Millet stalk	11	Nigeria	Jekayinfa and Scholz (2009)
	2.5	Ghana	Bayitse et al. (2013)

or maize cob yield is about 14% of grain yield, which represents about 16% of the total corn stover in a field (Zheng et al. 2014). Therefore, the multi-million metric tonnes of corn produced by the United States, Argentina, Brazil, India, South Africa, and Nigeria among others will leave behind an enormous corn cob waste (Ayeni and Daramola 2017; Badmus and Ariyo 2011; Narra et al. 2018; Soltani et al. 2016). Table 15.1 shows the estimates and range of different cereal wastes at different geographical locations.

### 15.2.2 Nuts and Byproducts

A nut is a fruit that contains edible seed and inedible hard shell. Nuts are described as a food class which is dry seed with ovary walls that becomes hard at maturity stage. They include almond, brazil nut, walnut, cashew nut, hazel nut, pine nut, and peanut according

to consumers' classification (Chang et al. 2016; Zhao et al. 2012). Nuts are rich sources of vitamins, minerals, and dietary fibers. Some nuts are also very rich in fat. For instance, pecan, macadamia, Brazil, and almond nuts have 70%, 66%, 65%, and 55% fat, respectively (Blomhoff et al. 2006). Nuts have protein content as high as 30% w/w and are known to be rich in antioxidants such as tocopherols and polyphenols (Alvarez-Parrilla et al. 2018; Davidson 2014; Wu et al. 2004).

Increasing demand for nuts has led to increased byproducts including leaves, cake and principally nutshell, which have high contents of bioactive compounds, making them important raw materials for nutraceutical products with a broad range of benefits (Alvarez-Parrilla et al. 2018). Global almond production in 2009 was around 2.31 million tonnes and only shell accounts for between 35% and 75% of the total fruit weight. This suggest that annual shell wastes could be as high as 1.7 million (Ebringerová et al. 2007; Pirayesh and Khazaean 2012). Global production of walnut was reported to be 1.48 million tonnes and the top three producers These are China (0.4 million tonnes) North America (0.3 million tonnes) and Iran (0.15 million tonnes) (Pirayesh and Khazaean 2012). Since walnut shell alone is about 67% of the total weight of the fruit (Martinez et al. 2003), shell waste as high as 1 million tonnes could be produced annually. Global peanut production in 2009 was around 35 million tonnes and this has grown to 44 million tonnes in 2014, indicating a significant increase in production (Adhikari et al. 2018; Zhang et al. 2012). China is the largest producer of peanut with an annual production of about 13 million tonnes (Zhang et al. 2012). This weight includes the shell. Peanut shell comprises about 33% of peanut pod (Adhikari et al. 2018; Gao et al. 2011; Radhakrishnan et al. 2013) consequently, around 14.5 million tonnes of peanut shell is left annually.

### 15.2.3 Fruits, Vegetables and Their Byproducts

Fruits and vegetables are a diverse group of plant foods that are good for human health. They are high in minerals, antioxidants vitamins, phytochemicals, and fiber which help lower the risks of developing health problems such as cardiovascular diseases, cancer, diabetes, obesity, and diverticulosis (Aranceta 2004). These benefits made most countries develop dietary recommendations for fruits and vegetables. For instance, Canada's Food Guide recommends eating at least one dark green vegetable and one orange per day and having vegetables and fruits more often than juice (Canada Food Guide). The Eatwell Plate in the United Kingdom recommends eating five portions of fruits and vegetables per day with one portion being 80 g ([eatwell.gov.uk](http://eatwell.gov.uk)). The United States Department of Agriculture (USDA) recommends eating a variety of vegetables, especially dark green, red, and orange vegetables, it also recommends having at least one cup of leafy vegetables (84 g) per day.

Fruit and vegetable wastes are generated during harvesting, transportation, storage, marketing, and processing. A study found that about 13 million tonnes of fruit and vegetable wastes are generated annually in Chinese cities (Shen et al. 2013). The organic content in some municipal solid wastes in China are >60% due to high percentage of fruits and vegetable wastes (Lin et al. 2011). Annually, the United States produce about 1 million tonnes of vegetable crop residues, while Brazil, and Spain produce 9.4 million and 0.6 million tonnes of citrus residues, respectively (Lin et al. 2013). In Ivory Coast, up to 20 million tonnes per annum of cocoa pods have been reported (Lin et al. 2013).

**Table 15.2** Summary of fruit and vegetable wastes generated in selected countries.

Country	Fruits and vegetables produced (million tonnes)	Losses and wastage (Percentage, %)			Waste generated (million tonnes)
		Processing	Distribution	Consumption	
India	221.43	25	10	7	1.81
China	595.25	2	8	15	31.98
Philippines	22.48	25	10	7	6.53
Malaysia	2.28	25	10	7	0.68
Thailand	14.08	25	10	7	1.57
United States	60.67	2	12	28	14.95

Source: Summarized from Wadhwa and Bakshi (2013).

A combined total of about 55 million tonnes of fruit and vegetable wastes were generated in India, the Philippines, China, and the United States during processing, packaging, distribution, and consumption (Wadhwa and Bakshi 2013). Annual global production of wastes reported for some fruits and vegetables were: citrus peel residue (15.6 million tonnes), apple pomace (3–4.2 million tonnes), grape pomace (5–9 million tonnes), banana peels (9 million tonnes), and kiwi residues (0.3 million tonnes) (Lin et al. 2013). Table 15.2 summarizes the fruit and vegetables generated during processing, distribution and consumption in some selected countries.

## 15.3 Extraction of Bioactive Compounds from Plant Wastes

Several extraction methods have been used to obtain bioactive compounds from plant wastes. The commonly used methods being organic solvent-based, energy-assisted and fluid-based methods will be discussed in this section.

### 15.3.1 Solvent Extraction Method

Solvents have been found to be highly effective for extracting bioactive compounds from plant wastes. For bioactive lipid extraction, hexane is the widely used solvent, however because of its neurotoxicity, several alternative solvents or solvent combinations have been used. For instance, ethanol has been used for extracting lipids from flaxseed cuticle (Holser and Akin 2008). Samples of flaxseed cuticle fragments were soaked in ethanol at different extraction temperatures ranging from 50 to 100 °C for specific time. Results showed that hot ethanol effectively extracted lipid compounds from the flaxseed cuticle fragments (Holser and Akin 2008). In another study, the authors found that 20% more rice bran oil was extracted with ethanol than with hexane (Terigar et al. 2011). In other studies, rice bran oils were extracted using methanol (Kale et al. 1999) and water (Hanmoungjai et al. 2000). Soxhlet Method has also been reported for the extraction of soybean and rice bran oil (Terigar et al. 2011). A combination of water and ethanol

have been used to extract polyphenols and flavonoids from grape fruit wastes (Aliakbarian et al. 2012). Solvent extraction of carotenoids from tomato wastes (seeds and skin) have been reported. The authors found that ethyl lactate, an environmentally friendly solvent gave the highest carotenoid yield ( $243 \text{ mg kg}^{-1}$ ) compared to ethyl acetate, acetone, ethanol, and hexane (Strati and Oreopoulou 2011a). Water was the preferred solvent for extracting phenolic compounds from pomegranate peels (Wang et al. 2011).

### 15.3.2 Microwave and Ultrasonic-Assisted Extraction

Microwave-assisted extraction has been reported to be highly effective for extraction bioactive compounds from plants. This method is advantageous, because bioactive compounds can be extracted more selectively and rapidly with lower energy consumption, less byproducts and reduced solvents (Aryee et al. 2018; Letellier and Budzinski 1999; Paré et al. 1994; Terigar et al. 2011). The amount of oil recovered from rice bran using a continuous microwave-assisted extraction (CMAE) system was 82% compared to 37% obtained using the conventional solvent extraction method involving hexane and ethanol (Terigar et al. 2011). MAE methods have been used to extract bioactive compounds including catechin, ascorbic acid and quercetin 3-O-glucopyranoside from cauliflower, celery, chicory, and asparagus wastes (Baiano et al. 2014). A recent study on ultrasound-assisted extraction of carotenoids from pomegranate peels using vegetable oil found that the efficient extraction period for achieving maximum yield of carotenoids was about 30 minute (Goula et al. 2017). Similar methods have also been used to extract polyphenolic compounds and ascorbic acids from pomegranate and (Pan et al. 2011) mandarin peels (Ma et al. 2008).

### 15.3.3 Fluid-Based Extraction Methods

Supercritical fluid and subcritical water extraction methods have been widely reported for extracting bioactive compounds from nut, fruit, and vegetable wastes. Both extraction methods are effective for selective extraction of compounds. They offer the possibility of mild extraction conditions combined with low energy requirement and often do not require toxic solvents (Aliakbarian et al. 2012; Baysal et al. 2000).

Subcritical water extraction of phenolic compounds from grape pomace has been reported. This method led to the extraction of significantly higher polyphenols and flavonoids than the conventional ethanol-water extraction method (Aliakbarian et al. 2012). Subcritical water extraction has also been used for the extraction of quercetin from onion waste (Turner et al. 2006). Eight phenolic compounds including gallic, chlorogenic, caffeoic, protocatechuic, syringic, *p*-hydroxyl benzoic, ferulic, and coumaric acids were extracted from potato peel using subcritical water extraction. High recoveries were obtained using this technique than when the conventional solvent extraction method involving ethanol: methanol mix was used (Singh and Saldaña 2011). Extractions of bioactive compounds from plant wastes using supercritical critical fluids have also been reported. Polyphenolic compounds from grape seeds were extracted using supercritical carbon dioxide ( $\text{CO}_2$ ) with ethanol as co-solvent. Gallic acid, epigallocatechin and epigallocatechin gallate were the phenolic compounds extracted at their maximum

levels (Yilmaz et al. 2011). A number of studies have been carried out on the use of supercritical CO<sub>2</sub> for the extraction of grape seed oil (Cao and Ito 2003; Fiori 2007; Paschos et al. 2009). Extraction of α-tocopherol enriched oil from grape seeds using supercritical CO<sub>2</sub> has also been reported (Bravi et al. 2007). Supercritical fluid extraction with CO<sub>2</sub>, ethyl acetate and ethanol as co-solvents was used to obtain phenolic compounds from guava seeds (Castro-Vargas et al. 2010). Lycopene and β-carotene were extracted from tomato paste waste using supercritical CO<sub>2</sub> with the addition of 5% ethanol (Baysal et al. 2000).

## 15.4 High-Value Products from Plant Wastes

### 15.4.1 Bioactive Lipids

Bioactive lipids can be obtained from cereal wastes and byproducts of fruits and vegetables. For instance, studies have shown that a variety of lipid compounds have been extracted from the cuticles of flaxseed, rice, sorghum, and other cereals (Amarasinghe et al. 2009; Holser and Akin 2008; Kim et al. 2003). Rice bran is a rich source of oil with an oil content of between 15% and 25% (Sounders 1985). Rice bran oil contains about 43% phytosterols, 10% steryl esters, and 1% tocopherol (Orthofer and Nicolosi 1993). The lipid compounds in flax cuticle include long chain fatty acids, alcohols, and wax esters (Holser and Akin 2008). Free and esterified sterols and triterpenols as well as sterol glucosides were found in flax fibers (Gutiérrez and del Río 2003). The majority of these lipids are in the cuticularized epidermis. Food grade waxes from cereals have reported (Nagendra Prasad et al. 2011). Studies have shown that rice bran wax can be used in pharmaceutical, cosmetic, polymer, and leather industries (Ito 2003; Nagendra Prasad et al. 2011).

Most of the cereal cuticular lipid compounds display bioactive properties in human systems. For instance, long chain alcohols such as policosanols have been reported to help in the prevention and treatment of cardiovascular disease by lowering elevated cholesterol levels as well as exhibit antithrombotic effects (Janikula 2002; Varady et al. 2003). Rice bran oil was found to be highly effective in decreasing low density lipoprotein than the oils obtained from safflower, sunflower, cottonseed, soybean, sesame, corn, and groundnut oils (Nagendra Prasad et al. 2011). Rice bran oil is rich in phytosterols, sterolins, and gamma-oryzanol, as such it can modulate immune system (Nagendra Prasad et al. 2011). Previous studies have shown that rice bran gamma oryzanol significantly reduced high serum levels in hypothyroid patients while also helping in preventing high blood pressure, hyperlipidaemia, and hyperglycaemia (Nagendra Prasad et al. 2011; Patel and Naik 2004). Oryzanol was found to be highly effective in alleviating menopausal symptoms like hot flashes (Nagendra Prasad et al. 2011). Dietary phytosterols have been reported to be highly effective in decreasing the epithelial cell proliferation thereby altering the levels of fecal cholesterol, cholesterol breakdown products and bile acids (Fuchs et al. 1999; Pietinen et al. 1999). Phytosterols have been reported to inhibit the growth and metastasis of human breast cancer as well as preventing colon cancer development (Awad et al. 2000; Awad et al. 2003; Llaverias et al. 2013; Rao and Janezic 1992).

### 15.4.2 Bioactive Polyphenolic Compounds

Several studies have shown that cereal wastes, byproducts from nuts, fruits, and vegetables are rich sources of polyphenolic compounds. These compounds exist in the forms of phenolic acids, stilbenes, flavonoids, lignans, coumarins, and tannins (Holland et al. 2017). Table 15.3 shows the different classes and types of polyphenolic compounds that have been extracted from plant wastes. Phenolic compounds have been shown to reduce the incidence of oxidative stress and associated conditions such as chronic fatigue syndrome, neurodegenerative, and cardiovascular diseases (Holland et al. 2017). Phenolic compounds have also been reported to have anti-inflammatory, anti-tumor, and anti-viral properties (Parvathy et al. 2009; Umesalma and Sudhandiran 2010). The potent antioxidant and radical scavenging activities of polyphenolic compounds are known to be responsible for most of their bioactive properties.

Tyrosol and oleuropein extracted from olive pomace were found to be highly effective in preventing endothelial dysfunction. Both compounds prevented the proliferation and migration associated with anoxia in human endothelial cells, down-regulated the levels of matrix metalloproteinase (MMP)-2, MMP-9 and membrane type-1 matrix metalloproteinase (MT1-MMP) and increased tissue MMP inhibitor-1 (TIMP-1) (Palmieri et al. 2012). Olive waste (pomace) could be used as a cheap source of high-value bioactive

**Table 15.3** Major polyphenolic compounds from plant wastes.

Classes	Types	Plant waste sources	References
Phenolic acids	Gallic acid, caffeic acid, syringic acid, ellagic acid, ferulic acid, benzoic acid, chlorogenic acid, protocatechuic acid, <i>p</i> -hydroxy acid, coumaric acid	Potato peels, grape seeds, apple pomace, olive pomace, rice husk and bran, triticale bran and straw, mango seed kernels	Abdalla et al. (2007), Butsat and Siriamornpun (2010), García et al. (2009), Hosseinian and Mazza (2009), P. P. Singh and Saldaña (2011), Yilmaz et al. (2011)
Stilbenes	Resveratrol, viniferin, astringinin, piceid, trans-pterostilbene	Grape pomace and skin	Casas et al. (2010), Paini et al. (2016), Peralbo-Molina et al. (2012)
Flavonoids	Epigallocatechin, catechin, epicatechin, kaempferol, myricetin, isorhamnetin, procyanidin, quercetin, epigallocatechin gallate	Pomegranate peels, grape seed, skin, and pomace	Peralbo-Molina et al. (2012), Wang et al. (2011), Yilmaz et al. (2011), Yu and Ahmedna (2013)
Lignans	Secoisolariciresinol diglucoside, pinoresinol	Wheat bran, triticale bran, and straw	Hosseinian and Mazza (2009), Qu et al. (2005)
Coumarins	Coumarin	Mang seed kernels	Abdalla et al. (2007), Maisuthisakul and Gordon (2009)
Tannins	Procyanidins	Apple pomace, olive pomace	L. Y. Foo and Lu (1999), Schieber et al. (2003)
Phenylethanoid	Tyrosol, hydroxytyrosol, oleuropein	Apple pomace, olive pomace, and leaves, winery waste	Cardoso et al. (2005), Jemai et al. (2009), Lafka et al. (2007)

compounds for the treatment and prevention of endothelial dysfunction-associated diseases. A combination of hydroxybenzoic acids, proanthocyanidins, flavan-3-ol monomers and anthocyanins from grape pomace showed significantly high anti-inflammatory effects by reducing the levels of TNF- $\alpha$  and IL1- $\beta$  in the peritoneal fluid (Denny et al. 2014). The potent anti-inflammatory and immunomodulatory properties of resveratrol, the main polyphenol extracted from grape skin and seeds have been widely reported (Denny et al. 2014; Xia et al. 2010; Yadav et al. 2009). Epicatechin, catechin, phloridzin, quercetin glucosides, and procyanidins extracted from apple pomace were found to be potent anti-inflammatory phenolics (Lauren et al. 2009; Yue et al. 2012). A broad range of polyphenols from cereal wastes such as millet, corn, and sorghum brans have been reported (Burdette et al. 2010; Chen et al. 2011; Kim et al. 2012).

### 15.4.3 Natural Pigments

Pigments such as carotenoids are responsible for the bright colors in fruits and vegetables. They play important roles in the health of plants and provide protective health benefits to human. However, the human body cannot produce them, so they need to be obtained from fruits and vegetables including apricots, carrot, pink grapefruit, guava, watermelon, tomatoes, and processed tomato products (Topal et al. 2006). Interestingly, significant amount of pigments is found on the outer layers (shells) of fruits and vegetables and are often lost during processing. Although there are diverse groups of natural pigments, only prominent ones will be discussed in this section.

#### 15.4.3.1 Carotenoids

Carotenoids constitute an important component of waste originating from tomato processing plants (Strati and Oreopoulou 2011b). The industrial processing of tomato produces up to 40% byproducts including tomato peels, pulp, and seeds (Topal et al. 2006). These wastes contain a substantial amount of carotenoids. For instance, the total carotenoid content of dry tomato peels collected from a commercial processing plant was  $793.2 \mu\text{g g}^{-1}$ . These were lycopene ( $734 \mu\text{g g}^{-1}$ );  $\beta$ -carotene ( $41.00 \mu\text{g g}^{-1}$ ), lutein ( $14.5 \mu\text{g g}^{-1}$ ), and zeaxanthin ( $3.7 \mu\text{g g}^{-1}$ ) (Knoblich et al. 2005). Lycopene remains the most abundant carotenoid in tomato. It has been shown that lycopene represents about 85% of the total carotenoid content of tomato waste (Strati and Oreopoulou 2011b). Tomato skin contains about five times more lycopene than the pulp (Sharma and Le Maguer 1996). In carrot pulp waste, total  $\beta$ -carotene content was  $41.00 \mu\text{g g}^{-1}$ , followed by  $\alpha$ -carotene ( $28.77 \mu\text{g g}^{-1}$ ) and lutein ( $4.66 \mu\text{g g}^{-1}$ ) (Chen and Tang 1998). These pigments have also been extracted from the byproducts of water melon, banana, mango, and pawpaw (Arumugam and Manikandan 2011; Tarazona-Díaz et al. 2011).

The health benefits of carotenoids have been widely reported. Studies have shown that a high intake of lycopene and other carotenoids is associated with a lower risk of oesophageal cancer (Ge et al. 2013). Intake of higher levels of  $\alpha$ -carotene,  $\beta$ -carotene, lutein, zeaxanthin, and lycopene have been reported to help in reducing the risk of breast cancer (Eliassen et al. et al. 2012). Carotenoids help prevent some cardiovascular and neurodegenerative diseases because of their antioxidant activity, protecting cells and tissues from oxidative damage (Stahl and Sies 2003). Most of these carotenoids are being used for food coloring and as antioxidants.

### 15.4.3.2 Chlorophyll

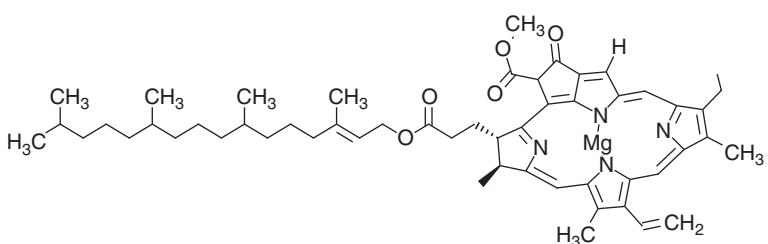
Chlorophyll is a natural amphiphilic green pigment found in most plants (Cubas et al. 2008). Chlorophyll has a hydrophilic head formed by a porphyrin group and a long hydrocarbon tail or phytol group (Derrien et al. 2017). The chemical structure of chlorophyll is presented in Figure 15.1. Chlorophyll has been extracted from spinach byproducts using aqueous ethanol and supercritical CO<sub>2</sub> (Derrien et al. 2018; Derrien et al. 2017). Chlorophyll is a major pigment generated during olive oil processing. A significantly high amount of chlorophyll is carried over into the pomace (Endeshaw et al. 2015). Other reported sources of chlorophyll include mulberry and kiwi fruit leaves (Mingyou 2006; Xiangyuan et al. 2011).

Dietary chlorophyll has been reported to be useful for the prevention of cancer. It was found that chlorophyll provides cancer chemoprotection by reducing carcinogen bioavailability (McQuistan et al. 2012). It has been found to prevent the growth of tumor cells (Chernomorsky et al. 1999). Studies have shown that chlorophyll is a potent anti-inflammatory pigment (Okai et al. 1997; Subramoniam et al. 2012). Because of its health benefits, chlorophyll supplements are now available commercially.

### 15.4.3.3 Anthocyanins

Anthocyanins are a well-known natural pigment derived from fruits and vegetables. They are not just pigments, they also possess a broad range of bioactive properties. Five types of anthocyanins including pelargonidin, cyanidin, delphinidin, peonidin, and malvidin were isolated from a fruit and vegetable waste collection center in China (Yuan et al. 2016). A study found that anthocyanins exist exclusively in the skins of blueberry, which is regarded as a byproduct of blueberry processing. Its content in the skin was 188.5 mg g<sup>-1</sup> while it was just 5.8 mg g<sup>-1</sup> and 0.1 mg g<sup>-1</sup> in the flesh and seeds, respectively (Lee and Wrolstad 2004). A higher concentration of anthocyanins was extracted from red grape pomace (Makris et al. 2008) and purple corncobs (Jing and Giusti 2005).

Studies have shown that increased consumption of anthocyanins can reduce the risk of cardiovascular diseases, the most common cause of mortality among men and women (Wallace 2011). Delphinidin was found to decrease the extent of apoptotic and necrotic cell death (Wallace 2011). Malvidin and pelargonidin inhibited the growth of human cancer cells in the stomach, colon, breast, lung, and central nervous system (Zhang et al. 2005). Meanwhile, cyanidin, delphinidin, and petunidin significantly inhibited breast cancer cell growth (Zhang et al. 2005). The antioxidant and anti-inflammatory properties of anthocyanins have also been reported (Wang et al. 1999).



**Figure 15.1** Chemical structure of chlorophyll.

#### 15.4.4 Addition to Cereal Brans to Food Products

Cereal brans are rich sources of dietary fibers. Dietary fiber is used in the formulation of foods, because it can help in modifying the texture of food while also enhancing the food stability during production and storage (Lebesi and Tzia 2011). Most cereal bran products have high insoluble fiber, ash, vitamins, lipids, and pigments. Cereal brans are now added to bread, pasta, cakes, and breakfast meals. Rice bran has been used as a functional ingredient added to bakery products (Abdul-Hamid and Luan 2000). A study found that rice bran protein can provide nourishment to pre-schoolers (Khan et al. 2011). Wheat-based noodles supplemented with varying amount of rice bran up to 15% had a lower cohesiveness and higher contents of polyphenols, flavonoids, anthocyanins, and higher antioxidative stability (Kong et al. 2012). Another study also found that pasta supplemented with rice bran retained most of its quality even after four months of storage (Kaur et al. 2012). Supplementation of goat feed with cereal bran improved milk production and its fatty acid composition (Park et al. 2013). This suggests that cereal bran can help improve the quality and storage stability of food.

The health benefits of cereal bran-derived dietary fiber have been reported. Brans derived from rice, wheat, oat, barley, sorghum, millet, rye, and maize have been characterized as health promoting ingredients (Patel 2015). They have shown beneficial effects against chronic diseases such as cardiovascular diseases, diverticulosis, diabetes, and colon cancer (Abdul-Hamid and Luan 2000). They also help in the prevention of constipation and help in reducing the risk of colorectal cancer (Bingham et al. 2003).

#### 15.4.5 Nut and Cereal Byproducts as Nutrient Resource in Microbial Cultivation

Nuts and some cereals are good sources of oil. However, after oil extraction, the resulting cake is discarded as waste, used as organic fertilizers or fed to animals. Some of these protein-rich cakes can also be used for cultivating enzyme-producing microorganisms. Oil cakes have been used as substrates in solid state fermentation. Soybean cake was used for the production of lipase by *Penicillium simplicissimum* (Di Luccio et al. 2004). Olive oil cake was used as a nutrient source for *Rhizomucor pusillus* and *Rhizopus rhizophodiformis* during the production of lipase. This is an interesting alternative to valorization of olive oil cake (Cordova et al. 1998). Several nut and cereal oil cakes from sunflower, sesame, soybean, coconut, mustard, palm kernel, groundnut, cottonseed, canola, olive, and rapeseed have been used to produce a broad range of enzymes. These include proteases, amylases, inulinases, phytases, glutaminases, mannases, tannases, and glucoamylase (Ramachandran et al. 2007). The use of these byproducts to produce mostly food-grade enzymes shows that wastes can be put to better use.

### 15.5 Conclusion

Bioactive compounds can be obtained from cereal, nuts, fruits, and vegetables wastes, and disposing them is a sheer waste of a valuable nutrient resource. This chapter has shown that these byproducts can be effectively utilized. Research efforts should be

concentrated on investigating novel applications of these wastes products in the development of functional foods.

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## 16

# Advances in Plant-Based Waste-to-Energy Conversion Technologies

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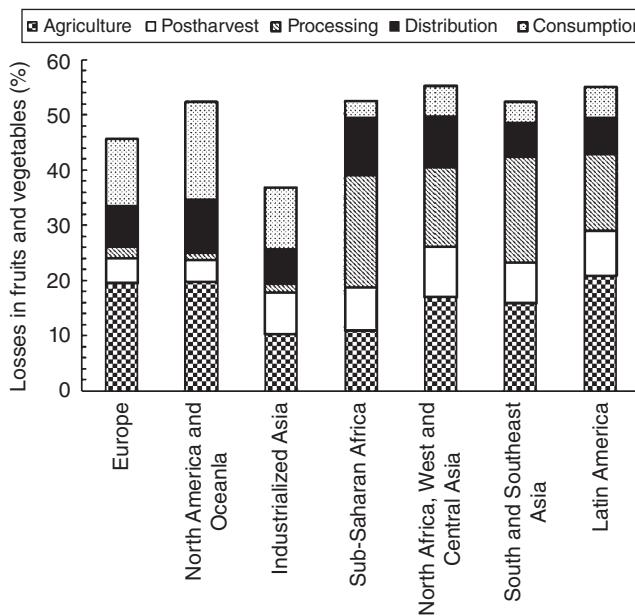
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## 16.1 Introduction

Sustainable food systems should provide high-quality foods at affordable costs and minimal environmental impact without compromising the natural resources needed for future generations. Food losses and waste impact the sustainability of food systems. They occur in all the steps involved in a food supply chain from farm to fork. Globally, approximately one-third of the food produced is either lost or wasted, which represents nearly 1.7 billion tons per year (FAO 2011, 2013). The estimated value of food waste is approximately US\$750 billion per year. According to Kader and Rolle (2004), post-harvest losses depend on the type of crop, production area, and production season. They also mentioned that about one-third of the produced horticultural crops were never consumed by humans. The losses of vegetable commodities during harvest and processing are due to mechanical damage, spillage, and/or degradation. The losses and wastes of these commodities could also occur during processing operations, such as washing, slicing, peeling, and blanching, as well as wholesale markets, retailers and supermarkets. Losses and waste at the consumer level could be due to undesirable commodities and leftovers. In addition to the losses of the valuable products, food waste and field residues could cause negative environmental impacts if they are not properly managed. The emissions of greenhouse gas (GHG) were estimated to be 4.14 ton of CO<sub>2</sub> equivalent (CO<sub>2</sub>e) per ton of food wasted (Oelofse and Nahman 2013).

Most of the food loss in low-income countries occurs during harvest and postharvest due to the application of manual processes and low-efficiency technologies. Food waste is not much at the consumer level. Figure 16.1 shows the losses in fruits and vegetables in different stages of the food chain in different regions. In developing countries, food waste occurs mainly in agriculture, post-harvest, and processing stages. The losses in agricultural production represent the major source of losses in vegetable and fruits. Losses during postharvest and distribution stages represent major sources of losses due to the deterioration of these crops during handling of these perishable commodities.



**Figure 16.1** Losses in fruits and vegetables commodities in different regions and stages of the food chain (WRAP 2015).

In industrial countries, more than 40% of food waste occurs at retail and consumption stages (WRAP 2015).

Bakas (2010) stated that reducing the climate impact from food consumption can be achieved by either reducing unnecessary production of food or changing dietary by reducing the consumption of meat and dairy products. If the second option is adopted, more vegetables and fruits would be expected, which could result in more plant food waste than the current quantities. Reduction and prevention of food losses and wastes are priorities as they are more effective in reducing GHG emissions than treatment processes (WRAP 2015). The waste management hierarchy, from low to high impact on the environment, is divided into several levels: reduction of waste at sources, recovery, and reuse of waste on site, off-site recycling, and waste treatment to reduce mass and volume, and landfill disposal (Boye et al. 2012). Since food wastes cannot be completely avoided, they need to be economically and environmentally managed. There are several technologies that can be employed to manage food waste for producing bioenergy and other valuable products. Each technology has an environmental footprint, advantages, and disadvantages. Table 16.1 shows lifecycle emissions of GHG for selected waste management options (WRAP 2015).

Several methods are available for the disposal of food wastes and surplus foods in markets. Eriksson and Spångberg (2017) performed a life cycle assessment of incineration, anaerobic digestion, conversion to various products and donation as means for disposal of waste and surplus food in the supermarkets. In the conversion scenario, unsellable and small damage or unappealing fruits and vegetables were sorted out from the waste stream and, then they were cooked and mixed with sugar and vinegar to produce chutney.

**Table 16.1** Emissions of greenhouse gas from selected waste management options (WRAP 2015).

Management or disposal technology	Emissions (kg/[ton of food waste]) <sup>a</sup>	Rank in waste hierarchy
Redistribute to people from processors and retail	-3090	Preventing
Redistribute to animal from processor	-220	Preventing/recovery
Anaerobic digestion	-162	Recovery
Incineration (with energy recovery)	-89	Recovery
Incineration (without energy recovery)	0	Disposal
Composting	-39	Recovery
Land spreading	-39	Recovery
Landfilling	536	Disposal

<sup>a</sup> Incurred net emissions (+) or avoided or savings, (-).

Chutney was then transported to supermarkets for retail. Their results showed that the reduction of global warming potential was in the range of 0.04 to -0.23 kg CO<sub>2</sub>e/kg for incineration and anaerobic digestion and -0.35 to -0.98 kg CO<sub>2</sub>e/kg for conversion and donation. The primary energy use for incineration and anaerobic digestion ranged from 1.2 to -1.2 MJ/kg and for conversion and donation ranged from -5.1 to -16 MJ kg<sup>-1</sup>. This indicates that incineration and anaerobic digestion had higher levels of global warming potential and primary energy inputs than other scenarios. This confirms the preference of conversion of the fruit and vegetable wastes into chutney and the donation as compared with other scenarios.

In addition to the aforementioned technologies and methods for disposal of food waste, there are several other technologies for bioenergy production from these wastes. The selection of a certain technology depends on material characteristics, advantages and disadvantages of a technology, and social and economic factors. The objectives of this chapter are to review: (i) the characteristics of plant food wastes; (ii) collection, storage and pretreatments of plant food wastes for bioenergy production; and (iii) the operational concepts, advantages and disadvantages of various bioenergy production technologies.

## 16.2 Characteristics of Plant Food Wastes

Plant food wastes can be solid, semisolid or liquid. Proximate and elemental analyzes of different plant wastes have been published in the literature. Asquer et al. (2013) characterized vegetables and fruits from a wholesale market in Sardinia, Italy, during a period of six months (Figure 16.2). They measured total solids (TSs), volatile solids (VSs), carbon to nitrogen ratio (C/N), and macro and micronutrients. Their results showed that fruit residues had higher TS, VS, and C/N compared to vegetable wastes. Calcium, potassium, magnesium, and sodium were the most abundant elements in fruits and vegetable wastes. Fruits contained less sulfur than vegetable wastes. Figure 16.2 shows the VS

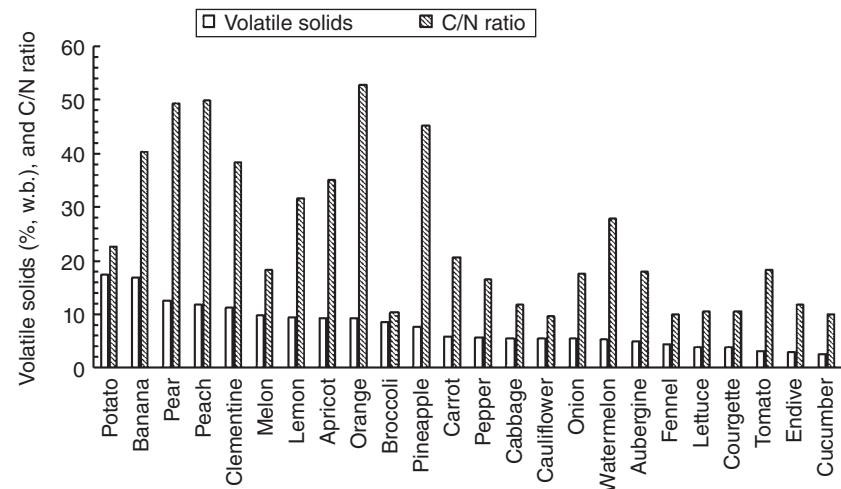


Figure 16.2 The VS and C/N of some fruits and vegetables (Asquer et al. 2013).

and C/N of some fruits and vegetables. Table 16.2 shows proximate analysis and lignocellulosic components of selected plant waste and residues. The compositions of solid vegetable and fruit wastes vary significantly with the crop type. Processing of fruits and vegetables consume large amounts of water in washing, peeling, and blanching. Most of the water used in these unit operations is discharged as wastewater rich in organic matter and nutrients. Table 16.3 shows the characteristics of selected wastewaters from fruits and vegetable processing (Soderquist et al. 1972; PBK Engineering Ltd. 1996). As can be seen, most of the wastewaters from fruits and vegetables contain high concentrations of chemical oxygen demand (COD) and biological oxygen demand (BOD). The characteristics of wastewater depend on the processed crop, unit operation, and equipment type.

### 16.3 Collection of Plant Food Wastes

Collection and transportation of field residues often impose for using them as feedstock for bioenergy production. This is due to the low densities of these materials and most of the crop harvesting machines are not designed to collect vegetable crop residues. For example, tomato harvesters separate the fruits from the plants and leave the residues on the field as shown in Figure 16.3. In addition, in cases where vegetable residues are mechanically collected, large amounts of soil can be collected along with vegetable residues, causing serious problems during their processing and conversion (Agneessens et al. 2014). Innovation is needed in order to improve the methods and equipment for plant biomass collection from vegetable production.

The systems and costs for collecting plant wastes and residues at the farm level depend on the crop type and the physical characteristics of its waste, harvesting method, harvesting losses, and the application of field drying. At the consumption stage, mixed food waste is generally collected as part of the municipal solid waste (MSW). In many

**Table 16.2** Composition of some plant biomass materials<sup>a</sup>.

Biomass material	Moisture content (%)	VS/TS (%)	TC (%)	TN (%)	C/N	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Reference
Wheat straw	na	na	36.4	0.38	95.8	35.7	43.7	6.0	Hills and Roberts (1981)
Barley straw	na	na	37.8	0.30	126.0	34.9	43.2	6.3	
Rice straw	na	na	33.6	0.39	86.2	37.4	44.9	4.9	
Tomato pomace	na	na	48.9	2.25	21.7	50.5	43.7	3.7	
Tomato waste	70.5	95.7	54.9	4.2	13.0	Na	na	na	Viswanath et al. (1992)
Mango waste	73.6	96.4	39.8	0.5	76.5	na	na	na	
Orange waste	73.4	94.2	42.5	1.0	40.5	na	na	na	
De-oiled orange waste	75.14	93.6	43.1	1.3	33.2	na	na	na	
Pineapple waste	87.69	93.8	38.9	0.9	42.3	na	na	na	
Banana waste	88.14	95.1	40.5	1.9	21.3	na	na	na	
Jackfruit waste	80.15	92.3	46.3	1.4	33.1	na	na	na	
Olive pomace	30.4	83.6	na	0.7	na	na	na	na	Tekin and Dalgiç (2000)
Onion processing residues	92.6	96.1	39.3	1.82	21.6	na	na	0.4	Romano and Zhang (2008)
Cauliflower residue	12.0	83	40.2	3.10	13.0	25.8	4.5	3.2	Madenoğlu et al. (2011)
Acorn	12.9	97	48.5	0.40	121.3	24.1	16.0	12.1	
Tomatoes residue	7.5	96	54.7	2.90	18.9	23.0	16.3	21.0	
Hazelnut shell	6.8	98	52.2	0.45	116.0	37.4	11.9	39.2	
White grape stem	69	92.4	43.6	1.36	32	8.5	3.7	8.2	Chen (2011)
White grape skin	69.3	92.7	42.5	1.07	40	10.5	5.1	9.6	
White grape seed	38.1	96.8	51.7	1.55	33	7.8	9.8	30.2	
White grape pomace	61.4	93.6	45.7	1.34	34	8.9	5.8	14.9	
Red grape stem	61.5	88.6	52.0	1.75	30	10.8	7.0	10.8	
Red grape skin	68.1	90.7	43.5	1.54	28	17.3	8.3	8.8	

(Continued)

**Table 16.2** (Continued)

Biomass material	Moisture content (%)	VS/TS (%)	TC (%)	TN (%)	C/N	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Reference
Red grape seed	48.1	94.7	50.7	2.19	23	9.4	9.8	21.9	
Red grape pomace	60.1	90.7	51.2	1.93	27	10.5	8.2	14.9	
Fresh grape pomace	65.5	87.4	44.3	1.2	36.9	9.2	4.0	11.6	Zheng et al. (2012)
Fermented grape pomace	66.4	92.0	48.2	2.5	19.3	14.5	10.3	17.2	
Mixed fruits and vegetable waste	92.6	88.1	43.3	2.8	15.6	na <sup>b</sup>	na	na	Wang et al. (2014)
Albizia leaves	63.5	93.7	49.7	5.1	9.8	10.9	4.9	26.9	Ge et al. (2014)
Taro skin	85.2	83.5	47.5	2.0	23.8	19.0	4.4	13.0	
Papaya	89.3	92.4	52.0	4.2	12.4	6.3	1.5	5.1	
Bagasse	10.4	85.6	49.8	0.2	249	na	na	na	Kang et al. (2014)
Coconut shell	4.4	73.7	51.1	0.1	511.0	na	na	na	
Corn stover	10.6	87.4	42.5	0.8	53.1	na	na	na	
Groundnut shell	7.9	73.9	50.9	1.2	42.4	na	na	na	
Hazelnut shell	7.2	77.0	51.5	1.4	36.8	na	na	na	
Olive husks	6.8	79.1	50	1.6	31.3	na	na	na	
Rice husks	10.6	62.8	49.3	0.8	61.6	na	na	na	
Soya husks	6.3	74.3	45.4	0.9	50.4	na	na	na	
Sunflower husks	9.1	76.0	50.4	1.1	45.8	na	na	na	
Tea wastes	7.3	75.8	48.6	3.8	12.8	na	na	na	
Mixed vegetable waste	72.8	60.0	na	na	10–14	na	na	na	Gulhane et al. (2016)
Corn stover	12.9	87.5	38.9	1.0	37.5	20.4	31.8	20.0	Li et al. (2016)
Tomato residues	87.5	81.8	38.0	2.4	15.8	5.1	12.2	9.7	
Apple pomace	8.9	81.3	47.9	0.78	61.5	47.5	27.8	22.4	Baray Guerrero et al. (2016)

<sup>a</sup> All components are dry base except moisture content that is in wet base.<sup>b</sup> na, not available.

**Table 16.3** Characteristics of selected wastewaters from selected fruits and vegetable processing (Soderquist et al. 1972; PBK Engineering Ltd. 1996).

Wastewater	Flow (m <sup>3</sup> ton <sup>-1</sup> )	COD (kg ton <sup>-1</sup> )	BOD (kg ton <sup>-1</sup> )	Total solids (kg ton <sup>-1</sup> )	Total N (kg ton <sup>-1</sup> )	Total P (kg ton <sup>-1</sup> )	Organic N (kg ton <sup>-1</sup> )	Ammonia (kg ton <sup>-1</sup> )	pH
Beet wash effluent	1.2	0.85	0.74	—	—	0.017	0.005	0.001	—
Beet blanching and peeling effluent	2.8	31.82	27.68	—	—	0.078	0.038	0.072	—
Green bean washing and grading effluent	2.2	0.64	0.55	1.548	0.028	0.006	0.028	0.000	7.1
Green bean water blanching effluent	0.4	1.50	1.30	1.468	0.041	0.007	0.039	0.002	6.0
Royal Ann cherry pitter effluent	8.3	13.19	11.47	12.772	0.081	0.026	0.052	0.025	4.3
Royal Ann cherry stemmer effluent	2.4	0.34	0.29	0.456	0.000	0.003	0.000	0.000	6.1
Lambert cherry pitter effluent	4.5	18.17	15.81	18.399	0.071	0.035	0.058	0.005	4.6
Bartlett pear mechanical peeler (Ewald) effluent	5.0	6.87	5.98	6.639	0.020	0.006	0.015	0.000	4.6
Bartlett pear mechanical peeler (Contour) effluent	2.1	15.82	13.76	14.836	0.073	0.010	0.058	0.000	4.2



Figure 16.3 Tomato harvest.

countries, the MSW is usually disposed of in landfills. In the U.S., food waste accounts for 16% of the MSW by weight. The tipping fee for disposing of MSW in the landfill could reach up to \$200 per ton depending on the regions, with \$35–50 per ton being common. In the recent years, landfilling is under scrutiny and many countries have enacted regulations that are aimed at reducing the landfill due to the limited spaces for landfills and their negative effects on environmental quality. For example, the State of California has issued the Assembly Bill 341 to divert at least 75% waste from landfill by the year 2020 (CLI 2011). According to the bill, public entities and commercial businesses that generate  $3.1 \text{ m}^3$  of organic waste per week and multi-family housing complexes that generate  $3.8 \text{ m}^3$  per week to adopt recycling practices. Efficient and economic separation and sorting are necessary for the efficient and economic production of valuable products from plants food waste.

Many countries have employed methods for residents and commercial businesses to separate food waste from other refuse materials. However, the behavior of residents may affect the quality and quantity of food waste that is separated in situ. Efficient and easy methods for the separation of food waste should be applied. Woon and Lo (2016) proposed a simple and consumer-acceptable sorting process to separate food waste from MSW at the consumer level with minimal change in consumer behavior. The process involves the use of the optic bag, with a certain color, to collect food waste and the rest of MSW is collected in common garbage bag. All the bags are transferred by refuse collection vehicle to refuse transfer stations wherein food waste bags are separated from other bags using optic sensors that pick up the colored bags. Then the separated food waste bags are sent to anaerobic digestion facilities.

In the US, most of the residential communities and small businesses collect food waste together with yard waste. Food wastes from commercial food businesses and food processing operations are normally collected separately. The food waste or mixed food and yard waste are treated at composting operations or anaerobic digestion facilities.



**Figure 16.4** Food waste collected in Sacramento and trucks used to collect food waste.

For example, food waste collected in the Sacramento region is treated at two anaerobic digestion facilities developed by CleanWorld. Some food waste collected in San Francisco is treated at an anaerobic digestion facility owned and operated by East Bay Municipal Utility District in Oakland. In some places, drop-off sites are used by residents and small businesses to dispose of their food waste (Yepsen 2012). The drop off sites minimize the driving distance and the transportation costs and road maintenance. In 2008, San Francisco passed the Mandatory Recycling and Composting Ordinance that requires everyone in the city, including tourists, to separate their wastes into one of three bins recycling, compost, and landfill. It is mandated to compost food waste to reach the goal of zero waste by 2020. The produced compost is sold in bulk to farms. The zero waste mandate means diverting wastes from landfill, incineration, or high-temperature technologies. In 2012, San Francisco achieved the highest landfill diversion rate of 80% in the country (EPA 2017). Figure 16.4 shows the food waste collected in Sacramento and trucks used to collect food waste.

Bernstad and la Cour Jansen (2012) described four systems for collection of household food waste in Sweden. In the first system, food waste is separated by the consumer in paper bags and disposed of in designated waste bins that are transferred via garbage vehicles to the anaerobic digestion facilities. Prior to digester feeding, paper bags are mechanically separated. The second system is similar to the first one, but food waste is dried at low temperature prior to the transportation to the anaerobic digestion facilities where it is directly fed to the digester without the separation of paper bags. Therefore, the paper bags are anaerobically digested and contributing to the biogas produced. In the third system, food waste is ground in kitchen sinks that have piping systems separated from the sewer system. Ground food waste is then transported to a settling tank. The supernatant is pumped to wastewater treatment plant and settled waste is transported to anaerobic digestion facilities. In the fourth system, food waste is transported from kitchen sinks to a central grinder that grinds food waste from various households. Settled sludge is stored underground and then collected and transported to anaerobic digestion facilities.

The first system has the lowest investment cost and is currently the most common system for food waste collection in Sweden. However, the food waste collected in the other systems had higher biogas yields than the first system, respectively.

In England, McManus (2011) mentioned that for small-scale biodiesel processing facilities, waste cooking oil was collected from hotels, restaurants, and schools using flatbed trucks running on the produced biodiesel, while oil was obtained from national oil collectors and delivered in oil tankers to the large-scale biodiesel facilities. In the U.S., there are independent renderers who collect used cooking oil from food processing facilities in specialized trucks that deliver the collected oil to processing facilities (NRA 2008). In some states, there are also collection centers and aggregation points for collecting used cooking oil (Environmental Assistance Center 2007). Collection centers accept used oils from households and other used oil generators who transport their own used oil. The aggregation points are central locations that consolidate oil facilities at multiple locations.

## 16.4 Pretreatments of Plant Food Waste

Food waste collected at the consumption stage may be contaminated with other materials such as plastics, glass, and metals. These materials should be removed before biochemical conversion processes in order to save the reactor volume occupied by these inert materials, prevent floatation of low-density materials in reactors, prevent the down operational time, and reduce maintenance requirements for reactors and handling equipment. Depending on the type and characteristics of food waste and the process to be applied to bioenergy production, these contaminants can be separated. Wet and dry processes are suitable for biochemical and thermochemical conversion processes. The dry separation processes include screens, depackagers, extrusion, and air separation. Figure 16.5 shows a bioseparator used at the anaerobic digestion facility in Sacramento for separating the contaminants from food waste prior to anaerobic digestion.



Figure 16.5 Bioseparator for separating contaminants from food waste prior to anaerobic digestion.



**Figure 16.6** Plastics and other materials separated from food waste.

Figure 16.6 shows the plastics and other materials separated from food waste. The wet separation processes include pulping, filtration, cyclonic classification, flotation, and settling. Coker (2014) described these separation processes in detail.

Pretreatment of lignocellulosic materials including some of the plant food waste is important to increase the efficiency of the biochemical conversion processes of these materials into bioenergy. Several pretreatments have been applied, including mechanical, thermal, and chemical pretreatments. These pretreatments can be applied individually or in combination. The main objectives of the pretreatments are increasing the surface area to material volume, decrystallization of cellulose and solubilization of lignin and hemicellulose so that the bioconversion rate and efficiency increase. In addition, biomass grinding can also help in homogenizing biomass materials and facilitate mixing and agitation during the bioprocessing. On the other hand, some of the pretreatment may produce inhibitors (e.g. furfural and HMF [hydroxymethylfurfural]) to microorganisms used in bioconversion processes. The operational principles, advantages, and disadvantages of different pretreatment methods have been reviewed by many authors (Mosier et al. 2005; Hendriks and Zeeman 2009; Zheng et al. 2009). Palamae et al. (2014) studied the effect of alkaline peroxide and peracetic acid as delignifying agents to minimize the loss of hemicellulose from oil palm empty fruit bunch. Peracetic acid was more effective in lignin removal with fewer losses of hemicellulose compared with the treatment with alkaline peroxide. A maximum lignin removal of 53% was achieved using 20 l of peracetic acid per each kilogram of the empty fruit bunch after treatment at 35 °C for nine hours. Liu et al. (2015a,b) studied pretreatment of wheat straw with potassium hydroxide and developed a continuous treatment process with black liquor recycle. Table 16.4 shows a comparison of selected pretreatment methods.

## 16.5 Storage of Plant Food Wastes

The seasonal nature of the production of vegetable and fruit crops entails the need to store their waste in order to assure a year-round supply of this waste to bioenergy production facilities. Most of the plant wastes are wet materials. They undergo biodegradation during storage, leading to the losses of organic matter and increase the number of

**Table 16.4** Comparison of the effects of selected pretreatments for lignocellulosic materials (Mosier et al. 2005; Hendriks and Zeeman 2009).

Pretreatment	Surface area increase	Cellulose decrystallization	Hemicellulose solubilization	Lignin solubilization	Lignin structure alteration	Furfural/HMF formation
Mechanical comminution	+	+				
Steam pretreatment/steam explosion	+		+	-	+	+
Hot water	+	ND	+	-	-	-
Acid	+		+	-	+	+
Alkaline	+		-	±	+	-
Oxidative	+	ND		±	+	-
Thermal + acid	+	ND	+	±	+	+
Thermal + alkaline	+	ND	-	±	+	-
Thermal + oxidative	+	ND	-	±	+	-
Thermal + alkaline + oxidative	+	ND	-	±	+	-
Ammonia fiber explosion	+	+	-	+	+	-
CO <sub>2</sub> explosion	+		+			

+ = major effect.

- = minor effect.

ND = not determined.

microorganisms that could affect the bioenergy production. Biomass storage should be economic, safe, and result in minimal losses of organic matter and environmental impact. Plant waste could be stored as dry or wet materials. Dry materials can be baled or compressed before storage to save storage space. Figure 16.7 shows the baled corn stover and stored on a farm in Iowa and baled wheat straw in California.

During the outdoor storage of other biomass materials such switchgrass and canary-grass, for 293–334 days, Shinners et al. (2010) found that the average dry matter losses were 3.8%, 4.8%, 7.5%, 8.7%, and 14.9% for bales wrapped with plastic film, breathable film, net wrap, plastic twine, and sisal twine, respectively. A loss of 3.0% dry matter was found in bales stored under cover while 1.1% dry matter loss was found for ensilage storage. The average initial moisture contents of the stored baled and ensilage biomass were 16.1% and 39.9% (w.b.), respectively.

Drying is a common practice for wet biomass to extend the shelf life of biomass material and facilitate their storage. However, it is an energy-intensive process that increases



Figure 16.7 Baled corn stover and stored on a farm in Iowa and baled wheat straw in California.

the cost of biomass materials. Ensiling is a preservation process that is traditionally used for storing wet biomass especially for producing animal feeds. However, in recent years ensiling has been proposed to store wet biomass for bioenergy production. In this process, materials are stored under anaerobic conditions that provoke naturally present organisms and plant enzymes to hydrolyze crop cell walls and ferment sugars producing organic acids (i.e. mainly lactic acid) that leads to pH decrease. Low pH in silage prevents microbial growth that is responsible for the losses of organic matter during biomass storage. In addition to the preservation of organic matter during ensiling, ensiling can also provide pretreatment for lignocellulosic materials. According to Chen et al. (2007), application of ensilage as a pretreatment of biomass materials is more economical, needs less energy, and produces fewer inhibitory substances to fermentation processes than other pretreatments. Additives such as enzymes can also be used to achieve better conditions for ensiling. Ren et al. (2006) found that the addition of hemicellulase and cellulase during the ensiling of corn stover significantly increased lactic acid production rates and reduced the pH to 3.9–4.5, and guaranteed a storage time of six months under anaerobic conditions. Méndez-Llorente et al. (2014) applied ensilage for discarded tomato from fields with and without additives such as brewer's yeast and cane molasses. After 140 days of ensiling, little changes in the biomass composition was observed for all the treatments. However, there was an increase in the crude protein content with using the additives. Figure 16.8 shows the corn stover ensilage on a farm in Germany for use in anaerobic digesters.

Zheng et al. (2012) ensiled fresh and fermented grape pomace in bench scale, inoculated with lactic acid bacteria, and in pilot scale (1200 l) without inoculation. Unlike fermented grape pomace that is produced after red wine fermentation, fresh grape pomace is the residue after juice is extracted from grapes for white wine production. Prior to ensiling, water was used to leach and remove ethanol and water-soluble carbohydrates from the fermented and fresh pomace, respectively. For the bench-scale experiments, their results indicated that inoculated and not inoculated ensiled pomaces were equivalent in most of the measured matrices. For both materials ensiled in pilot scale without inoculation with lactic bacteria, the pH remained stable and relatively small increases in lactic acid were observed after one year of storage. Fresh pomace contained a lower concentration of acetic acid than the fermented one. Butyric acid was undetectable in fresh pomace but decreased slightly in the fermented pomace over the course of ensilage.



**Figure 16.8** Corn stover ensilage on a farm in Germany for use in anaerobic digesters.

Water soluble carbohydrate declined to undetectable levels over the ensiling period. Ethanol concentration increased over the ensiling period for fresh grape pomace. In addition to ensiling of materials individually, co-ensiling of two or more materials is desirable because it can be possible to control the properties of ensiling materials (e.g. moisture contents and nutrients needed for the growth of lactic acid bacteria), and the fermentation products. Garcia and Kalscheur (2004) studied the co-ensiling of wet distiller's grains, soybean hulls, and beet pulp with results showing that co-ensiling resulted in more acetic acid production than ensiling corn alone.

## 16.6 Technologies for Converting Plant Food Waste to Bioenergy

Several technologies are available for the production of the bioenergy from plants food waste. They include biochemical conversion technologies (e.g. anaerobic digestion, and fermentation), thermochemical conversion technologies (e.g. pyrolysis, and gasification), and chemical conversion technologies such as transesterification for biodiesel production from used cooking oil. Although individual technologies are applied for the production of bioenergy from plant wastes, combined technologies are also applied to produce different bioenergy products.

### 16.6.1 Anaerobic Digestion

Anaerobic digestion is a biochemical process in which organic matter is converted, in the absence of oxygen, by anaerobic microorganisms into biogas. Biogas is composed mainly of methane (60–70% v/v) and carbon dioxide (30–40%) with trace amounts of other gases (e.g. hydrogen sulfide) and water vapor. Removal of water, hydrogen sulfide, and volatile organic compounds is necessary before using biogas as fuel for heat and

electricity generation or used as a transportation fuel. The performance and stability of anaerobic digesters depend on system configuration and operational parameters such as organic loading rate (OLR), temperature and retention time, and the characteristics of the biomass material. Anaerobic digestion can be applied under psychrophilic ( $<25^{\circ}\text{C}$ ), mesophilic ( $25\text{--}45^{\circ}\text{C}$ ), or thermophilic ( $>45^{\circ}\text{C}$ ) temperature. In the absence of inhibitory compounds, the degradation rates of organic material increase with increased temperature. There are several anaerobic digestion systems that are suitable for solid and liquid substrates. Detailed descriptions of these systems have been presented by many researchers (e.g. Zhang and El-Mashad 2007). The costs of the anaerobic digestion system depend on the types and characteristics of feedstock, digestion system configuration, climate conditions, end-use of biogas and digester effluent management. The success of anaerobic digestion depends strongly on the balance of the carbon with nitrogen and other nutrients, and biodegradability of the feedstock. The desirable C/N is in the range of 15–30, depending on the biodegradability of feedstock. Most plant food wastes are carbon-rich and easily biodegradable. OLR in the anaerobic digester needs to be carefully controlled. Otherwise, the digestion process may not be stable due to the accumulation of volatile fatty acids (VFAs) that could produce inhibition or complete cessation of the methanogenic activity. Different measures can be applied to reduce the negative effects of high VFA concentrations on the degradation of food wastes: dilution of the food waste before digestion, increase of the hydraulic retention rate and decrease of loading rates, codigestion of food waste and other substrates, and application of two-phased anaerobic digesters.

Fruit and vegetable wastes contain low nitrogen contents and high C/N ratios that ranged from 15.4 to 35.7 (Bouallagui et al. 2004). This may indicate that fruits and vegetables can be successfully digested with the need to adjust their C/N ratios. Asquer et al. (2013) presented the macro, micro, and trace elements in 24 fruit and vegetable wastes. These wastes contained macro and microelements making each of them a suitable feedstock for anaerobic digestion without the need for adding nutrients or codigestion with other substrates. However, it is preferable to codigest both vegetable and fruit wastes to use the benefits of the balanced nutrients in the mixture that prevent the instability of the anaerobic digestion process. Moreover, the characteristics of feedstocks affect the quality of biogas. Scano et al. (2014) mentioned that wastes of leafy vegetables contain high levels of sulfur that could increase the hydrogen sulfide content in the biogas that in turn increase the costs of biogas cleaning and upgrading.

Gulhane et al. (2016) studied the anaerobic digestion of vegetable market waste for one year in an anaerobic baffled reactor at an OLR of 0.5 kg[VS]/m<sup>3</sup>/ day and a hydraulic retention time (HRT) of 30 days. Biogas yields ranged from 0.7 to 0.8 l/g[VS added] and methane content ranged from 55% to 58%. Viswanath et al. (1992) studied the anaerobic digestion of mango, pineapple, tomato, jackfruit, banana, and orange wastes at different HRTs and OLR of 38 kg[VS]/m<sup>3</sup>/day. The maximum biogas and methane yields were 0.6 and 0.37 m<sup>3</sup>/kg[VS], respectively, at an HRT of 20 days and OLR of 40 kg[TS]/m<sup>3</sup>/day. The total VFAs was 322 mg l<sup>-1</sup> (as acetate). At HRT of eight days, there was an accumulation of VFAs at 6430 mg l<sup>-1</sup> with a very low methane yield of 0.37 m<sup>3</sup>/kg[VS]. Nguyen et al. (2016) employed semi-continuous anaerobic digester for treating food waste. The system temperature increased from 38 to 55 °C at the rate of 1 °C every two days. The OLR increased from 2.1 to 8.62 kg[VS]/m<sup>3</sup>/day in four steps during 100 days of operation. Depending on the conditions applied in each phase through

the experimental work, biogas yields ranged from 43.3–162.1 l kg<sup>-1</sup>[waste] and methane content ranged from 43% to 63%. The greatest VS reduction (87.01%) was achieved at OLR of 8.62 kg[VS]/m<sup>3</sup>/day, although high concentrations of ammonia (3700 mg l<sup>-1</sup>) and VFAs (7101 mg l<sup>-1</sup>) were found. The successful digestion might be due to the acclimation of anaerobic microorganisms to high ammonia and VFAs. In addition to the inhibition of anaerobic digestion by VFAs at high concentrations, vegetable and fruit wastes could be inhibited by phenolic compounds. For the digestion of cherry stillage, Alvarez et al. (2005) found that the anaerobic sequencing batch digestion at 30 °C was inhibited by phenolic compounds at OLR of 0.3 g[COD]/g[VSS]/day. They applied ozone as a pre-treatment to remove more than 75% of polyphenols. Li et al. (2016) studied the anaerobic batch codigestion of tomato residues with dairy manure and corn stover at 20% total solids under 35 °C for 45 days. Mixing tomato residues with dairy manure and corn stover increased methane yields. The highest methane yield and VS reduction of 415 m3/ton[ton] and 46.2% could be obtained, respectively, for a mixture consisting of 33% (based on VS) corn stover, 54% dairy manure, and 13% tomato residues. Using batch digestion tests at 35 °C, Qu et al. (2009) determined the methane yield of pomegranate marc, a byproduct of pomegranate juice processing, before and after extraction of antioxidants and oil. At an initial loading of 5.0 kg[VS/m3/day and a digestion time of 20 days, methane yields of the raw pomegranate peels, seeds, and their mixture were 207, 249, and 221 l/kg[VS], respectively. While after extraction of antioxidants and oils, methane yields were 148, 183, and 161 l/kg[VS], respectively.

Biogas production yields from selected plants biomass and operational conditions of digesters are shown in Table 16.5. As can be seen from this table, biogas and methane yields depend on the type of substrate, reactor type, and operational conditions. Anaerobic digestion has been widely applied to field residues. However, the recalcitrant nature of the lignocellulosic materials requires the application of a pretreatment to increase the biodegradability of these materials. Zhou et al. (2017) studied the effect of pre-aeration on the biodegradability of rice straw under different temperatures regimes (25, 35, and 45 °C) for 2, 4, 6, and 8 days. Methane production yield of 355 ml/g[VS] could be obtained after aeration for two days at 35 °C. This yield is higher than that (306 ml/g [VS]) obtained for the untreated straw. Results showed that increasing the substrate-to-inoculum ratios decreased methane yield and increased lag phase due to the VFAs inhibition. After a batch digestion time of 20 days at 50 °C, Chen (2011) determined biogas yields of approximately 520 and 430 ml/g[VS] and methane content of 65% and 69%, respectively, from the skin and stem, respectively. He also found a biogas yield of 308 and 146 ml from the white and red grape seeds regardless the smaller particle size of the seeds than the skin. The author attributed the lower biogas yield to the high lignin and hemicellulose contents in the seeds. He also found that alkaline pretreatment was more effective with red grape pomace than white grape pomace.

A two-phase anaerobic digester system was developed in the laboratory and scaled up to pilot and full scales to treat food waste and field residues (Zhang and Zhang 1999; Zhang and Rapport 2011). The system is called Anaerobic Phased Solids (APSs) digester. The system has been applied to treat easily biodegradable materials such as food and green wastes. The system was further developed and used as a foundation to develop a three-stage thermophilic anaerobic digestion system. In a collaboration with our industry partner, CleanWorld, the newly developed three stage system was scaled up and installed at the campus of California, Davis (Figure 16.9) and in the city of Sacramento

**Table 16.5** Biogas production from selected plants biomass materials.

Feedstocks	Reactor type/Size	Temperature (°C)	Biogas yield (l kg <sup>-1</sup> ) <sup>a</sup>	Methane content (%)	Reference
Bananas (fruit and stem)	Continuous, 20 l	35 °C	497 <sup>b</sup>	53	Stewart et al. (1984)
Potatoes (peels, rejects)			350–410 <sup>b</sup>	44–50	
Oats			227–257 <sup>b</sup>	51–54	
Carrot processing wastewater	UASB, 2–3 l	55 °C	315 <sup>c</sup>	49 for carrot wastewater	Lepistö and Rintala (1997)
Potato and swede processing			347		
Wastewater					
Rice straw	APS	35 °C	380–470	49–52	Zhang and Zhang (1999)
Onion processing waste	APS	35 °C	0.51–0.62	50–57	Romano and Zhang (2008)
	Batch		0.69	55	
<i>Acorus calamus</i> Linn			510		Jiang et al. (2014)
<i>Typha orientalis</i> Presl.			513		
<i>Pontederia cordata</i>			473		
<i>Canna indica</i>	Batch	37	555		
<i>Colocasia tonoimo</i> Nakai			629		
<i>Thalia dealbata</i>			578		
<i>Hydrocotyle vulgaris</i>			539		
Raw fruit and vegetable waste	Tubular, 18 l	20	386.3–625.0	56–58	Bouallagui et al. (2004)
		35	183.2–705.9	54–65	
		55	733.8–997.5	58–62	
Mixed fruit and vegetable wastes	Tubular, 950 l	35	773	55.9	Scano et al. (2014)
Potato peel waste	Batch	35	367	65	Liang and McDonald (2015)
Mixed vegetable wastes	Anaerobic baffled reactor, 60 l		700	52.0	Gulhane et al. (2016)

<sup>a</sup> All values per kg VS.<sup>b</sup> Per kg of TS.<sup>c</sup> l/kg COD.



Figure 16.9 UC Davis high rate anaerobic digestion system.



Figure 16.10 Anaerobic digestion and renewable natural gas facility in Sacramento, CA.

(Figure 16.10). Both systems are operated at thermophilic conditions ( $55^{\circ}\text{C}$ ). The UC Davis anaerobic digestion system treats 20 000 ton of mixed food wastes per year. The biogas produced is used in combined heat and power generation. The electricity generated (approximately 16 000 kWh/d) is provided to the university. It has been operational since January 2014. The Sacramento anaerobic digestion facility treats approximately 40 000 tons of food waste per year. The biogas produced is upgraded into renewable natural gas for vehicles.

Wu et al. (2016) applied a two-phase system consisting of a completely stirred tank acid reactor and an up-flow anaerobic sludge bed methane reactor to treat a simulated mixture of fruits and vegetable waste. The mixture contained 57% watermelon, 29% apple,

and 14% potato by wet weight. The effluent of the first phase contained a lactic acid as a predominant intermediate at an average concentration of  $14.8 \text{ g l}^{-1}$ . *Lactobacillus* and *Methanosaeta* were the predominant organisms in first and second phase, respectively. The two-phase AD system was successfully operated at a low HRT of 3.56 days yielding a methane yield of  $244.2 \text{ ml/g[VS added]}$ .

Downstream processing and handling of anaerobic digestion effluents are applied to recover valuable products such as biofertilizers and reduce potential air emissions from remaining biodegradable materials in the digestate (Barzee et al. 2017). Moraes et al. (2017) applied anaerobic digestion to reduce the emissions of GHG from vinasse during handling and storage. Vinasse was mixed with cow manure and straw and anaerobically codigested at  $35^\circ\text{C}$  and 35 days HRT. The mixture volatile solids contained 61% of vinasse, 2% manure, and 37% straw. The emission from digestate and untreated vinasse was measured during the storage in closed containers, with an opening of 1 cm diameter in the container lid, for 21 days at  $37^\circ\text{C}$ . For the storage of the untreated vinasse, 10% v/v of digestate was added to the vinasse to simulate the inoculation with a microbiota that is retained in the anaerobically digested vinasse stores. An accumulative emission of  $43.8 \text{ kg[CO}_{2\text{eq}}]/\text{kg}$ , of carbon contained in vinasse, could be determined for the material that was not anaerobically treated material. During storage, no  $\text{CH}_4$  emissions could be measured from the digestate produced from the codigestion of vinasse, manure, and straw. The authors attributed the absence of the methane emission during the storage to the depletion of biodegradable organic matter during the digestion process. Vinasse is a byproduct of the distillation of ethanol produced from molasses fermentation. Each cubic meter of ethanol produces  $10\text{--}15 \text{ m}^3$  of vinasses that contain  $50\text{--}150 \text{ g[COD]}/\text{l}$  (Nair and Taherzadeh (2016).

### 16.6.2 Fermentation Processes

Ethanol is the main biofuel that is currently produced in several countries such as the United States and Brazil. It is produced mainly from corn and sugarcane in the United States and Brazil, respectively. Corn ethanol represented about 94% of all biofuel production in the United States (USDA 2013). The cost of the corn and sugarcane as feedstocks for ethanol production represented 70–90% of the total costs of ethanol production (McAlloon et al. 2000). Plant wastes can be a cheap source for the production of ethanol and butanol that can also be used as a fuel as a chemical for industrial applications (Singh et al. 2012). Production of ethanol from food waste is more valuable than biogas production (Huang et al. 2015). This is due to the fact that ethanol is easy to handle and store. Moreover, it can be used directly as an engine fuel. Plant food wastes could be either starch-rich or lignocellulosic materials. Wei et al. (2015) used cassava stem as a source of starch for the production of ethanol. They mentioned that using cassava stem starch could increase ethanol yield by 26% over that of the roots only. There are several challenges for the commercial production of ethanol from lignocellulosic materials including some plant food waste and field residues: (i) reducing lignin contents of feedstocks; (ii) developing effective pretreatments for lignocellulosic feedstocks; (iii) developing efficient processes for simultaneous conversion of pentose and hexose; (iv) developing genetically modified microorganisms that can tolerate high concentrations of ethanol; and (v) developing efficient fermenters for ethanol production at high sugar concentrations (i.e. high solid contents), and slip-stream processes for alcohol recovery during the

fermentation. Several pretreatments have been developed for lignocellulosic materials, as described above, and research has been conducted to solve these challenges (Vane 2008; Singh et al. 2012). Song et al. (2017) used cruciferous vegetable residue for the production of fermentable sugars and D-psicose. Results showed that this residue did not require pretreatment to enhance saccharification. After yeast fermentation, ethanol and D-Psicose yields were 166.7 and 49.4 g/kg[residue]. Ethanol yield represented 85.7% of the theoretical value.

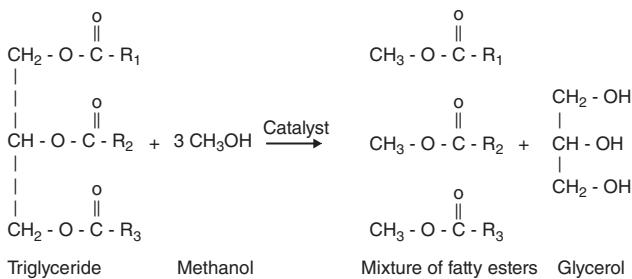
Onion skin waste (OSW) was used to produce sugars for bioethanol production using xylanase and pectinase at a fixed concentration 1.0 and 0.16 mg/g[OSW], respectively (Choi et al. 2015). Glucose yield of 400 mg g<sup>-1</sup> was obtained at cellulase loading of 0.72 mg/g[OSW]. Pourbafrani et al. (2013) determined an ethanol yield of 39.5 l/[ton of citrus waste]. Matsakas and Christakopoulos (2015) used household food waste for the production of cellulase that was used in a second step for the saccharification of that waste at a high dry material 30% w/w. After saccharification, fermentation was carried out using bakery yeast. Results showed that ethanol production reached 38.6% of the maximum theoretically possible based on both soluble and insoluble carbohydrates. Nair and Taherzadeh (2016) applied *Aspergillus oryzae* and *Neurospora intermedia* fungi to convert vinasse or stillage into ethanol and protein-rich fungal biomass. *N. intermedia* fungi have a potential to produce ethanol. Their results showed that ethanol and fungal biomass yield ranged from 2.28 to 10.57 g[ethanol]/l[vinasse] and 37.9 to 222.8 g[dry biomass]/l[vinasse], after applying *A. oryzae* and 3.52 to 12.14 g[ethanol]/l[vinasse] and 31.7 to 202.4 g[dry biomass]/l[vinasse], after applying *N. intermedia*, respectively. The yield of ethanol and fungi biomass depended on vinasse concentration, cultivation time, temperature, and the media pH.

Most of the fermentation processes are carried out at low solid contents to alleviate the inhibition of yeast. Izmirlioglu and Demirci (2016) employed biofilm reactors for the production of bioethanol from potato waste hydrolysate using *Saccharomyces cerevisiae*. They found that the optimum pH, temperature, and mixing speed for ethanol production were 4.2, 34 °C, and 100 rpm, respectively. The ethanol yield was 0.459 g[ethanol]/g[glucose] that represented 92.1% of the theoretical yield and ethanol concentration was 37.1 g/l. Kalmokoff and Ingledew (1985) found that at 12% ethanol (v/v), yeast growth was completely inhibited while fermentative ability was reduced by 50%. Huang et al. (2015) investigated the effect of incorporating vacuum recovery system to remove ethanol during the fermentation of food wastes at high solids content (35%, w/w). This reduces yeast ethanol inhibition. Food waste consisted of mashed potatoes, sweet corn, and white bread. Without applying vacuum recovery unit, a high concentration of ethanol (144 g/l) could be produced. However, applying the vacuum recovery the ethanol concentration was controlled below 100 g/l in the fermentation broth. Ethanol yield was found to be 358 and 327 g/kg[food waste (dry basis)], for the system with and without applying the vacuum unit, respectively. Ethanol was produced from different plant wastes. Bello et al. (2014) conducted experiments to produce ethanol from banana wastes. They investigated the effect of the fermentation byproducts on membrane performance for the recovery of ethanol by using pervaporation. Results showed that the presence of fermentation byproducts (e.g. lactic acid) increased the hydrophilicity and the separation efficiency. Aramrueang et al. (2017) characterized sugar beet roots, and poor-quality watermelon, and tomato as sources for bioenergy. Results showed that sugar beet roots, tomato, and melons had high soluble sugar contents in a range of

66.8–74.9%, 42.1%, and 53.0–69.0% dry basis, respectively. The theoretical ethanol production potential yield from sugar beet roots was 591 l/[dry ton], when all carbohydrates were used during fermentation, while the ethanol potentials for melon and tomato was in a range of 448–545 l[ethanol]/[dry ton].

### 16.6.3 Physicochemical Processes

Biodiesel is another major biofuel produced and used in several countries. It is mainly produced from vegetable oils and animal fats. Used cooking oils can be a cheap feedstock for the production of biodiesel. They are usually disposed of in the sewage system, which increases the organic loads on treatment facilities and the treatment cost. Production of biodiesel from the used oils could achieve combined economic and environmental benefits (de Araújo et al. 2013). Biodiesel has several advantages such as its renewability and biodegradability, and lower emission of CO<sub>2</sub> than diesel. However, it may increase the emissions of NO<sub>x</sub>. Biodiesel also has minimum emissions of SO<sub>x</sub> because it does not contain sulfur (EPA 2002). Biodiesel (fatty-acid alkyl esters) is mainly produced via transesterification that is a chemical reaction of a lipid with an alcohol in the presence of a catalyst. During the transesterification, an alcohol cleavages esters. Transesterification consists of three consecutive reversible reactions: triglycerides is firstly converted to diglycerides that are then converted to monoglycerides. The latter are finally converted methyl esters and glycerol that is a byproduct. The transesterification reaction can be written as follows:



Several factors affect the biodiesel yield and process economics including reaction temperature and time, alcohol type, alcohol/oil molar ratio, catalyst type, and the contents of water and free fatty acid (FFA) in the oil. Different temperatures in the range of 20–60 °C, molar ratios of alcohol to triglyceride in the range of 3 : 1–30 : 1, and amounts of catalyst ranged from 0.3% to 1% (w/w) have been applied for the transesterification of different oils (Ma and Hanna 1999). The catalyst improves reaction rate and biodiesel yield. The high temperature applied during frying and the water released from fried foods accelerate the hydrolysis of triglycerides to diglycerides, monoglyceride, glycerol, and FFA (Choe and Min 2007). In addition, used frying oils may contain high polymer content, and food residues (i.e. suspended solids). The presence of suspended solids, high FFA and moisture contents in the used cooking oil could reduce biodiesel yield compared with virgin oils, and cause hurdles and increase the processing costs during the separation and purification of biodiesel (Dias et al. 2008; Talebian-Kiakalaieh et al. 2013). Pretreatments could be applied to used cooking oils before transesterification.

to remove water using heat drying, magnesium sulfide (Felizardo et al. 2006); silica gel (Issariyakul et al. 2007); calcium chloride (Predojevic 2008); anhydrous sodium sulphate and remove suspended matter using filtration under vacuum (Dias et al. 2008); and filtration by cellulose filter (Predojevic 2008); remove FFA by neutralization and separate it as soap and remove polymers by adsorption using clay or active charcoal (Cvengroš and Cvengrosova 2004).

Alkalies (e.g. sodium hydroxide, sodium methoxide, potassium hydroxide, potassium methoxide, sodium amide, sodium hydride, potassium amide, and potassium hydride), acids (sulfuric acid, phosphoric acid, hydrochloric acid, or organic sulfonic acid), or enzyme (e.g. immobilized lipase) catalyst are commonly used in the transesterification reactions (Ma and Hanna 1999). When using base catalysts, oils should be anhydrous and contain low concentrations of FFAs to avoid the formation of soap and the reduction of biodiesel yields (Vicente et al. 2004). A successful alkaline transesterification could occur using oil with  $3 \text{ mg[KOH]}/\text{g[oil]}$  that is equivalent to 1.5% FFA (El-Mashad et al. 2008). However, other authors (e.g. Van Gerpen 2005) mentioned that the reaction could still be catalyzed with an alkali catalyst up to 5% FFA, but additional catalyst must be used to compensate for the catalyst lost to soap. The soap formation could decrease the biodiesel yield, complicate the separation of glycerol and purification of biodiesel product, and can increase the costs of the treatment of alkaline wastewater (Anitha and Dawn 2010). Two-step transesterification is usually applied for oils with FFA contents. The first step is an acid-catalyzed pre-treatment to esterify the FFA and therefore reduce the acid value before the second step wherein the triglycerides is transesterified with an alkaline catalyst. However, the acid-catalyzed reaction is much slower than the alkaline-catalyzed reaction (Ramadhas et al. 2005).

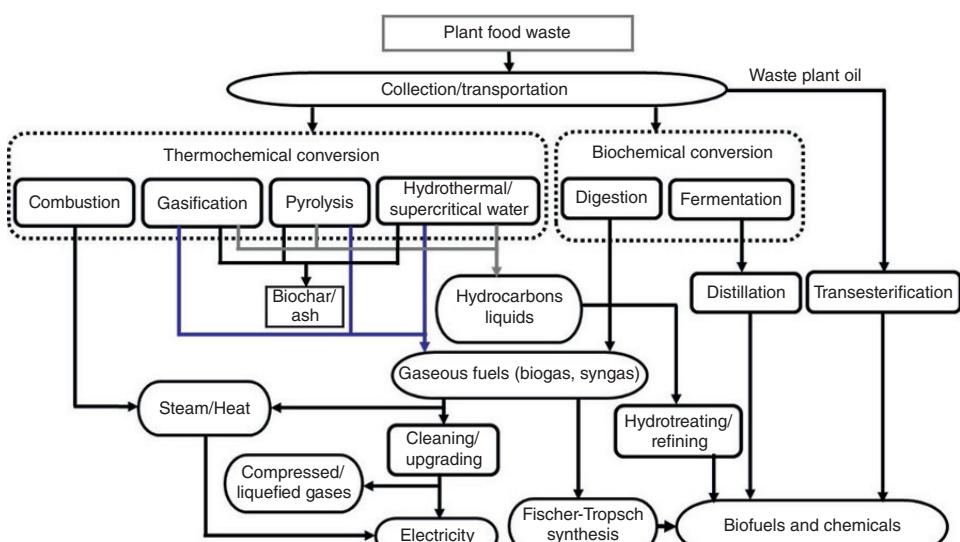
In addition to the homogeneous catalysts, heterogeneous acid and base catalysts have also been used for the production of biodiesel. These catalysts can have a high tolerance to high FFA and water contents and can be reused that could positively affect the economics of the commercial biodiesel production (Talebian-Kiakalaieh et al. 2013). Anitha and Dawn (2010) used Cesium Tungstophosphoric acid as a catalyst (w/w) for the transesterification of waste cooking oil. A maximum yield of 90.5% was obtained after one-hour reaction time using a 0.03% catalyst, and methanol: oil molar ratio of 6.6 : 1. The produced biodiesel had properties that meet the American Society for Testing and Material (ASTM) standard. Enzymatically catalyzed transesterification is more attractive for oils with high moisture contents and high acid values. Enzymatically catalyzed transesterification has more moderate reaction conditions, high recovery yields, and simplified purification processes than chemically catalyzed processes. However, the high costs and stability of enzymes affect their industrial applications. Enzyme immobilization could help in reducing the costs, increasing the stability of enzymes; increasing recovery efficiency; and facilitates the product separation thus enabling the reuse of enzymes (Singh et al. 2015). Wang et al. (2017) used immobilized MAS1 lipase for the transesterification of cooking oils with one-step addition of methanol. High biodiesel yield (Ca 90%) was obtained. This yield was higher than that obtained with immobilized Novozym 435, Lipozyme RM IM, and Lipozyme TL IM. The immobilized MAS1 was resistant to high methanol concentration and good reusability.

Waste cooking oil can be used to produce jet biofuel using meso-Y, SAPO-34, and HY zeolites (Li et al., 2015). Zeolite Meso-Y exhibited a high jet range alkane yield of 40.5% and a low jet range aromatic hydrocarbon yield of 11.3% at 400 °C. The produced jet fuel

with Ni/HY and Ni/SAPO-34 exhibited the lowest and high aromatic hydrocarbon selectivity, respectively. Low and high aromatic hydrocarbon selectivity lead to reduced heating value and poor lubrication, respectively. Martinez-Guerra and Gude (2014) studied the effect of pulse sonication and the type of alcohol (methanol and ethanol) on the transesterification reaction of waste vegetable oil without heating or mechanical mixing. They found that the optimum conditions for transesterification using ethanol or methanol were: alcohol to oil ratio of 9 : 1, catalyst amount of 1% wt, reaction time of 1–2 minutes at a power output rate between 75 and 150 W. The highest yield of biodiesel (>99%) was obtained using ethanol–methanol mixtures (50–50%). The latter was attributed to the combination of ultrasonic effect and the better solubility of ethanol at lower power density and ultrasound intensity. Razaghi et al. (2016) used a combined thermochemical treatment for the hydrolysis of fruits and vegetable waste for the growth of red yeast *Rhodotorula glutinis* for biodiesel production. They also used the residual solids for biogas production. It was observed that there was incomplete carbohydrate utilization due to the limitation of nitrogen. Combined thermo-chemical treatment yielded the highest biomass, total fatty acids, and RS-derived biogas yields. A biomethane potential of 140–160 l/kg[VS of residues] was determined.

#### 16.6.4 Thermochemical Processes

There are several thermochemical technologies that are commonly employed for converting plant food wastes into solid, liquid and gas bioenergy (Figure 16.11). The selection of certain technology depends on the characteristics and availability and continuous supply of the material, desired bioenergy products, easiness, and control of process operation, operation safety and environmental impacts, capital and operation costs, and the



**Figure 16.11** Main technologies for converting plant food waste into bioenergy. Source: Adapted from Turkenburg et al. 2000.

need for downstream processing of products and effluents. Since most of the food plant wastes have low densities and contain high volatile organic matter, faster consumption in the reactor and easy ignition are expected (de Oliveira et al. 2013). Thermochemical processes produce solid, liquid, and gaseous (syngas) products. Gaseous and liquid products can be used for electricity generation after the removal of impurities such as tar, and sulfur-containing and alkali compounds. The produced syngas from biomass gasification can be fermented to produce methanol and higher alcohols or catalytically converted to light diesel (Cantrell et al. 2007). It can also be used to produce alkanes via Fischer Tropsch process using catalysts such as Co, Fe, and Ru-based. The biochar (solid product) can be used as a soil amendment and an energy source. Although the biochar has better characteristics, as a bioenergy source, than its biomass origin, it has a low density that increases the cost of transportation and storage. Therefore, densification of biochar could increase its energy intensity. Bai et al. (2017) investigated the co-pelletizing of wheat straw biochar and peanut shells. The latter was an effective and inexpensive binding agent for high-quality biochar pellets. Optimum pelletizing was obtained with 15% peanut shell and 10% water content. The produced biochar pellets can be used as renewable biofuels. The ash produced can be used as road paving or bulking materials.

Thermochemical processes include direct combustion (i.e. incineration), pyrolysis, gasification, and hydrothermal degradation. Compared with biochemical processes, thermochemical processes have the following advantages (Cantrell et al. 2007): (i) thermochemical reactors are more compact, (ii) processing time is short (in the order of minutes), (iii) the applied high temperatures destroy pathogenic microorganisms and most pharmaceutically active compounds, (iv) these processes are flexible and can simultaneously process several materials such as manure and crop residues, and (v) possibilities of nutrient recovery. On the other hand, thermochemical processes, except for hydrothermal processes, require low moisture (<40%). Therefore, drying process should be applied prior to most of the thermochemical processes. The design of a thermochemical reactor depends on the characteristics of the material and the kinetics material pyrolysis under different pyrolysis conditions.

Combustion (i.e. incineration) is a complete oxidation of organic matter in the presence of air under high temperatures. Its main products are heat and flue gas that consists of carbon dioxide and water vapor. The heat can be used for the production of steam that can be used as a heating medium or can be used for electricity generation. Incineration can reduce the volume and weight of waste by 90% and 70%, respectively; reduce the demand for landfill; and have higher efficiencies of energy recovery than anaerobic digestion and landfill gas. However, incineration can cause air and water pollution (Allobergenova 2006). Flora and Riahi-Nezhad (2006) revealed that direct combustion is the simplest economical thermal technology for energy production from organic matter in large-scale installations. However, the efficiency of incineration is low, and the process is not economically feasible when applied for fruits and vegetable wastes (Allobergenova 2006). This is due to the high moisture contents (>70%).

Pyrolysis and gasification are two main thermochemical processes are carried out at high temperatures and ambient pressure. These processes can be carried out at different temperatures (Table 16.6). While pyrolysis is carried out in the absence of oxygen, gasification occurs at a partial oxidation and atmospheric pressure. Oxygen, air, or steam is usually used as an oxidative agent. At low temperatures 200–300 °C, pyrolysis is called torrefaction, that mainly produces solid product; biochar. The products of pyrolysis are

**Table 16.6** A comparison of different thermochemical processes (Priyadarsan et al. 2004; Roos 2010; Morrin et al. 2012; Tanger et al. 2013; Elliott et al. 2015).

Parameter	Combustion	Gasification	Pyrolysis	Hydrothermal liquefaction and gasification	Supercritical water oxidation and power generation
Desired moisture content of biomass	Preferably 5%	20–30% But common 15%	20–30%	>30%	>30%
Typical temperature range	800–1200 °C	500–1400 °C	350–600 °C	273–342 °C	>342 °C
Pressure	Atmosphere	Atmosphere to 33 bar	Atmosphere to 20 bar	4–22 MPa	>22 MPa
Oxidation agent	Oxygen supplied at greater than stoichiometric	Steam, CO <sub>2</sub> or air; oxygen supplied at less than stoichiometric	Absence of steam or oxygen	Oxygen and water	Oxygen and water
Catalysts	Non	Catalysts (e.g. limestone, olivine, alkali carbonates, Ni-based catalysts) may be used	Catalysts such as zeolite and active metals like, Na, Fe, Ca, and K	Colemanite, sodium perborate monohydrate, disodium octaborate tetrahydrate, and boric acid	Hydrochloric acid, Boron, N, S, P, and C Water may be added
Main products	Heat	Heat and combustible gas	Heat and combustible biooil and gas	Heat and combustible biooil and gas	Heat and combustible biooil and gas

gases (H<sub>2</sub>, CO<sub>2</sub>, CO), liquid (biooil), and solid (biochar). The proportion of the products of the pyrolysis depends on the pyrolysis temperature, heating rate, and residence time. Ali et al. (2017) studied the kinetics of the pyrolysis of coconut shells. The pyrolysis process was kinetically described as four independent reactions that simultaneously occur and matches pseudo-water, pseudo-hemicellulose, pseudo-cellulose, and pseudo-lignin. Apparent activation energy increased with the difficulty in the decomposition of complex components. Pseudo-cellulose and pseudo-hemicellulose degraded faster (five to six times) than lignin. Zabaniotou et al. (2008) studied the batch pyrolysis of sunflower shells at temperatures of 300 to 600 °C at a heating rate of 40 °C/s under atmospheric pressure and helium as sweeping gas. Results showed that the maximum gas yield of around 53% (w.b.) at 500 °C; whereas the maximum gas yield of approximately 21% was obtained at a pyrolysis temperature of 400 °C. Increasing the pyrolysis temperature increases the gas yield and the gas contents in hydrogen and methane. For tomato plant waste, the energy yield in the form of syngas increased from 0.44 to 7.92 MJ/kg of

material by increasing the pyrolysis temperature from 400 to 800 °C, respectively. The energy yield in the form biochar increased from 24.2 to 25.7, respectively (Encinar et al. 2008). Baray Guerrero et al. (2016) performed pyrolysis experiments in a tubular reactor at temperature 300–450 °C at a heating rate of 5–20 °C/min. Results showed that a maximum of 71.5% (w) of non-condensable volatile matter, 25.4% (w) of condensable volatile matter and 3% (w) of residual matter. The produced gas contained 49.8% (v), 26.8% and 23.4% of CO, CO<sub>2</sub>, CH<sub>4</sub>, respectively. The produced gas was used to produce H<sub>2</sub> by the absorption enhanced reforming of methane. A maximum hydrogen yield was obtained at reforming temperature of 715 °C and a steam to methane ratio of 3.5 and 3.5 mol[CaO]/mol[CH<sub>4</sub>]. While the maximum hydrogen purity of 84% was obtained at reforming temperature of 660 °C.

Gasification occurs at temperatures ranging from 800 to 1100 °C. The products of gasification are syngas and biochar byproduct. Oxygen, air, or steam is usually used as an oxidative agent. Syngas is a mixture consisting primarily of H<sub>2</sub>, CO and lesser amounts of CO<sub>2</sub> and CH<sub>4</sub>. Although the long-time application of gasification, it has a few challenges (Pereira et al. 2012): it needs expensive equipment to separate syngas from contaminants; it needs dry materials, and the presence of tar in the syngas that necessitates the application of special equipment for gas treatments. According to de Oliveira et al. (2013), gasification can be a sustainable technology for bioenergy production, reduce GHG emission, and help in regional economic, social, and agricultural development. As reviewed by Hossain et al. (2016), production of hydrogen from oil palm waste via gasification and hydrolysis could be cost-effective in comparison to water electrolysis and coal gasification. Community Power Corp. (CPC) developed a modified downdraft gasifier (Figure 16.12) that was applied to produce power using walnut shells at a farm near Winters, California (Williams and Kaffka 2015). The produced gas passes through bag filters to remove particulate matter and part of condensed tar. The clean gas is used to fuel spark ignition engine-generator sets for power production.

To avoid the costs of the drying of wet biomass materials before combustion, pyrolysis, and gasification, hydrothermal and supercritical water treatments are being applied.



Figure 16.12 Walnut shell gasification system in Winters, CA (Williams and Kaffka 2015).

Hydrothermal treatment of biomass is carried out at temperatures of 250–374 °C and pressures of 4–22 MPa. These conditions are comparable to those of supercritical water processing that is carried out at temperatures and pressures of >647 and >22 MPa (Elliott et al. 2015). Depending on the temperature, hydrothermal processing is divided into (Elliott et al. 2015): hydrothermal carbonization that is occurred at temperatures below 520 K and its main product is hydrochar; hydrothermal liquefaction that is carried out at temperatures in the range 520–647 K, and its products are liquid fuel known as biocrude; and hydrothermal gasification that is carried out at temperatures above 647 K and its main product is a synthetic fuel gas. Hydrothermal carbonization is a thermochemical process to convert moist biomass into carbon-rich char (hydrochar), bioliquid, and gases mainly CO<sub>2</sub>. It is performed under an inert atmosphere and at temperatures of 180–280 °C, pressures slightly higher than water saturation pressure, and reaction time ranging from one minute to several hours with a common residence time of 20 minutes (Toufiq Reza et al. 2014). The produced hydrobiochar can be used as fuels and soil amendments, and the production of supercapacitors, carbon nanospheres, and adsorbents. At short retention times, the bioliquid produced contains potentially toxic substances like phenols, furfurals, and their derivatives. Anaerobic digestion could be a suitable technology to produce biogas from the bioliquid. Nanda et al. (2016) studied the effect of temperature (400–600 °C), biomass to water ratio (1 : 5 and 1 : 10), and reaction time (15–45 minutes) at a pressure range of 23–25 MPa on the hydrothermal of selected plant wastes. Results showed that using K<sub>2</sub>CO<sub>3</sub> (2% wet weight), coconut shells achieved the highest hydrogen yield of 4.8 mmol/g and total gas yield of 15 mmol g<sup>-1</sup> at hydrothermal temperature of 600 °C, a reaction time of 45 minutes and biomass to water ratio of 1 : 10. Pereira et al. (2012) revealed that supercritical water gasification is a promising technology for the production of H<sub>2</sub> and methane. Supercritical water gasification occurs at temperatures above 374 °C and pressures higher than 2.2 MPa. Madenoğlu et al. (2011) applied a supercritical water gasification at 600 °C and 35 MPa for cauliflower residue, acorn, tomatoes residue, extracted acorn, and hazelnut shell using K<sub>2</sub>CO<sub>3</sub> and Trona (Na<sub>2</sub>CO<sub>3</sub>·NaHCO<sub>3</sub>·2H<sub>2</sub>O) as catalysts. Both catalysts increased hydrogen yields and enhanced carbon gasification efficiencies. A comparison of different thermochemical processes is shown in Table 16.6.

## 16.7 Conclusions

Food waste is produced through all steps of food production chain. It can cause negative environmental impacts if it is not well managed. Plant food waste can be produced as individual streams (e.g. wastes from fields and food processors) or mixed with other wastes such as MSW. Field residues could contribute to the increase in food sustainability if they are well managed and reused as a feedstock for bioenergy production. In many countries, the common disposal methods for food wastes are landfilling or composting and for field residues are either burning or application in the fields. Food waste can be a good feedstock for the production of bioenergy. The composition of food waste significantly varies from the starchy, lignocellulosic, and fatty stream. The collection of food wastes depends on their characteristics. Storage of plant food wastes is important to assure a year-round supply of these materials that are seasonally produced. Controlled

storage of biomass materials is important to prevent fermentation and self-ignition. While dried materials are easily transferable and storable as bales, wet materials can be stored by ensiling.

Several technologies can be used for the production of bioenergy from plant food wastes. The selection of a technology depends on the characteristics of feedstocks, including anaerobic digestion for the production of biogas from wastes especially with high moisture contents; transesterification for biodiesel production from used cooking oils; fermentation for the production of alcohols from carbohydrate-rich wastes; combustion, pyrolysis, and gasification for the production of syngas, biochar and biooils from feedstocks with low moisture contents; and hydrothermal processes for the production of syngas, biochar, and biooils from feedstocks with high moisture contents. Many waste to energy conversion technologies are available for commercial application. Favorable waste management and renewable energy policies and markets as well as government financial assistance are crucial for wide adoption of these technologies. Often integrated systems with multiple products are required for achieving favorable economics. More demonstration and business case studies are needed to show the technological and financial information needed to assist with the project development. Continuing advances are also needed through research and development efforts to improve these technologies with respect to increasing the efficiencies and lowering the costs of waste to energy conversion technologies.

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## 17

# Biological and Microbial Technologies for the Transformation of Fruits and Vegetable Wastes

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## 17.1 Introduction

Recent statistical data have reported the yearly production of fruits and vegetables to be over 675 million metric and 1 billion metric tonnes respectively ([www.statista.com](http://www.statista.com)). Over 50% of the world's vegetables which is worth above 25 billion US dollars is produced by China. The total contribution of India, USA, Turkey, and Iran, who follow immediately after China is a little about above 18% (FAOSTAT 2015). Fruit and vegetables are utilized either fresh or processed. Processed alternatives include canned, bottled preserve, frozen, dried products. The global market share for these processed products are as follows: canned fruits and vegetables (40%), frozen fruits and vegetables (36%), juices, pre-cut vegetables and ready-to-eat salads (14%), and dried and dehydrated fruits and vegetables (10%) (IBISWORLD 2016). There is the perception that fresh fruits and vegetables are more nutritious and healthier than processed forms. The processed fruits and vegetable market are much stabilized in the developed than developing countries. Consumers in the developing countries prefer fresh produce, which is usually cheaper than the processed forms. The world leading consumers of fruits and vegetable products are North America, Europe, and Asia (IBISWORLD 2016). The fruit and vegetable industry in the developing countries is still developing and there is the need for expansion.

Approximately a third of the fruits and vegetables produced never get consumed. They all go waste along the food production and consumption system (Gustavsson et al. 2011). In the low-income countries, most of the waste are caused by poor storage facilities, infrastructure and transportation, inadequate market facilities and poor packaging. In the high-income industrialized countries, factors such as quality standards which are focused on visual appeal, unit processes aimed at obtaining perfectly shaped products (e.g. trimming of potatoes), poor temperature conditions during retail, overemphasis on best-before-dates and use-by dates, leftovers, militate food waste (Gustavsson et al. 2011). Disposal of this huge amount of food waste is an environmental threat. A lot

of methane gasses and carbon dioxide are exposed to the environment during the decomposition of these crops. The methane gas is even more harmful than carbon dioxide in the production of heat (Adhikari et al. 2006; Papargyropoulou et al. 2014). Standard practice in waste management requires the use of the waste hierarchy, of which waste disposal is the least favorable option and should be the last choice after all other options have been exploited (Papargyropoulou et al. 2014). In the Europe Waste Framework Directive 2008/98/EC, requirements are that generation of the waste must be prevented as much as possible. Otherwise, the waste generator must put steps in place to reuse the waste generated, recycle, or recover (Papargyropoulou et al. 2014; Sakai et al. 2011; Vandermeersch et al. 2014). Using these standard practices to govern the management of waste in the fruit and vegetable industry, it becomes imperative that harvesting and processing byproducts that may not be fit for human consumption but contain certain vital substances must be salvaged to promote good health and protect the environment, thereby enhancing the utilization of the global fruits and vegetables to the maximum without necessarily increasing production. Vandermeersch et al. (2014) have outlined a detailed food waste management hierarchy proposed by the Public Flemish Waste Management Company (from Belgium) (Table 17.1).

Fruit and vegetable processing and preservation can be considered as one of the largest industrial sectors in the food processing industry, which is not without waste (Di Donato et al. 2011). Fruits and vegetables are seasonal crops, but their demand by consumers is all year round. This consumer need is met by selling fresh produce when the crop is in season and the excess is processed for the lean season (Chandrasekaran 2012). Consumer quest for healthy but convenient foods requires that food processor comes out with innovative products very frequently. In food production, raw materials entering the food production process exists either as the desired product or product specific waste. This results in an increase in the already existing waste (Chandrasekaran 2012; Ravindran and Jaiswal 2016; Russ and Schnappinger 2007). Fruits and vegetables are mainly processed by canning, freezing, juicing, pre-cutting, or drying. These processing methods involve unit operations such as

**Table 17.1** Food waste heirarchy.

Step	Action
1	Avoid waste creation
2	Incorporation of waste into human nutrition
3	Waste transformation for human utilization
4	Feeding livestock
5	Industrial utilization (in a bio-based economy)
6	Process into fertilizer (by anaerobic digestion or composting)
7	Energy generation
8	Incineration
9	Landfill

Source: (Vandermeersch et al. 2014).

peeling, cutting, crushing, and expressing and are intended to separate the unwanted parts (residue) of the fruits and vegetables from the parts of interest. Depending on the fruits and vegetables, and the product of interest, the processing waste may contain the stems, leaves, stalk, peels, pomace, seeds, cores, rinds, unused pulp, skins, pits, pulp, twigs, and spoiled fruits and vegetables which constitutes about 30–50% of the original material (Ajila et al. 2007; Di Donato et al. 2011).

The European legislations (Commission Regulations 442/1975/EEC; 689/1991/EEC), define food waste as high organic load residues derived usually during raw materials processing of foodstuff. The processing waste is usually the non-product flow of raw materials which can be part of the raw materials or processing intermediate. The waste generated may be due to a lack of conformance to processing specifications which may result in finished products being part of waste (Chandrasekaran 2012). The waste generated may be solid or liquid waste depending on the unit processes involved in the production: i.e. harvesting and grading; cleaning; sorting; blanching; washing and cooling; packaging; clean up; juicing; cutting, etc. These waste materials may not be of direct interest to the processor but can be very useful in other applications. In the food industry, waste prevention is achieved by the application of more efficient production technique, which involves the efficient use of raw materials, very good process design and reduces operational cost (Darlington et al. 2009).

The food processing industry is considering environmental issue as an essential part of their corporate image by adopting the ISO standardized Life Cycle Assessment (LCA) methods. This is to ensure the production of food product in a safe and environmentally friendly way (Chandrasekaran 2012). By the LCA methods, manufacturers are required to thoroughly utilize their raw materials. Hence in recent times, valuable chemicals and metabolites are recovered from food waste through chemical and biotechnological processes. Factors necessitating valorization of food processing byproducts which includes fruits and vegetables are summarized in Table 17.2. There is the prospect of using biological and microbial technologies to harness the tremendous organic resources in fruit and vegetable waste. As such, these waste products will become a valuable natural resource for wealth creation. This chapter seeks to examine the researched biological and microbial technologies with the potential for the transformation of waste from fruits and vegetables into high value products namely food, feed, fuel, and diverse of chemicals for pharmaceutical and nutraceutical products.

**Table 17.2** Factors necessitating valorization of food processing byproducts.

S/N	Factor
1	Population growth resulting in increase in consumers
2	Decline in agricultural Production
3	Increase in the cost of productivity
4	Underutilization of available resources

Source: (Chandrasekaran 2012).

## 17.2 Sources and Components of Some Global Fruits and Vegetable Waste

Fruit and vegetable waste constitute about 20–50% of municipal waste (Kosseva 2013). The main components of fruit and vegetable wastes are complex and simple carbohydrates, pigments, flavor compounds and antioxidants. The moisture content of fruit and vegetable waste is very high, constituting about 80–90%, with solids ranging from 8% to 18%. The main solid fractions are sugars, hemicellulose, cellulose, pectin, lignin, flavor-active compounds, and natural pigments. Fruits and vegetables pigments include chlorophyll, carotenoids, anthocyanins, and betalains. The key minerals found in fruits and vegetables are potassium, phosphorus, calcium, and iron. Vitamins such as thiamine, riboflavin, vitamin B6, niacin, folate, vitamin A and E are also predominant. Major sources and components of some fruit and vegetable residue are presented in Tables 17.3 and 17.4.

Potatoes are processed mainly into chips or French fries. Their main processing solid wastes are the peels and/or cull potatoes (Schieber et al. 2001). Potato peels are rich in phenols and the extraction of these phenols have been investigated (Oreopoulou and Tzia 2007). The citrus fruit is consumed either fresh or in the form of juice. The byproducts obtained after juice processing including peel, pulp, membranes, cores, and seeds, all of which account for 50% of fresh fruit weight. The main composition of citrus fruits comprises nitrogen, lipid, sugar, acid, insoluble carbohydrate, enzyme, flavonoid, bitter principle, peel oil, volatile constituent, pigment, vitamins, and minerals (Dijlas et al. 2009). The amount of these compounds present are affected by maturity, variety, climate, and cultivation conditions. The pomace and peel of citrus fruits are rich in pectin, flavonoids, essential oils, and carotenoids (Chedea et al. 2010; Di Donato et al. 2011; Farhat et al. 2011; Kim et al. 2004; Masmoudi et al. 2008; Sharma et al. 2017).

Apple pomace obtained after apple juice processing is made up of water, insoluble carbohydrate (cellulose, hemicellulose, and lignin), simple sugar (fructose, glucose, and sucrose), mineral, protein, vitamin, and polyphenol compound (Mamma et al. 2009; Vendruscolo et al. 2008). Main polyphenols present in apple peel are catechin, epicatechin, hydroxycinnamate, phloretin glycoside, quercetin glycoside, procyanidin, chlorogenic acid, phloridzin, and 3-hydroxy-phloridzin, (Mamma et al. 2009).

The pomace and stone are the main waste product of peach and apricot. The pomace is rich in protein, pectin, and polyphenol, with good gelling property due to a high degree of methoxylation in pectin (Arvanitoyannis 2008).

Grape pomace, obtained after wine making, contains seed, stalk, and skin, all of which account for 20% of the total grape weight. The chemical composition of grape pomace includes alcohol, acid, aldehyde, ester, pectin, polyphenol, minerals, and sugar (Dijlas et al. 2009).

The main products from mango are juice, puree, nectar, leather, pickle, and canned slices. The main mango wastes are peel and seed. The mango seed is a rich source of carbohydrate. It also contains a significant amount of protein, fat, and fiber (Zein et al. 2005). There is also the presence of functional components in mango peels such as polyphenol, carotenoid, enzyme, dietary fiber, and vitamins E and C (Ajila et al. 2007).

The major byproduct obtained during processing of pineapple juice is pulp, containing sucrose, starch, and hemicellulose. The main wastes from tomato are skin, pulp, seed, and plant (Encinar et al. 2008), which are generated during the processing of the vegetable into juice, soup, puree, ketchup, and paste. Dried tomato pomace contains soluble

**Table 17.3** Sources of some fruit and vegetable waste and their estimated percentage waste.

Fruit/vegetable	Waste	% Waste
Apple	Pomace, leaves, peels, cores, defective culls	25
Banana	peels	30–35
Berries	Defective berries, stem, leaves	
Cabbage	Cores, defective heads, defective leaves	
Carrots	Peels, cull	12
Cherries	Leaves, culls, undersized pits,	
Citrus	Culls, leaves, peel, and seeds	50
Cucumber	Undersized, cull	
Grapes	Stem waste, pomace, cull	20
Guava		10
Mango	Peels, stone	50
Spinach and other green leafy vegetables	Loose leaves, weeds, off grade material	
Beans and peas	Leaves, stem, pods, off grade material	40
Onions	Tops, roots, skin, undersized, culls, loose skin, onion with root hairs	10
Peaches	Leaves, undersized pits, culls and trim waste, small pieces	
Pears	Defective pears, leaves, peels, core, cull, trim waste, small pieces	
Peppers	Culls, pomace, small pieces	
Pineapples	Peels, cores, and rejected fruits	33
Potatoes	Peeled waste and culls	15–40
Squash	Culls and undersize, stem, leaves, end cuts, off grade materials	
Sweet potato	Peel waste, trimming waste,	
Tomato	Defective tomato, pomace	20–40

Sources: (Ajila et al. 2012; Chandrasekaran 2012; Schieber et al. 2001).

sugars such as fructose, glucose, and sucrose (Del Valle et al. 2006). The skin of tomato is also very rich in lycopene as the lycopene content in tomato peel is fivefold higher than that in tomato pulp,

Carrot waste, also called carrot residue is made up of the pomace and the peel. It is obtained mainly from carrot juice processing (Chantaro et al. 2008). Carrot residue accounts for 12% of fresh carrot weight. Carrot residue contains a large amount of fiber, with cellulose making up the largest portion (51.6%), followed by lignin (32.2%), hemicellulose (12.3%), and pectin (3.88%). It also contains some amounts of uronic acid and sugar (Nasernejad et al. 2005; Nawirska and Kwasniewska 2005; Singh et al. 2006). Carotenoids are also present in the carrot peel as in tomato skin and pomace (Chantaro et al. 2008; Strati and Oreopoulou 2011).

**Table 17.4** Potential byproducts of some fruit and vegetable waste.

Fruit/vegetable	Byproducts
Apple	Pectin, polyphenols, catechins, hydroxycinnamates, phloretin glycosides, quercetin glycosides, and procyanidins
Banana	Anthocyanin pigments: delphinidin, cyanidin, pelargonidin, peonidin, petunidin, and malvidin Carotenoids: xanthophylls
Carrot	carotenes, fiber, uronic acids, and neutral sugars
Citrus fruits	Fiber pectins, hesperidin, narirutin, naringin, and eriocitrin
Grape	Ethanol, tartarates, citric acid, grape seed oil, hydrocolloids, dietary fiber, anthocyanins, catechins, flavonol glycosides, phenolic acids, alcohols, and stilbenes
Guava	Guava seed oil, antioxidant dietary fiber
Mango	Mango seed kernel fat, gallic ellagic acids, and gallates gallotannins and condensed tannin-related polyphenols, dietary fiber containing
Onion	Fiber, quercetin glycosides, fructans, and fructooligosaccharides
Papaya	Papain, pectin, seed meal (high in crude fiber and protein)
Peach and Apricot	pectin, persipan
Pineapple	sucrose, starch, and hemicellulose, bromelain
Potato	phenolic acids: chlorogenic, gallic, protocatechuic, and caffeic acids
Red beet	Betalains, betacyanins, and betaxanthins
Tomato	Tomato seed oil, lycopene
Red beet	Betalains, betacyanins, and betaxanthins

Source: (Schieber et al. 2001).

### 17.3 Biological and Microbial Processes Used on Fruit and Vegetable Waste

There are various biological and microbial processes used in the transformation of fruit and vegetable waste into valuable products. These processes include fermentation, the use of enzymes and a biorefinery approach. Microbial action or enzymatic processing is often needed for converting byproducts of food processing to valuable products. The components in the waste stream can be directly converted into the desirable product by enzyme action alone, or, a combination of physicochemical or enzymatic preprocesses which is followed by microbial fermentation to produce the desired end product. The latter process is used for wastes containing polymeric compounds such as starch, cellulose hemicellulose, and lignin.

#### 17.3.1 Fermentation

Fermentation is described as the process that involves the mass culturing of microorganisms to manufacture products (Chandrasekaran 2012). Microorganisms that are used in

food fermentations belong to the Generally Recognized as Safe (GRAS) category. In industrial biotechnology production, the types of fermentation processes employed are submerged fermentation (SmF) and solid state fermentation (SSF) (Couto et al. 2006). In general, industrial fermentation processes require sugar or starch (hydrolyzed into fermentable sugars) substrate. Even though fruit and vegetable waste may contain some sugars, there is also substantial amount of lignocellulosic matrix. This will require conversion either by mechanical, chemical, physical, and enzymatic pre-treatments before microbial fermentation to improve yield of the product of interest (Couto et al. 2006). With the emergence of SSF technology, lignocellulose waste are now ready substrate for the support of microbial growth and production (Robinson et al. 2001). The solid and liquid waste generated from the processing of fruits and vegetables contain vital nutrients making them suitable substrate for the growth of microorganism i.e. bacteria, yeast, and mold (Nigam and Pandey 2009; Thassitou and Arvanitoyannis 2001).

### 17.3.1.1 Solid State Fermentation (SSF)

This type of fermentation is a downstream process, and it is very simple and easy to perform. According to Pandey et al. (2000), SSF is the fermentation technique which allows the growth of microorganisms on a moist solid substrate. The moisture in the substrate is expected to be bound to solids present and not free flowing. The SSF has been used in the preparation of most fermented foods such as corn dough, *agbelima* (cassava dough), fermented sorghum, fermented millet, dairy products, soya sauce, and tempeh (Couto 2008).

There are two different categories of substrates used for SSF; inert (synthetic materials) and non-inert (organic materials). The later can also be referred to as support substrate because it provides the microorganism the necessary nutrients to support their existence and multiplication. However, inert substrates are merely surfaces for attachment (Pandey et al. 2000). Food waste is a potential organic material to be utilized as non-inert substrate for SSF. The advantage in utilizing this food waste is that, more useful materials are produced from the raw materials, thereby, reducing the total production cost which leads to maximized profit (Table 17.5). During SSF process, a more consistent product is produced, with lower energy requirements. SSF could be carried out in smaller fermenters and the polluting effluents are in smaller volumes, compared to SmF (Ajila et al. 2012). The choice of a substrate during SSF is influenced by the particles size, chemical (nutritional) composition, availability, and cost of the material.

#### 17.3.1.1.1 Particle Size

A substrate with small particle size provides a larger surface area for the growth of the microorganism. However, when the substrate particle size is too small it also impedes the

**Table 17.5** Advantages SSF over SmF.

1	Require lower energy
2	There is a lower risk of contamination
3	Very simple machinery are required with simple control systems
4	Requires small fermenter since there is no free water present

Source: (Robinson et al. 2001).

microbial growth. This is because there is the formation of agglomerates which may inhibit microbial respiration due to poor aeration, retarding their growth (Pandey et al. 2000). Alternatively, when the substrate particle size is large, there turn to be large interparticle space, allowing for aeration, nonetheless, there is limited surface for microbial attachment (Pandey et al. 2000). A proper balance in the particle size of the substrate will optimize the growth of the microbes.

#### **17.3.1.1.2 Chemical (Nutritional) Composition**

Fruit and vegetable waste are characterized by their high nutritional contents. The high moisture contents present in this waste accelerates decomposition upon accumulation, therefore conducive for the multiplication of microorganisms including disease causing ones (Ravindran and Jaiswal 2016). Fruit and vegetable wastes are rich in simple and complex carbohydrates and other bioactive compounds necessary for the growth of microorganisms. The relatively high sugar content of the substrate supports fungal growth. Different species of fungi have been used on different fruit and vegetable waste in order to produce high value biological products such as color and flavor compounds, organic acids, enzymes, biodegradable plastics, nanoparticles, etc.

#### **17.3.1.1.3 Availability and Cost**

According to Ravindran and Jaiswal (2016), about 90 million tonnes of food waste are generated annually from food manufacturing sector. A significant amount (20–50%) of this waste is from fruits and vegetables (Kosseva 2013), which pose a threat to the environment. There is abundant fruit and vegetable waste which is generated from the farm (harvesting and grading), through processing (cleaning, blanching, washing, cooling, cutting, juicing, packaging, etc.) retail and consumption. The increase in food product development to provide consumers with diverse food products to meet their demand for convenience, have also resulted in the increase in processing waste. Thus, food processing waste including fruit and vegetable waste readily available and very cheap.

### **17.3.2 Applications of SSF to Fruit and Vegetable Waste**

Application of SSF to fruit and vegetable waste for production of valuable products are summarized below. Valuable products of fermentation derived from fruit and vegetable waste are natural flavors, organic acids, polymer, enzymes, antibiotics.

#### **17.3.2.1 Natural Flavours**

Natural flavors are aromatic chemical substances that are obtained from feedstock of plant or animal sources (Couto et al. 2006; Nigam and Pandey 2009). These compounds can be synthesized physically as well as enzymatically and microbiologically. The conventional extraction of natural aroma compounds from plants are tedious and costly. The production of natural aroma compounds by fermentation or bioconversion using microorganisms is an economic alternative (Daigle et al. 1999). It will also satisfy the consumer demand for natural products additives (Longo and Sanromán 2006). Both SmF and SSF method have been used in the production of natural flavors. However, the SSF approached is considered to a cheaper alternative to SmF. These simple biotechnology procedures afford manufacturers to produce less expensive natural food additives for today's, knowledgeable and sophisticated consumer.

Aromatic compounds with strong fruity aroma have been produced by *Ceratocystis fimbriata* using apple pomace as substrate. Other substrates such as amaranth, cassava bagasse, and soy bean have been exploited. But the intensity of the aroma produced is dependent on the substrate used (Bramorski et al. 1998; Panesar and Marwaha 2013; Ray et al. 2008). Other aromatic compounds with some alcoholic note have been produced by *Rhizopus* strains using apple pomace substrate (Christen et al. 2000).

### 17.3.2.2 Organic Acids

Citric acid is an important commercial product. Globally, it is the most produced organic acid solely by microbial fermentation. Over 800 000 tonnes are produced annually with an estimated 5% increase (Kamzolova et al. 2005; Nigam and Pandey 2009). It is widely used in the food, pharmaceutical and beverage industries as an acidifier and flavor enhancer (Couto 2008) Citric acid has been produced from apple pomace (Shojaosadati and Babaeipour 2002) and pineapple (Imandi et al. 2008; Kumar et al. 2003) using *Aspergillus niger* by SSF process, with a recorded yield of at least 50% of sugar consumed in the substrate. Imandi et al. (2008) also produced citric acid using *Yarrowia lipolytica* with pineapple waste as the substrate.

Other organic acids produced solely by microbial fermentation are acetic acid, and tartric acid (Papargyropoulou et al. 2014). Apple pomace has also been used in the production of essential fatty acids such as  $\gamma$ -linolenic acid (GLA) using *Thamnidium elegans*. The total yield recorded was up to 3.50 g kg<sup>-1</sup> of the moist substrate (Stredansky et al. 1999).

Citric acid and tartrates have been recovered from grape pomace. Cassava bagasse and sugarcane bagasse have been utilized to produce L(+) lactic acid by *Lactobacillus delbrueckii* under SSF conditions. The process was able to convert almost the entire amount of sugars present (99%) into lactic acid. Lactic acid is a very important food additive used as both an acidulant and a preservative (Couto 2008 Table 17.6).

### 17.3.2.3 Heteropolysaccharides

Through SSF, polysaccharides such as xanthan gum and heteropolysaccharide-7 have been produced (Jin et al. 2002; Stredansky and Conti 1999). These long chain

**Table 17.6** Use of fruit and vegetables as substrate for the production of certain organic acids.

Fruit/vegetable	Microorganism	Fermentation product
Sweet potato	<i>Aspergillus niger</i>	Citric acid
Pineapple waste	<i>Aspergillus Foetidus</i> <i>Aspergillus niger</i>	Citric acid Citric acid
Carrot-processing waste	<i>Aspergillus niger</i> <i>Rhizopus Sp.</i>	Citric acid Lactic acid
Sweet potato	<i>Rhizopus Sp.</i> <i>Aspergillus niger</i>	Oxalic acid
Cassava	<i>Aspergillus niger</i> <i>Aspergillus niger</i> <i>Streptococcus thermophilus</i>	Citric acid Lactic acid Furmaric acid
Kiwifruit peel	<i>Aspergillus niger</i> NRRL567	Citric acid

Source: (Pandey et al. 2001).

polysaccharides are very useful in food product development. They contribute useful properties as emulsification, stabilization, suspension of particulates, control of crystallization, inhibition of syneresis, encapsulation, and film formation to formulated products. However, factors limiting their production include their high cost of recovery and the use of nonfood bacteria for their production (De Vuyst and Degeest 1999). There is the potential of using apple pomace in the production of pectin. Apple pectin has better gelling properties than citrus pectin. However, mango pectin has also been found to have superior properties than apple pectin. Dietary fiber from grape pomace with antioxidant activity has been developed similar to that of mango dietary fiber (Larrauri et al. 1996; Saura-Calixto 1998) due to the significant amount of antioxidants found in the pomace. However, the antioxidant activity is reduced by exposure to high temperature during drying as a result of reduction in the extractable polyphenol levels (Larrauri et al. 1997). Guava waste which includes the peels and some pulp and lime peels are good sources of antioxidant dietary fiber and fiber pectins respectively (Jimenez-Escriv et al. 2001; Schieber et al. 2001).

#### 17.3.2.4 Biopolymers and Other Useful Substances

Biopolymers are long chain macromolecules of importance in food processing. Examples of biopolymers with their respective monomers are starch (sugars), protein and peptides (amino acids), DNA and RNA (nucleic acid) (De Vuyst and Degeest 1999). The studies of Streit et al. (2009) indicated that fungal chitosan can be extracted from *Gongronella butleri* with biomass ( $0.1783 \text{ g g}^{-1}$  of apple pomace) for a medium supplemented with  $40 \text{ g l}^{-1}$  of reducing sugars and  $2.5 \text{ g l}^{-1}$  of sodium nitrate. Chitosan produced by *G. butleri* CCT 4274 on the watery extract of apple pomace have been reported (Panesar and Marwaha 2013; Vendruscolo et al. 2008).

Other useful products obtained from fruit and vegetable pomace include mushroom, baker's yeast oleaginous microorganisms, etc. The most attractive material for mushroom production is lignocellulose materials mostly obtained from agricultural residue (Vendruscolo et al. 2008). When different types of mushrooms were produced using apple pomace and sawdust individually and as mixtures, the yield of mushroom from apple pomace alone was found to be higher than that of sawdust alone (Worrall and Yang 1992). However, the addition of ash to apple pomace improved the yield.

The production of baker's yeast using agroindustrial residues as a substrate has become an interesting alternative for reducing production costs. Bhushan and Joshi (2006) used apple pomace extract to produce baker's yeast with equivalent dough-rising capacity as commercial baker's yeast. The use of apple pomace extract as a substrate can be considered as a fitting alternative to molasses, traditionally used as a carbon source for baker's yeast production.

Oleaginous microorganisms are microorganisms capable of producing oil of up to 20% of its biomass weight and above (Thevenieau and Nicaud 2013). The oil produced by these microorganisms are called microbial or single cell oil. Oleaginous microorganisms such as bacteria, yeast, mold, and microalgae are capable of producing oil comparable to that produced from oilseeds (Thevenieau and Nicaud 2013). Among the factors needed to produce microbial oil, the cost of raw material, i.e. suitable substrate for the growth of this microbe is of great importance. Oleaginous yeast, for example, is able to grow on varied carbon sources of which fruit and vegetable residue is key. Fruit and vegetable residues are good carbon sources with lignocellulosic or sugar-based biomasses will form

cheap substrates for yeast and fungi cultivation. Thereby solving the limitation of the application of oleaginous oils for high-value products. The best-known oleaginous yeasts are typically found in the genera *Candida*, *Cryptococcus*, *Lipomyces*, *Rhodosporidium*, *Rhodotorula*, *Rhizopus*, *Trichosporon*, and *Yarrowia*. Generally, yeast microbial oils have higher yields than other oligoenoious microorganisms. Fungi species, such as *Aspergillus terreus*, *Claviceps purpurea*, *Tolypocladium longisegmentum*, *Mortierella alpina*, *Mortierella isabellina*, also have lipid reserve that can be utilized. *Chlorophyta* and *Bacillariophyceae* are microalgae also with high oil contents. Lipids from oleaginous fungi are comparable to cocoa butter, therefore they are potential substitutes. Cocoa butter has a high saturated fatty acid content of up to 60%; of this 35% is stearic acid and 25% is palmitic acid (Dyal and Narine 2005).

#### 17.3.2.5 Enzymes

Industrial enzymes have been among most commercially successful products produced by SSF (Thomas et al. 2013). Couto (2008) and Pandey et al. 2000 are of the opinion that enzymes produced by SSF are more active and potent than those produce through SmF. Moreover, the production cost of enzymes using SSF is by far, cheaper than using SmF. Some of these enzymes suitable for the hydrolysis of the lignocellulolitic components of fruit and vegetable waste. The presence of these enzymes in animals feed are useful for proper digestion of the feed and bioavailability of the nutrients.

Amylase have been from apple pomace and banana waste by *Aspergillus oryzae* using SSF (Nigam and Singh 1994). This enzyme has also been produced from potato peel using *Bacillus licheniformis* and *Bacillus subtilis* also under SSF (Shukla and Kar 2006).

Pectin hydrolyzing enzymes, pectinase, which include pectin esterase, polygalacturonase, pectate lyase, and pectin lyase, occur as structural polysaccharides in the middle lamella and primary-cell walls of higher plants. The application of theses enzymes are for clarification of juices during the processing of fruit juices and also as feed additives to eliminate all the antinutritional effect of pectin and cellulose, enhances the viscosity of plant based products, and improves digestibility in animals (Ajila et al. 2012). Pectinases production by *A. niger* strains using different agro-industrial residues such as, cranberry and strawberry pomace, citrus peel, orange bagasse, sugarcane bagasse, and wheat bran as substrates have been reported (Dhillon et al. 2004; Zheng and Shetty 2000).

#### 17.3.2.6 Animal Feed

In developing countries where protein supplementation of food for both human and animal usage is expensive, SSF has been proposed as a good alternative for improving the nutritional value of animal feed (Robinson and Nigam 2003). During SSF of agricultural waste, there is the overall increase in protein, total fat, and fatty acids. The bioavailability of nutrients increases, and antinutritional factors, such as phytic acid, polyphenols, and tannins decrease, which generally enhance the nutritional quality of the feed (Ajila et al. 2012).

A number of researchers have used apple pomace to produce protein rich feed. The fermentation process results in enzymatic breakdown of the lignocellulosic matter present in the pomace into hexoses and pentoses for subsequent utilisation. Different fungi used for the enrichment of apple pomace via fermentation are *Candida utilis* and *A. niger* (Bhalla and Joshi 1994); *Candida utilis* and *Kloeckera apiculata* (Rahmat et al. 1995) *C. utilis* and *Pleurotus ostreatus* (Villas-Bôas et al. 2003). SSF have also been used

to enrich waste from orange, potato pulp, and Chinese cabbage for utilization as animal feed (Ajila et al. 2012).

### 17.3.3 Enzymatic Pretreatment of Fruit and Vegetable Waste

Enzymes are biological catalysts, available from plant, animal, and microbial sources, and play key roles in metabolism, and biosynthesis of macro molecules (Chandrasekaran 2012). Enzymes are very important in the food industry operations. Most industrial applications now employ the use of microbial enzymes due to the ease with which they can be obtained and their stability. Conventionally, commercial-scale production of microbial metabolites (including enzymes) carried out by SmF allows better process control and reduced risk of contamination. SSF, are used for the large-scale manufacture of microbial enzymes. Especially the low-value high volume enzymes such as amylases and cellulases.

Fruit and vegetable waste which are high in lignin and cellulose may require some level of pretreatment before further processing to transform them into very valuable product. One biological pretreatment method employed is the use of enzymes. Patle and Lal (2007) used different pretreatment methods such as acid, alkaline, and enzymatic hydrolysis to hydrolyze different fruit and vegetable waste, and the hydrolysates subsequently use to produced alcohol by fermentation. They observed that enzymatic hydrolysis yielded maximum reducing sugars from fruit and vegetable wastes, which implies more alcohol yield. Pectin from citrus processing waste has been extracted through enzymatic treatment (Donaghy and McKay 1994; Tripodo et al. 2004).

Even though the cost of enzyme is very high and may not be economical, the recent use of fruit and vegetable waste as substrates for SSF, has led to producing these enzymes at relatives lower cost. However, the industrial use of these enzymes will be more cost effective if these enzymes are immobilized for reuse. Trevan (1980) describes immobilization as the association of enzymes with a distinct phase that allows exchange with, but is separated from, the bulk phase in which the substrate, effectors, or inhibitor molecules are dispersed and monitored. Chandrasekaran (2012) categorizes the common methods for immobilization of enzymes into physical methods (which include adsorption, entrapment, and encapsulation) and chemical methods (include covalent bonding and cross-linking). Even though all these methods have the advantage and disadvantages, they are used solely for the purpose of retrieving enzymes used in industrial process for reuse. Some products produced from waste products by the help of enzymes are discussed below.

#### 17.3.3.1 Biofuel Production

Production of ethanol and other biofuels from sugars and starches from sugarcane, cereals, and other food sources of sugar and starch create an unhealthy competition between the food and fuel industry. Thus, production of alcohol from food waste is a cheap alternative with advantages to the environment (Mtui 2009).

It has been reported that fermentation of food waste alone results in lower yield of alcohol as compared to pretreatments which hydrolyzes the waste into simple sugars before fermentation. A simultaneous process of saccharification and fermentation have been reported to increase yield significantly (Mtui 2009). One biological pretreatment employed is the use of enzymes. The type of enzymes used are dependent on the type

**Table 17.7** Microorganism responsible for the production of various biofuels.

Microorganism	Biofuel	Reference(s)
Native or engineered <i>Saccharomyces</i> , <i>Zymomonas</i> , or <i>E. coli</i>	Bioethanol	Chandrasekaran (2012)
<i>Clostridium</i> strains	Biobutanol	Khedkar et al. (2017) Nimbalkar et al. (2018) Procentese et al. (2017)
<i>Clostridium pasteurianum</i>	Biohydrogen	Chandrasekaran (2012)

of waste being used. Production of biofuel such as bioethanol, biobutanol, biohydrogen requires simple sugars such as glucose and sucrose which are present in fruit and vegetable waste either freely or in complexes. Enzymes such as amylases, cellulases and hemicellulases are used to hydrolyze starch, cellulose and hemicellulose respectively. Lignin-hydrolyzing enzymes such as lignin peroxidases, manganese peroxidase, and laccases, used for delignification; require substrates with lignin. Fermentable sugars which are produced as a result of enzyme hydrolysis can now be converted into specific biofuels using specific microorganism (Table 17.7).

#### 17.3.3.2 Food

Fruit and vegetable waste are rich sources of dietary fibers, carotenoids, phytoestrogens, natural antioxidants, and functional compounds. Extraction of these all important bioactive compounds from fruit and vegetable processing wastes requires the use of cell wall-degrading enzymes such as cellulases, xylanases, pectinases, and feruloyl esterases, etc. (Çinar 2005; Laroze et al. 2010; Lavecchia and Zuorro 2008; Meyer et al. 1998).

## 17.4 Conclusion

In this era of increasing world population, it is the natural tendency to increase food production to meet the food needs of the society. But the total utilization of agricultural produce to derive as much valuable products from them as possible is key to sustainable agriculture. Fruit and vegetable wastes are important sources of bioactive compounds and a rich source of dietary fiber, edible oils, and bio pigments. They are also a good substrate for the production of organic acids, enzymes, and biofuel. There is, therefore, the need to shift focus from increasing the production of new raw materials to meet the needs of consumers to promoting diversification of resources available. This will help solve the huge municipal waste problem. There is government commitment in some countries to ensure the total utilization of agricultural produce through the formation and implementation of particular policies. This policy approach is necessary to task food manufactures to make frantic efforts to ensure the wholistic utilization of their raw materials. The fruit and vegetable industry makes a significant contribution to the municipal waste problem. They need to be tasked to be responsible for the transformation and valorization of their waste to reduce their burden on the environment in terms of pollution.

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## 18

# Bioethanol from Waste – Prospects and Challenges of Current and Emerging Technologies

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## Abbreviations

BSG	Brewers' spent grain
CBP	Consolidated bioprocessing
CW	Cheese Whey
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization
FFVs	Flexible Fuel Vehicles
GHG	Greenhouse Gas
IEA	International Energy Agency
IRENA	International Renewable Energy Agency
OPW	Orange peel waste
RFS	Renewable Fuel Standard
SCG	Spent Coffee Grounds
SHF	Separate Hydrolysis and Fermentation
SSF	Simultaneous Saccharification and Fermentation
TRL	Technology Readiness Level
US	United States

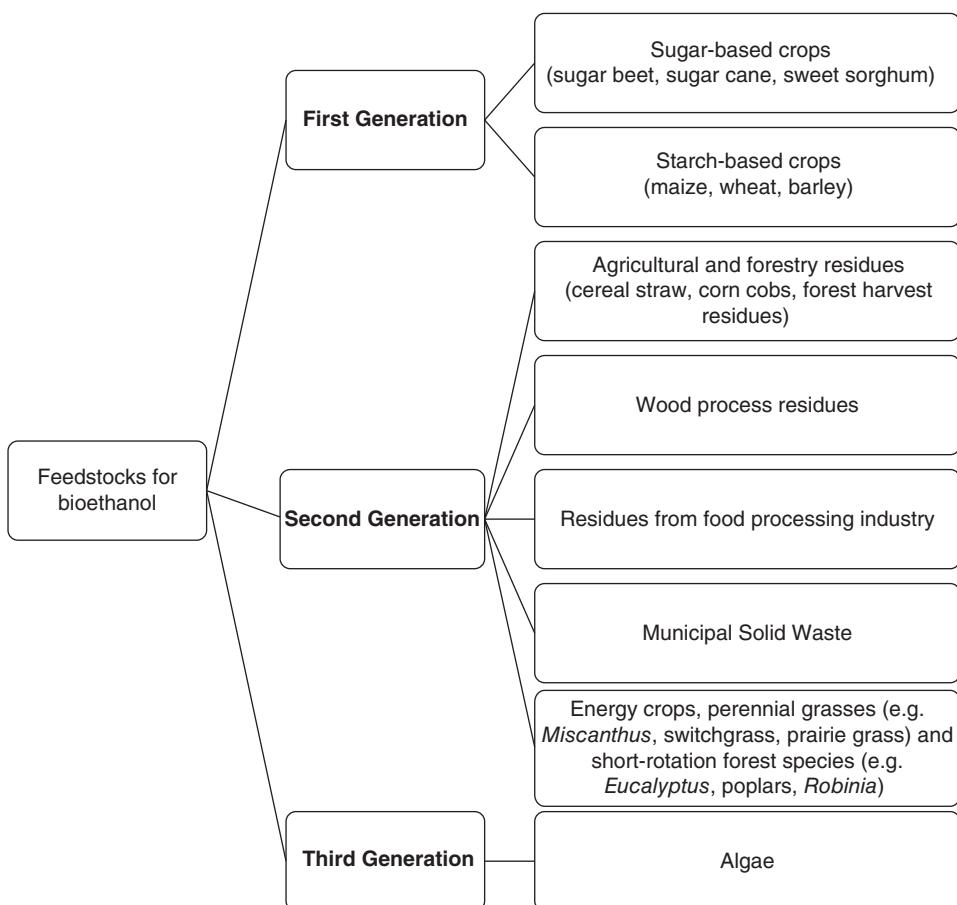
## 18.1 Introduction

According to the International Renewable Energy Agency (IRENA), renewable energy includes all forms of energy produced from renewable sources in a sustainable manner such as bioenergy, geothermal energy, hydropower, ocean energy, solar, and wind energy

(International Energy Agency and the World Bank 2014). The International Energy Agency (IEA) identifies bioenergy as the largest source of renewable energy today, providing heat and electricity, as well as transport fuels. As defined by IEA, bioenergy is the energy generated from the conversion of solid, liquid, and gaseous products derived from biomass. Biomass is any organic matter, i.e. biological material, available on a renewable basis, and it includes feedstock derived from animals or plants, such as wood and agricultural crops, and organic waste from municipal and industrial sources. Biomass is highly utilized for the production of biofuels.

IEA and Food and Agriculture Organization (FAO) defined biofuels as liquid fuels that derive from biomass including bioethanol and biodiesel (International Energy Agency and Food and Agriculture Organization 2017); however, in a previous report of IEA (International Energy Agency 2011) biofuels were defined as liquid and gaseous fuels produced from biomass. On the same side, in EU level Directive 2009/28/EC, biofuels are defined as both liquid and gaseous fuel for transport produced from biomass. Today three generations of biofuels are generally distinguished, i.e. first, second, and third-generation biofuels; still, there is not a strict technical definition for these terms and the distinction between them hinges around the feedstock used in production (Carriquiry et al. 2010). In particular, first-generation biofuels are produced from the conversion of food crops, such as corn, rapeseed, sugar beet, cereals, and others (EurObserv'ER 2016), also called "conventional," which includes bioethanol and biodiesel outputs. This category also includes the production of vegetable oil that can be used pure and directly by specific engines. The production of biogas fuel (generally in the form of biomethane), obtained by the anaerobic digestion process followed by purification, is a somewhat special category, because it can be produced both from fermentable waste and food crops. Second-generation biofuels are those produced from residual agro-industrial waste feedstocks that do not rely on agri-food crops. They are more environmentally friendly in terms of Greenhouse Gas (GHG) emissions because they utilize the lignocellulose contained in the plant cell walls. The raw materials range from straw, green waste (tree cuttings, etc.) or even fast-growing energy plants such as miscanthus. They enable mainly bioethanol production, but some of the processes produce biodiesel as well. Finally, third-generation biofuels incorporate biofuel produced from algae, also known as "algofuel," that present the advantage of not competing with food or energy crops (EurObserv'ER 2016). The second and third-generation biofuels constitute the "advanced biofuels" that promise to be more sustainable with lower carbon footprint. Both generations have the advantage to be independent of the land used for other primary needs, such as food production and farming. Feedstock includes lignocellulosic residues from agriculture and forestry, fast-rotation non-food crops (possibly grown on marginal, non-arable land), organic fraction of urban waste and microalgae (IEA-ETSAP and IRENA 2013).

In line with the above definitional framework, first-generation bioethanol refers to the bioethanol derived from agricultural edible products, either from sugar-based crops (sugarcane, sugar beet and sweet sorghum) or starch-based crops (wheat, maize, barley, and cassava). Second-generation bioethanol is ethanol produced from feedstocks which do not compete with food production. Second-generation biofuels are based on lignocellulosic material such as straw, grasses, wood, stovers, agricultural waste, organic waste from municipal solid waste, waste paper, etc. Third-generation bioethanol is focused on the use of marine organisms such as algae (Jambo et al. 2016). Potential feedstocks for bioethanol production are presented in Figure 18.1.



**Figure 18.1** Overview on potential feedstocks for the generation of first, second, and third-generation bioethanol.

This chapter is organized as follows. Section 18.2 presents an overview of the bioethanol market. Section 18.3 provides a literature review on the current status and emerging technologies for second generation bioethanol production from waste. More specifically, the examined feedstocks include lignocellulosic streams, industrial food processing waste streams and the organic fraction of municipal solid waste. The main processes studied are the bioconversion and the thermochemical conversion process at laboratory, pilot, and commercial level. Finally, in Section 18.4 the environmental sustainability of critical aspects of second-generation ethanol production processes is examined.

## 18.2 Overview of Bioethanol Market

Bioethanol ( $\text{EtOH}$ ,  $\text{CH}_3\text{CH}_2\text{OH}$ ) is a liquid biofuel. In particular, bioethanol is ethanol produced from biomass (Directive 2009/28/EC, OJ L 140, 5.6.2009). Bioethanol can be

produced from a wide variety of feedstocks that can be broadly classified as: (i) sucrose-containing feedstock; (ii) starch-containing feedstock; and (iii) cellulosic feedstock (IEA 2008).

Bioethanol is the most widely produced biofuel globally (Marelli et al. 2015). The production of bioethanol in the world was increased from 2005 to 2010 due to policies supporting biofuels' use in the transportation. Such policies were developed in more than 50 countries and specifically blending mandates were introduced in 27 countries at national level and in 27 states/provinces (Marelli et al. 2015). Biofuels are used as blends with conventional fuels in existing engines (NREL 2015). E10, known as "gasohol" in Brazil and the U.S.A., is a low-level blend of up to 10% bioethanol and at least 90% gasoline which can be used with no or small engine modification by more than 85% of the cars circulating in the EU and all cars manufactured after 2010 (Marelli et al. 2015; NREL 2015). It is classified as "substantially similar" to gasoline by the U.S. Environmental Protection Agency (EPA) and is legal for use in any gasoline-powered vehicle (Marelli et al. 2015; NREL 2015). In 2011, EPA approved E15 (15% ethanol, 85% gasoline) for use in light-duty conventional vehicles model year 2001 and newer (NREL 2015). Higher blends of bioethanol to petrol such as E85 (or flex fuel), which is a high-level ethanol-gasoline blend containing 51–83% ethanol, requires engine's modifications and cannot be legally used in conventional gasoline-powered vehicles (Marelli et al. 2015; NREL 2015). E85 can be used in flexible fuel vehicles (FFVs), which have an internal combustion engine and are designed to run on E85, gasoline, or any blend of gasoline and ethanol up to 83% (NREL 2015). Bioethanol can also be blended with diesel in diesel engines ("E-diesel"/ED95 fuel blends) or as a blend with biodiesel in diesel engines ("BE-diesel" fuel blends) (Marelli et al. 2015).

In 2016, the global production of fuel ethanol reached 99 billion liters while biodiesel production was 31 billion liters (REN21 2017). Worldwide, United States (US) and Brazil are the leading bioethanol producers covering 59% and 27% of global production in 2016, respectively (REN21 2017; Marelli et al. 2015). In 2016, China, Canada, and Thailand were the next largest producers (REN21 2017). The U.S.A. and Brazil are also the leading exporters of bioethanol (Marelli et al. 2015).

The constitutes the largest producer of first-generation bioethanol and is also the front-runner in the development and scaling-up of advanced biofuels technologies (Marelli et al. 2015). Corn is the primary U.S. feedstock used for bioethanol production (Marelli et al. 2015; Fernholz et al. 2017; Monceaux 2009; Lopes et al. 2016). In 2015, the U.S. produced an estimated 56 billion liters (Fernholz et al. 2017). U.S. extensive experience in first-generation production of bioethanol has certainly facilitated its quick response to the massive incentives, resulting in progressing on the development of second-generation cellulosic ethanol plants, commonly utilizing corn stover (Marelli et al. 2015). In the U.S., the governmental policy regulating biofuel production is the Renewable Fuel Standard (RFS) (Fernholz et al. 2017). It was established under the Energy Policy Act of 2005 and expanded under the Energy Independence and Security Act of 2007 (Fernholz et al. 2017).

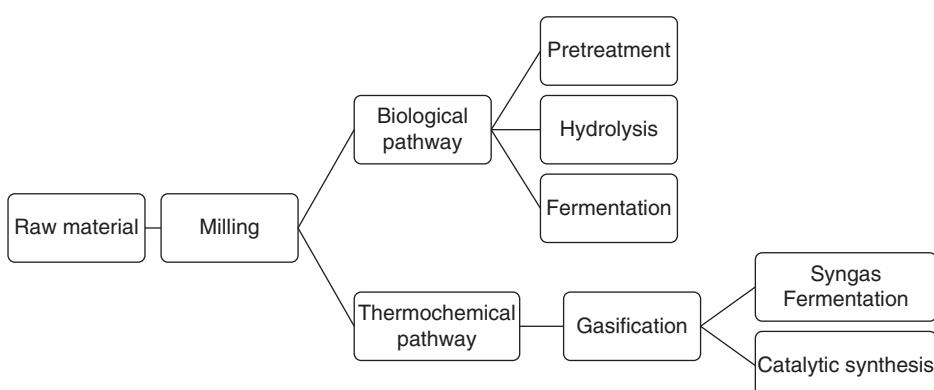
In Brazil, bioethanol has been produced from sugar cane and used as a transport fuel for about 40 years with continual improvements in technology (Marelli et al. 2015; Fernholz et al. 2017; Lopes et al. 2016). In Brazil, total ethanol production for 2016 was estimated at 31 billion liters. Total ethanol exports for 2017 were forecast at 1.4 million liters, similar to 2016. Fuel ethanol exports for 2016 were estimated at 750 million liters, mostly to the U.S. market (Barros 2016). Brazil has the most developed

biofuel program in the world (Lopes et al. 2016). It was initiated by the oil crisis of the early 1970s, and by 1975 the National Alcohol Program (Proalcool) had been introduced, offering government subsidies to the sugar cane and ethanol industry (Lopes et al. 2016). Government-support allowed large-scale investment in research and technology developments, which perfected the transformation processes and lowered manufacturing costs (Lopes et al. 2016). At the beginning of twenty-first century, the use of ethanol as fuel was resumed, mainly motivated by high oil prices in the international market and the development of flex-fuel technology (Lopes et al. 2016).

In the EU, in 2016 ethanol production was 4.8 billion liters (6% drop compared to 2015) (REN21 2017). An estimated, 75 million liters was cellulosic ethanol in 2015. The EU currently has 71 first-generation ethanol plants with a capacity of approximately 8.48 billion liters (REN21 2017). As a result of the Biofuels Directive of 2003 (Directive 2003/30/EC), both the use and the production of bioethanol rose steadily for a long time in the EU. The European biofuel market is now regulated by the Directive (EU) 2015/1513 whose wording focuses on the environmental impact of first-generation biofuel development. The main effect of this directive is to limit the energy share of biofuel produced from cereal, sugar, and oilseed crops on farming land to 7% by 2017 in Member States' renewable energy consumption for transport. The overall 10% renewable energy target in transport is retained, while the remaining 3% can be obtained through electric mobility or by using biofuel produced from specific feedstocks that benefit from double accounting (Marelli et al. 2015).

### 18.3 Second Generation Bioethanol Production Processes

Bioethanol can be produced through two major conversion pathways, i.e. the biochemical and the thermochemical (IEA-ETSAP and IRENA 2013; Sikarwar et al. 2017; Vohra et al. 2014; Naik et al. 2010; Achinas and Euverink 2016). The two major pathways are depicted in Figure 18.2. It must be mentioned that depending on the raw material the utilization pathway is modified so as to achieve a viable process.



**Figure 18.2** Basic pathways of raw material for the production of second generation bio-ethanol.

The biological pathway involves extraction of sugars followed by fermentation to obtain EtOH. In this pathway, bioethanol production is accomplished in four main steps, the details of which vary substantially depending on the type of source. Firstly, biomass undergoes a pretreatment step to render it more susceptible to the subsequent enzymatic hydrolysis step. During the hydrolysis step, lignocellulose is converted to monosaccharides, which are fermented to ethanol in the third step of the overall process. The final stage involves ethanol separation and purification, usually by distillation-rectification-dehydration (Vohra et al. 2014). On the other hand, the thermochemical process involves feedstock gasification to produce synthesis gas (also known as syngas and includes CO, H<sub>2</sub>, and CO<sub>2</sub>), which is then converted into ethanol. Bioethanol from syngas can be produced either directly or indirectly. Bioethanol can be synthesized directly from syngas through selective hydrogenation, while it can be also produced indirectly by employing methanol as intermediate through reductive carbonylation (methanol homologation) or through the ENSOL process including acetic acid generation, followed by ethanol synthesis (Sikarwar et al. 2017). Another promising indirect method is the syngas fermentation during which syngas is converted to bioethanol using microbial catalysts (Sikarwar et al. 2017).

In the following paragraphs literature review on the production of second-generation bioethanol is presented per type of feedstock used.

### 18.3.1 Lignocellulosic Feedstock

The main sources of lignocellulosic feedstock for the production of second-generation bioethanol involve agriculture and forestry waste, such as wheat straw, rice husks, wood trimmings and sawdust, but also dedicated energy crops, which are however beyond the scope of the present chapter.

Agricultural crop residues involve harvesting crop residues and processing crop residues, with the former being the most abundant fraction. Wheat, rice, maize, and sugarcane provide the majority of lignocellulosic biomass in the agricultural sector (Saini et al. 2015). Wheat is the world's most widely grown crop, and leads to the annual generation of approximately 430 Tg of straw for the production of about 120 GL bioethanol (Talebnia et al. 2010). Corn stover, which is left over after harvesting corn kernel, is produced at a rate of 1 dry kg per dry kg of corn grain. A study conducted in 2004 showed that 73 GL of bioethanol were annually derived from corn stover and wasted corn, which was equivalent to about 4.7% of the annual gasoline consumption (Kim and Dale 2004).

Forestry residues originate from biomass not harvested or removed from sorting regions in commercial wood production, but also through forest management operations such as precommercial thinning and removal or dead trees (Kang et al. 2014). Forestry waste, such as sawdust, bark, and wood chips have also been used as bioethanol feedstocks (Limayem and Ricke 2012). In the U.S., more than 50% of the cellulosic biomass is derived from woody material (Schwab et al. 2016). There are mainly two types of wood materials, classified as hardwoods and softwoods. The latter originate from conifers and gymnosperm trees, such as pine, cedar, spruce, cypress, fir, hemlock, and redwood (Hoadley 2000). Hardwoods are angiosperm trees, such as poplar, willow, oak, cottonwood, and aspen, and are mostly deciduous (Markwardt and Wilson 1935). Compared to agricultural biomass, woody biomass has higher lignin content and is consequently more recalcitrant to enzymatic degradation. However, it also has

significant advantages: the flexible harvesting times eliminate long-term storage and its higher density results in lower transportation cost. In addition, woody biomass has lower pentoses content, which is favorable for bioconversion to ethanol (Zhu and Pan 2010).

The composition of lignocellulosic biomass, energy content and physical characteristics vary substantially, depending on various parameters such as harvest year, climate, harvest, and collection techniques and soil composition. Consequently, process economics are hard to determine without previously determining accurately the chemical composition of feedstocks (Sluiter et al. 2010). Lignified secondary plant cell walls are the major source of plant biomass and are typically composed of cellulose (40–80%), hemicellulose (10–40%), lignin (5–25%) and cell wall proteins (Kumar et al. 2015). Cellulose consists of long chains of  $\beta$ -(1 → 4)-linked D-glucose units, that gather together to form microfibers, stabilized via hydrogen bonds (Brodeur et al. 2011). Hemicelluloses are a heterogeneous group of polysaccharides, including xyloglucans, xylans, mannans and glucomannans, and  $\beta$ -(1 → 3, 1 → 4)-glucans. Their main role is to associate cellulose microfibrils, resulting in a more rigid cell wall (Scheller and Ulvskov 2010). Lignin is an amorphous, phenolic polymer that also provides mechanical strength to the cell wall. It is heterogeneous and is formed from the polymerization of the hydroxycinnamyl alcohols, namely *p*-coumaryl, coniferyl, and sinapyl alcohol (Kumar et al. 2015).

### 18.3.1.1 Bioconversion Process

The production of bioethanol via biochemical processes (fermentation) involves four stages, namely pretreatment, hydrolysis, fermentation, and distillation. Biochemical processes can be applied in the first three stages, as presented in the following paragraphs.

Pretreatment: Biomass recalcitrance to enzymatic or microbial degradation is the main reason for the increased production cost of second-generation bioethanol (Himmel et al. 2007). Consequently, various pretreatment techniques are applied prior to hydrolysis in order to expose cellulose and hemicellulose to the degrading activity of enzymes. They can be classified as chemical, physical, biological or a combination of them (Vohra et al. 2014). Each pretreatment process has a specific effect on the cellulose, hemicellulose, and lignin fraction of the biomass. Physical (mechanical) treatment increases the available surface area by reducing the size of the biomass (Maurya and Singla 2015). Chemical methods involve the use of dilute acids (such as sulfuric or hydrochloric acid), alkalis (such as calcium hydroxide) or liquid ammonia. The use of dilute acids results in the removal of hemicellulose, while lignin remains unaffected (Mussatto et al. 2010). Steam explosion is the most commonly used physico-chemical method. It subjects lignocellulosic biomass to high temperature and pressure for a few seconds to several minutes, and then the pressure is released to atmospheric level, causing an explosion (Capolupo and Faraco 2016). Compared to other pretreatment techniques, it requires lower investment capital, has lower environmental impact, less hazardous process conditions and results in an almost complete sugar recovery (Capolupo and Faraco 2016).

Biological pretreatment employs biomass degrading microorganisms, with the aim to render lignocellulosic feedstock more susceptible to enzymatic treatment. The most commonly used microorganisms are brown rot fungi, attacking cellulose, and white and soft rots that degrade both cellulose and lignin (Sarkar et al. 2012). The absence of harmful chemicals and harsh conditions renders biological pretreatment an environmentally sustainable method. However, one of the main barriers for its implementation on an industrial scale are the low hydrolysis rates and low yields (Aditiya et al. 2016).

In addition to microorganisms, enzymes have also been applied as milder and more environmentally-friendly pretreatment of lignocellulosic feedstocks. Laccase, combined with a mediator, has been successfully applied for the delignification of woody and non-woody substrates resulting in higher saccharification degrees (Martinez et al. 2017).

Hydrolysis of lignocellulosic biomass breaks down polysaccharides to their corresponding monomers (sugars), which is a prerequisite for their use by microorganisms in the subsequent fermentation step. There are two major methods applied for hydrolyzing biomass, dilute-acid hydrolysis and enzymatic hydrolysis. The former is performed with mineral acids such as dilute  $H_2SO_4$  or HCl at temperatures of about 160 °C and pressures of about 10 atm (Sun and Cheng 2002). The main drawback of the method is the generation of toxic compounds that inhibit the fermenting microorganisms (Mussatto and Roberto 2004).

Enzymatic hydrolysis relies on the usage of enzymes for the decomposition of polysaccharide polymers into their constituents. Cellulose, the most abundant component of the plant cell wall, is remarkably recalcitrant to degradation due to its increased crystallinity. However, since it is the main source of glucose for the subsequent fermentation step, its efficient decomposition is of outmost importance for the sustainability of the overall process. The enzymes implicated in cellulose degradation are endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91),  $\beta$ -glucosidases (EC 3.2.1.21) and the recently discovered lytic polysaccharide monooxygenases (EC 1.14.99.53-56) (Horn et al. 2012). Hemicellulose is more amorphous than cellulose and due to its heterogeneous nature, it is degraded by a wider variety of enzyme activities, such as xylanases, endoglucanases, mannanases, feruloyl esterases, and arabinofuranosidases (Rytioja et al. 2014). The use of enzymes instead of acids for the decomposition of plant polymers results in milder reaction conditions and hence, less equipment maintenance cost. The main disadvantages of the method involve the high cost of the enzymes as well as their inhibition by substances released during the pretreatment step or by other enzymes present in the hydrolysis mixture (Aditiya et al. 2016; Ximenes et al. 2011).

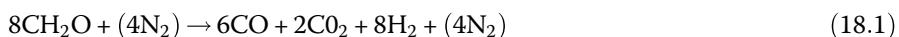
Fermentation: The most commonly used microorganisms for the fermentative conversion of sugars to ethanol are the yeast *Saccharomyces cerevisiae* and the bacterium *Zymomonas mobilis* (Aditiya et al. 2016). *S. cerevisiae* is the first choice for industrial ethanol production, due to its high fermenting capacity and its relative tolerance to low pH values and to high sugar and ethanol concentrations. In addition, it can be fairly resistant to inhibitors present in biomass hydrolysates and can grow under the anaerobic conditions occurring in the fermentation vessels (Nevoigt 2008). Even though the commonly used fermenting microorganisms convert efficiently hexose sugars, they are incapable of fermenting pentose sugars. To overcome the limitations emerging from the use of natural biocatalysts for the fermentation step, microorganisms are engineered to alter their fermenting properties toward the utilization of pentoses in order to increase ethanol yield. In past decades, metabolic, and evolutionary engineering strategies have been extensively performed with the aim to expand the ability of *S. cerevisiae* to ferment different sugars simultaneously (Zhou et al. 2012; Peng et al. 2012; Kuyper et al. 2003).

There are two main alternative processes for the production of ethanol; separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). In industrial conditions, the treatment of biomass has to be performed under high-gravity conditions (high dry matter content). The accumulation of sugars in the case of separate pre-hydrolysis and hydrolysis steps has an inhibitory effect to the enzymes, such

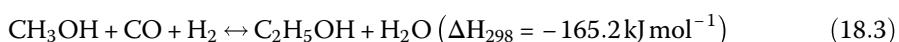
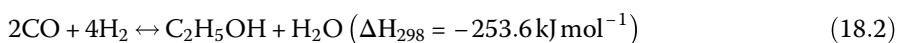
as  $\beta$ -glucosidases and cellobiohydrolases. This problem is alleviated in SSF, where the glucose formed is continuously fermented to ethanol, and ethanol is continuously removed during the fermentation (Viikari et al. 2012). Simultaneous saccharification and co-fermentation (SSCF), where mixed culture microbes are the fermenting agents, does not require sugar separation, sterilization, while allowing the use of various materials as substrate (Aditiya et al. 2016). In consolidated bioprocessing (CBP), the same microorganisms perform saccharification and fermentation. The production of enzymes, the hydrolysis step and the fermentation of hexose and pentose sugars are performed in the same reactor and consequently, the CBP method offers the benefits of low total production cost. In the case of CBP, the engineering of naturally occurring microbes is required in order to equip them with cellulase-coding genes when they are naturally carrying fermentation related genes or fermenting properties when they are biomass degraders (Olson et al. 2012). In Table 18.1, an overview of operational units worldwide for the production of cellulosic ethanol via fermentation is presented.

### 18.3.1.2 Thermochemical Conversion Process

The thermochemical pathway of bioethanol production constitutes an alternative to the classical biological production. The procedure includes the conversion of lignocellulosic material to synthesis gas through gasification and the subsequent catalytic synthesis of ethanol using syngas as raw material. Gasification materializes the complete depolymerization of biomass with limited oxygen at high temperatures, typically above 850 °C, to a gaseous intermediate (syngas) (Foust et al. 2009). According to Clausen and Gaddy (1993) synthesis gases, consisting of CO, H<sub>2</sub>, and CO<sub>2</sub>, may be produced from biomass according to the approximate reaction:



According to Sikarwar et al. (2017) ethanol can be synthesized either directly or indirectly (Sikarwar et al. 2017). In particular, direct synthesis of ethanol is performed through selective hydrogenation of CO at the catalyst surface (18.2). Indirect synthesis of ethanol is implemented through the generation of methanol from syngas which in turn is converted to ethanol. One approach for indirect synthesis of ethanol involves the reductive carbonylation of methanol in the presence of a catalyst. This process yields bioethanol by joining C–C bonds over the surface of the catalyst and is known as MeOH homologation (18.2). According to Subramanian et al. (2010), MeOH homologation via reductive carbonylation has lower ethanol yields and selectivity vis-à-vis commercial levels (Subramanian et al. 2010). Both direct synthesis and production of ethanol via methanol homologation are accompanied by several side reactions, such as the highly exothermic methanation reaction. Another approach of indirect synthesis of ethanol is the so called ENSOL process. This three-step process includes methanol synthesis from syngas (18.4), acetic acid production via methanol carbonylation (18.5) and ethanol synthesis through hydrogenation of acetic acid (18.6). The ENSOL approach has three steps and different catalysts are needed for each, making the process both expensive and complex. Reactions, as presented by Sikarwar et al. (2017) are given below.



**Table 18.1** An overview of operational units worldwide for the production of cellulosic ethanol via fermentation (IEA 39, Task Energy 2017).

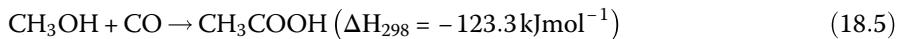
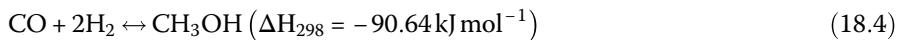
Project owner	Project name	Country	City	State	Technology Readiness Level (TRL)	Feedstock	Input	Ethanol ( $t\text{ yr}^{-1}$ )
Aemetis	pilot	US	Butte	Montana	4–5	Lignocellulosic crops	Switchgrass, grass seed, grass straw and corn stalks	500
American Process	Thomaston GP3+ Biorefinery	US	Thomaston	Georgia	6–7	Forest residues	Any woody or non-woody biomass	180
Anhui BBCA Biochemical	BBCA	China	Bengbu	Anhui	6–7	Lignocellulosic crops	Corncob/corn stover	5 000
Beta Renewables	Biochemtex	Italy	Rivalta Scrivia	—	4–5	Lignocellulosic crops	—	50
Beta Renewables	IBP-Italian Bio Fuel	Italy	Crescentino	—	8	Lignocellulosic crops	Wheat straw, rice straw, Arundo donax, poplar	40 000
Borregaard AS	BALI Biorefinery Demo	Norway	Sarpsborg	—	6–7	Lignocellulosic crops	Sugarcane bagasse, straw, wood, energy crops, other lignocellulosics	110
Borregaard Industries AS	ChemCell Ethanol	Norway	Sarpsborg	—	8	Lignocellulosic crops	Sulfite spent liquor (SSL, 33% dry content) from spruce wood pulping	15 800
Cane Technology Center (CTC)	CTC	Brazil	Piracicaba, SP	—	8	Lignocellulosic crops	Bagasse	2 400
Chempolis Ltd	Chempolis Biorefining Plant	Finland	Oulu	—	6–7	Lignocellulosic crops	Non-wood and non-food lignocellulosic biomass such as straw, reed, empty fruit bunch, bagasse, corn stalks, as well as wood residues	5 000
Clariant	Sunliquid	Germany	Straubing	Bavaria	6–7	Lignocellulosic crops	Wheat straw	1 000
COFCO Zhaodong Co.	COFCO Demo	China	Zhaodong	Heilongjian	6–7	Agricultural residues	Corn stover	500

DuPont	DuPont Cellulosic Ethanol Demonstration plant	US	Vonore	Tennessee	6–7	Agricultural residues	Corn stover, cobs, and fiber; switchgrass	750
Energy and Chemical Department of East China University of Science and Technology	Energy Pilot	China	Shanghai	—	4–5	Lignocellulosic crops	Crop and forestry residues	600
Gevo	Gevo	US	Luverne, MN	—	8	Forest residues	Corn	54 000
GranBio	Bioflex1	Brazil	Sao Miguel	Alagoas	8	Lignocellulosic crops	Sugarcane bagasse and straw	65 000
Greenfield Ethanol	Greenfield	Canada	Chatham, ON	—	4–5	Lignocellulosic crops	—	30
Henan Tianguan Group	Henan 1	China	Zhenping	Henan	8	Lignocellulosic crops	Wheat/corn stover	10 000
Henan Tianguan Group	Henan 2	China	Zhenping	Henan	8	Lignocellulosic crops	—	30 000
Logen Corporation	Demo	Canada	Ottawa	Ontario	6–7	Lignocellulosic crops	Wheat, barley, and oat straw; corn stover, sugar cane bagasse and other agricultural residues	1 600
Iowa State University	BioCentury Research Farm	US	Boone	Iowa	4–5	Lignocellulosic crops	Grains, oilseeds, vegetable oils, glycerin	200
Jilin Fuel Alcohol	Jilin2	China	Jilin	Jilin	6–7	Lignocellulosic crops	Straw	3 000
Lignol	Pilot	Canada	Vancouver, BC	—	4–5	Sugarcane bagasse	—	30
Lignol	Lignol	US	Grand Junction, IA	—	4–5	Forest residues	Woody Biomass	60
Lignol Innovations Ltd.	Pilot	Canada	Burnaby	British Columbia	4–5	Lignocellulosic crops	Hardwood and softwood residues	30
Longlive Bio-technology Co. Ltd.	Longlive	China	Yucheng	Shandong	8	Lignocellulosic crops	Corn cob	60 000

(Continued)

**Table 18.1** (Continued)

Project owner	Project name	Country	City	State	Technology Readiness Level (TRL)	Feedstock	Input	Ethanol (t yr <sup>-1</sup> )
Petrobras	Pilot	Brazil	Rio de Janeiro	Rio de Janeiro	4–5	Sugarcane bagasse	—	270
POET	Scotland	US	Scotland	South Dakota	4–5	Lignocellulosic crops	Corn fiber, corn cobs and corn stalks	60
POET-DSM Advanced Biofuels	Project Liberty	US	Emmetsburg	Iowa	8	Agricultural residues	—	75 000
PROCETHOL 2G	Futurol Project	France	Pomacle	—	4–5	Lignocellulosic crops	Flexible: woody and agricultural byproducts, residues, energy crops	2 700
Quad-County Corn Processors	Quad-County Biorefinery	US	Galva	—	8	Lignocellulosic crops	Corn kernel fiber	6 000
Raizen Energia	Brazil	Brazil	Costa Pinto, Piracicave	—	8	Lignocellulosic crops	Bagasse	31 600
Renmatix	Demonstration Plant	US	Rome	NY	6–7	Lignocellulosic crops	Wood Chips, Switchgrass, and other raw materials	500
Shandong Zesheng Biotech Co.	Zesheng	China	Dongping	Dongping	6–7	Lignocellulosic crops	Straw	3 000
SP/EPAP	Biorefinery Demo Plant	Sweden	Ornskoldsvik	—	6–7	Lignocellulosic crops	Primary wood chips; sugarcane bagasse, wheat, corn stover, energy grass, recycled waste	160
Woodland Biofuels	Pilot	Canada	Sarnia, Ontario	—	4–5	Organic residues and waste streams	Wood waste	60
Woodland Biofuels	Demo	Canada	Sarnia, Ontario	—	6–7	Organic residues and waste streams	Wood waste	601
ZeaChem	Demonstration scale biorefinery	US	Boardman	Oregon	6–7	Lignocellulosic crops	Poplar trees, wheat straw	750



The catalyst activity is closely related with its composition, structure, and reaction conditions, such as temperature, pressure, solvent, syngas feedstock, reactor, catalyst preparation and activation methods, etc. (He and Zhang 2008). The catalysts applied for the conversion of syngas to bioethanol can be classified as Rh-based, Cu-based, Co-based, and Mo-based catalysts (Lopez 2017). Rh-based catalysts have the highest selectivity to ethanol (Lopez 2017) and they have been tested with a variety of metal promoters (e.g. Fe, La, V, Zr, Ce, and Mn) (Lopez 2017) in order to increase ethanol selectivity. Cu-based catalysts are less selective for ethanol formation; however, metal promoters such as Fe, Co, Ni, La, Mn, or Pd are active for increasing the yield to alcohols (Nina et al.). According to Subramanian et al. (2010) the Rh-La-V/SiO<sub>2</sub> catalyst was able to increase the ethanol selectivity from 16.7% to 39%, as compared to unpromoted Rh/SiO<sub>2</sub>. Moreover, based on Mei et al., Rh-Mn/SiO<sub>2</sub> catalyst has shown a high syngas conversion of 42% (Mei et al. 2010), but low ethanol selectivity (<9%). Liu et al. (2011) have shown that Rh-Ce-Zr/SiO<sub>2</sub> catalyst can achieve both a high ethanol selectivity and a high syngas conversion of 35% and 27%, respectively (Liu et al. 2011). The same result has been produced using a Rh-CeO<sub>2</sub>/TiO<sub>2</sub> catalyst, showing a selectivity to ethanol of 33% and a syngas conversion of 32% (Li et al. 2013). Experiments conducted with Cu-based catalyst containing Cu-Pd-Fe-Co metals, has shown a syngas conversion as high as 84% and an alcohol selectivity of 37% with the following alcohol distribution: 26% methanol, 38% ethanol, 27% propanol and 9% butanol (Yang et al. 2011). Another approach is the combination of gasification and biological fermentation (Van Kasteren 2011; Acharya et al. 2014). After gasification, anaerobic bacteria such as *Clostridium ljungdahlii* are used to convert the CO, CO<sub>2</sub>, and H<sub>2</sub> into ethanol (Van Kasteren 2011). The components of synthesis gas may be converted into ethanol according to the following reactions (18.7) and (18.8) (Clausen and Gaddy 1993):



The gasification/fermentation pathway is an interesting alternative way of producing bioethanol. Via traditional fermentation processes, lignin, an important component of biomass, cannot be fermented. Gasification and subsequent fermentation of the produced gas enables fermentation of all carbon and hydrogen containing material and also non-biodegradable materials like plastics.

There are number of mesophilic and thermophilic microorganisms available for the fermentation of syngas (Acharya et al. 2014). Fermentation of syngas by *Alkalibaculum bacchi* strain CP15 and propionic acid producer *Clostridium propionicum*, resulted in the double production of alcohol (Liu et al. 2014). The microbial catalysts mostly used in fermentation of syngas are *Moorella thermoacetica*, *Butyribacterium methylotrophicum*, *C. ljungdahlii*, *Clostridium autoethanogenum*, *Acetobacterium woodii*, *Clostridium carboxidivorans* and *Peptostreptococcus* (Subramanian et al. 2010). The production of

bioethanol from syngas using biological catalysts such as *A. woodii*, *C. carboxidivorans* and *Peptostreptococcus productus* is more efficient based on the conversion yield compared with chemical catalysts such as copper, cobalt, or iron (Heiskanen et al. 2007). Advantages of syngas fermentation include feedstock flexibility, high rate of energy, carbon capture and manufacturing costs in comparison with conventional fermentation and thermochemical approaches (Daniell et al. 2012).

The possibilities of converting syngas into valuable ethanol through the thermochemical process have been confirmed and there are currently operating facilities. In Table 18.2, the current status worldwide of syngas to ethanol production is presented.

### 18.3.2 Industrial Food Processing Waste Streams

#### 18.3.2.1 Apple Pomace

Apple pomace is the solid waste fraction produced from the industrial process of fruit pressing for juice extraction. Apple pomace consists of the fruit pulp, peel and core and it constitutes 25–35% of the apple fruit weight (Joshi et al. 2012).

In most cases, apple pomace is treated as waste, while there are cases where it is also used as animal feed. However, the latter is not widely applied due to the low nutritional value, spoilage, and microbial growth of apple pomace (Hang et al. 1982; Joshi 1998; Magyar et al. 2016; Vendruscolo et al. 2008). Considering that apple pomace has a high content of simple sugars and polysaccharides, it constitutes a good substrate for bioconversion to ethanol.

The most common method for the production of ethanol from apple pomace is solid state fermentation, since the substrate is not readily amenable to submerged yeast fermentation (Hang et al. 1982). In addition, several studies have revealed that natural biota leads to low ethanol production and therefore the addition of inoculum is considered necessary in order to increase production (Hang et al. 1982; Jarosz 1988; Sandhu and Joshi 1997).

High amounts of pectin, xylose, and lignin in apple pomace may hinder the process or call for special treatment (Dhillon et al. 2012; Magyar et al. 2016; Parmar and Rupasinghe 2013; Villas-Bôas et al. 2003). In particular, pectin and lignin form a shield around cellulose and hemicellulose preventing their hydrolysis to sugars. To address this, carbohydrolases are used to break down the shield and to enable liquefaction and saccharification of apple pomace (Parmar and Rupasinghe 2013).

The most commonly employed microorganism for bioethanol production is the yeast *S. cerevisiae* because of its ability for high ethanol yield. However, there are some filamentous fungi which are also able to utilize sugars from lignocellulosic biomass (including xylose) directly through fermentation for the production of ethanol, although with lower yields (Evcan and Tari 2015). For that reason, several researchers have tried coculturing *S. cerevisiae* with these fungi for the production of ethanol from apple pomace (Chatanta et al. 2008; Evcan and Tari 2015; Gulhuane et al. 2015).

Finally, Magyar et al. (2016) examined the conversion of apple pomace to ethanol through different pretreatment methods followed by SHF or SSF using commercial enzymes and native *S. cerevisiae* strain at high solids loading (20% and 30%). They concluded that autoclave with no chemical pretreatment showed better or similar results compared to acid and alkali pretreatments. With no chemical pretreatment, inhibition of fermentation from sugar and lignin degradation products associated with

**Table 18.2** An overview of operational units worldwide for the production of bioethanol production from syngas (IEA 39, Task Energy 2017).

Project owner	Project name	Status	Country	City	State	TRL	Technology	Feedstock	Input	Output
Tembec Chemical Group	Synthesis Tembec Chemical Quebec	Operational	Canada	Temiscaming	Quebec	6–7	Gasification	Lignocellulosic crops	Spent sulphite liquor feedstock	Cellulosic ethanol (13 000 t yr <sup>-1</sup> )
Vanerco (Enerkem and Greenfield Ethanol)	Varennes Cellulosic Ethanol	Under construction	Canada	Varennes, PQ		6–7	Fuel Synthesis	Organic residues and waste streams	Sorted industrial, commercial, and institutional waste	Ethanol (planned capacity: 30 000 t yr <sup>-1</sup> )
Enerkem	Westbury commercial demonstration facility	Operational 2009	Canada	Westbury	Quebec	6–7	Fuel Synthesis	forest residues	Treated wood (i.e. decommissioned electricity poles, and railway ties), wood waste and municipal solid waste (MSW) (48 t d <sup>-1</sup> )	<ul style="list-style-type: none"> <li>• Cellulosic ethanol (4 000 t yr<sup>-1</sup>)</li> <li>• Methanol (1 000)</li> <li>• Various chemicals</li> </ul>
Enerkem	Synthesis Enerkem Sherbrooke	Operational 2003	Canada	Sherbrooke	Quebec	4–5	Gasification	Lignocellulosic crops	Municipal solid waste, wood chips, treated wood, sludge, petroleum coke, spent plastics and wheat straw	<ul style="list-style-type: none"> <li>• Cellulosic ethanol (375 t yr<sup>-1</sup>)</li> <li>• Methanol (475 m<sup>3</sup> yr<sup>-1</sup>)</li> <li>• Syngas</li> </ul>
Enerkem Alberta Biofuels LP	Edmonton Waste-to-Biofuels Project	Operational 2014	Canada	Edmonton	Alberta	8	Fuel Synthesis	Organic residues and waste streams	Post-sorted MSW (100 000 t yr <sup>-1</sup> )	<ul style="list-style-type: none"> <li>• Ethanol (30 000 t yr<sup>-1</sup>)</li> <li>• Methanol</li> <li>• Various chemicals</li> </ul>
LanzaTech	Waste gas to fuel	Operational 2008	United States	Chicago		1–3	Gasification	Organic residues and waste streams	Steel waste gas	Cellulosic ethanol
Aemetis	Aemetis Commercial	Planned	United States	Keyes	California	8	Fermentation	Biomass syngas	Biomass syngas	Biomass syngas ethanol

pretreatment were avoided thus allowing simple sugars to be completely consumed. Lastly, no significant difference in ethanol generated with SHF and SSF was observed.

#### 18.3.2.2 Orange Peel Waste

Orange peel waste (OPW) comprise of the processing residues from orange juice extraction such as the peels, segment membranes, cores, seeds, and juice sacs (Zhou et al. 2008). It is estimated that OPW is about 50–60% of the total fruit weight (Wilkins et al. 2007a,b).

The main management methods reported for these waste streams is drying followed by pelletization for the production of cattle feed or, alternatively, disposal (Wilkins et al. 2007a,b). However, in the case of animal feed, considering its low price and the associated costs for drying, it is not an economical option and therefore, it is not widely applied (Grohmann et al. 1994).

OPW contain significant amounts of both soluble carbohydrates (glucose, fructose, sucrose) and insoluble carbohydrates (cellulose, hemicellulose, pectin), while lignin is usually absent or in very low content (Grohmann et al. 1994).

The most common microorganism used for fermentation is again the yeast *S. cerevisiae* which however is not able to ferment pentoses or galacturonic acid (Grohmann et al. 1995). Alternatively, the bacteria *Escherichia coli* KO11 and the yeast *Pichia kudriavzevii* KVMP10 have been used from Grohmann et al. (1995) and Koutinas et al. (2016) respectively with very good results.

To ferment monomeric sugars from citrus peel waste, it is necessary first to reduce the concentration of D-limonene below the threshold level, above which inhibition of yeast growth during fermentation is observed (Grohmann et al. 1994; Wilkins et al. 2007a; Awan et al. 2013). D-limonene is a terpene found in very high concentrations in citrus peel oil and can be removed or recovered with appropriate pretreatment (Awan et al. 2013). Among the cases studied, Grohmann et al. (1994) and Grohmann et al. (1995) applied filtration, Wilkins et al. (2007b) and John et al. (2017) applied steam explosion, Choi et al. (2015) applied sorption to raw cotton and activated carbon followed by liquid extraction with hexane, while the review of Ruiz et al. (2016) also mentions biological treatment, aeration, and steam distillation among the relevant pretreatment methods.

Finally, it is worth noting the study of Awan et al. (2013) toward increasing cost effectiveness of the process. In specific terms, they used *Xanthomonas axonopodis* pv. *citri* strain 306, which produces a cocktail of enzymes with cellulolytic, hemicellulolytic, and pectinolytic properties presenting sugar yields very similar to that of commercial enzymes. In addition, they used co-cultures of *S. cerevisiae* with *Candida parapsilosis* strains, which resulted in a reduction of fermentation time to six hours.

#### 18.3.2.3 Potato Peel Waste

The byproducts of the potato processing industry for the production of food products such as potato chips, French fries, and instant mashed potatoes consist mostly of potato peel waste (Arapoglou et al. 2010; Kawa-Rygielska et al. 2012; Yamada et al. 2009). The most common management method of potato processing byproducts is use in animal feed (Kawa-Rygielska et al. 2012; Radunz et al. 2003). However, potato processing waste are rich in starch, as well as in other carbohydrates and therefore they are a suitable substrate for bioethanol production.

The most widely applied method for ethanol production from potato waste is liquefaction and saccharification followed by fermentation by *Saccharomyces* yeasts. The first two stages refer to the hydrolysis of starch, where during liquefaction amylases are employed to decrease viscosity or to produce dextrins, while during saccharification the enzymes utilize dextrins to produce glucose (Arapoglou et al. 2010).

Izmirlioglu and Demirci (2012, 2015) investigated the substitution of yeast extract in the fermentation process by more economic alternates, in order to increase economic efficiency. In particular, they concluded that the yeast extract may be substituted by animal-based nitrogen sources (poultry meal, feather meal) or by a combination of malt extract and magnesium sulfate with higher ethanol production compared to yeast.

#### 18.3.2.4 Brewers' Spent Grain

Brewers' spent grain (BSG) consist of the husk, pericarp, seed coat and other solid residues of the barley grain generated during the brewing process of mashing and lautering of barley malt for the extraction of starch and the preparation of wort (Mussatto and Roberto 2006; White et al. 2008; Xiros et al. 2008).

BSG are a rich source of polysaccharides, such as cellulose and hemicellulose (approximately 50%), with the other 50% mainly comprising of lignin and proteins (Carvalheiro et al. 2004a,b; Mussatto and Roberto 2006; Wilkinson et al. 2014; Wilkinson et al. 2017; Xiros et al. 2008). The BSG composition varies depending on the barley cultivar, the harvest period and the brewing process followed.

BSG has been traditionally used by farmers as animal feed mainly for cattle at no or low cost, while a number of other potential applications are currently being investigated and promoted, such as use in energy production or in chemical and biotechnological processes (Mussatto and Roberto 2006; Mussatto 2014).

The majority of the research studies reviewed on BSG conversion to ethanol have applied alkali pretreatment prior to enzymatic hydrolysis (Table 18.3). This is due to the fact that alkaline reagents are quite efficient in removing lignin, which is found in relatively high concentrations in BSG compared to the other residues of the food industry examined here. The removal of lignin enables physical access of enzymes to cellulose and hemicellulose and reduces enzyme binding to lignin (Wilkinson et al. 2014).

To address the issue of increased process costs incurred by the large enzyme quantities consumed for the degradation of polysaccharides, two research studies (Xiros et al. 2008; Xiros and Christakopoulos 2009) have applied on-site enzyme production with the application of the solid-state and submerged culture techniques. In particular, in both studies the fungus *Fusarium oxysporum* was used, while the BSG substrate was enhanced by corn cob – a cheap agricultural by product – in order to provide the necessary physical support and energy for the fungus to grow. *F. oxysporum* is known for its ability to directly convert cellulose and hemicellulose through consecutive hydrolysis and fermentation (Xiros et al. 2008). In addition, Wilkinson et al. (2017) employed the fungus *Aspergillus oryzae*, which is known inter alia for its ability to produce lignocellulolytic enzymes (endocellulases and xylanases) when cultured in lignocellulosic substrate (Acharya et al. 2010).

Xiros and Christakopoulos (2009) and Wilkinson et al. (2017) adopted the CBP approach, which combines all steps required for the production of ethanol, such as the production of enzymes, lignocellulose degradation, and fermentation in one step. The only drawback of CBP is the low productivity rates, which however, may be counterbalanced by the low energy and water inputs.

**Table 18.3** Review of production processes, inputs, and ethanol yields per food waste stream.

Feedstock	Production process	Saccharification [S] and Fermentation [F] enzymes/ microorganisms	Maximum ethanol yield	References
Apple pomace	Solid State Fermentation	[F]: <i>S. cerevisiae</i> Montrachet strain 522	4.3 g/100 g solids	Hang et al. 1982
Apple pomace	Solid State Fermentation	[F]: <i>S. cerevisiae</i> ATCC 24702	19.3 g/100 g solids	Ngadi and Correia 1992
Apple pomace	Acid pretreatment and Solid State Fermentation	[F]: <i>S. Cerevisiae</i>	4.5% v/v	Joshi et al. 1995
Apple pomace	Solid State Fermentation	S&[F]: Coculture of <i>A. foetidus</i> MTCC 151 (pectinase), <i>F. oxysporum</i> MTCC 1755 (cellulase) and <i>S. cerevisiae</i> MTCC 173	12.7 g/100 g solids	Chatanta et al. 2008
Apple pomace	Solid State Fermentation	[S]: $\alpha$ -amylase, cellulase; [F]: Y51 strain from natural fermentation of apple pomace	5.2% v/v	Mahawar et al. 2013
Apple pomace	Acid pre-treatment, Enzymatic hydrolysis and fermentation	[S]: Pectinex 3XL (pectinase), Novozyme 188 (b- glucosidase), Celluclast 1.51 (cellulase); [F]: <i>S. cerevisiae</i>	4% v/v or 19 g/100 g solids	Parmar and Rupasinghe 2013
Apple pomace	Acid hydrolysis and direct fermentation	[F]: Coculture of <i>T. harzianum</i> NRRL31396, <i>A. sojae</i> ATCC 20235 and <i>S. cerevisiae</i>	8.75 g l <sup>-1</sup>	Evcan and Tari 2015
Apple pomace	Solid State Fermentation	[F]: Coculture of <i>S. cerevisiae</i> , <i>F. oxysporum</i> and <i>A. foetidus</i>	1.37 g l <sup>-1</sup>	Gulhane et al. 2015
Apple pomace	Separate hydrolysis and fermentation (30% solids loading)	[S]: CTec3 (cellulase), HTec3 (hemicellulase), Pectinex (pectinase); [F]: Native <i>S. cerevisiae</i> strain ATCC 4124	55.1 g l <sup>-1</sup> or 13.4 g/ 100 g solids	Magyar et al. 2016
Orange peel waste	Enzymatic hydrolysis and submerged fermentation	[S]: Pectinex Ultra SP (pectinase), Celluclast 1.51 (cellulase), Novozym 188 (b-glucosidase); [F]: <i>S. cerevisiae</i> strain	45 g l <sup>-1</sup>	Grohmann et al. 1994
Orange peel waste	Simultaneous saccharification and fermentation (SSF)	[S]: pectinase, cellulase, f-glucosidase; [F]: <i>S. cerevisiae</i>	40.5 g l <sup>-1</sup>	Zhou et al. 2008

Orange peel waste	Simultaneous saccharification and fermentation (23.5% solids loading)	[S]: Pectinex Ultra SP (pectinase), Celluclast 1.5 I (cellulase), Novozym 188 (b-glucosidase); [F]: <i>K. marxianus</i>	$34.5 \text{ g l}^{-1}$	Widmer et al. 2009
Orange peel powder	Two-Stage acid Hydrolysis and Fermentation	Not stated	$33 \text{ g l}^{-1}$ or $25 \text{ g}/100 \text{ g}$ solids	Oberoi et al. 2010
Orange peel waste	Autoclave pretreatment and submerged fermentation	[F]: <i>S. cerevisiae</i>	$9.8 \text{ g l}^{-1}$	Mishra et al. 2012
Orange peel waste	Simultaneous saccharification and fermentation (SSF)	Co-culture of <i>A. niger</i> [S] and <i>S. cerevisiae</i> [F]	$33 \text{ g l}^{-1}$	Shilpa et al. 2013
Orange peel waste	Enzymatic hydrolysis and submerged fermentation	[S]: <i>X. axonopodis</i> pv. <i>citri</i> (Xac 306 -IBSBF 1594); [F]: Co-culture of <i>S. cerevisiae</i> with <i>C. parapsilos</i> strain NRRL-12969	$54 \text{ g l}^{-1}$	Awan et al. 2013
Orange peel powder	Dilute acid hydrolysis (steam explosion-autoclave) and fermentation	[S]: <i>S. cerevisiae</i> NCIM 3495	$41 \text{ g l}^{-1}$	Joshi et al. 2015
Orange peel waste	Enzymatic hydrolysis and continuous fermentation with immobilized yeast cells	[S]: <i>A. citrisporus</i> and <i>T. iongibrachiatum</i> ; [F]: <i>S. cerevisiae</i> KCTC 7906	$27.1 \text{ g l}^{-1}$	Choi et al. 2015
Orange peel waste	Submerged fermentation ( $42^\circ\text{C}$ )	[F]: <i>P. kudriavzevii</i> KVMP10	$54 \text{ g l}^{-1}$	Koutinas et al. 2016
Orange peel waste	Submerged fermentation ( $37^\circ\text{C}$ )	[F]: <i>S. cerevisiae</i>	$40.9 \text{ g l}^{-1}$	Wilkins et al. 2007a,b
Orange peel waste	Simultaneous saccharification and fermentation (SSF)	[S]: Pectinex Ultra SP (pectinase), Celluclast 1.5 I (cellulase), Novozym 188 (b-glucosidase); [F]: <i>S. cerevisiae</i>	$39.6 \text{ g l}^{-1}$	Wilkins et al. 2007a,b
Potato peel waste and mash	Enzymatic hydrolysis and fermentation	[S]: Thermamyl ( $\alpha$ -amylase), AMG 300 L (glucoamylase), NS50012 (fungal $\beta$ -glucanase), Pectinase HL (pectinase); [F]: <i>S. cerevisiae</i>	$48.6 \text{ g l}^{-1}$	Yamada et al. 2009
Potato peel waste	Acid hydrolysis, enzymatic hydrolysis and fermentation	[S]: Viscozyme L ( $\beta$ -glucanase), Ternamyl 120 L ( $\alpha$ -amylase), Celluclast 1.5 I (cellulase); [F]: <i>S. cerevisiae</i> var. <i>bayanus</i>	$7.6 \text{ g l}^{-1}$	Arapoglou et al. 2010

(Continued)

**Table 18.3** (Continued)

Feedstock	Production process	Saccharification [S] and Fermentation [F] enzymes/ microorganisms	Maximum ethanol yield	References
Potato mash waste	Liquefaction, saccharification and fermentation	[S]: EC 3.2.1.1 ( $\alpha$ -amylase), EC 3.2.1.3 (amyloglucosidase); [F]: Poultry meal	35 g l <sup>-1</sup>	Izmirlioglu and Demirci 2012
Potato peel and dust waste	Liquefaction, saccharification and fermentation	[S]: Termamyl SC ( $\alpha$ -amylase), San Super (glucoamylase), Optimash BG ( $\beta$ -glucanase); <i>T. reesei</i> (xylanase); [F]: <i>S. cerevisiae</i> (Red industrial yeast strain)	61.7 g l <sup>-1</sup>	Kawa-Rygielska et al. 2012
Potato mash waste	Liquefaction, saccharification and fermentation	[S]: EC 3.2.1.1 ( $\alpha$ -amylase), EC 3.2.1.3 (amyloglucosidase); [F]: Malt extract and magnesium sulfate (yeast substitutes)	24.6 g l <sup>-1</sup>	Izmirlioglu and Demirci 2015
Potato peel waste	Microbial liquefaction, saccharification, and fermentation	Sequential culture of <i>A. niger</i> [S] and <i>S. cerevisiae</i> [F]	67.3 g l <sup>-1</sup> (supernatant)	Bekele et al. 2015
Brewer's Spent grains	Acid and enzymatic hydrolysis and Fermentation (20% solids loading)	[S]: cellulase, b-glucosidase, hemicellulase, xylanase; [F]: <i>P. stipitis</i> NCYC 1540	8.3 g l <sup>-1</sup> or 32 g/100 g solids	White et al. 2008
Brewer's Spent grains	Alkali and enzymatic hydrolysis and Coupled solid-state and submerged fermentation	S&[F]: <i>F. oxysporum</i> F3 with corn cob as co-substrate	6.5 g/100 g solids	Xiros et al. 2008
Brewer's Spent grains	Alkali and enzymatic hydrolysis and Submerged fermentation with consolidated bioprocessing	S&[F]: <i>F. oxysporum</i> F3 with corn cob as co-substrate	10.9 g/100 g solids	Xiros and Christakopoulos 2009
Brewer's Spent grains	Alkali and enzymatic hydrolysis and Fermentation (25% solids loading)	[S]: CelliCTec2 (cellulase); [F]: <i>S. cerevisiae</i> strain NCYC479	17.3 g l <sup>-1</sup>	Wilkinson et al. 2014
Brewer's Spent grains	Alkaline-acid pretreatment, enzymatic hydrolysis and Fermentation	[S]: Cellulase, $\beta$ -glucosidase; [F]: <i>S. cerevisiae</i> NRRL YB 2293	12.8 g l <sup>-1</sup>	Liguori et al. 2015

Brewer's Spent grains	Consolidated bioconversion process (25% solids loading)	[S]: <i>A. Oryzae</i> ; [F]: <i>S. cerevisiae</i> (NCYC479)	$37 \text{ g l}^{-1}$	Wilkinson et al. 2017
Coffee husks	Fermentation	[F]: <i>S. cerevisiae</i>	$13.6 \text{ g l}^{-1}$ or $8.5 \text{ g / 100 g solids}$	Gouvea et al. 2009
Coffee pulp and mucilage waste	Acid hydrolysis and fermentation	[F]: Exponential phase <i>S. cerevisiae</i> and whole panela	$25.44 \text{ g l}^{-1}$	Navia et al. 2011
Spent coffee grounds	Popping pretreatment, Enzymatic hydrolysis and Simultaneous Saccharification and Fermentation	[S]: Cellulast 1.5 l (cellulase), Pectinex SP-L (pectinase); [F]: <i>S. cerevisiae</i> strain KCTC 7906	$15.3 \text{ g l}^{-1}$	Choi et al. 2012
Spent coffee grounds	Acid hydrolysis and fermentation	[F]: <i>S. cerevisiae</i> (RL-11)	$11.7 \text{ g l}^{-1}$	Mussatto et al. 2012
Spent coffee grounds	Acid hydrolysis and fermentation	[F]: <i>S. cerevisiae</i> (KTCT7226)	$22 \text{ g l}^{-1}$	Kwon et al. 2013
Coffee pulp waste	Fermentation	[F]: <i>H. uvarum</i> UFLA CAF76	$14.67 \text{ g l}^{-1}$	Bonilla-Hermosa et al. 2014
Coffee pulp and mucilage waste	Acid hydrolysis and fermentation	[F]: <i>Pichia anomala</i> (M4)	$6.12 \text{ g l}^{-1}$	Woldesenbet et al. 2016
Coffee husks	Acid and enzymatic hydrolysis and fermentation	[S]: NS50013 (cellulase), C6105 (b-glucosidase); [F]: <i>S. cerevisiae</i> GSE16-T18	$36.6 \text{ g l}^{-1}$ or $4.3 \text{ g / 100 g solids}$	Dadi et al. 2017
Spent coffee grounds	Acid and enzymatic hydrolysis and fermentation	[S]: NS50013 (cellulase), C6105 (b-glucosidase); [F]: <i>S. cerevisiae</i> GSE16-T18	$47.9 \text{ g l}^{-1}$ or $7.1 \text{ g / 100 g solids}$	Dadi et al. 2017
Municipal food waste (cafeteria)	Enzymatic hydrolysis and fermentation	[S]: Spirizyme Plus FG (glucoamylase); [F]: <i>S. cerevisiae</i> KA4	$57.5 \text{ g l}^{-1}$	Kim et al. 2008
Municipal food waste (cafeteria)	Simultaneous saccharification and fermentation	[S]: $\alpha$ -amylase, glucoamylase, cellulase, protease, phytase, pectinase, lipase; [F]: <i>Z. mobilis</i> 10225	$53.2 \text{ g l}^{-1}$	Ma et al. 2008
Municipal food waste (cafeteria)	Enzymatic hydrolysis and Continuous fermentation	[S]: Nagase N-40 (glucoamylase); [F]: <i>S. cerevisiae</i> strain KF-7	$30.9 \text{ g l}^{-1}$	Tang et al. 2008
Municipal food waste (cafeteria)	Simultaneous saccharification and fermentation with distillery waste recycling	[S]: glucoamylase, protease; [F]: <i>Z. mobilis</i> 10225	$50 \text{ g l}^{-1}$	Ma et al. 2009

(Continued)

**Table 18.3** (Continued)

Feedstock	Production process	Saccharification [S] and Fermentation [F] enzymes/ microorganisms	Maximum ethanol yield	References
Municipal food waste (cafeteria)	Enzymatic hydrolysis and Fermentation	[S]: multi enzyme of arabinase, cellulase, $\beta$ -glucanase, hemicellulase, xylanase; [F]: <i>S. cerevisiae</i> strain KCTC 7107	29.1 g l <sup>-1</sup> or 46 g/ 100 g solids	Moon et al. 2009
Municipal food waste (canteen)	Enzymatic liquefaction and repeated-batch Simultaneous saccharification and fermentation	[S]: Termamyl ( $\alpha$ -amylase), glucoamylase XL-4, cellulase SS; [F]: <i>S. cerevisiae</i> KF-7	44 g l <sup>-1</sup>	Koike et al. 2009
Municipal food waste (cafeteria)	Thermal pretreatment and Simultaneous saccharification and fermentation (25%w/w solids loading)	[S]: NS50013 (cellulase), NS50010 ( $\beta$ -glucosidase); [F]: <i>S. cerevisiae</i> (Fermentis Ethanol Red)	30 g l <sup>-1</sup>	Ballesteros et al. 2010
Municipal food waste (cafeteria)	Enzymatic hydrolysis and fermentation	[S]: Viscozyme L. (cocktail of arabanase, cellulase, $\beta$ -glucanase, hemicellulase, xylanase), Spirizyme Plus FG (glucoamylase), Alcalase 2.41 FG, Subtilisin (protease), Novozym (cellulase); [F]: <i>S. cerevisiae</i>	46 g l <sup>-1</sup> or 43 g/100 g solids	Kim et al. 2011
Municipal food waste (cafeteria)	Enzymatic hydrolysis and Fermentation (20% solids loading)	[S]: A6211-1MU ( $\alpha$ -amylase), AMG 10115 (amyloglucosidase), C1794-10KU (cellulase), 49290 ( $\beta$ -glucosidase); [F]: <i>S. cerevisiae</i>	32.2 g l <sup>-1</sup> or 16 g/ 100 g solids	Uncu and Cekmecelioglu 2011
Municipal food waste-restaurant	Enzymatic hydrolysis and Fermentation	[S]: $\alpha$ -amylase, glucoamylase; [F]: <i>S. cerevisiae</i> H058	81.5 g l <sup>-1</sup>	Yan et al. 2011
Municipal food waste (cafeteria)	Simultaneous saccharification and fermentation	[S]: SAN Super 240 L ( $\alpha$ -amylase, amyloglucosidase, protease); [F]: <i>S. cerevisiae</i> (KCCM No. 50549)	36 g l <sup>-1</sup>	Hong and Yoon 2011
Municipal food waste (cafeteria)	Simultaneous saccharification and fermentation	[S]: Spirizyme Plus (glucoamylase), Viscozyme (multi-enzyme of beta-glucanase, xylanase, cellulase, hemicellulase); [F]: <i>S. italicus</i> KJ	30 g l <sup>-1</sup>	Li et al. 2011
Municipal food waste (cafeteria)	Enzymatic hydrolysis and co- fermentation	[S]: Spirizyme Plus FG (glucoamylase), Viscozyme L (cocktail of arabanase, cellulase, $\beta$ -glucanase, hemicellulase, xylanase) [F]: <i>S. coreanus</i> and <i>P. stipitis</i>	48.6 g l <sup>-1</sup>	Jeong et al. 2012

Municipal food waste (cafeteria)	Enzymatic hydrolysis and Fermentation	[S]: Liquozyme SC ( $\alpha$ -amylase), Spirizyme (glucoamylase); [F]: <i>S. cerevisiae</i>	$8 \text{ g l}^{-1}$	Walker et al. 2013
Municipal food waste (household)	Enzymatic hydrolysis and Fermentation with recycling of solid residue after hydrothermal pretreatment (45% w/v solids loading)	[S]: Celluclast <sup>®</sup> 1.51 (cellulase), Novozym 188 ( $\beta$ -glucosidase); [F]: <i>S. cerevisiae</i>	$42.78 \text{ g l}^{-1}$ or $10.8 \text{ g / 100 g solids}$	Matsakas et al. 2014
Municipal food waste (retail store)	Acid and enzymatic hydrolysis and Vacuum fermentation (35% solids loading)	[S]: GHSE-Stargen 002 ( $\alpha$ -amylase and glucoamylase), GC 212 (protease); [F]: <i>S. cerevisiae</i>	144 or $35.8 \text{ g / 100 g solids}$	Huang et al. 2015
Municipal food waste (household)	Enzymatic hydrolysis and fermentation (30% solids loading)	[S]: <i>M. thermophila</i> (cellulase) [F]: <i>S. cerevisiae</i>	$19.3 \text{ g l}^{-1}$	Matsakas and Christakopoulos 2015
Municipal food waste (household)	Hydrothermal acid pretreatment and Non-isothermal Simultaneous Saccharification and Fermentation (40% solids loading)	[S]: Celluclast 1.5 l (cellulase), Novozym 188 ( $\beta$ -glucosidase); [F]: <i>S. cerevisiae</i>	$42.7 \text{ g l}^{-1}$ or $10.7 \text{ g / 100 g solids}$	Alamanou et al. 2015

### 18.3.2.5 Coffee Residues

The production process of coffee generates a great amount of residues, which differ depending on the type of process followed. In particular, coffee berries are transformed into coffee beans with the wet or the dry processing method producing coffee pulp and mucilage or coffee husks as residues, respectively. Furthermore, during the production of instant coffee, spent coffee grounds (SCGs) are produced as process residues.

Coffee pulp represents 29% of the coffee berry on a dry weight basis (Murthy and Naidu 2012), while it is estimated that for every unit of coffee produced, 0.5 units of coffee pulp are generated (Roussos et al. 1995). It contains 23–27% (db) fermentable sugars (Woldesenbet et al. 2016), as well as proteins and minerals, but it also contains tannins and polyphenols that are toxic (Pandey et al. 2000).

Coffee husks account for 12% of the coffee berry on a dry basis (Murthy and Naidu 2012), have a low moisture content (15%) and are mainly composed of carbohydrates (72%) and proteins (7%) on a wet weight basis (Gouvea et al. 2009). Among polysaccharides, cellulose, hemicellulose, and lignin are present at equally high concentrations (Bekalo and Reinhardt 2010).

SCG are the solid residues produced during the extraction of soluble solids and volatile compounds from coffee powder with hot water or steam for the production of instant/soluble coffee (Mussatto et al. 2011a). It is estimated that one unit of SCG corresponds to 1.5 units of raw coffee and to 0.5 units of instant coffee (Pfluger 1975). SCG are also generated during coffee brewing, the process of soluble extraction from roasted coffee grounds with the use of hot water for the preparation of coffee drink. They have a high moisture content (80–85%) (Mussatto et al. 2011a) and are rich in carbohydrates, which constitute approximately half of the residue dry weight (Mussatto et al. 2011b). Hemicellulose, which is the main polysaccharide of SCG followed by lignin, is mainly composed of mannose and galactose, while xylose is found in very low or non-detectable concentrations (Choi et al. 2012; Mussatto et al. 2011b). Finally, SCG contain considerable amounts of protein, oils, and polyphenols (Kwon et al. 2013; Mussatto et al. 2011b,c).

Direct disposal of coffee processing residues is not advisable due to their toxic nature and their high organic load, which are responsible for serious environmental problems, if pretreatment is not applied (Murthy and Naidu 2012; Mussatto et al. 2011a). SCG is used in some cases as fuel in industrial boilers in order to exploit its high calorific power (Silva et al. 1998). Research is ongoing for the use of SCG in other applications with the aim to exploit its valuable characteristics and at the same time to address the limiting factors to their spread.

All the above-mentioned coffee residues constitute a good substrate for bioethanol production. An initial pretreatment step is necessary, though, in order to minimize hemicellulose and lignin (Redgwell et al. 2002). As it can be seen in Table 18.3, the majority of the researchers using this substrate have applied acid hydrolysis as pretreatment, while some of them applied complementary to this enzymatic hydrolysis. Choi et al. (2012) applied popping treatment and enzymatic hydrolysis, with the former to contribute in increasing the surface area of the substrate with the application of pressure and therefore, facilitating the latter.

However, there are cases where direct fermentation was applied (Bonilla-Hermosa et al. 2014; Gouvea et al. 2009), which presented quite satisfactory ethanol production yields without any pretreatment step. For the case of Bonilla-Hermosa et al. (2014) it

should also be noted that *Hanseniaspora uvarum* UFLA CAF76, an apiculate yeast isolated from coffee fermentation, was employed during fermentation, in contrast to most of the studies reviewed where *S. cerevisiae* was employed. Finally, Dadi et al. (2017) concluded that the use of cellulolytic enzymes (after acid hydrolysis) and of “lignocellulose yeast” resulted in approximately 7.5 times higher ethanol yields on average, compared to the use of commercial baker’s yeast only.

#### 18.3.2.6 Cheese Whey

Cheese is produced by coagulation of casein (the milk protein) in a way that traps milk solids and fat into a curd matrix. The curd is cut into cubes. The cheese yield is approximately 10%, with the remaining 90% being a liquid byproduct called “whey.” The liquid whey is separated and drained from the curd (Valta et al. 2015, 2017). Fifty years ago, cheese whey (CW) was considered as wastewater from the processing of cheese and it was mainly disposed along with wastewater. Today, cheese whey is used for the production of white or as a functional ingredient in food and pharmaceutical applications, and as a nutrient in dietetic and health foods (Valta et al. 2017). Apart from the aforementioned applications, there is an increasing interest in CW valorization for alcohol production. Bioethanol production through batch fermentation from ricotta cheese whey (scotta) was studied by Sansonetti and coworkers (Sansonetti et al. 2009). The yeast *Kluyveromyces marxianus* was used for the fermentation process under anaerobic conditions. The study has shown that scotta constitutes an excellent substrate for fermentation, with complete lactose consumption in 13 hours and high ethanol yield (97% of the theoretical value). CW bioethanol production through fermentation using *K. marxianus* biofilm has been also investigated concluding that such a process can be a promising solution for both environmental protection and energy production (Joshi et al. 2010). Another study using *K. marxianus* for fermentative production of bioethanol from CW was performed by Hadiyanto and coworkers (Hadiyanto et al. 2014), who examined the effect of various temperatures (30, 35, 40 °C) to the ethanol yield through fed-batch fermentation experiments. Results showed that the highest biomass and ethanol concentrations were achieved at 30 °C, with  $\mu$  (0.186/hour),  $Y_p/s$  (0.21 g/g), and  $Y_x/s$  (0.32 g/g). Moreover, the production of ethanol from cheese whey powder (CWP) using *K. marxianus* UFV-3 was also examined, (Diniz et al. 2014; Silveira et al. 2005). Diniz and coworkers concluded that using *K. marxianus* UFV-3 to convert lactose from CWP into ethanol is promising, since yields close to the theoretical values, were achieved, at a wide range of temperature and pH values (Diniz et al. 2014).

#### 18.3.3 Organic Fraction of Municipal Solid Waste

In this waste stream category, all food waste generated from the household and tertiary sector is included. As a consequence, these may include raw or cooked food discarded from households, cafeterias, restaurants, etc. The composition of food waste may vary considerably depending on the nutritional habits of people, the dietary patterns of a society, the food production availability in the area and the season, as well as on the type of source generating them, i.e. households or cafeterias and restaurants.

The management method of food waste also varies depending on the municipal waste management methods applied in each country and on whether there is in place a source separation system for food waste. For example, in many Eastern countries the main waste

management method applied is incineration, in some European countries the main method is landfilling, while in other countries where food waste is separated at source, such as in many northern European countries, other biological management methods are applied, e.g. composting and anaerobic digestion. However, the incineration method is not appropriate for this kind of waste due to its high moisture content and the associated environmental concerns, while the energy recovery potential is lower due to the energy consumed for removing the moisture. In the case where landfilling is exercised without any pollution control, it results in the emission of significant amounts of GHGs (and especially methane which is a very potent GHG), as well as in water pollution through leachate run-off. In addition, direct landfilling has started being banned in many countries during the last decade. On the other hand, the biological management methods may also cause soil and water pollution due to the leaching of substances from the application of the produced soil conditioner on land. Finally, the use of food waste as animal feed is mostly an informal management method applied directly by householders that breed animals, while the wider and centralized application of this method needs caution due to the safety and hygiene risks that it may imply.

The main characteristics of the processes applied within the studies reviewed on the production of ethanol from food waste are presented in Table 18.3. As it can be seen, the main method process applied is enzymatic hydrolysis followed by fermentation or SSF by *S. cerevisiae*.

Jeong et al. (2012) in their effort to convert both hexoses and pentoses that are not converted by *S. cerevisiae*, successfully used a combination of *Saccharomyces coreanus* and *Pichia stipitis* during fermentation, which are able to convert hexoses and pentoses respectively. However, given that the microorganisms require different growth conditions, *S. coreanus* was used during the first part and *P. stipitis* during the second part of fermentation. To further increase the efficiency of the process and ethanol yield, the liquid and solid residues of the fermentation stage have been recycled into the hydrolysis stage (Ma et al. 2009; Matsakas et al. 2014). Aiming in the decrease of high enzyme loadings required by the SSF process due to the different temperature optima for saccharification and fermentation, a non-Isothermal SSF was applied (Alamanou et al. 2015), which was suggested by Wu and Lee (1998) with a modification for adaptation to a fed batch mode. Huang et al. (2015) in order to address reduced ethanol yields caused by high solids loading and high ethanol concentration inhibiting yeast activity (Wang et al. 1999), applied vacuum stripping for the in situ ethanol removal (Shihadeh et al. 2014).

## 18.4 Environmental Sustainability

The production of biofuels has emerged from the need to decarbonize our economy in order to mitigate climate change and to restrain depletion of fossil resources. However, the production of bio-based products may sometimes lead to opposite results, unless attention is being paid so as the lifecycle impacts of the examined products are lower than those of the respective conventional products. Therefore, Life Cycle Analysis (LCA) is a useful tool for assessing the environmental performance of ethanol production processes and systems.

Mu et al. (2010) conducted a comparative LCA for the biochemical and thermochemical production processes of lignocellulosic ethanol. These two methods produce similar

ethanol yields and present the same energy efficiency at plant level. However, when examining it from a lifecycle perspective and taking into account all material and energy flow data, they found that the biochemical production method is slightly better than the thermochemical method with respect to the GHG emissions and the consumption of fossil fuels. On the other hand, the thermochemical method was superior with respect to water consumption. Furthermore, they showed that if the thermochemical process produced mixed alcohols for chemicals as well, then it would perform better in all impact categories examined due to the substitution of conventional chemicals.

Stichnothe and Azapagic (2009) found that bioethanol produced from biodegradable municipal waste results in significant GHG savings compared to petrol, as for a 95% biogenic carbon content in waste 92.5% savings are achieved, while for over 97% biogenic carbon content, the bioethanol system becomes a carbon sequester with over 100% savings over petrol.

Spatari et al. (2010) conducted an LCA for comparing emerging lignocellulosic ethanol conversion technologies. In general, they found that all examined technologies presented negative impacts (savings) with respect to fossil energy inputs and GHG emissions, while the technologies with lower ethanol yields performed better. This was attributed to the fact that the lower ethanol yield is associated with higher quantities of electricity as a co-product, since more of the feedstock is devoted to electricity production. As a result, it can be said that the credits achieved by the substitution of conventional coal-based electricity, outperform the credits from the substitution of conventional transport fuel.

MacLean and Spatari (2009) investigated the contribution of enzymes and process chemicals in the lifecycle performance of ethanol. Their research shows that while enzyme contribution for 1st generation ethanol is 3% of total fossil energy use and GHG emissions from the whole production process, this trend increases up to 30–40% for 2nd generation ethanol. Although the overall impacts from 2nd generation ethanol are substantially lower compared to 1st generation (three to sixfold), consumption of enzymes is higher, mostly because 2nd generation feedstock is more difficult to be hydrolyzed. Therefore, the development of enzymes with high specific activity and on-site enzyme production are among the solutions proposed.

## 18.5 Conclusions

Bioethanol production has been mainly affected by the policy context promoting the achievement of specific targets for reduction in fossil fuel consumption; penetration of renewable energy sources in the energy market; and climate change mitigation, while at the same time the technological readiness and viability of the related production processes have been also crucial parameters. Those considerations played an important role in increasing the interest toward 2nd generation bioethanol from waste sources. Based on the review conducted, several types of waste streams are currently investigated for their potential to produce bioethanol, since they are considered to have significant energy content that remains unexploited. Such feedstocks include lignocellulosic streams, such as the industrial food processing waste streams and the organic fraction of municipal solid waste. The lignocellulosic stream is the most frequently used and its exploitation mainly through the bioconversion but also through the thermochemical

process has currently reached the highest technological readiness level. On the other hand, the progress toward commercial valorization of other waste streams is still very limited. Among the challenges of the reviewed processes are the high energy and enzyme consumption, which in turn affects the environmental and economic sustainability of the process. Therefore, further study needs to be undertaken, particularly toward the utilization of food waste, which will resolve waste disposal problems aiming in a sustainable society.

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## 19

# Applicability of Agro Waste for Remediation of Chemical Contaminants in Water

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## 19.1 Introduction

The important natural source of water is an essential and major component for all living things in various ways. It is also an important constituent of all plants and animals. It covers almost 70.9% of the Earth's surface. On Earth, 96.5% of the planet's water is found in oceans, 1.7% in groundwater and 1.7% in glaciers and the ice caps of Antarctica and Greenland, a small fraction in other large water bodies, and 0.001% in the air as vapor, clouds and precipitation. Only 2.5% of the Earth's water is fresh water, and 98.8% of that water is in ice and groundwater. Less than 0.3% of all freshwater is in rivers, lakes, and the atmosphere, and an even smaller amount of the Earth's freshwater (0.003%) is contained within biological bodies and manufactured products. Its uses may include drinking and other domestic uses, industrial cooling, power generation, agriculture, transportation, and waste disposal. Interestingly, coastal zones and estuaries are the most productive ecosystems of the world, with equally high ecological and economic values (Emerton 2006). A coastal zone is the interface between the land and water which are important because a majority of the world's population inhabit such zones. Coastal zones are continually changing because of the dynamic interaction between the oceans and the land (Gazeau et al. 2004; Ramanathan et al. 2010). Within these zones, the coastal waters are of ecological importance for several reasons, perhaps the most important being that they support 25% of global primary production and 80% of global carbon production (UNEP 1992).

The water environment is disturbing a wide variety of pollutants that includes heavy metals, inorganic anions as well as organic compounds which are released by metal based and metal processing industries. Particularly, estuaries, rivers and their tributaries are major reservoirs of pollutants which are carrying enormous amounts of wastes of both natural and anthropogenic sources from upper stretches to coastal region (Singh 2001).

Unregulated direct disposal, discharge of partially and untreated waste waters from industries, accidental spills and surface run-offs cause pollution of the waterways, rivers and ultimately the coastal environment. These wastes affect the qualities as well as biodiversity of aquatic ecosystems.

Water quality is important in aquatic systems because its imbalances can cause stress, poor growth, and mortality of aquatic organisms (Boyd and Tucker 1998). Many factors influence water quality including climate and precipitation, soil type, geology, vegetation, groundwater, flow conditions, and human activities (Florescu et al. 2011). Water quality is mainly determined by physico-chemical and biological parameters of aquatic systems (Sachidanandamurthy and Yajurvedi 2006; Flo et al. 2011). Extensive land-use activities like agriculture, mining and urban development can also significantly impact water quality parameters. Properties of water such as temperature, pH, dissolved oxygen, and the concentration of nitrates and phosphates are important indicators of water quality. These properties of water can change as a result of natural and human related processes including discharges of industrial or agricultural wastes. Changes in these parameters may be detrimental to the organisms in and around the water source. These properties can be used to determine the effects of pollution on aquatic ecosystem health and can sometimes be used to identify sources of pollution in water.

Various techniques have been used for the removal of heavy metals from contaminated waters and these include: reverse osmosis, electro-dialysis, ultrafiltration, ion-exchange, chemical precipitation, etc. However, all these methods have disadvantages like incomplete metal removal, high reagent and energy requirements, generation of toxic sludge or other waste products that require careful disposal (Ahalya et al. 2003; Sannasi et al. 2006). With increasing environmental awareness and legal constraints being imposed on discharge of effluents, a need for cost-effective alternative technologies is essential. In this endeavor, Agro-waste byproducts have emerged as an option for developing economic and eco-friendly waste water treatment processes.

## 19.2 Determination of Chemical Contaminants in Aquatic Environment

Both terrestrial and aquatic environments have suffered due to the effects of heavy metal pollution (Nriagu and Pacyna 1988). Various types of pollutants released into the aquatic ecosystem may significantly affect the biodiversity, and in extreme cases, may lead to the destruction of whole ecosystems. The pollutants can be either in organic and inorganic form such as metals, metalloids, pesticides, herbicides, oil spills, etc. Amongst them, heavy metals are common water pollutants which are released by metal-based industries. Contamination is especially concentrated in coastal areas and aquatic environments, which are sometimes polluted by several heavy metals, because most industries such as those involving electroplating, plastics manufacturing, nickel-cadmium battery manufacturing, fertilizers, pigments, mining, and metallurgical processes are usually located in aquatic environments (Arica et al. 2001; Norton et al. 2004).

Heavy metals are persistent in nature, extremely low concentrations in fresh and marine water. Heavy metals accumulate in aquatic organisms, and concentrations in their body tissue can be hundreds of times greater than levels in surrounding water

(Kamaruzzaman et al. 2011). Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. They may enter the human body through food, water, air, or absorption through the skin when they come in contact with humans in agriculture and in manufacturing, pharmaceutical, industrial, or residential settings. Industrial exposure accounts for a common route of exposure for adults. Ingestion is the most common route of exposure in children (Roberts 1999). Thus, consumption of contaminated fish can be harmful; some well-documented examples are Hg, Cd, and Cu poisoning (Goldberg 1992). The outbreak of previously unknown severe bone pain diseases among the populations living around Toyama Prefecture, Japan was reported in 1930 (Kaji 2012). Lead poisoning, which is so severe as to cause evident illness, is now very rare. For as is known, lead fulfills no essential function in the human body, it can merely do harm after uptake from food, air or water. Lead is a particularly dangerous chemical, as it can accumulate in individual organisms, but also in entire food chains. Lead disturbs hemoglobin synthesis, renal function and causes neurological and behavioral disturbances in children (WHO 1995). Even low blood lead concentrations have been associated with intellectual impairment in children (Canfield et al. 2003).

Metals have been used in all aspects of human life. Several macro- and micronutrients are essential for all life forms although many others have not been assigned any biological function. The activities such as restricted mining, extensive industrialization, large scale urbanization, modern agricultural methods, and faulty waste disposal practices have resulted in the release of high levels of toxic pollutants including heavy metals in the environment. Based on the chemical distribution of metals, Zn is the most mobile, i.e. it can pass easily into the water under changing environmental conditions and showed the highest percentages in the acid-soluble fraction, especially in the central coastal area, where the samples contained over 50% of this element associated with this fraction. Later on, Singh et al. (2005) studied the concentrations of heavy metals in water and bed sediments of the Gomti river from a fairly long stretch of 500 km from Neemsar to Jaunpur. Based on the geo accumulation indices, the Gomti river sediments from Neemsar to Jaunpur are considered to be unpolluted with respect to Cr, Cu, Fe, Mn, and Zn. It is unpolluted to moderately polluted with Pb. In the case of Cd, it varies from moderately polluted to highly polluted.

Duman et al. (2007) studied the seasonal variations of heavy metal concentrations in water and sediment samples of Lake Sapanca, İstanbul, Turkey. Lake Sapanca is exposed to heavy urbanization and industrialization because of its natural beauty and its proximity. Heavy metal concentrations (Cd, Pb, Cr, Cu, Mn, Ni, and Zn) in this study area are varied seasonally. The river receives waste from the Ergani Copper Plant that is situated in this region. In general, the average Cd, Cu, Mn, Ni, Zn, and Fe values in study sites were found to be high in the spring and summer seasons. Jonathan et al. (2009) studied physicochemical parameters and dissolved trace elements (Fe, Mn, Cr, Cu, Ni, Co, Pb, Zn, and Cd) of Uppanar River water (RW) and surface and bottom coastal waters (CWs) off Cuddalore, southeast coast of India. High concentrations of Fe and Mn in RW and CW indicate enrichment above the normal level. Physicochemical parameters do not exhibit a significant relationship with trace metals. The results signify that industrial growth has affected the aquatic environments, and regular monitoring will help to adopt stringent pollution control measures for better management of the aquatic region. Later on, Chakraborty et al. (2009) investigated the metal concentration in the aquatic phase and underlying surface sediment from three stations (viz. Shankarpur, Canning,

and Bali Islands) of the coastal zone of West Bengal. The order of the heavy metal level in the ambient media of the selected stations is Zn > Cu > Pb. Highest concentrations of heavy metals were recorded in the surface water during monsoon and lowest concentrations in the pre-monsoon season. A correlation result elucidates a sharp exchange of selected metals between the aquatic phase and sediment in the study area. Kumar and Patterson Edward (2009) assessed the trace metal (Cr, Cu, Ni, Co, Pb, Zn, and Cd) concentration in the sediment cores of the Manakudy estuary, on the south west coast of India. The metal concentrations showed peak values at sulfide phase and it is related to anthropogenic activities. The correlation of trace metals with sulfur indicates that they were precipitated as metal sulfides.

Cevik et al. (2009) determined the heavy metal concentration in sediment samples of Seyhan Dam reservoir, Turkey. Correlation analyzes showed that metal content of Seyhan dam sediment was affected by organic matter and grain size. The results of geoaccumulation index reveal that sediments of Seyhan Dam were strongly polluted with Cd. Karbassi et al. (2008) studied the sedimentation rate, contents of heavy metals (Zn, Co, and Ni), and its speciation in sediment core from Gorgan Bay. Sedimentation rate in the study area has given an ample opportunity to track contents of Zn, Co, and Ni with different sedimentary phases for the past 500 years (1500–2002). Sedimentation rate of  $1.4 \text{ mm yr}^{-1}$  was obtained based on  $^{210}\text{Pb}$  activity study of sediment core. Chemical portioning studies revealed that percentiles and amounts of Zn, Ni, and Co in non-lithogenous phases increase slightly toward the top of the core sediment samples. Pandey et al. (2009) investigated the metal contamination of Ganga River in relation to atmospheric deposition. The concentration of Cr, Cu, Cd, and Pb remained below their maximum admissible concentrations. Metal concentrations in river water showed significant correlation and seasonal synchrony with atmospheric deposition. Akbulut and Akbulut (2010) studied the accumulation of heavy metals such as Pb, Hg, Co, Cr, Cu, Zn, and Br were determined in water, sediment, muscle, and gill of three fish species (*Leuciscus cephalus*, *Capoeta tinca*, *Capoeta capoeta*) which were collected in Kizilirmak River Basin (Delice River). Olubunmi and Olorunsola (2010) evaluated the status of heavy metal pollution in sediment of Agbabu Bitumen deposit area, Nigeria. Mercury was not detected in all the samples. The mean concentrations of the heavy metals in sediment ( $0.38 \pm 0.03$  to  $6619 \pm 290$  ppm dry season and  $0.24 \pm 0.05$  to  $8144 \pm 229$  ppm wet season) were lower than the values recommended in Consensus-Based Sediment Quality Guidelines of Wisconsin. The Geo-accumulation Index (Igeo) calculated in both dry and wet seasons gave values indicating no pollution to moderately polluted. Mohiuddin et al. (2011) investigated the spatial distribution, seasonal and temporal variations of different heavy metal concentration in water and sediment samples of Buriganga river of Bangladesh. Concentrations of total chromium, lead, cadmium, zinc, copper, nickel, cobalt, and arsenic in water samples were greatly exceeded. Enrichment factor values demonstrated that the lead, cadmium, zinc, chromium, and copper in most of the sediment samples were enriched sever to very severely. These extents of heavy metals pollution in the Buriganga river system implies that the condition is much more frightening and may severely affect the aquatic ecology of the river.

Prabhahar et al. (2011) studied the seasonal distribution of heavy metals in Vellar river, South east coast of India whereas; Laxmi Priya et al. (2011) studied the accumulation of six heavy metals (Cr, Cd, Cu, Zn, Pb, and Ni) in sediment, water and in tissue parts of *Mugil cephalus* and *Crassostrea madrasensis* was studied in two locations of Pulicat lake,

Southeast coast of India, which receives considerable quantity of effluents from industries located in North Chennai coastal region. Metals were highly concentrated in sediments when compared to water and biota. The geoaccumulation index ( $I_{geo}$ ) for Pulicat lake sediments indicate that the sediments are extremely contaminated with Cd and moderately contaminated with Cu and Ni. Deepulal et al. (2012) studied the distribution and accumulation of trace metals in the sediments of the Cochin estuary. The study indicated that the spatial variation for the metals like Mg, Cr, Fe, Co, Ni, Cu, Zn, Cd, and Pb were predominant unlike Mn which shows a temporal variation. The strong association of trace metals with Fe and Mn hydroxides and oxides are prominent along the Cochin estuary. The anthropogenic inputs of industrial effluents mainly control the trace metals enrichment in the Cochin estuary. Satpathy et al. (2011) studied the seasonal variations of heavy metals in the marine sediments off Kalpakkam, East Coast of India. The coastal areas of Kalpakkam is one of the most important and sensitive area in the India which hosts a nuclear power plant (Madras Atomic Power Station, MAPS) and a desalination plant. Alongside the canal, a large number of small- to medium-scale industries have begun in the recent past. Associated with these is the discharge from their outlet to the canal, which obviously contaminated the already polluted canal. Heavy metal values showed maximum variation for Fe and minimum for Cd.

Baisha Bay, Nan'ao Island, one of Guangdong Province's largest aquaculture bases in Southern China. The metal concentrations in surface sediments of Baisha Bay were (expressed in  $\text{mg kg}^{-1}$ ): 0.040–0.220 (Cd), 24.22–39.61 (Pb), 25.30–42.66 (Cr), 10.83–19.54 (Ni), 15.06–39.24 (Cu), and 55.12–141.73 (Zn), respectively. The highest concentrations and the greatest increasing rates of heavy metals were found in a sediment core in a fish cage culture area due to receiving sewage discharge, uneaten fish bait, and boat gasoline combustion. Cd was preferentially associated with the acid-soluble fraction and Pb mainly with the reducible fraction in surface sediments. Meanwhile, Cd and Pb displayed greatest labile fractions, indicating anthropogenic origin (Gu et al. 2012). Sany et al. (2012) investigated the temporal and spatial distribution of heavy metals in water and sediment samples of Port Klang, Malaysia. The contamination status of study area was estimated by contamination factor (Cf) and contamination degree (Cd) using the heavy metal concentrations data. Results show that concentrations of metals in sediment and water were significantly higher than the background values at which these metals are considered hazardous. The main sources of heavy metal contamination in Port Klang were industrial wastewater and port activities. Saygi and Yigit (2012) studied the distribution and accumulation of heavy metals in the water, sediments, plankton of Yeniçağa Lake, and its potential sources (creeks, sewage, artesian well, soil). Results showed that the trace and toxic elements (Al, As, Mn, Pb, and Fe) concentration in lake water and/or its feeding sources were above the recommended water standards (WHO 2004). It was found that the maximum accumulation of the heavy metals iron, aluminum, manganese, zinc, and barium in the sediment of Yeniçağa Lake. In addition to that, metals like Pb, Cd, Zn, Ni, Co, and Cu were analyzed and found in sediment. The presence of such metals might be due to discharge of industrial effluents, land-based anthropogenic inputs and municipal sewage through the Ennore estuary. The results of the present study suggested the need for a regular monitoring program of the Chennai coast which will help to improve the quality of near shore coastal environment (Ramanibai and Shanthi 2012). Kumar et al. (2013) studied the seasonal variations in quality of Sabarmati River and Kharicut canal with respect to heavy metal contamination in water and

sediment samples. The concentrations of heavy metals were found to be higher in the pre-monsoon season than in the monsoon and post-monsoon seasons in water samples. The pollution load index, contamination factor, and degree of contamination (Cd) in sediments were calculated to know the extent of anthropogenic pressures.

### 19.3 Possibilities of Bioconversion of Agro-Waste Using Microorganisms

New method of biological treatment and conversion of agro-waste with the use of intensively cultivated phototrophic microorganisms is reviewed in this part. Wastewaters from farming areas and safe utilization of manure and processing facilities are problematic impairing the development of agricultural production. Agricultural wastewaters are characterized by the high content of nitrogen (N) in the forms of ammonium and nitrate and phosphorus (P) thereby representing a considerable threat to the environment. Discharging of untreated agro- and farm waste are prohibited as it contamination of soils and causes eutrophication and water bodies with pathogenic microbes (Mulbry et al. 2008; Kim et al. 2007; Ntp 2001.). Current regulations allow the application of agricultural wastes as fertilizers only after chemical and thermal disinfection; further, biotechnologies are used for treatment of farm waste only rarely (Ntp 2001). However, multistage biological ponds including the aerobic microalgae ponds and biofilters with aerotanks, sand and/or gravel filters are used for biological treatment of farm waste in traditionally (Ntp 2001). Still, these methods are inefficient during cold seasons and their large land area requirement hinders their implementation. Few farms have efficient treatment facilities violating in many cases the operation regulations. The above-mentioned problems make apparent the necessity of alternative, more efficient technologies. In this regard, microbial-based bio conversion of agro-waste treatment represents a potent candidate.

The possibility of bio conversion of agro-waste treatment with photosynthetic microorganisms has been studied for more than half a century (Oswald and Gotaas 1957), but the history of the investigation of deep treatment of waste and wastewater with microalgae in closed photo bioreactors is relatively short. The microbial-based treatment technologies have certain advantages, such as the growth rate of the microbes and efficient sequestration of biogenic elements (Sivakumar et al. 2011). In addition, microbes provide the photosynthetic aeration with the photosynthetically evolving oxygen readily oxidizing organic molecules and supporting growth of heterotrophic bacteria consuming organic substances from the wastes (Munoz and Guiessse 2006). The treatment with photosynthetic microorganisms is highly environment friendly since it does not produce secondary wastes such as sludge.

Special attention should be paid to the ability of microbial aggregation to synthesize value added products such as essential polyunsaturated fatty acids including eicosapentaenoic acid (Cohen and Khozin-Goldberg 2010; Guschina and Harwood 2013), arachidonic (Crawford et al. 2003), linolenic (Wang et al. 2012). Microorganisms also synthesize carotenoid antioxidants, such as astaxanthin (Dhankhar et al. 2012) and  $\beta$  carotene (Takaichi 2011). The biomass of microorganism cultivated in wastewater is enriched with carbohydrates and lipids (Park et al. 2011) and it could be converted to

biodiesel, biohydrogen, and biogas (methane) (Georgianna and Mayfield 2012). The most promising microbial strains have a high growth rate, which is essential for the efficient nutrient removal (Olguin 2003). The ability of mixotrophic growth, rapid cell sedimentation, tolerance to eutrophic conditions, and accumulation of value-added compounds are also very significant in the selection of candidate strains. Unicellular chlorophytes were showed the highest tolerance to eutrophic conditions (Aslan and Kapdan 2006; Ruiz Marin et al. 2010). Representatives of the genera *Chlorella* and *Scenedesmus* commonly dominate phytoplanktonic communities in oxidation ponds (Kebed Westhead et al. 2006; Lincoln et al. 1996). Moreover, Cyanobacterial species also showed the highest nutrient removal in certain systems (Pizarro et al. 2006).

Further, the tolerance to highest concentrations of P, N, and organic components are varied widely in chlorophyte species. For example, *Chlorella vulgaris* outperformed *Chlorella kessleri* at sequestering P and N from waste water (Travieso et al. 1992) whereas *Scenedesmus obliquus* showed good growth than *C. vulgaris* (Ruiz Marin et al. 2010). Another green alga *Botryococcus braunii*, a significantly produced lipids and hydrocarbons, featured prompt growth in farm wastewater with higher nitrate concentration (Hoffman 2002). Cucumber and maize fertilized with microorganisms grown on dairy wastewater showed same yields as the same crops fertilized with the synthetic fertilizers; the concentrations of the available P and N were paralleled in both cases (Kim et al. 2007). In spite of the ability of microorganisms to accumulate heavy metals in significant quantities, there was no measurable build-up of heavy metal concentration in the soils fertilized with the microbial biomass (Wilkie and Mulbry 2002).

## 19.4 Different Parameters Involvement in Chemical Contaminant Biosorption

The bioremediation process of heavy metals from wastewater might be influenced by different kinds of physical and chemical factors, such as pH, temperature, initial concentration, biosorbent dose, biosorbent size, ionic strength, co-ions, etc. These factors regulate the overall biosorption process through affecting the uptake rate, selectivity and amount of heavy metals removed. Extensive research has been undertaken to investigate the effects of these operating parameters. These physical and chemical factors that are involved in biosorption are briefly discussed in this section.

### 19.4.1 Influence of pH

Among bioremediation process factors, pH played a significant role in controlling the biosorption of heavy metals. The pH values can affect the surfaces charge of agricultural waste-based biosorptions, the degree of ionization and speciation of heavy metals (Park et al. 2010). This pH dependence could be described by the involvement of functional groups in metal uptake (Kumar et al. 2012). As solution pH increases, the biosorptive removal of cationic metals also increases, whereas that of anionic metals decreases. However, at lower pH, the overall surface charge of agricultural waste-based biosorbents will be positive. The pH<sup>+</sup> ions compete effectively with the metal cations, causing a reduced biosorption capacity. When pH values high, the agricultural waste based biosorbents

surface becomes increasingly negatively charged which favors the heavy metal ions uptake due to electrostatic interaction. At very high pH, the biosorption stops and the hydroxide precipitation will starts (Taha et al. 2011; Njoku et al. 2011; El-Sayed et al. 2011). Similarly, Giri et al. (2012) reported the effect of pH ion on the removal of Cr (VI) metal using *Eichhornia crassipes* root activated carbon. It could remove Cr(VI) efficiently and removal factor increased from 41.22% to 85.52% for  $10\text{ mg l}^{-1}$ , 45.34–89.23% for  $50\text{ mg l}^{-1}$  and 50.23–92.24% for  $100\text{ mg l}^{-1}$  with the increase of pH value from 1.5 to 4.5. Taha et al. (2011) studied the significant role of pH ion in the biosorption of Cd(II), Pb(II), and Zn(II) ions using potato peels. These peels could remove the metals very efficiently and values are increased with increasing pH values from 2 to 6. Further, after pH 6, there was a decrease in metal ion biosorption. Later on, Reddy et al. (2011) studied the removal of Ni(II) with *Moringa oleifera* bark. They noticed that, under high pH ion conditions, the removal percentage of Ni(II) metal was very low. However, the adsorption rate was increased with the increase in pH value from 3.0 to 6.0 and further decreased in the range of pH between 7.0 and 8.0. Recently, Sivaperumal et al. (2017a,b) also reported that, the  $\text{Cs}^+$  ion biosorption using marine actinobacterium *Nocardiopsis* sp. 13H with different concentrations of pH (1–10) and it could remove the  $\text{Cs}^+$  ion very effectively at pH 8.0.

#### 19.4.2 Influence of Temperature

Bioremediation researchers have accomplished a huge number of studies on the effects of temperature on organic/inorganic uptake. The changes in solutions temperature affect not only distribution rate of metal ions but also the solubility of metal ions (Park et al. 2010). Based on surface functional groups of a given agricultural waste-based biosorbents, temperature has a given influence on the adsorption capability. However, it is a mutual conclusion of many reports that the influence of temperature is to a limited extent and only in a definite temperature range (Sahmoune et al. 2011). The biosorption process could be affected by temperature in different ways depending on the endothermic or exothermic nature of the remediation process. Many investigators are reported that biosorption processes are exothermic, which means that adsorption capacity is inversely proportional to the temperature (Sahmoune et al. 2011). Kumar et al. (2012) found that the biosorption of Cd (II) ion by cashew nut shell decreased from 80.13% to 74.32% with the rise in temperature from 30 to 60 °C. They recognized this to the decrease in surface activity of agricultural waste based biosorbents. Similar trend was noticed by El-Sayed et al. (2011) in case of Zn(II), Cd(II), and Mn(II) biosorption onto maize stalks. Giri et al. (2012) also reported the related trend in case of adsorption of Cr(VI) ion by the activated carbon originated from *E. crassipes* roots. They explored that the percentage removal of Cr(VI) increased from 79.24% to 92.24% as the temperature increased from 25 to 55 °C. In the same way, García-Rosales and Colín-Cruz (2010) observed that the adsorption of Pb(II) and Cd(II) by the stalk sponge of *Zea mays* increased 1.1–1.8 times with increasing temperature from 20 to 40 °C. Banerjee et al. (2012) reported similar observation in case of adsorption of Cu (II) by watermelon shell. They attributed this trend to either increase in number of available active sites or decrease in the boundary layer thickness surrounding the agricultural waste based biosorbents. Recently, Sivaperumal et al. (2017a,b) studied the difference of  $\text{Sr}^+$  adsorption rate at different temperatures and it was found a maximum ( $91.3\% \pm 1.2\%$ ) adsorption of

$\text{Sr}^+$  at 35 °C and further increasing the temperature values, the biosorption rate was gradually decreased.

#### 19.4.3 Effect of Biosorbent Dosage

Many researchers report that the removal percentages of heavy metal ions increase with increasing agricultural waste based biosorbents dosages. Some few examples are described here. Boota et al. (2009) studied that there was a remarkable decrease in the adsorption capacities of Zn(II) and Cu(II) as *Citrus reticulata* dose increased. They ascribed this effect to overlapping of adsorption sites leading to a decrease in the total surface area. Kumar et al. (2012) found that the Cd(II) ion removal increased quickly as cashew nut shell dosage increased. Further, the highest percentage removal of Cd(II) ion was 75.35% at the cashew nut shell concentration of 3 g l<sup>-1</sup>. They endorsed this behavior might be higher number of available adsorption sites presented in the agricultural waste-based biosorbents. Similarly, Gala and Sanak-Rydlewska (2011) also observed the maximum adsorption capacities of agricultural waste based biosorbents with higher doses.

#### 19.4.4 Effect of Ionic Strength and Co-ions

Generally, real industrial wastewater contains not only heavy metals but also other metal ions, such as  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{K}^+$ , and  $\text{Mg}^{2+}$ . Therefore, these metals are usually added into heavy metal solutions to explore the effects of ionic strength on the biosorption process of heavy metal ions. Reddy et al. (2010) explored that the presence of common metal ions like  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$  had no important effects on the biosorption of Pb(II) by *M. oleifera* leaves. However, El-Sayed et al. (2011) noted that a rise in ionic strength led to a decrease in the metal uptake ability. They concluded that this to the decrease in the activity of metal ions and the increase in concentration of competing cations. Moreover, Njoku et al. (2011) also observed that the effect of  $\text{Ca}^{2+}$  on the biosorption of the metal ions was higher significant than that of  $\text{Na}^+$  ion. They clarified this by the fact that the divalent  $\text{Ca}^{2+}$  ion had a higher affinity to the active sites and thus might compete more effective for binding sites than the monovalent of  $\text{Na}^+$  ions.

Diverse effects of co-existing ions on the biosorption of heavy metals are reported by different authors. Goyal and Srivastava (2009) observed that the removal percentages of heavy metals ions by *Z. mays* in single metal solutions (Cd 79.36%, Pb 87.34%, Cr 76.43%) and Ni 71.98% were higher than those in a multi-metal solution (Cd 73.72%, Pb 81.21%, Cr 68.91%) and Ni 64.03%. They concluded that this result was due to the competition between cations. However, García-Mendieta et al. (2012) reported the contrary results and the removal percentages of Mn and Fe from a binary system were similar to the values found in single systems. This behavior indicated that Mn and Fe did not compete for the adsorption sites on the green tomato husk. Chibani et al. (2012) studied the removal of arsenate using *Withania frutescens* and *Cyperus rhizome* plants. They observed that the presence of  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{Zn}^{2+}$  ions in the metal solution had no major effects. Therefore, authors are concluded that the interfering effects on the arsenate biosorption became stronger with the increasing valence of competing anions.

## 19.5 Agro-Waste Byproducts and Their Potential Application for Remediation

### 19.5.1 Cellulose, Chitin and Chitosan

Cellulose is a natural polymer and its nanomaterial forms are efficient to remove lead ( $Pb^{2+}$ ) and cadmium ( $Cd^{2+}$ ) from the aquatic environment (Yu et al. 2013). Conversion of carboxylic group in cellulose nanomaterial will produce sodiated carboxylate, which enhances the ability of metal chelating activity. In the same way Srivastava et al. (2012) also reported that the carboxyl group modified cellulose nanomaterial has 10% higher efficiency in the removal of nickel ( $Ni^{2+}$ ), cadmium ( $Cd^{2+}$ ), chromium ( $Cr^{3+}$ ), and lead ( $Pb^{2+}$ ) from the aqueous solution (Yang et al. 2014). Further Ma et al. (2012) reported that the cellulose nanofibers can remove  $167\text{ mg g}^{-1}$  of radioactive uranyl ions ( $UO_2^{2+}$ ) from the water. Hemicellulose was extracted by alkaline treatment from Pine wood, switch grass, and coastal Bermuda grass. These extracted hemicelluloses, which are used for the water desalination and heavy metal removal (Ayoub et al. 2013). Chitin is the most abundant polysaccharide extracted from crab shell, fish scale, and prawn scale which produced much cellulose. The nanostructured forms of chitin film from crab shell waste are potentially used for remediation (Pandharipande and Bhagat 2016). The chitin byproduct of chitosan has been used as an adsorption agent in water clarification system for the adsorption of dyes (Guibal et al. 2006; Sye et al. 2008; Rodrigues et al. 2008; Mouz-dahir et al. 2010) and heavy metals (Li and Bai 2002; Tran et al. 2010; Wan et al. 2010; Dinu and Dragan 2010; Li et al. 2013). Chitosan is cationically charged, and it can be exploited to remove the negative charged organic and inorganic impurities from water treatment system (Szygula et al. 2009). Further, Zeenat et al. (2013) also extracted the chitosan from prawn shell and used it for the wastewater treatment from ghee industry. The experiment showed that chitosan reduced 80.1% of chemical oxygen demand (COD), 91.8% turbidity, 72.5% of dissolved solid, and 73.7% of conductivity from the wastewater. Recently, chitosan-based metal particle composites have been used increasingly as an alternative adsorbent in water treatment, such as using metal oxides (Vaishnavi Sureshkumar et al. 2016), bimetals (Moradi et al. 2014), metals (Zainal et al. 2009), and magnetite (Thakre et al. 2010), to adsorb heavy metals and dyes from wastewater. In addition to that, chitosan-coated magnetite nanoparticles were prepared and used as bactericidal agent to remove organic contaminants and bacteria from water (Abd-Elhakeem and Alkhulaqi 2014). Moradi et al. (2014) and their research team were developed and testing of sorbent performance of chitosan-ZnO nanoparticle composites materials (as beads) for pesticide adsorption from aqueous solution of permethrin 25%. It was demonstrated that, the chitosan-ZnO nanoparticle beads had an excellent adsorption performance and its removal efficiency was increased from 49% to 99%. Based on the high sorbent capacity, these beads could explore a new biocompatible and eco-friendly strategy for pesticide removal and could be used in water treatment process.

### 19.5.2 Extra Cellular Polymeric Substance

Generally, microorganisms carry endogenous genetic, biochemical and physiological properties that make them perfect agents for pollutant remediation in soil and water. Microbes possess immense diversity and unique capacity to produce natural products

and their importance for biotechnology was broadly realized by the scientific communities recently. The structural diversity with various interesting properties of Extracellular Polymeric Substances (EPSs) produced by microorganisms could be explored for bioremediation applications. The EPS represent with different classes of macromolecules such as polysaccharides, proteins, nucleic acids, lipids and other polymeric compounds presented in the interior of various microbial aggregates (Wingender et al. 1999). Moreover, the EPS are mainly the high-molecular-weight secretions from microorganisms and the products of cellular lysis and hydrolysis of macromolecules. Humic substances may also be a key component of the EPS in sludge in biological wastewater treatment reactors, accounting for approximately 20% of the total amount (Frolund et al. 1995, 1996). Some organic matters from wastewater can also be adsorbed to the EPS matrix (Nielsen and Jahn 1999; Liu and Fang 2003). Several studies have shown that EPS also play a crucial role in biosorption of heavy metals and radionuclides (Brown and Lester 1979, 1982; Ledin 2000; Sivaperumal et al. 2017a,b). The sorption of heavy metals is part of a protection strategy against toxic effects (Decho 2000). When there are changes in the growth medium, such as the presence or not of Cu, cells of *Pseudomonas aeruginosa* respond by producing EPS which have large Cu binding capacities (Kazy et al. 2002). The EPS synthesis by cells resistant to Cu showed elevated Cu binding ability ( $320 \text{ mg g}^{-1}$  EPS) compared to that produced by cells sensitive to Cu presence ( $270 \text{ mg g}^{-1}$  EPS). Moreover, biofilms in the presence of Cu have a high EPS to cell ratio, suggesting that EPS production may provide an important defence strategy against toxic effects, perhaps through sequestering toxic copper ions (Keevil 2003).

### 19.5.3 Microbial Enzymes

To minimize industrial wastes, enzymes could constitute a novel alternative in terms of waste treatment. Nowadays, the evidence on the mechanisms of bioremediation-related enzymes from microorganisms has been extensively studied. Enzymes derived from marine microorganisms have more advantages when compared with other derived enzymes from plants or animals. It has various catalyzing activity, solvent and temperature stability, high production in less time and is easy to harvest. Such enzymes may have various industrial applications in the form of protease, amylase, and lipase enzymes (Kumar and Takagi 1999). In addition, enzyme facilitated practices are promptly gaining attention due to the less process time, energy input will be very low, less toxic, cost effective, and eco-friendly features (Li et al. 2012; Choi et al. 2015). Most of the enzymes are functioning for waste treatment such as amylases, amyloglucosidases, amidases, glucoamylases, cellulases, lipases, proteases, and pectinases (Riffaldi et al. 2006; Karigar and Rao 2011). Likewise, agarase, amylase, cellulase, carragenases, chitinase, lipase, and lignocellulase are also isolated from marine microorganisms and are used in the production of bioethanol and other purposes. Later on, Gao et al. (2014) reported the hydrolysis of enzymes and the strong track relation between maximum adsorption capacity and the enzymatic hydrolysis. Oxidoreductases enzymes, like manganese peroxidase, laccase, lignin peroxidase and tyrosinase catalyze or eliminate the industrial effluents of chlorinated phenolic compounds (Le Roes-Hill and Prins 2016). Recently, Karimi and Taherzadeh (2016) critically reviewed the pre-treatment of lignocelluloses and changes of the different properties during the adsorption and accessibility of the enzymes.

## 19.6 Agro-Waste Materials: Dye Removal Agents

Water contamination due to dyes constitutes a major problem and removal of these dyes from water is very expensive. To remediate this kind of contamination, an alternate solution is required, and the Agricultural waste materials have played significant role in this regard. Till now, various agricultural waste materials are being studied for the removal of different dyes from aqueous solutions at different operating conditions (Banerjee et al. 2012). Earlier, Sivaraj et al., (2001) reported that the orange peel can remove  $19.88 \text{ mg g}^{-1}$  concentration of Aid violet. Methylene blue adsorbed by various agriculture products like Guava leaf ( $185.2 \text{ mg g}^{-1}$ ),  $192.7 \text{ mg g}^{-1}$  by broad bean peel,  $289.26 \text{ mg g}^{-1}$  by durian shell (Ponnusami et al. 2008). Similarly, basic blue ( $17.3 \text{ mg g}^{-1}$ ) was removed by date pit and  $19.82 \text{ mg g}^{-1}$  removed by straw Banat et al. (2003). Later on, Bharathi and Ramesh (2012) reported that, *Citrullus lanatus* rind could remove the Crystal violet dye efficiently. Likewise  $90.09 \text{ mg g}^{-1}$  of direct red dye adsorbed by almond shell (Ardejani et al. 2008), Malachite green was removed  $149.25 \text{ mg g}^{-1}$  by oil palm fiber (Hameed and El-Khaiary 2008) and the  $55.5 \text{ mg g}^{-1}$  of reactive dye were adsorbed by peanut hull (Tanyildizi 2011). Moreover, an agricultural waste hazelnut shell and pineapple steam could remove Methylene blue from aqueous solution, without any pretreatment (Dogan et al. 2009; Hameed et al. 2009).

The sawdust materials remove the dye malachite green from aqueous solutions very effectively (Shukla et al. 2002; Garg et al. 2004). Similarly, beach wood sawdust adsorbed six reactive dyes from the water and the percentage removal of direct brown in acidic pH range between 4 and 7 (Dulman and Cucu-man 2009). Wang et al. (2008) studied the mechanism and maximum capacity of Malachite green and methylene blue dyes are adsorption by rice bran and wheat bran. These positively charged dyes are influenced by surface charges which manipulate the pH level in solution. In this study, authors suggested that higher level of dye was removed at high pH. Broad bean peel removed cationic dye (Methylene blue)  $192.7 \text{ mg g}^{-1}$  and ground nut shell was the good absorbent of malachite green. In this experiment 94.5% of malachite green dye was removed in 30 minutes by groundnut shell (Malik et al. 2007). Further, Ngoh et al. (2015) studied the removal of coomassie brilliant blue R-250 from the water treatment using the agriculture waste of *Parkia speciosa* pod. It showed highest rate of adsorption (83.4%) in acidic pH ranges from 5 to 0.10 at 70 minutes. Recently, Ghorbani et al. (2015) reported that, high purity silica nanoparticles from rice husk which has  $200 \pm 20 \text{ nm}$  in size with 97% purity and it could remove the metals very efficiently.

Enzymes have a number of applications in different fields and so, they can virtually catalyze a mixture of diverse pollutants from level of soluble toxic to insoluble non-toxic materials. The most relevant enzymes for the detoxification of organic pollutants are oxidative enzymes (Gianfreda et al. 1999; Duran and Esposito 2000; Torres et al. 2003; Gianfreda and Rao 2004; Rodríguez Couto and Toca Herrera 2006; Gianfreda et al. 2006). In addition, it is significant to gain knowledge about effective enzyme selection protocol and sequence based description for worldwide marine environments through metagenomic tools (Feller 2013), which may have promising novel biotechnological applications (Niehaus et al. 2011), in extreme conditions (Vester et al. 2014; Alcaide et al. 2015; Ferrer et al. 2016). The creation of industrially related enzymes from marine environs genomes has novelty and potential applications, supported by advanced technologies such as metagenomics and culturing (Drepper et al. 2014). In addition, biocatalysts both in

the form of immobilized or free enzyme, cell free system and complete cell catalysts was highly applicable for the remediation process (Schrewe et al. 2013; You and Zhang 2013; Jeon et al. 2015; Schmidt et al. 2015).

## 19.7 Conclusion

This chapter discusses the potential use biosorbents from agricultural waste-based for sequestering chemical contaminants in terms of their adsorption capacities, operating factors and different remediation methods. The use of these low-cost materials is suggested since they are relatively cheap or of no cost, easily accessible, renewable, and show high affinity for chemical remediation. Further, agricultural waste-based products can successfully eliminate heavy metals from contaminated water. Therefore, these agricultural waste products should be further explored for novel, cost-effective and more selective remediation process by the researchers in future.

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## 20

# Biopesticides and Biofertilizers: Types, Production, Benefits, and Utilization

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## 20.1 Introduction

It is well established that world population will reach nine billion by 2050. However, significant crop production to meet the increasing human population is yet to be achieved. Global sustainable food production is still a challenge for mankind while public concern over the impact of synthetic chemicals including fertilizers and pesticides both directly and indirectly on the environment and human health is alarming. In the past two decades, the global total area of organic farmlands has steadily risen with 50.9 million ha of land reportedly found under organic production (Willer and Lernoud 2017). The largest growth of organic land based on continent from 2006 to 2015 was recorded by Oceanic region, followed by Europe and Latin America. The current global trend shows a continuous rise in the proportions of organic producers, processors, and consumers throughout the world because of increased consumer awareness, pressure from environmental stewards and premiums paid for organic produce and organic products. Thus, the increasing high demand for organically grown food and its associated products by consumers is one of the major factors for the increasing research interests and utilization of biopesticides and biofertilizers.

The use of biopesticides and biofertilizers in agriculture enhance crop protection and productivity. It also minimizes the dependence on synthetic chemical pesticides and fertilizers, which are widely used in conventional agricultural systems throughout the world. In modern agriculture, where it seems difficult to achieve potential and stable yield, and to maintain soil natural productivity simultaneously, the twin approach of biopesticide and biofertilizer applications, have produced remarkable results. These include sustainable crop productivity, increased yield, and improved overall soil bio-physicochemical conditions. Hence, biopesticides and biofertilizers are invaluable concepts in integrated pest management (IPM), integrated nutrient management and nutrient cycling processes. Biopesticide is a modern crop protection tool used to reduce

crop damage or crop yield loss from weed, insects, diseases, and nematodes. These chapter will closely look at the various types of biopesticides and biofertilizers, their general production methods, mechanism of action and utilization in agriculture and benefits.

## 20.2 What is Biopesticides?

The term biopesticides defines compounds that are used to manage agricultural pests by mechanisms that involve specific biological effects rather than being used as broader chemical pesticides. These bioproducts contain biocontrol agents i.e. natural organisms or substances derived from natural materials such as animals, plants, bacteria, or certain minerals including their genes or metabolites specially formulated for use as a safe and effective pest controlling product. The United States Environmental Protection Agency (EPA) describes biopesticides as a broad term that includes naturally occurring substances that control pest (biochemical pesticide), microorganisms that control pests (microbial pesticides) and pesticidal substances produced by plants containing added genetic material. The added genetic materials are referred to as plant-incorporated protectants (PIPs). Other terms associated with biopesticide includes, biological control agents and biorationals. The United Nations Food and Agriculture Organization (FAO) explained that biopesticides include those biocontrol agents that are passive agents, in contrast to biocontrol agents that actively seek out the pest, such as parasitoids, predators, and many species of entomopathogenic nematodes. Biopesticides are largely microbial pathogens of the pest in need of control, which can be processed and marketed in the same way as chemical pesticides, and some have enjoyed considerable success in crop protection systems. The formulation of biopesticides is classified into solid formulation and liquid formulation. Comparatively, solid formulations increase crop yield but offer a low shelf-life while liquid formulations provide an increased shelf-life and are preferred to solid formulations.

The global market for biopesticide was estimated at USD 2466.7 million in 2015 and is expected to reach 3.2 billion in 2017. Notwithstanding the low toxicity and environmental impact levels, the adoption of biopesticides is constrained by high costs, scarcity, short shelf-life and high specificity. Consequently, it is unaffordable to most growers and potentially adds to the total cost of production. Increasing research and discovery and easing registration and licensing procedures in addition to subsidies will facilitate its adoption and reduce product cost to growers. Furthermore, an increase in awareness about advantages of biopesticides among growers in addition to the ease of access will further drive the market forward.

## 20.3 Major Biopesticide Companies

The major companies that produces commercial amounts of various biopesticides are: Bayer Crop Science AG, BASF SE, Certis LLC, Agraquest, Koppert Biological systems, Marrone Bio-innovations, and Valent Biosciences.

## 20.4 Benefits of Biopesticides

Biopesticides are pest management tools that are based on beneficial microorganisms (bacteria, viruses, fungi, and protozoa), beneficial nematodes or other safe biologically based active ingredients (Sharma and Malik 2012). They are a group of crop protection tool compatible with the IPM method (Chandler et al. 2011). They play a key role in crop protection, especially in areas where pesticide resistance, niche markets, and environmental concerns limit the use of chemical pesticide products. The most commonly used biopesticides are living organisms, which are pathogenic for the pest of interest. These include biofungicides (*Trichoderma*), bioherbicides (*Phytopthora*) and bio-insecticides (*Bacillus thuringiensis*) (Gupta and Dikshit 2010). The growing interests in the use of biopesticide can be linked to its associated benefits which include the following:

- 1) Ecological benefit; they are less toxic and harmful than conventional pesticides thus reducing exposure of consumers to regulated pesticides.
- 2) Target specific; designed to affect only the target pest and closely related organisms, in contrast to conventional pesticides that may affect other different organisms such as birds, insects, and mammals.
- 3) Environmentally beneficent; they are often effective in small quantities, decompose quickly, thereby resulting in lower exposure and limited adverse effects on the environment, flora and fauna and largely avoiding pollution problems.
- 4) Suitability; greatly reduces the use of conventional pesticides when used as a component of IPM programs while crop yields remain high (U.S. Environmental Protection Agency, EPA, 2017 [www.epa.gov](http://www.epa.gov)).

Additional benefits include the fact that biopesticides have the capacity to assist the maintenance of beneficial insect populations due to the nonhazardous application process and current use in resistance management programs (Hassan and Gökçe 2014). It can be surmised that the use of biopesticides provides farmers and growers with the opportunities to satisfy the needs of today's consumers, who are now well-informed and enlightened about their health and the foods they eat. To meet demands to produce first-rate vegetables and fruits, growers are now more than ever, mindful of the fact that consumers are aware that produce grown with little or no synthetic chemical inputs are safer to eat, healthier and friendlier to the environment.

## 20.5 Precautionary Measures in the Use of Biopesticides

The occurrence and use of synthetic chemical pesticides in crop protection is expected to continue. But it is likely the trend and intensity of use of synthetic chemical pesticides will be determined by external forces concern with human, animal, and environmental health. These forces will inevitably drive the growth of the global biopesticides market size as evidenced in the current wave of policies, Acts and regulations in many countries. In response to the rising demand for regulated food safety and quality, growers are encouraged to be proactive and adopt conservative practices without compromising crop productivity and quality. These practices and adoption of acceptable technologies are often backed by government and private funded multidisciplinary research in various jurisdictions.

## 20.6 Categories of Biopesticides

According to literature information (Gupta and Dikshit 2010; Mazid et al. 2011a, b; Sharma and Malik 2012; Moshi and Matoju 2017), biopesticides can be classified into three major categories based on the active substance present. These are microbial pesticides, biochemical pesticides, and PIPs. Each of these class of biopesticide has specific function as described below.

### 20.6.1 Microbial Pesticide

Microbial pesticides consist of microorganisms (e.g. bacteria, fungi, viruses, algae, and protozoans) as the active-ingredient. These classes of biopesticides have been successfully used in controlling insect pests, plant pathogens, and weeds. Though each microbial active ingredient is relatively specific for its target pest, microbial pesticides are relatively broad spectrum and can control many kinds of pests. For instance, there are fungi that kill specific insects while other fungi can control certain weeds. Microbial pesticides suppress pests through the production of a toxin specific to the pest that causes the disease to be controlled (Sharma and Malik 2012). Typically, the effect of microbial entomopathogens occurs by invasion through the integument or gut of the insect, which is usually followed by multiplication of the pathogen resulting in the death of the host such as insect pest. Studies have demonstrated that microbial pathogens produce insecticidal toxin important in pathogenesis. Most of the toxins produced by microbial pathogens were identified to be peptides (Burges 1981). These peptides vary greatly in terms of chemical structure, toxicity, and specificity.

The most widely used microbial biopesticide is the insect pathogenic bacterium, *B. thuringiensis* (Bt). It produces a protein crystal termed as the Bt δ-endotoxin during bacterial spore formation. This compound can cause the lysis of gut cells when consumed by susceptible insects (Gill et al. 1992). Binding of the Bt crystalline protein to the insect gut receptor determines the target insect species (Kumar 2012). Some Bt ingredients control moth larvae on plants, other Bt ingredients are specific for larvae of flies and mosquitoes ([www.epa.gov](http://www.epa.gov)). Other microbial insecticides include bioproducts based on entomopathogenic baculoviruses and fungi. Baculoviruses are highly selective, host specific, infecting mainly insects and some arthropods. They are divided into two genera; *Nucleopolyhedrovirus* (NPV) and *Granulovirus* (GV). Worldwide, there are approximately 13 NPV registered virus based biopesticides (Thakore 2006). In Brazil, NPV of the soya bean caterpillar *Anticarsia gemmatalis* was used on up to 4 million ha, i.e. approximately, 35% of the existing soya bean crops in the mid-1990s (Moscardi 1999). At least 170 different biopesticide products based on entomopathogenic fungi have been developed for use against at least five insect and acarine orders in the horticulture industry comprising greenhouse crops, fruits, and field vegetables as well as broad-acre crops. About half of all these bioproducts developed and supplied by companies in Central and South America as documented by De Faria and Wright (2007). Examples of microbial biopesticides that have been used against plant pathogens include *Trichoderma harzianum*, which is an antagonist of *Rhizoctonia*, *Pythium*, *Fusarium* and other soil-borne pathogens (Harman 2006). However, there is the need to continuously monitor microbial pesticides to ensure that they do not become capable of harming non-target organisms, including humans and other beneficial organisms.

## 20.6.2 Biochemical Pesticides

Biochemical pesticides are naturally occurring substances that control pests by non-toxic mechanisms. In Canada, this class of biopesticide is referred to as semiochemical (Agriculture and Agri-Food Canada 2018). They may originate from plants, animals, or insects. These classes of biopesticides include substances that interfere with mating and population build-up. The control is manifested when signals that supposed to lead to behavioral response is rather sent to another organism. An example of this is the insect sex-pheromones used in monitoring traps (Agriculture and Agri-Food Canada 2018) and various scented plant extracts that attract insect pests to traps (Mazid et al. 2011a, b). Secondary metabolites produced by plants deter herbivores from feeding on them and thus, are used as biopesticides. A common example of such secondary metabolites are pyrethrins, which are fast acting insecticidal compounds produced by *Chrysanthemum cinerariaefolium* (Silvério et al. 2009). The most widely used botanical compound is neem (*Azadirachta indica*) oil, which is an insecticidal extracted from the seeds of the neem tree (Schmutterer 1990). The neem tree supplies at least two organic compounds that kill insects, namely; azadirachtin and salannin. Azadirachtin acts as an insect feeding deterrent and growth regulator. Azadirachtin treated insects will be unable to molt to the next life stage and die within a few days. Azadirachtin acts as a repellent, primarily when applied to a plant and may kill an insect within 24 hours (Buss and Park-Brown 2002). Commercial Azadirachtin products from this category include Spined Soldier Bug Attractors™, which is an aggregation hormone of *Podisus maculiventris* can be used against *Leptinotarsa decemlineata* (Aldrich and Cantelo 1999). Some insect growth regulators have been identified for use against stored products pests either alone or in combination with insecticides, for example, methoprene and hydroprene against *Sitophilus oryzae* Linnaeus, *Sitophilus granarius* Linnaeus (Gupta and Mkhize 1983).

## 20.6.3 Plant-Incorporated Protectants

PIPs are biopesticidal substances produced by plants from genetic material that has been added to the plant. It is also referred to as the non-conventional pest control product (Agriculture and Agri-Food Canada 2018). For example, a gene for a specific Bt pesticidal protein can be introduced into the plant genetic material. Subsequently, the plant, instead of the Bt bacterium, manufactures the pesticidal protein which makes it resistant to pest attack. The production of transgenic plant that express insecticidal δ-endotoxins derived from the soil bacterium *B. thuringiensis* (Bt plants) were first commercialized in the US in 1996 (Usta 2013). The expression of these toxins conferred protection against insect crop destruction. According to the study by Zhang et al. (2006), the lethality of Bt endotoxins is seen to be highly dependent upon the alkaline (i.e. high pH) environment of the insect gut; this is a feature that assures that these toxins are not active in vertebrates, especially in humans. These proteins or genes have been commercially produced and incorporated into some crops. Examples of these crops include corn (*Zea mays*), rice (*Oryza sativa*), soybean (*Glycine max*), tobacco (*Nicotiana tabacum*), sugarcane (*Saccharum officinarum*), potato (*Solanum tuberosum*), alfalfa (*Medicago sativa*), tomato (*Solanum lycopersicum*), brassica (*Brassica* spp.) and cotton (*Gossypium hirsutum*). These allow greater coverage by reaching locations on plants that are inaccessible to foliar sprays (Usta 2013). Other examples of this class of biopesticides are plant extracts,

oils, fertilizer, plant growth supplements and inert materials (Agriculture and Agri-Food Canada 2018).

## 20.7 Types of Biopesticides

There are four major types of biopesticide based on its use and target pest. These different types of biopesticides, namely: bioinsecticides, biofungicides, bioherbicides, and bionematicides are described below.

### 20.7.1 Bioinsecticides

Among all the biopesticide groups, bioinsecticide is one of the most studied biological control tool. Several microbes such as bacteria (*Bacillus*, *Enterobacter*, and *Pseudomonas*; Sharma and Malik 2012), fungi (*Beauveria*, *Aschersonia*, *Coelomomyces*; Rao 2016), viruses (Granulosis viruses, Nuclear polyhedrosis viruses, NPV; Senthil-Nathan 2015) and plant-based extracts (neem oil) have been tested for their insect control abilities (Sharma and Malik 2012). Leading in the area of bioinsecticide studies is the use of the bacteria *B. thuringiensis* (Bt). Bt has shown insecticidal activity against more than several insects including cabbage (*Brassica oleracea* var. *capitata*) worm, grape (*Vitis vinifera*) leaf folder and bollworm (*Pectiniphora gossypiella*). Bt produces different classes of toxin or proteins which act against insects and a number of products have been formulated from these Bt toxins (Kumar 2012). Similarly, entomogenous fungi have successfully been used to protect crops from insects both in storage and on the field. Example is the use of *Verticillium lecanii* for the control of whitefly and aphids, and *Beauveria brassiana* for Colorado potato beetle control (Mazid et al. 2011a, b). The virus, NPV (Baculovirus), has also been found to be very effective in the management of butterflies and moths (Senthil-Nathan 2015).

### 20.7.2 Biofungicides

Biofungicides is the general name given to microorganisms and naturally occurring compounds that possess the ability to control plant diseases specifically, fungal pathogens. Several biocontrol agents, especially fungi and bacteria, have demonstrated great potential for fungal control both on the field and in storage. Among the biofungicides, *Trichoderma* spp. is the most studied and have demonstrated effective control of different pathogens on different crop species. Other fungi demonstrated to control plant diseases include *Clonostachys* spp., (Reeh and Cutler 2013; Borges, et al. 2015), *Gliocladium* spp. (Elmhirst et al. 2011), *Penicillium* sp. and *Aureobasidium pullulans* (Schmid et al. 2011; Parafati et al. 2015). *Bacillus* spp., *Streptomyces* spp. and *Pseudomonas syringae* are also well-known bacteria that are effective against fungi. Several plant extracts (*Allium* spp. and *Capsicum* spp.; Wilson et al. 1997), essential oils such as peppermint (*Mentha piperita*) and sweet basil (*Ocimum basilicum*) (Ziedan and Farrag 2008), proteins such as Banda de Lupinus albus doce or BLAD from sweet lupine (*Lupinus* sp.) plants have been demonstrated to effectively control plant diseases (Monteiro et al. 2015). A number of commercial products have been developed from various fungi, bacteria, and plant-based products for the control of various diseases in agriculture.

### 20.7.3 Bioherbicides

Bioherbicides are plant pathogenic microorganisms or microbial plant toxins beneficial for biological weed control applied in similar ways to conventional herbicides. Several fungi and bacteria have been demonstrated to control weeds effectively. In biological weed control, fungi are the most used, hence, in some cases bioherbicides are referred to as mycoherbicides. One of the leading and most successful weed management tools is the use of the rust fungus, *Puccinia chondrillina*, for the management of rush skeleton (*Chondrilla juncea*) in Australia and the United States (Cullen et al. 1973; Emge et al. 1981). Also, *Fusarium oxysporum* was effectively used to control prickly pear cactus (*Opuntia ficus-indica*) in Hawaii (Zvonko 2015). Pathogenic bacteria including *P. syringae* pv. *tagetis* and *Xanthomonas campestris* pv. *poannua* have also been used to control weeds such as annual bluegrass (*Poa annua*) in bermudagrass golf greens and Asteraceae weeds (i.e. Canada thistle, *Cirsium arvense*; common ragweed, *Ambrosia artemisiifolia*; and Jerusalem artichoke, *Helianthus tuberosus*; Johnson et al. 1996). Also, essential oils and extracts from plants such as eucalyptus (*Eucalyptus* spp.), rosemary (*Rosmarinus officinalis*) and Lawson cypress (*Chamaecyparis lawsoniana*) have demonstrated significant weed inhibition against amaranth (*Amaranthus retroflexus*) and knapweed (*Acropitilon repens*) (Ramezani et al. 2008). Like bioinsecticides and biofungicides, a number of commercial bioherbicides have been developed for weed management.

### 20.7.4 Bionematicides

Several microbes, especially fungi and plant-based compounds have been studied and well known for their nematode control abilities. Fungus such as *Paecilomyces lilacinus* (Hashem and Abo-Elyousr 2011), *Muscodor albus* (Riga et al. 2008), *Syncephalastrum racemosum* (Huang et al. 2014), *T. harzianum* (Sahebani and Hadavi 2008) and *Dactyliella oviparasitica* (Olatinwo et al. 2006) have been studied and used as bionematicides. Plants extracts from *Cucumis myriocarpus* (Mashela 2002; Mashela et al. 2011), lemon grass (*Cymbopogon citratus*), Siam weed (*Chromolaena odorata*), castor bean (*Ricinus communis*) (Adegbite and Adesiyen 2005), *Tagetes erecta* (African marigold), *Allium sativum* (garlic) and neem (*A. indica*) (Abo-Elyousr et al. 2010) have demonstrated nematicidal properties. A notable example is the use of *A. indica* in the control of root-knot nematode in soybean. This plant extract demonstrated 100% inhibition of egg hatch and larval mortality (Adegbite and Adesiyen 2006). In biological nematode control, the cultivation of specific plants such as marigold (*Tagetes* spp.) can also be used to control nematode such as root-knot in the soil at the pre-planting stage to protect the main crop (Hooks et al. 2010).

## 20.8 Mode of Action of Biopesticides

Understanding the mechanisms of biological control of pest through the interactions between biocontrol agents and their target pests or the effect of natural compounds on pests can allow us to employ strategies that will create conditions conducive for successful biocontrol or improved biocontrol of pests. The modes of action of some biopesticides are well understood while others are understudied. Biopesticides are associated

with interconnected and puzzling collection of mechanisms in achieving pest control. The various mechanisms employed by biopesticides in control of pests are described in brief below.

### 20.8.1 Production of Toxins and Antibiotics

Meticulous and controlled production of toxins is the most important step in the biological pest control scheme. Many biopesticides such as Bt produces different classes of compounds that are toxic to the target pests. For example, Bt produces a crystalline protein that is toxic to the target pests (Whalon and Wingerd 2003). The Bt crystalline protein binds to receptors in the gut of the insect pest after ingestion leading to activation of cell death pathways (Kumar 2012). Several phytotoxins are produced by microbes with herbicidal prospective. For example, *Phoma macrostomina* produces phytotoxic metabolites (tetramic acids) which causes bleaching and chlorosis when applied to broadleaf species (Graupner et al. 2006).

Antibiosis or production of antibiotics is another well-known mode of action of biopesticides. These antibiotics act directly or indirectly on pests. Compounds such as Bacillomycin D, and Mycostubilin (Leclère et al. 2005) have been identified with *Bacillus*. Three types of antibiotics are known to be produced which are volatile, non-volatile and water soluble (Junaid et al. 2013). Among the types of antibiotic, volatile is the most effective one as it diffuses to act away from the point of production (Junaid et al. 2013). The production of toxins and antibiotics are mostly associated with bacteria biopesticide. However, a few fungi including *Trichoderma* spp. have been identified to produce antibiotics (Wilhite et al. 2001).

### 20.8.2 Competition for Space and Nutrients

Molecules of biopesticides, especially microbial pesticides occupy the same host surfaces, which brings about competition with nutrients for the same location. This mechanism was identified as one of the common mode of actions of biopesticides. The biopesticides are adapted to this competition and as such, they (fungal or bacteria sources) rapidly proliferate and colonizes host surfaces and wound areas. Fungal mycelia also form loops and mesh-like web in the soils which traps and prevent the free movements of nematode (Siddiqui and Mahmood 1996; Zhang and Hyde 2014). The colonization of host surfaces and soils by biopesticides, deprives pest of establishing themselves in available spaces and thereby, creating unfriendly conditions for the target pests to multiply. The proliferation of biopesticides on host surfaces is accompanied by an increased demand for nutrients since the host is deprived of nutrients. Thus, due to the rapid growth and uptake of nutrients by the biopesticide, pests, and pathogens are prevented or deprived of nutrients leading to their growth disruption and subsequent demise. For effective use of biopesticide to compete with the target pests, the biopesticides should adapt to different environmental and nutritional conditions for rapid growth and colonization.

### 20.8.3 Parasitism

Some biopesticides act by directly parasitizing on the target pest. For example, entomopathogenic fungi such as *Lecanicillium dimorphum* and *Lecanicillium cf. psalliotae* have

been found to parasitize on *Phoenicococcus marlatti* on date palm (Asensio et al. 2005). Entomopathogenic fungi identify and attack insects through fungal spore adhesion and formation of appressoria that penetrate the insect cuticle (Hajek and Leger 1994). Upon successful entry into the body cavity (hemocoel) of an insect, fungal filaments go through budding for rapid propagation. *Trichoderma* spp. are also known to act on other pathogens including nematodes by degrading and feeding on them for their growth (Sahebani and Hadavi 2008). Viral biopesticides basically parasitize on their host when they are ingested by insects. The virus then spread throughout the insect's body. However, in some insects, infection may be limited to the midgut or body fat (Senthil-Nathan 2015).

#### 20.8.4 Other Modes of Action

Although numerous studies have pointed out the antimicrobial activity of plant extracts and essential oils on different pests, the precise mode of action of these essential oils and plant extracts are still unclear (Tajkarimi et al. 2010). They certainly vary from source to source given diversity in the chemical compositions of the different plant species. These differences can be ascribed to influential factors in the growing environment and management practices. Nevertheless, it is thought that essential oils act on the cytoplasmic cell membranes of microbes. Due to the hydrophobic nature of essential oils, it is believed that they accumulate in cell membranes of pests. Therefore, disrupting the cell structure and increasing permeability, leading to leakage of cell constituent (Diao et al. 2014). Several other modes of action have been identified with bio pesticides some of which are specific. These include, the production of attractants, adhesives, allelopathy, and induced resistance.

### 20.9 Production of Biopesticide

Presently, several biopesticides are commercially available for pest control in various fields. Nevertheless, their production and commercialization are similar to any multi-step processes, which involve a wide range of activities. Rules and regulations governing commercialization vary according to the active ingredient (microbial or biochemical) and from country to country. In view of this, it is important to be aware of specific regulations governing commercialization of biopesticides in one's region or country.

Activities involved in the production of biopesticides are:

- isolation and identification of the target microorganism from the natural environment;
- evaluation of the target microorganism *in vitro* and under controlled environment conditions;
- identification and testing of the best isolate under field conditions; d) mass production;
- product formulation;
- test of the delivery mode;
- compatibility test; and
- product registration and release (Junaid et al. 2013).

The major obstacles to the production of biopesticides are encountered mostly after the identification of the best isolates for pest control. Mass production and formulations are very important stages in this process and can have great impact on the outcome of the final product. Biopesticides have been positive and successful, but there are other challenges that are associated with its use on commercial fields. These include product formulation, storage, and inconsistency between laboratory and field results. Though there are some challenges to the use of biopesticides including product formulation, storage and inconsistency, these do not prevent the use of biopesticides for disease management.

## 20.10 What is Biofertilizer?

Biofertilizers are biological formulations that composed of living or potent microbial cells that has the potential to improve plant nutrient bioavailability and bioaccessibility through enhancement of plant nutrient uptake when added to the growing medium, i.e. soil and soil-less media. A common carrier for the dry form of biofertilizer is lignite, a material with high organic matter and a water-holding capacity of more than 200% with respect to its dry weight. The availability and efficacies of biofertilizers are affected by many known and unknown, and direct and indirect factors. Consequently, although cost of production of biofertilizers is generally low, research and development are limited by poor quality biofertilizer products, lack of high potent multifunctional biofertilizer products, lack of suitable microorganism carrier and incompatible microbial isolates, and little to no field research under different soil, plant, and economic situations.

Globally, the market share for biofertilizer was estimated at US\$ billion in 2011. This figure is expected to double between 2017 and 2022 (<https://www.bio-fit.eu/q9/lo10-bio-fertilizers-technology-%E2%80%93-awareness,-marketing-and-future?showall=&start=3>). The global biofertilizer market is divided into two major classes; namely, organic residue-based and microorganism-based biofertilizers, which is dependent on the source of nutrients and raw materials. More than half of the market share is covered by the latter. For example, the nitrogen-fixing bacteria, *Rhizobium* and *Azotobacter*, are the major subsector and widely applied in agriculture. This group of bacteria-based biofertilizers account for the major market share. Organic residue-based fertilizers are classified into farmyard manure, crop residues, and green manure depending on the source of the feedstock or raw material. Biofertilizer is largely applied to cereal crops seconded by horticultural crops (i.e. fruits and vegetables).

## 20.11 Precautionary Measures in the Use of Biofertilizers

The rigorous regulatory framework for maximum residue level and growth in organic farming make Europe a major market, which accounts for approximately 40% of the global organic fertilizer consumption. Overall, research has proven that biofertilizers have no known harmful effects on soil-living organisms and environment. It contains microorganisms that help in the restoration of the natural soil fertility status through

healthy nutrient cycle processes and building of soil organic matter. The beneficial microorganisms in biofertilizers can be referred to as plant growth-promoting rhizobacteria due to the functions they play in plant growth. Many species of microorganisms are used to formulate biofertilizers depending on the types of biofertilizer as described in detail below.

## 20.12 Major Biofertilizer Companies

The major companies that produces commercial amounts of various biofertilizers are: CBF China Bio Fertilizers A.G., Labiofam S.A., and Novozymes A.S.

## 20.13 Types of Biofertilizers and Mode of Action

The major types of biofertilizers are nitrogen fixing, phosphorus solubilization, potassium solubilization, zinc solubilization, and iron sequestration biofertilizers. Each of these biofertilizers has its purpose and functions in plant growth and development and must be used appropriately.

### 20.13.1 Nitrogen Fixing Biofertilizer (NBF)

Nitrogen (N) is an important nutrient element for plant growth and productivity. It promotes vegetative growth and is a constituent of chlorophyll. The atmosphere contains 78% of N<sub>2</sub> that is unavailable for plant use. For plants to utilize atmospheric N, N needs to be converted to an utilizable form (NH<sub>3</sub>) via biological N-fixation (Tairo and Ndakidemi 2013). Nitrogen fixing biofertilizer (NBF) contains bacteria called *Rhizobium* (N-fixer) from the family of *Rhizobiaceae*, which can help convert atmospheric N for plant usage. *Rhizobium* is symbiotic in nature and can fix 50–100 kg N/ha per year (Mahdi et al. 2010; Mazid and Khan 2014; Majumdar 2015). This group of bacteria is common to leguminous crops alone. The *Rhizobium* enters the root hairs, multiplies and colonizes the roots of legumes leading to the formation of root nodules (i.e. tumor-like growth), which helps in ammonia production. Furthermore, *Rhizobium* has been found to fix atmospheric N in symbiotic association between legumes and non-legume plants (Saikia and Jain 2007). For instance, *Rhizobium* isolates (*Rhizobium oryzae* sp. nov.) from wild rice (*Oryza alta*) have been shown to supply N, promote growth and development of rice plants. They also reported that the same strain of *Rhizobium* can help nodulate *Phaseolus vulgaris* (common bean) and soybean.

In many crops, *Rhizobium* inoculation have shown to significantly improve crop yields such as lentil (*Lens culinaris*), pea (*Pisum sativum*), alfalfa and sugar beet (*Beta vulgaris*) rhizosphere, berseem (*Trifolium alexandrinum*), groundnut (*Arachis hypogaea* L.) and soybean (Grossman et al. 2011; Sharma et al. 2011; Mazid and Khan 2014). *Sinorhizobium meliloti* 1021, a subspecies of *Rhizobium* has also been found to infect plants other than legumes such as rice (Chi et al. 2010). The population of these bacteria was reduced by the absence of legumes in the field, which suggested the need for artificial inoculation

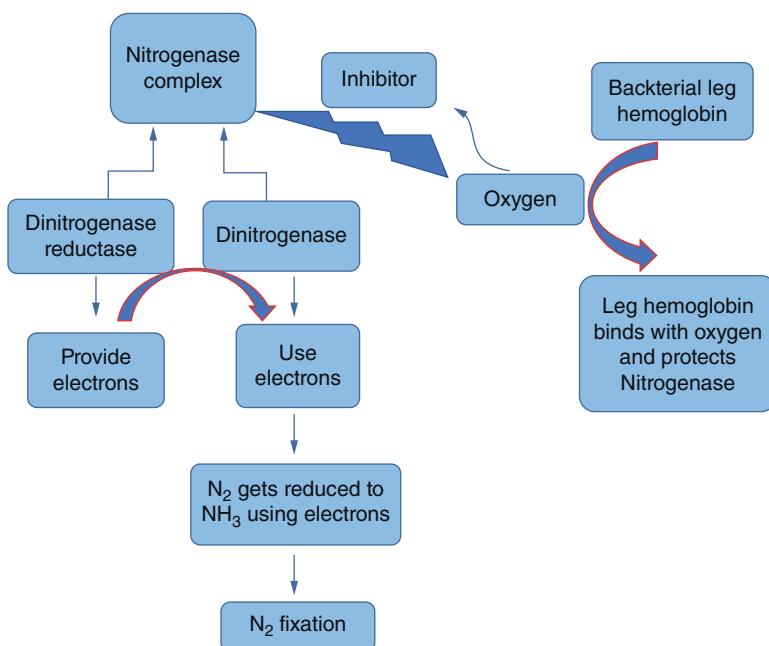
to restore the *Rhizobium* population near the root zone (rhizosphere) of non-leguminous plants to promote N-fixation. This can be achieved through the use and/or application of NBFs.

Apart from the *Rhizobiaceae* family, there are several other bacteria families that are known N-fixers. The *Spirillaceae* family are heterotrophic and associative in nature and can be beneficial to non-leguminous plants, especially plants with the C4-dicarboxylic pathway of photosynthesis (Mahdi et al. 2010; Mehnaz 2015). A member of the *Spirillaceae* family, *Azospirillum*, are free-living, motile and aerobic bacterium that can survive in flooded conditions. *Azospirillum* can fix approximately 20–40 kg N/ha per year. They can promote plant growth and development by improving lateral roots and root hair formation to provide more root surface area for adequate nutrients absorption (Mahdi et al. 2010). As a result, the water status and nutrient profile of the host plants are enhanced (Sarig et al. 1992). Among this genus, *Azospirillum lipoferum* and *Azospirillum brasiliense* are globally distributed and have shown to be beneficial. For example, a study conducted in India to evaluate the potential of non-symbiotic bacteria diazotrophs as a plant growth promoter in crop farming systems (Saikia et al. 2013) revealed that inoculation of *A. lipoferum* and *A. brasiliense* improved leaf area index and yield characteristics. Also, in India, root immersion of rice seedling in 2% solution of *Azospirillum* inoculant led to 100 kg/ha yield increase of rice plants (Sahoo et al. 2014). *Azospirillum* can survive, grow, and fix N on malic and aspartic acids. In crops like maize (*Z. mays*), sorghum (*Sorghum bicolor*), sugarcane and pearl millet (*Pennisetum glaucum*), the *Azospirillum* colonizes the roots and penetrates the root tissues to establish a mutual relationship with the plants without producing visible nodules or growth (Mahdi et al. 2010).

The mechanism of molecular N<sub>2</sub>-fixation by rhizobacteria is presented in Figure 20.1. The process of N<sub>2</sub>-fixation is achieved via the nitrogenase enzyme complex (dinitrogenase reductase and dinitrogenase). Electrons are provided by dinitrogenase reductase while dinitrogenase uses the electrons to reduce N<sub>2</sub> to NH<sub>3</sub>. Enzymes can bind to oxygen thereby becoming inactive (Mahanty et al. 2017). However, the bacterial leg hemoglobin binds more effectively to free oxygen due to its stronger affinity for oxygen. This enables the enzyme complex to remain active (Figure 20.1). Examples of *Azospirillum* species also includes *A. amazonense*, *A. halopraeferens*, and *A. irakense* (Mishra et al. 2013).

Other important groups of N-fixers include those belonging to the *Azotobacteriaceae* family (*Azotobacter*), *Cyanobacteria* (Blue Green Algae [BGA]) and *Azolla*. *Azotobacter* plays a key role in the N-cycle and metabolic functions. They are free-living non-symbiotic bacteria existing in neutral or alkaline soils. They are also aerobic and heterotrophic in nature. The most commonly occurring species is the *Azotobacter chroococcum*, which can be found in arable lands in tropical, temperate, arctic, and/or Mediterranean regions (Mahdi et al. 2010). However, species like *Azotobacter vinelandii*, *Azotobacter beijerinckii*, *Azotobacter insignis* and *Azotobacter macrocytogenes* have also been reported. *Azotobacter* has been found to fix 15–20 kg N/ha per year (Majumdar 2015). Besides N-fixation, they also produce certain plant hormones such as indole acetic acid, gibberellins, and cytokinins, which promote plant growth via enhancement of seed germination and advancement of root architecture.

In addition, they can produce vitamins (e.g. thiamine and riboflavin; Revillas et al. 2000) and anti-fungi antibodies that help in inhibiting pathogenic microorganisms such as *Fusarium*, *Alternaria*, and *Helminthosporium*, which can be found around the root system of plants (Subba Rao 2001; Arun 2007; Mahdi et al. 2010). However, the



**Figure 20.1** Schematic diagram showing the mechanism of molecular  $N_2$ -fixation by growth promoting rhizobacteria Mahanty et al. (2017).

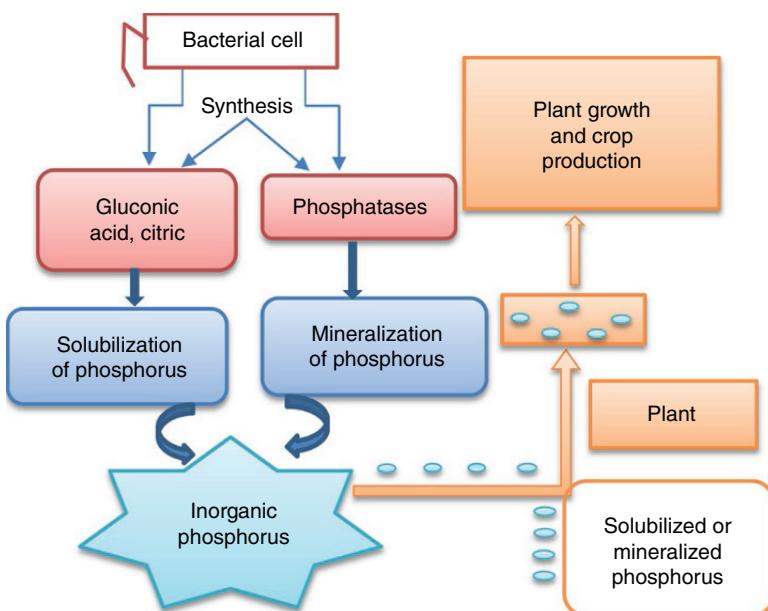
abundance of *Azotobacter* in cultivated soils and the rhizosphere of plants is generally low due to lack of soil organic matter and presence of antagonistic microorganisms. Therefore, they hardly exceed  $10^4$ – $10^5 \text{ g}^{-1}$  of soil. Consequently, the application of N-fixers biofertilizers will help improve the presence of these bacteria. They also promote the biological synthesis of active plant growth promoting substances and thereby, promotes seedlings germination and seedlings establishment.

*Azotobacter nigricans*, *Azotobacter armeniacus* and *Azotobacter paspali* are also utilized as biofertilizers for several crops such as wheat (*Triticum aestivum*), oat (*Avena sativa*), barley (*Hordeum vulgare L.*), mustard (*Brassica juncea*), sesame (*Sesamum indicum*), rice, linseeds (*Linum usitatissimum*), sunflower (*Helianthus annuus*), castor (*R. communis*), maize, sorghum, cotton, jute (*Cochchorus olitorius*), sugar beets (*B. vulgaris*), tobacco, tea (*Camellia sinensis*), coffee (*Coffee arabica*), rubber (*Ficus elastic*) and coconuts (*Cocos nucifera*).

BGA are phototrophic in nature and capable of producing growth promoting substances like auxin, indole acetic acid and gibberellins acids. In submerged rice fields where they can be found in abundance, they can fix 20–30 kg N/ha per year (Mahdi et al. 2010; Mishra et al. 2013). BGA N-fixers are filamentous in structure consisting of specialized cells (i.e. heterocyst) which serve as micro-nodules for the synthesis and the fixation of N (Mahdi et al. 2010). Examples of cyanobacteria includes, *Aulosira*, *Tolyphothrix*, *Scytonema*, *Nostoc*, *Anabaena*, and *Plectoneme*. They are the most commonly used biofertilizers. It has been reported that *Cylindrospermum musicola* release vitamins and growth promoting substances (as mentioned earlier) that improve the plant root growth and grain yield (Sahoo et al. 2013).

### 20.13.2 Phosphorus Solubilizing Biofertilizer (PSB)

Phosphorus (P) is the second essential mineral nutrient that is needed in large quantity by plants (Kaiser et al. 2016). Due to its immobile nature, P is extremely deficient in tropical and subtropical soils. It is very important in the synthesis of nucleic acids and phospholipids. As part of adenosine triphosphate (ATP), P aids the conversion of food energy into chemical energy during photophosphorylation in photosynthesis; and into chemical energy to be extracted during respiration. The amount of P present in the soil is enormous but is usually fixed (i.e. P-fixation) at the point of application and therefore, becomes insoluble and unavailable to plants. Also, the amount of P-fixation in calcareous or acidic soil conditions is usually high and can be minimized by adjusting the pH of the soil to an optimum level of P-availability (Mahdi et al. 2012). Phosphorus solubilizing biofertilizer (PSB) application is used to help remedy and make P more bioavailable and bioaccessible to promote plant growth and development. For instance, PSB contains bacteria called phospho-bacterin, which helps in solubilizing insoluble phosphate (e.g. di- and tri-calcium phosphates, hydroxyapatites, and rock phosphates) and making it more available to plants (Mahdi et al. 2010). They are usually found in considerable amounts within the soil and plant rhizosphere. The solubilization effect is achieved through the production of organic acids by the phospho-bacterin that lowers the pH of the soil leading to the dissolution of phosphate compounds and release of adequate P for plant use (Arun 2007; Glick 2012; Mahanty et al. 2017). Organic acids such as gluconic and citric acids synthesized by these bacteria have low molecular weights and characterized by hydroxyl and carboxyl groups that can chelate cations bound to phosphate (Glick 2012; McComb et al. 2013). This event leads to the alteration of insoluble P to soluble P forms (Figure 20.2). Examples of bacteria with the ability to solubilize and



**Figure 20.2** Schematic diagram showing the mechanism of inorganic phosphorus solubilization by phosphate-solubilizing rhizobacteria Mahanty et al. (2017).

mobilize P are *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Aerobacter*, *Flavobacterium*, *Aspergillus*, and *Erwinia* (Mahdi et al. 2010; Mahanty et al. 2017).

Besides solubilizing P, PSB enhances plant growth through the stimulation of biological N-fixation by N-fixing microorganisms. In a pot-experiment conducted in Nigeria using sandy-loam soil, two fungi i.e. *Aspergillus fumigatus* and *Aspergillus niger* were isolated from decaying cassava peels. These fungi were found to convert cassava waste through the process of semi-solid fermentation technique to phosphate fertilizer. It was also reported that *A. niger* significantly increased the growth of pigeon pea (*Cajanus cajan*; Ogbo 2010).

### 20.13.3 Potassium Solubilisation

Potassium (K) is an essential nutrient in crop production. It plays a key role in regulating the stomatal opening and closing and thereby, maintaining healthy water balance. The supply of total K in soil is high, but only small amounts are available for plant utilization. Potassium exists in three forms at the same time in the soil system; namely, unavailable, slowly available, and readily available forms (Kaiser et al. 2016). Biofertilizer application can help remedy this situation by solubilizing the unavailable K and therefore, making it more accessible to plants. For example, bacteria like *Frateuria aurantia* can mobilize and solubilize unavailable K for plant growth (Mazid and Khan 2014).

*Herbspirillum* sp. also belongs to the *Spirillaceae* family. They form symbiotic association responsible for atmospheric N-fixation in sugarcane plants (Mazid and Khan 2014). However, the inoculation of plant with *Herbspirillum* helps to enhance N-availability, promote nitrate, and importantly, K and phosphate uptake. It also helps in the production of growth promoting substance such as kinetin, auxin, and gibberellic acid (Khan et al. 2011). Examples of K solubilizing microorganisms that have been confirmed to mobilize and solubilize K efficiently belongs to the genus *Aspergillus*, *Bacillus*, and *Clostridium*.

## 20.14 Zinc Solubilization

Zinc (Zn) is a component of several organic complexes and DNA-protein. It also plays a vital role in protein synthesis, growth hormone production and seed development. In a submerged condition, the plant utilizes only 1–4% of total available Zn while 75% of the Zn applied will be fixed (i.e. Zn-fixation) making it unavailable for plant use (i.e. crystalline iron oxide bound and residual Zn, respectively). Both in flooded and submerged conditions, Zn availability declines due to changes in pH and the resultant formation of insoluble Zn compounds (Hafeez et al. 2013). However, the insoluble Zn compounds formed are most likely to be associated with Mn and Fe hydroxides from the breakdown of oxides and adsorption of carbonates such as magnesium carbonate (Hafeez et al. 2013).

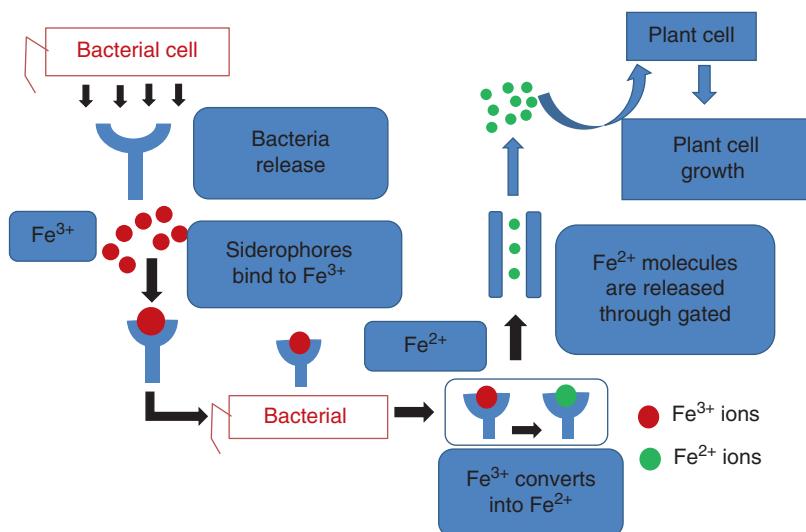
Zn-fixation can be explained under two soil conditions. Firstly, in acidic soil conditions, which is related to cation exchange capacity (CEC). For instance, CEC soils tend to have high clay and organic matter contents, which tend to bind Zn to soil colloids due to the formation of zinc hydroxide and zincates. This action reduces the availability of Zn

in the soil medium. Also, in the clay lattice magnesium can substitute Zn resulting in Zn-fixation. Secondly, in alkaline conditions where fixation occurs because of chemisorptions and complexation (i.e. a reaction between metal ion and ligands) by organic ligands, Zn can be made available and accessible for plant utilization through the application of biofertilizer (Alloway 2008; Mahdi et al. 2010; Hafeez et al. 2013). For instance, the application of *B. subtilis*, *Thiobacillus thioxidans*, and *Saccharomyces* sp. as biofertilizers have shown to mobilize and solubilize Zn into usable plant forms such as  $Zn^{2+}$  cation, which is the predominant form taken up by plants (Mahdi et al. 2010; Samoon et al. 2010; Mazid and Khan 2014).

#### 20.14.1 Iron Sequestration

Iron (Fe) is a micronutrient that is involved in photosynthesis, chlorophyll formation, respiration, and many enzymatic reactions. Plants can utilize Fe either  $Fe^{2+}$  (ferrous cation) or  $Fe^{3+}$  (ferric cation). Generally, Fe is required by all living organisms. It predominantly occurs as  $Fe^{3+}$  in an aerobic environment in which it forms insoluble hydroxides and oxyhydroxides (Mahdi et al. 2010). Hence, most of the Fe become inaccessible to both bacteria and plants (Rajkumar et al. 2010). Bacteria in general, acquire Fe by secreting iron chelators called siderophores (Rajkumar et al. 2010). The majority of the siderophores, i.e. extracellular and intracellular siderophores are water soluble, have low molecular weights and high affinity for complex Fe (Mahdi et al. 2010; Hider and Kong 2010; Rajkumar et al. 2010).

The schematic diagram in Figure 20.3 shows the mechanism of Fe sequestration through siderophores production by rhizobacteria. In the bacteria membrane,  $Fe^{3+}$  is converted into  $Fe^{2+}$  by both Gram-positive and Gram-negative bacteria. The  $Fe^{2+}$  molecules are then released from siderophores into the cell through a gating channel that links



**Figure 20.3** Mechanism of iron sequestration or solubilization through siderophores production by plant growth-promoting rhizobacteria Mahanty et al. (2017).

the inner and outer membranes (Mahanty et al. 2017). Consequently, siderophores can also act as Fe solubilizing agents under limiting conditions (Rajkumar et al. 2010; Ahemad and Khan 2011). There are various Fe assimilation mechanisms by plant from bacterial siderophores, namely: (i) chelating and releasing Fe; (ii) direct uptake of siderophores-Fe complexes; and/or (iii) by a ligand exchange reaction (Thomine and Lanquar 2011). Apart from Fe assimilation, one of the benefits of bacterial siderophores to host plant is that they help to alleviate plant stress caused by extreme levels of heavy metals in the soil (Rajkumar et al. 2012; Mahanty et al. 2017). This is achieved by the siderophores binding to heavy metals and thereby, reducing the soluble metal concentration in the soil (Glick 2012; Rajkumar et al. 2012).

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## 21

# Valorization of Agricultural Byproducts Through Conversion to Biochar and Bio-Oil

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## 21.1 Introduction

Agricultural byproducts refer to the organic residues generated from agricultural production activities. The materials extend to crop residues (e.g. corn stover, wheat straw, rice husks, and oat hulls), animal wastes (e.g. cattle manure, swine manure, and poultry litter), food processing refuses (e.g. fruit and vegetable peels, nutshells, bones, cotton-seed meal, and coffee grounds), spent mushroom substrate, green waste from yard trimming, and forestry debris from logging and thinning. The global agriculture (including forestry) generates annually 3.5–4.0 billion dry tons of crop residues (Smil 1999), 4.5–6.0 billion dry tons of animal manures (FAO 2017; Zhang et al. 2017), and 1.5–2.0 billion dry tons of woody debris (Baruya 2015). In the US, for example, a total of 813 million dry tons of agricultural byproducts are generated per annum, covering 500 million dry tons of crop residues (USDA 2006), 73 million dry tons of recoverable animal manure (Gollehon et al. 2016), and 240 million dry tons of forestry wood debris (McKeever 2004). There are additionally 210 million dry tons of municipal organic refuse and 7.2 million dry tons of sewage sludge/biosolids available each year as organic wastes (EPA 2007; Peccia and Westerhoff 2015). These agricultural byproducts are commonly discarded in the field for natural decay, collected as a raw fuel, applied to cropland as a soil conditioner and/or organic fertilizer, disposed of through landfilling, or simply burned for clearings. These treatments yield low values and may cause environmental pollution.

Conversion to biochar and bio-oil through pyrolysis may be a value-added approach to reuse agricultural byproducts (Ro et al. 2010; Guo et al. 2012). Pyrolysis is a simple, long-practiced technique for manufacturing charcoal by heating wood in the absence of air (oxygen) (Guo et al. 2015). Pyrolytic treatment of wood results in three products: a grayish black solid residue as charcoal, a brown flowing liquid as pyrolysis bio-oil, and an uncondensable gaseous vapor as syngas (Figure 21.1). While syngas (major components

$\text{CO}$  and  $\text{H}_2$ ) is combustible and can be used directly as a fuel to power the pyrolytic treatment, charcoal and bio-oil are valuable products that have numerous domestic uses (Guo et al. 2015). Pyrolysis of agricultural byproducts yields the same three products (Figure 21.1): the solid biochar, the liquid bio-oil, and the gaseous syngas (Guo et al. 2012). In particular, biochar is biomass-derived charcoal to be used as a soil amendment. The material by definition is “fine-grained or granular charcoal made from heating vegetative biomass, bones, manure solids, or other plant-derived organic residues in an oxygen-free or oxygen-limited environment and used as a soil amendment for agricultural and environmental purposes” (Guo et al. 2016). The most important characteristics of biochar are its extraordinary environmental persistence and high water and nutrient holding capacity. Relative to raw plant materials and humic substances that are generally biodegradable, biochar is much more recalcitrant, resisting to natural oxidation and microbial decomposition in field soils. Up to 86% of the pyrogenic carbon (C) in biochar is extremely stable and against stringent chemical oxidation (Song and Guo 2012), enabling biochar to persist in natural soils over thousands of years (Guo 2016a). Biochar is porous and possesses numerous acidic functional groups, engendering its great water retention and cation exchange capabilities (Guo et al. 2016). Application of biochar as a soil amendment in agricultural production and environmental restoration furnishes numerous long-term benefits, including soil fertility enhancement, crop production improvement, carbon sequestration enlargement, land rehabilitation promotion, greenhouse gas emission mitigation, and water contamination reduction (Guo et al. 2016). Pyrolysis bio-oil, on the other hand, is a mixture of various low-molecular-weight organic compounds such as alkenes, alcohols, aldehydes, acids, esters, ketones, phenols, furans, and sugars (Guo et al. 2015). It can be refined to produce transportation fuels (e.g. gasoline, kerosene, and diesel) and high-value industrial chemicals (e.g. methanol, dimethyl ether, formaldehyde, acetic acid, and isobutene) (Zhang et al. 2013a). The yield and quality of biochar and bio-oil from pyrolytic treatment of biomass materials, however, vary significantly with the feedstock type and pyrolysis conditions. Intensive research has been conducted to optimize the pyrolysis technique (in temperature, heating rate, reaction time, pressure, and other operating parameters) and to characterize the biochar and bio-oil products derived from different agricultural byproducts (Waheed et al. 2013; Windeatt et al. 2014; He et al. 2016; Arazo et al. 2017; Cong et al. 2018).

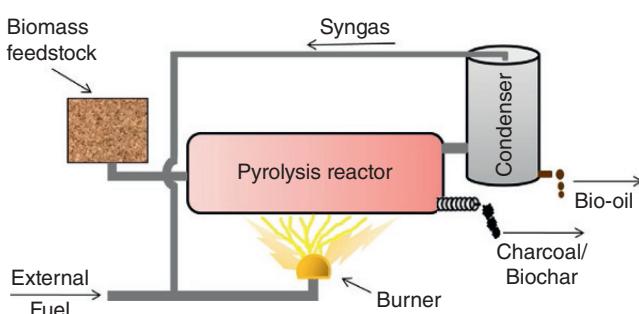
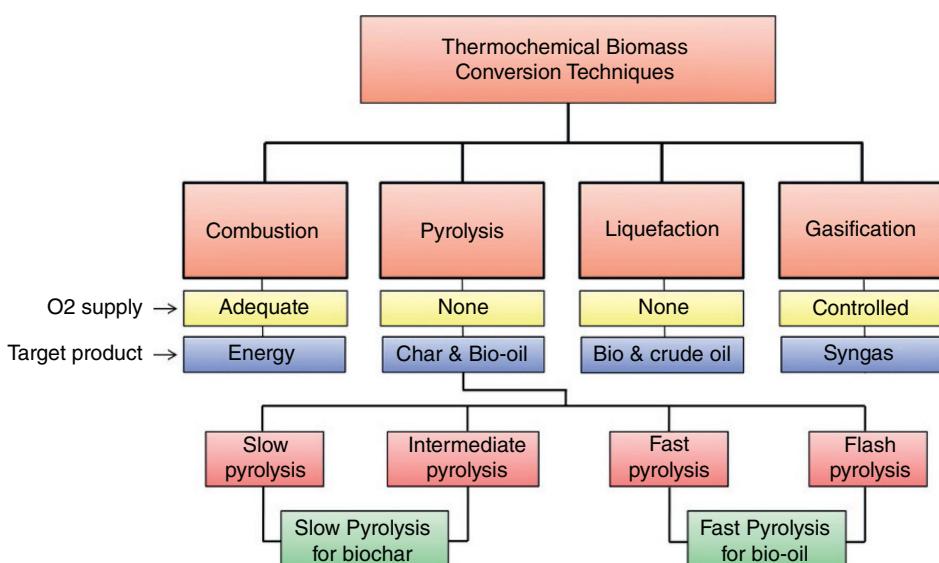


Figure 21.1 A diagram of pyrolysis systems for biochar and bio-oil production.

A variety of agricultural and environmental applications of biochar have been tested to explore the potential benefits (Guo et al. 2016). Furthermore, efficient refining techniques have been developed to recover advanced biofuels and industrial chemicals from pyrolysis bio-oil (Zhang et al. 2013a; Fermoso et al. 2017). This chapter is to review the pyrolysis technologies for converting agricultural byproducts to biochar and bio-oil and to summarize the research advances in characterization and application of different biowastes-derived biochars and bio-oils.

## 21.2 Pyrolysis Technologies for Biochar and Bio-Oil Production

Pyrolysis is a thermochemical process for treating organic materials in high temperature (e.g. >200 °C), O<sub>2</sub>-free environments. The technique is designed to produce charcoal and bio-oil from biomass materials. In practice, the pyrolysis reactor is maintained O<sub>2</sub>-absent to maximize the yields of biochar and bio-oil. Compared with other thermochemical biomass conversion technologies, pyrolysis differs primarily in the process O<sub>2</sub> supply and the target products (Figure 21.2). For instance, gasification is to heat organic materials at >700 °C with controlled O<sub>2</sub> availability for maximal syngas production, liquefaction (or hydrothermal processing) is to produce bio-crude oil by quick treating (e.g. 30–60 minutes) organic materials in 15–35% solid-water slurry at high temperature (e.g. 250–375 °C), high pressure (e.g. 4–22 MPa), and O<sub>2</sub>-free conditions, while combustion is to burn organic materials at >500 °C with adequate O<sub>2</sub> supply for most energy release (Tekin et al. 2014; Guo et al. 2015). Based on the residence time of organic solids



**Figure 21.2** A flow chart of available thermochemical biomass conversion technologies, with highlight on pyrolysis.

in the reactor and the heating rate, pyrolysis is divided into slow pyrolysis (hours to days in residence time;  $<10\text{ }^{\circ}\text{C s}^{-1}$  in heating rate), intermediate (minutes;  $10\text{--}100\text{ }^{\circ}\text{C s}^{-1}$ ), fast pyrolysis (seconds;  $>100\text{ }^{\circ}\text{C s}^{-1}$ ), and flash pyrolysis (ultrafast pyrolysis with  $>500\text{ }^{\circ}\text{C s}^{-1}$  heating rate and shortened vapor residence time [vapor moving from reactor to condenser] by vacuum or sweep  $\text{N}_2$  gas flow) (Figure 21.2; Onay and Kockar 2003; He et al. 2016). More broadly, pyrolysis is classified into two categories: slow pyrolysis (covering slow and intermediate pyrolysis) and fast pyrolysis (covering fast and flash pyrolysis). The present chapter follows this classification (Figure 21.2). In general, slow pyrolysis is practiced in order to produce charcoal, while fast pyrolysis is employed to generate bio-oil (Guo et al. 2012). The technology for torrefaction is actually low temperature (i.e.  $200\text{--}300\text{ }^{\circ}\text{C}$ ), incomplete slow pyrolysis (typical heating rate  $<1\text{ }^{\circ}\text{C/s}$ ) for converting waste wood and other lignocellulosic materials into a low moisture content, high heating value, hydrophobic, and easy-to-grind brownish product with improved combustion performance and storage convenience (Koppejan et al. 2012).

### 21.2.1 Slow Pyrolysis

Slow pyrolysis, sometimes also called carbonization, has been practiced for thousands of years to produce charcoal from wood and other organic materials. In slow pyrolysis, wood and other organic residues are heated in a closed kiln or retort (pyrolysis reactor; Figure 21.1). Thermal decomposition of biomass materials starts as the temperature exceeds  $190\text{ }^{\circ}\text{C}$  (Guo et al. 2015). At  $\geq 250\text{ }^{\circ}\text{C}$ , carbohydrates lose mass at significant rates with substantial evolution of  $\text{CO}_2$  and  $\text{CO}$  (Antal and Gronli 2003). The overall pyrolytic transformation can be separated into three stages: (i) dehydration, in which biomass loses moisture and becomes “oven” dry, (ii) initial charring, at which dry organic matter decomposes to generate primary char and tarry vapor, and (iii) further charring, during which primary char is transformed to ultimate char and tarry vapor (Guo et al. 2012). The tarry vapor is a mixture of water steam, condensable organic compounds, and uncondensable gases including  $\text{CO}$ ,  $\text{H}_2$ ,  $\text{CH}_4$ ,  $\text{C}_2\text{H}_4$ ,  $\text{C}_3\text{H}_6$ , and  $\text{CO}_2$ . The vapors are emitted from the vents installed in the kiln or retort and condense at room temperature to form bio-oil (Figure 21.1). Little pressure buildup takes place inside the reactor, allowing pyrolytic decomposition of biomass to occur at the ambient atmospheric pressure. The uncondensable vapor portion is combusted to provide heat for sustaining the pyrolysis process (Figure 21.1).

Pyrolytic decomposition of biomass is overall an endothermic reaction, especially at low temperature (e.g.  $<800\text{ }^{\circ}\text{C}$ ) (Basu 2010). External heat energy is required to initiate and maintain the pyrolysis process. Typically, the heat energy is furnished by partial combustion of the feedstock, by pyrolysis vapor recirculation, or by external heaters (Antal and Gronli 2003). The peak temperature (highest treatment temperature, HTT), reaction (solid residence) time, and heating rate (heat transfer rate or heat flux), and feed characteristics (e.g. particle size, density, and composition) influence the yield and quality of biochar and bio-oil (Basu 2010). A higher HTT or a smaller feed particle size boosts the heating rate. The yields of biochar and bio-oil are calculated as:

$$\chi = \frac{m_{\text{char}}}{m_{\text{feed}}} \times 100\% \quad (21.1)$$

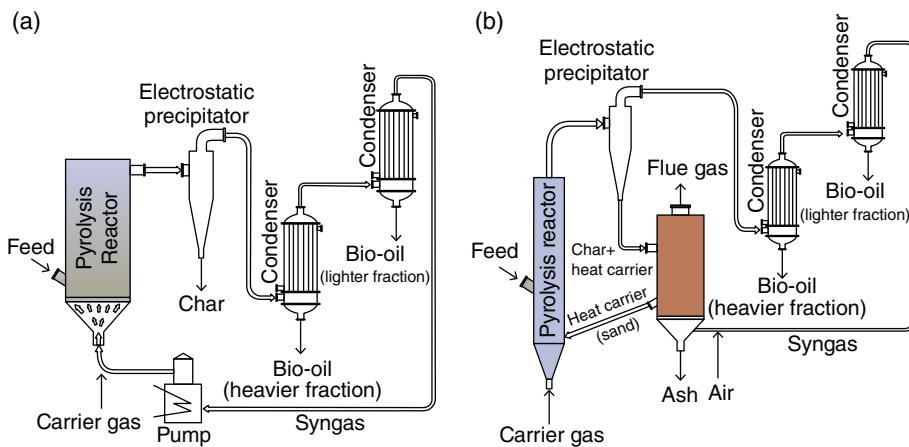
$$\eta = \frac{m_{\text{bio-oil}}}{m_{\text{feed}}} \times 100\% \quad (21.2)$$

where  $\chi$  and  $\eta$  represent biochar yield and bio-oil yield, respectively,  $m_{\text{char}}$  is the weight of biochar generated,  $m_{\text{bio-oil}}$  is the adjusted amount (subtracting contribution from the original feed moisture) of bio-oil recovered, and  $m_{\text{feed}}$  is dry weight of biomass feed consumed. The yield of biochar decreases while the yield of bio-oil increases with elevating the pyrolysis peak temperature (Demirbas 2004; Song and Guo 2012). The reaction time should be long enough to achieve complete pyrolysis, as indicated by no visible smokes further emitted from the reactor (Song and Guo 2012). Inadequate reaction time results in low-quality biochar products that contain significant contents of instable organic volatiles. The reaction time required for complete pyrolysis, however, increases as the HTT decreases or the heating rate drops (Song and Guo 2012). On average, the biochar, bio-oil, and syngas yields from slow pyrolysis of wood are 35%, 30%, and 35% of the dry feed mass, respectively (Laird et al. 2009).

Both fixed bed reactors (e.g. kilns and retorts) and moving bed reactors (e.g. screw/ auger and rotating drum pyrolyzers) have been designed to produce biochar from agricultural byproducts through slow pyrolysis (Ronsse 2016). Batch feed kilns and retorts are usually used in backyard biochar production practices or adopted in remote regions of developing countries owing to their low costs and operation simplicity. It takes hours to days to produce one batch of biochar. Emissions of pyrolysis vapors to the atmosphere are generally not controlled, causing air pollution concerns. In large-scale, industrial biochar production, moving bed pyrolyzers with a continuous feed mode are necessary to achieve uninterrupted generation of biochar. Such systems, however, involve significantly higher capital investments and stricter feed requirements (e.g. dry,  $<1$  cm particulates) (Ronsse 2016). Instead of harvesting bio-oil, pyrolysis vapors are mostly combusted to provide additional heat for supporting the pyrolysis operation. Regardless of the pyrolyzer design, a lower pyrolysis temperature, a slower heating rate, and a longer vapor residence time maximize the yield of biochar (Basu 2010). Low temperature slow pyrolysis (e.g. HTT  $\leq 450$  °C) is commonly recommended to manufacture biochar from agricultural byproducts (Song and Guo 2012; Li et al. 2018).

### 21.2.2 Fast Pyrolysis

Fast pyrolysis is pyrolytic decomposition of biomass materials at extraordinarily high heating rates. In operation, the biomass feed is passed in a continuous flow through a hot (700–1000 °C), O<sub>2</sub>-free chamber (pyrolysis reactor) to be heated to around 500 °C within 2 seconds, being rapidly converted to biochar and pyrolysis vapors. The pyrolysis vapors are efficiently quenched to recover bio-oil. The uncondensable vapor fraction is combusted to provide heat energy or circulated as fluidizing gas. Fluid bed reactors and circulating fluidized bed reactors are commonly used for fast pyrolysis, with generally N<sub>2</sub> as the carrier gas to fluidize the feed and sweep the pyrolysis vapor (Figure 21.3). These pyrolyzer systems demonstrate a desirable bio-oil yield up to 70% of the ash-free feed mass. In fluid bed systems, the resulting biochar is recovered, and syngas is combined with the carrier gas. In circulating fluidized bed systems, biochar and syngas are combusted in a burner to heat silica sand as the heat carrier that is conveyed to the reactor for sustaining the pyrolysis process (Figure 21.3). If the two systems are compared, fluid bed pyrolyzers are relatively simpler in construction and operation, yet they involve higher operating costs and smaller particle size requirements for feed (i.e.  $<1$  mm) (Clifford 2017). Other types of reactors are also available, including screw reactors,



**Figure 21.3** Fast pyrolysis systems with (a) a fluid bed reactor and (b) a circulating fluidized bed reactor for bio-oil production Clifford 2017.

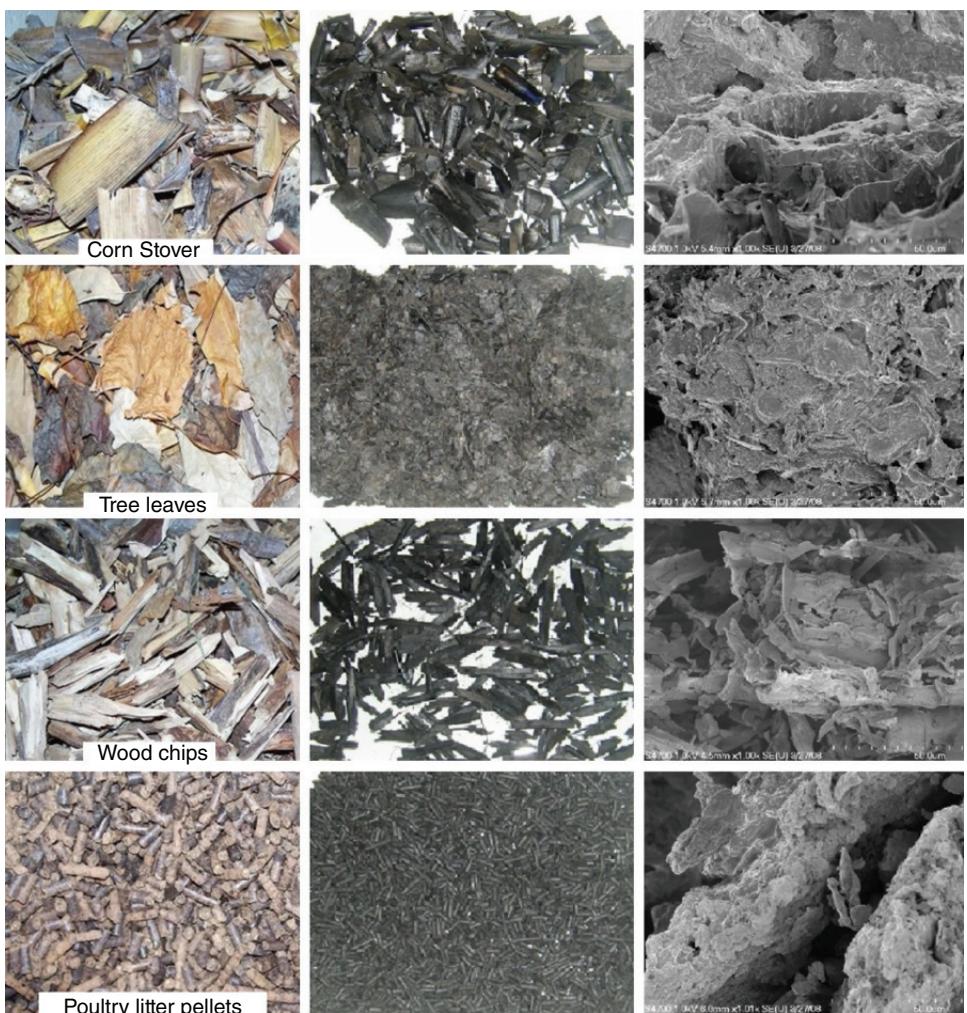
ablative plate reactors, rotating cone reactors, vortex reactors, pneumatic transport reactors, and vacuum reactors (Basu 2010; Vamvuka 2011).

Fast pyrolysis aims at bio-oil production, which could be maximized at an intermediate pyrolysis temperature, a higher heating rate, and a shorter vapor residence time (Basu 2010). To achieve a satisfactory bio-oil yield, fast pyrolysis has stricter requirements for the biomass feed: the moisture content should be <12% and the particle size be <2 mm. The solid residence time and the vapor residence time are limited to <2 seconds and <1 second, respectively. The heating rate is generally  $>300\text{ }^{\circ}\text{C s}^{-1}$  and can be as high as  $>1000\text{ }^{\circ}\text{C s}^{-1}$  (Basu 2010). At the upper end, for example, flash pyrolysis requires the pyrolysis temperature in the range of  $800\text{--}1000\text{ }^{\circ}\text{C}$ , the solid residence time <1 second, the vapor residence time < 0.5 second, a heating rate  $> 1000\text{ }^{\circ}\text{C s}^{-1}$ , and a feed particle size <0.2 mm (He et al. 2016). The yields of bio-oil, biochar, and syngas from fast pyrolysis of wood are normally in the range of 50–70%, 10–30%, and 15–20% of dry feed mass, respectively (Laird et al. 2009; Vamvuka 2011).

## 21.3 Quality Characteristics of Agricultural Byproducts-Derived Biochar and Bio-Oil

### 21.3.1 Quality Characteristics of Biowastes-Derived Biochars

Biochar was originally invented as a soil amendment to promote plant growth and crop production by improving soil quality and sustaining soil fertility (Guo 2016b). Other benefits such as enhancing carbon sequestration, mitigating greenhouse gas emissions, and reducing environmental pollution are complimentary. High quality biochars are products possessing (i) great environmental recalcitrance as indicated by the high proportion of inherent stable carbon and (ii) great nutrient and water retention capacities as indicated by the high values of cation exchange capacity (CEC) and specific surface area (SSA) or porosity. The International Biochar Initiative (IBI) proposes the following



**Figure 21.4** Biochars (middle column) generated from different organic residues (left column) through 400 °C slow pyrolysis and their scanning electron microscopy (SEM) images (right column) Guo et al. 2016.

parameters for biochar quality evaluation: particle size distribution, SSA, pH, electrical conductivity, mineral ash content, lime equivalency, germination inhibition assay, organic carbon (OC) content, H:OC ratio, total and available N, P, Ca, Mg, and S contents, and contents of polycyclic aromatic hydrocarbons, dioxins/furans, poly-chlorinated biphenols, and the common toxic elements (IBI 2015). Most of these quality characteristics are determined by the biochar feedstock type and influenced by the production conditions (Figure 21.4). In general, pyrolysis enriches OC and ash minerals in biochar and transforms the biodegradable feed OC and mineral salts into more stable, environmentally recalcitrant forms; the effects increase as the pyrolysis temperature is elevated. Accompanying the improvement in product stability is, however, a

compromised biochar yield at higher pyrolysis temperature (Song and Guo 2012). These two aspects need equivalent considerations upon determining the optimal pyrolysis temperature.

It would be desirable if biochar contained significant amounts of slowly releasable plant macronutrients including N, P, K, Ca, Mg, and S. In reality, however, biochar is generally low in plant nutrients; it cannot serve as a major nutrient source for plants and therefore, should not be used to replace chemical fertilizers in crop production. The liming potential of biochar originates from its base metal components (Ca, Mg, K, and Na) in the mineral ash and can be indicated by its pH and mineral ash content (Guo et al. 2012). Biochar is typically alkaline, with a pH value up to 11.0 in 1:5 solid/water slurries (Table 21.1). Poorly prepared biochars through incomplete pyrolytic decomposition may demonstrate a pH value below 7.0 as a result of accumulation of low molecular weight organic acids. Rectification can be achieved by adequately elongating the feedstock residence time in the pyrolysis reactor. Biochars from the alone pyrolysis treatment generally possess a low SSA (e.g.  $<20 \text{ m}^2 \text{ g}^{-1}$ ), especially when the feedstock is rich in ash minerals. Air cooling in sealed containers after pyrolysis leads to formation of organic condensate coatings on biochar surfaces, causing the hydrophobicity and likely the low SSA values of fresh biochars (Yi et al. 2015). The condensates are water soluble and biodegradable, implicating the potential increase of biochar in SSA following field weathering. Inadvertent activation by steam (e.g. cooling biochar by spraying water) immediately following pyrolysis may occur, opening and enlarging the inherent pores that were blocked by mineral ashes and organics and consequently, resulting in products with high SSA values (Table 21.1). Biochar possesses numerous surface acidic functional groups (Song and Guo 2012), engendering the material relatively high CEC values (Table 21.1).

Biochars derived from manures and food processing wastes (e.g. poultry litter, biosolids, swine manure, cattle manure, spent mushroom substrate, and animal bones) contain remarkably higher contents of nutrients than those derived from crop residues and herbaceous biomass, while wood-derived biochars carry marginal plant nutrients (Table 21.1). Significant losses of N occur during pyrolytic conversion of N-rich feedstocks (e.g. animal manures and biosolids) to biochar, especially at higher pyrolysis temperature (e.g.  $>400^\circ\text{C}$ ). Meanwhile, P is transformed into more recalcitrant, yet slowly-releasable species. For instance, when poultry litter was converted to biochar through fixed-bed slow pyrolysis at 300, 400, 500, and  $600^\circ\text{C}$ , the losses of N accounted for 18.2%, 55.8%, 95.3%, and 98.2% of the feed N, respectively, while the stable OC (against acidic dichromate oxidation) in the products accounted for 36.8%, 48.1%, 85.5%, and 86.3% of the total OC, respectively (Song and Guo 2012). The organic P in poultry litter was transformed to inorganic forms and the predominant orthophosphate-P in the biochars became less labile (Li et al. 2018). Overall, high quality biochar products can be manufactured from agricultural byproducts through complete slow pyrolysis at an intermediate temperature (e.g.  $400\text{--}450^\circ\text{C}$ ).

### 21.3.2 Quality Characteristics of Biowastes-Derived Bio-Oils

Bio-oil refers to pyrolysis vapor condensates, a dark brown liquid at room temperature that can be refined for biofuels and industrial chemicals. Crude pyrolysis bio-oil is a complex mixture of numerous organic compounds, water, and the impurities colloidal char

**Table 21.1** Quality characteristics of biochars generated from agricultural byproducts.

Feedstock	Pyrolysis conditions	Yield %	pH	Mineral ash, g kg <sup>-1</sup>	CEC, cmol <sub>c</sub> kg <sup>-1</sup>	SSA, m <sup>2</sup> g <sup>-1</sup>	TN, g kg <sup>-1</sup>	TP, g kg <sup>-1</sup>	Reference
Poultry litter	300 °C slow pyrolysis	60.1	9.5	478.7	51.1	2.7	41.7	22.7	Song and Guo 2012
Poultry litter	400 °C slow pyrolysis	56.2	10.3	566.2	41.7	3.9	26.3	26.3	Song and Guo 2012
Poultry litter	500 °C slow pyrolysis	51.5	10.7	605.8	35.8	4.8	12.1	27.9	Song and Guo 2012
Pig manure solids	420 °C slow pyrolysis	40.3	9.7	345.0			21.1	38.5	Marchetti et al. 2012
Cow manure	400 °C slow pyrolysis		9.0	703.0			13.5	4.36	Singh et al. 2010
Sewage sludge	487 °C fast pyrolysis	28.7	9.0	659.0			45.5		Arazo et al. 2017
Waste wood	400 °C slow pyrolysis	32.7	7.5	32.0	7.9	15.4	2.5	0.18	Tian et al. 2016
Waste wood	500 °C slow pyrolysis	25.8	8.2	42.0	7.5	26.6	3.0	0.34	Tian et al. 2016
Waste wood	420 °C slow pyrolysis	29.4	8.8	86.0			10.8	1.3	Marchetti et al. 2012
Poplar wood	400 °C slow pyrolysis	32.0	9.0	19.0	144.0	3.0	7.8		Kloss et al. 2011
Pine chips	400 °C slow pyrolysis	35.0	7.6		7.27		2.55	0.15	Gaskin et al. 2008
Pine chips	500 °C slow pyrolysis	30.0	8.3		5.03		2.23	0.14	Gaskin et al. 2008
Spruce wood and needle mix	400 °C slow pyrolysis	36.0	6.9	35.0	73.5	1.8	10.2		Kloss et al. 2011
Greenwaste	450 °C slow pyrolysis	33.0		107.6		7.3	11.7		Zheng et al. 2010
Greenwaste	450 °C slow pyrolysis		9.4		24.0		1.8		Chan et al. 2007

(Continued)

**Table 21.1** (Continued)

Feedstock	Pyrolysis conditions	Yield %	pH	Mineral ash, g kg <sup>-1</sup>	CEC, cmol <sub>c</sub> kg <sup>-1</sup>	SSA, m <sup>2</sup> g <sup>-1</sup>	TN, g kg <sup>-1</sup>	TP, g kg <sup>-1</sup>	Reference
Cotton stalk	600 °C slow pyrolysis		10.3	95.0		121	38.0		Windeatt et al. 2014
Cottonseed meal	300 °C slow pyrolysis	53.3	9.1	137.0		<1.0	89.8	22.7	Guo and He 2019
Cottonseed meal	400 °C slow pyrolysis	40.8	10.1	173.3		<1.0	58.7	26.3	Guo and He 2019
Peanut hulls	400 °C slow pyrolysis	36.0	10.5		14.2		24.3	1.83	Gaskin et al. 2008
Peanut hulls	400 °C slow pyrolysis	40.0	7.9	82.0		0.52	27.0	2.6	Novak et al. 2009
Peanut hulls	500 °C slow pyrolysis	35.0	8.6	93.0		1.22	27.0	2.6	Novak et al. 2009
Corn cobs	500 °C fast pyrolysis	18.5	7.8			<1.0		4.36	Mullen et al. 2010
Corn stover	500 °C fast pyrolysis	16.8	7.2			3.1		12.9	Mullen et al. 2010
Rapeseed straw	400 °C slow pyrolysis	39.4		122.2		16.0	14.3		Karaosmanoglu et al. 2000
Rapeseed straw	600 °C slow pyrolysis	32.2		138.5		17.6	15.3		Karaosmanoglu et al. 2000
Wheat straw	400 °C slow pyrolysis	34.0	9.1	97.0	161.6	4.8	10.5		Kloss et al. 2011
Switchgrass	250 °C slow pyrolysis	78.0	5.4	26.0		0.4	4.3	1.0	Novak et al. 2009
Switchgrass	500 °C slow pyrolysis	29.0	8.0	78.0		62.2	10.7	2.4	Novak et al. 2009

particles and mineral ash (Czernik and Bridgwater 2004). It is acidic, corrosive, viscous, unstable, and difficult to ignite (Xiao 2014). The water content of crude pyrolysis bio-oil ranges from 15 to 48 wt%. Moisture and particulate-free bio-oil consists typically of 45–60 wt% C, 5–7 wt% H, 30–50 wt% O, and minor contents of N, S, and alkali (Ruddy et al. 2014). Chemical and spectroscopic characterization has identified various organic acids, aldehydes, alcohols, esters ketones, phenols, guaiacols, syringols, alkenes, sugars, furans, aromatics, nitrogen-containing compounds, miscellaneous oxygenates, and ash minerals in bio-oil (Mullen and Boateng 2008; Vamvuka 2011). The molecular weight of these organic compounds is in the range of 30–2000 Da. Specific examples of the chemicals include formic acid, acetic acid, pentadecane, hydroxybutyric acid, toluene, 3-methyl phenol, 3-methyl benzoic acid, 1,2-benzenedicarboxylic acid, 2-hydroxyl benzaldehyde, and 1-(4-hydroxy-3-methoxy) acetophenone (Xu et al. 2008; Arazo et al. 2017). Hydroxyacetaldehyde is generally the most abundant organic constituent in bio-oil and may account up to 10 wt% of the total organic component, followed by acetic acid and formic acid (up to 5 wt% and 3%, respectively) (Vamvuka 2011). Due to the significant presence of carboxylic acids, bio-oils from low-ash feedstocks are strongly acidic (pH 2.4–3.2) and corrosive to metals such as copper, aluminum, and iron (Darmstadt et al. 2004). The high oxygen content and water content impart crude bio-oil with a relatively low calorific potential: the volumetric heating value is merely 50–60% to that of diesel fuel (Mohan et al. 2006). The instability of bio-oil is indicated by its viscosity increase and phase separation over storage, a result of interactions between the inherent reactive, oxygenated chemical components such as carboxylic acids, aldehydes, catecols, guaiacols, and syringols (Xiao 2014).

The yield of bio-oil and its quality as a fuel and chemical feedstock vary with the source material and the pyrolysis conditions (Table 21.2). Bio-oils derived from animal manures and food processing wastes show higher contents of N and relative composition of nitrogen compounds. The products from wood and herbaceous biomass are strongly acidic, while those from mineral ash-rich feedstocks demonstrate neutral to slightly alkaline (Table 21.2). The pyrolysis temperature also significantly influences the chemical composition of pyrolysis bio-oil (Vamvuka 2011). Particular chemical transformation and purification is needed to upgrade crude pyrolysis bio-oil into transportation fuels and industrial chemicals.

## 21.4 Potential Utilization of Biochar and Bio-Oil

### 21.4.1 Agricultural Applications of Biochar

As a porous, environmentally stable solid with high water and nutrient holding capabilities, biochar is destined to be an ideal soil conditioner for persistently improving soil quality and promoting plant growth. The fertile *Terra Preta* soils in the Amazonian Basin are believed a legacy of ancient inhabitants who amended local barren soils with charcoal prepared from wood, animal manures, and fish bones (Sombroek 1966). Intensive laboratory and field trials have shown that amendment with quality biochar at appropriate rates significantly ameliorates soil physical, chemical, and biological properties. The long-term soil fertility and overall health are improved, leading to enhanced crop productivity (Guo et al. 2016). In field application, biochar in 0.25–2 mm grains are typically

**Table 21.2** Quality characteristics of pyrolysis bio-oils generated from agricultural byproducts.

Feedstock	Pyrolysis conditions	Yield, %	Specific gravity	Moisture %	HHV, MJ kg <sup>-1</sup>	pH	C, %	O, %	N, %	Reference
Wood	500 °C fast pyrolysis	60.1	1.18	22.0	17.1	2.5	56.4	2.0	0.2	Huffman et al. 1991
Wood	500 °C fast pyrolysis		1.20	31.8	16.3	2.4	55.5		0.1	Ikura et al. 2003
Wood	400 °C slow pyrolysis	43.7	1.08	39.0	12.5	2.3				Xiao 2014
Pine wood	450 °C slow pyrolysis	31.9	1.15	19.5	20.8	3.4				Ravindran 2011
Pine wood	500 °C slow pyrolysis	37.8	1.14	18.9	22.1	3.7				Ravindran 2011
Corn stover	500 °C fast pyrolysis	61.6			22.1		54.0	37.9	1.2	Mullen et al. 2010
Corn stover	400 °C slow pyrolysis	40.8	1.08	46.5	19.3	2.7				Xiao 2014
Corn cobs	500 °C fast pyrolysis	61.0			19.5		55.1	36.9	0.6	Mullen et al. 2010
Rice husks	500 °C fast pyrolysis	56.0	1.16	33.0	14.3	2.8	39.9	6.1	0.6	Xu et al. 2008
Poultry litter	450 °C slow pyrolysis	33.3	1.06	31.7	12.8	9.3				Ravindran 2011
Poultry litter	500 °C slow pyrolysis	28.9	1.06	29.2	13.7	9.4				Ravindran 2011
Poultry litter	400 °C slow pyrolysis	30.4	1.03	39.2	19.7	8.6				Xiao 2014
Sewage sludge	488 °C fast pyrolysis	35.8	1.10	11.5	35.4	6.8	65.2	17.4	8.4	Arazo et al. 2017

mixed with the top soil at 2–5 wt% (or 30–75 ton ha<sup>-1</sup>). For clayey soils, coarser biochar (e.g. 0.5–5 mm) may be used. It has been reported that biochar incorporation reduced soil bulk density while it increased the microtomographic (i.e. >70 µm) porosity, macropore (>1500 µm) volume, macropore connectivity, soil water retention, and plant available water capacity as well as saturated hydraulic conductivity (Eastman 2011; Quin et al. 2014; Hardie et al. 2014). The soil aggregate stability was also improved (Herath et al. 2013). The alkaline nature of biochar and its inherent, slow-releasable nutrients enable the material to reduce soil acidity and enhance soil immediate fertility following land application (Novak et al. 2009; Chintala et al. 2014). The results are more evident in strongly acidic (i.e. pH <5.5), highly leached infertile soils (Yamato et al. 2006; Steiner et al. 2008). The long-term effect of biochar amendment on enhancing soil fertility is primarily achieved by increasing soil CEC and reducing nutrient leaching losses (Lehmann et al. 2003; Knowles et al. 2011). Biochar contains significant contents of labile OC (Song and Guo 2012). Soil amendment with biochar provides additional biodegradable OC to the colonized microbes and furnishes a more favorable inhabiting environment, improving soil microbial communities and other biological properties. Notable enhancements in phosphate-solubilizing bacteria abundance, microbial N<sub>2</sub>-fixing activity, arbuscular mycorrhizal colonization, plant root development, soil basal respiration, and overall microbial biomass were observed when strongly acidic, infertile soils were amended with agricultural byproducts-derived biochars (Steiner et al. 2008; Anderson et al. 2011; Van Zwieten et al. 2015). The soil quality improvements through biochar amendment lead to promoted plant growth and crop productivity. In infertile soils amended with biochars at significant rates (e.g. >15 ton ha<sup>-1</sup>), remarkable increases in corn grain yield, wheat grain yield, and peanut yield were achieved (Kimetu et al. 2008; Baronti et al. 2010). Crop responses to biochar amendment may not be evident in fertile soils, especially when wood-derived biochar is applied at low rates (e.g. <15 Mg ha<sup>-1</sup>) (Blackwell et al. 2007; Asai et al. 2009; Guo et al. 2016). The reported positive results were mostly observed in short-term experiments with acidic to neutral, coarse to medium texture soils amended with nutrient-rich biochars. For modern agriculture that relies on heavy chemical fertilization to secure high crop yield, biochar amendment helps sustain soil quality and reduce the fertilization rates yet cannot replace the role of chemical fertilizers. Combined chemical fertilization and soil biochar amendment is recommended.

Biochar has also been assessed for uses as a feed additive or a manure amendment to improve animal health and reduce ammonia and odor emissions. Charcoal addition to animal feed has long been practiced in animal production systems (Toth and Dou 2016). Van et al. (2006) found that adding bamboo-based biochar to the goat feed at 0.5–1.0 g kg<sup>-1</sup> goat body weight facilitated the feed protein digestibility and the overall N retention by animals. Mixing biochar with the floor beddings at 5–10 vol% remarkably reduced litter wetness and caking, coop odor, and ammonia air concentrations in broiler houses (Gerlach and Schmidt 2012). Incorporation of biochar in animal manures in feeding lots and composting facilities greatly helped retain N and P nutrients through adsorption and ammonia emission reduction, resulting in better quality organic fertilizers (Steiner et al. 2010; Sarkhot et al. 2013). Taghizadeh-Toosi et al. (2012) reported that land application of a wood-based biochar at 15–30 ton ha<sup>-1</sup> reduced NH<sub>3</sub> volatilization losses by 45% from a silt loam soil spiked with ruminant urine. It is noteworthy that acidified biochars are rather efficient to scrub NH<sub>3</sub> from the air stream (Ciahotný et al. 2006; Chou et al. 2006,

Chaisongkroh et al. 2012). Asada et al. (2006) observed that the NH<sub>3</sub> adsorption capacity of a bamboo-based biochar was greatly enhanced through the acidification treatment with diluted sulfuric acid. Although no attempts of applying biochar as a poultry litter amendment during broiler grow-out have been reported, preliminary laboratory trials suggest the promising use of acidified biochars to effectively control NH<sub>3</sub> emission from animal feed operations (Zhang et al. 2016).

#### 21.4.2 Environmental Applications of Biochar

Biochar has been explored for environmental rehabilitation uses. It is well known that biochar amendment enhances soil carbon sequestration and reduces cropland greenhouse gas emissions. Through conversion to biochar via slow pyrolysis, 40–45% of the biodegradable OC in raw agricultural byproducts is transformed into recalcitrant forms and sequestered in the black solid (Guo and Shen 2011). In biochar, up to 86% of the OC is highly stable, resistant to acidic dichromate oxidation (Song and Guo 2012). The stable carbon can persist in the natural environment for thousands of years, substantially enlarging the soil carbon pool if biochar soil amendment is widely practiced. Biochar amendment also demonstrates the potential for reducing CH<sub>4</sub> and N<sub>2</sub>O emissions from the treated soils by 15–55% (Saarnio 2016). Research shows that soil emissions of greenhouse gases could be initially stimulated by biochar amendment, yet over time the suppression effect became manifested (Mukherjee and Lal 2013).

Biochar amendment has been attempted to mitigate soil contamination from heavy metals and organic pollutants. Attributing to the high SSA and abundant surface functional groups, biochars are sorptive to nutrients, metal ions, and organic compounds. Experimental studies indicated biochars were able to immobilize the heavy metals Cu, Cd, Ni, Pb, and Zn in soils and remove the organic compounds atrazine, simazine, phenanthrene, and naphthalene from water (Gomez-Eyles et al. 2013). Biochar incorporation attenuated organic contaminants and adsorbed heavy metals in soils, minimizing their mobility and bioavailability (Zhang et al. 2013b; Rees et al. 2014).

The environmental persistence and the great capability for simultaneously ameliorating soil health and decontaminating polluted soil make biochar a promising material in land reclamation. Amending abandoned mine land soil with poultry litter-derived biochar at 2 wt% dramatically promoted the germination and growth of the native plant Poverty Oatgrass (*Danthonia spicata*) (Buyantogtokh 2013). Field trials at an abandoned mine land site in San Juan County, Colorado showed impressively promoted vegetation growth and establishment through amending the strongly acidic, heavy metals-contaminated soils with biochar in comparison with straw mulch (Peltz and Harley 2016).

Biochar has been evaluated as a filter medium in bioretention basins to treat urban storm water (Guo 2013). Tian et al. (2016) observed that sand infiltration columns amended with 10% wood-based biochar or poultry litter-based biochar prepared by ≥500 °C slow pyrolysis removed more than 90% of the nitrogen from the influent containing 2 mg l<sup>-1</sup> NH<sub>4</sub><sup>+</sup>. Biochars with high CEC and SSA may be used as effective filter media in stormwater treatment facilities to remove nutrients, heavy metals, pesticides, and other contaminants (Shimabuku et al. 2016).

### 21.4.3 Refining Pyrolysis Bio-Oil for Biofuels and Chemicals

Pyrolysis bio-oil, similar to fossil petroleum oil, serves as a source material for transportation fuels and industrial chemicals. Low moisture content (e.g. <25%) pyrolysis bio-oil can be directly combusted for energy using specialized burners (Vamvuka 2011). Physical blending with No. 2 diesel fuel or ethanol rectifies such bio-oil to a readily combustible fuel that can be used as a quality liquid fuel for residential heating, electricity generation, and powering stationary engines (Ikura et al. 2003; Nguyen and Honnery 2008). Through particular refining technologies such as catalytic cracking and hydrodeoxygenation, pyrolysis bio-oil can be upgraded into gasoline, kerosene, and diesel fuels. Furthermore, pyrolysis bio-oil contains more than 300 organic compounds (Czernik and Bridgwater 2004); these chemicals can be separated and purified for different industrial purposes (Zhang et al. 2013a). In addition, pyrolysis bio-oils derived from plant materials have been used to manufacture liquid smoke products for the food industry to prepare “barbecue” flavor foods (Montazeri et al. 2013). Worldwide there are a number of operating plants to convert agricultural byproducts to pyrolysis bio-oil. More efficient catalysts and refining techniques have been under development to facilitate transportation fuel production from pyrolysis bio-oil with cost-competitiveness (Cheng et al. 2017).

## 21.5 The Global Scenario of Biochar and Bio-Oil Production from Agricultural Byproducts

According to IBI, worldwide there were 326 active biochar manufacturing companies in 2015, of which 132 were located in the US, 79 in Europe, 27 in Australia and New Zealand, 23 in China, 16 in Canada, and 9 in Africa (IBI 2016). Globally a total of 283 200 tons of biochar was produced in the year (GVR 2017). In 2016, the US biochar production was 117 300 tons, mainly from pyrolysis and gasification of wood and other agricultural byproducts (GVR 2017).

Commercial production of bio-oil via fast pyrolysis has not been realized due to its low economic viability. Globally and in the US, there are a number of pilot-scale operating plants to produce bio-oil from woody biomass (Guo et al. 2015). The bio-oil is upgraded to heating fuels and industrial feedstock materials.

The global biochar market has been expanding annually at an average rate of 13.1%. It was predicted that the global biochar market would reach \$3.14 billion by 2025 (GVR 2017). Different varieties of pyrolysis units have been designed to produce biochar, bio-oil, and syngas from agricultural byproducts at backyard, small, and industrial scales (Figure 21.5). The feasible feedstocks include crop residues, waste wood, forestry debris, food processing residues (e.g. nut shells, fruit pits, bagasse, etc.), animal manures, sewage sludge, yard trimmings, food waste, and municipal organic solid waste (IBI 2018). At present the global production and utilization of biochar is still in its infancy. Considering the current high growth rate, IBI set up a One-Billion-Ton vision in 2017, striving to promote the global annual production of biochar to 1 billion ton within the next 50 years.



**Figure 21.5** A mobile pyrolyzer designed by Virginia Polytechnic Institute and State University (Blacksburg, VA) for small scale production of biochar and bio-oil from agricultural byproducts IBI 2018.

## 21.6 Summary and Conclusions

Globally more than 10 billion dry tons of agricultural byproducts are generated each year, including crop residues, animal manures, food processing refuses, forestry debris, and green waste. These biomass residues can be valorized through thermochemical conversion to biochar and bio-oil, two valuable products for agriculture, industry, energy, and the environmental sector. The pyrolysis technique, that has long been practiced to manufacture charcoal from wood, is ready to be used to produce biochar and bio-oil from agricultural byproducts: Heating biomass residues in the absence of air results in a black solid as biochar and a liquid vapor condensate as bio-oil. Biochar is porous, environmentally stable, and nutrient and water retentive. Applied to cropland as a soil amendment, biochar persistently improves soil physical, chemical, and biological properties, sustains the overall soil quality, and promotes crop production. Biochar amendment also facilitates soil carbon sequestration, mitigates soil greenhouse gas emissions, and deactivates soil contaminants. Incorporation of biochar in animal feed enhances animal performance while in manure waste reduces nutrient losses. Biochar can also be used as a soil amendment in abandoned mine land reclamation and as a filter medium in stormwater treatment. Bio-oil can be used as a heating fuel, upgraded to transportation fuels, and refined for industrial chemicals and materials.

The yield and quality of biochar and bio-oil from pyrolysis of agricultural byproducts vary significantly with the feedstock type and the pyrolysis conditions. Slow pyrolysis at lower temperature (e.g. 400 °C) maximizes the biochar yield while fast pyrolysis at moderate temperature (e.g. 500 °C) peaks the bio-oil yield. Given its lower feedstock pre-treatment requirements, lower infrastructure investment, and less operating complexity, slow pyrolysis is recommended to convert agricultural byproducts to biochar and bio-oil. Stability, CEC, and porosity are the most important parameters to evaluate the biochar

quality, while moisture, C and O contents are those to assess the bio-oil quality. Global production and utilization of biochar emerged a decade ago and has been expanding at a high rate, striving toward a \$3 billion market by 2025.

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## 22

# Polymers and Adsorbents from Agricultural Waste

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## 22.1 Introduction

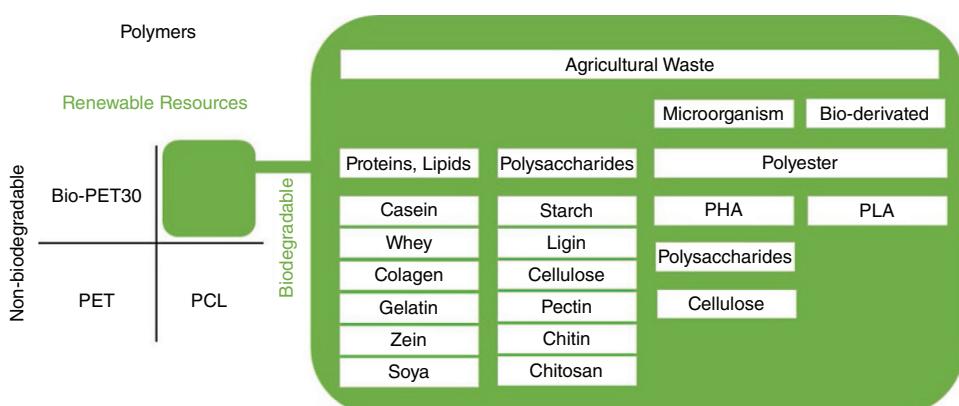
Environmental pollution, especially water pollution of lakes, rivers, and oceans, is one of the major challenges that humanity is facing in its history. The environment, and also human health, are at risk due to a worldwide increase in pollution (Schwarzenbach et al. 2010). There are several resources of pollution, such as heavy metals from industrial processes or petroleum-based plastic materials. Millions of tons of plastic are polluting the sea each year. Synthetic plastic is non-biodegradable, instead it degrades through mechanical and physical influences to form so-called microplastics (<1 mm) or nanoplastics ( $\leq 0.1 \mu\text{m}$ ). These particles can enter the human food chain with unknown consequences (Wagner and Lambert 2018). Eriksen et al. calculated that a minimum of 5.25 trillion plastic particles with a total weight of 270 000 tons are polluting our seas (Eriksen et al. 2014). Almost the half of the plastic goods sold are only used for a short time and could be substituted easily by society (Chen and Patel 2012), potentially easing the increasing burden of plastic waste accumulation.

The agricultural industry produces annual waste in the billion-ton scale, additionally 1 billion tons of produced food is wasted (Obi et al. 2016; Gustavsson et al. 2013). This organic biomass is full of useful compounds like proteins, lipids, or polysaccharides. This chapter focuses on the production of biodegradable polymers, which are favorable substitutes of petroleum-based plastics, and on the production of adsorbents, which can be used for the removal of heavy metals and other toxic chemicals from wastewater, both from agricultural waste streams.

## 22.2 Polymers

Polymers are large molecules consisting of repeating sequences of either one monomer (homopolymers) or different monomers (heteropolymers) in linear or interconnected structure. Depending on their length and composition, they have various thermal and mechanical properties (Gnanou and Fontanille 2007). Polymers can be divided into four groups: (i) non-biodegradable polymers from petrochemical resources (e.g. polyethylene terephthalate, PET); (ii) non-biodegradable polymers from renewable resources (e.g. Bio-PET30); (iii) biodegradable polymers from petrochemical resources (e.g. polycaprolactone, PCL); and (iv) biodegradable polymers from renewable resources (e.g. polyhydroxyalkanoate, PHA) (Siebert-Raths and Endres 2011). The last group, biodegradable polymers from renewable resources plays an increasing role in the society to replace sustainable common non-biodegradable polymers from petrochemical resources (Sillanpää and Ncibi 2017). Biodegradable polymers can be grouped in three clusters; (i) polymers directly recovered from agricultural waste as polysaccharides, proteins and/or lipids; (ii) polymers synthesized from microorganisms fed on renewable carbon sources, such as PHA; and (iii) chemically fabricated polymers from bioderived monomers, such as poly lactic acids (PLAs). An overview of the different polymer groups and clusters of biodegradable polymers is shown in Figure 22.1.

Biopolymers with similar properties compared to thermoplastics are so called bioplastics, regardless of their resource or their biodegradability. The global production of bioplastics is predicted to increase from 4 million tons in 2016 to 6 million tons in 2020, wherefrom only 1 million tons are represented by bio-based and biodegradable polymers (Aeschelmann and Carus 2015). At the same time, the annual petrochemical plastic production of 300 million tons is increasing yearly by 4% (Plastics Europe 2015). This sub-chapter focuses on the production of biodegradable bioplastics from agricultural waste streams, since the costs for the feedstocks are one of the main reasons why these materials still play a minor role in industrial applications. Using low-cost feedstocks like agricultural waste has the potential to push the current high market prize of bioplastics ( $2\text{--}15 \text{ } \text{€ kg}^{-1}$ ) to a level that could result in a higher acceptance of these materials in society (Siebert-Raths and Endres 2011; Rodriguez-Perez et al. 2018; Lettner et al. 2017; Dietrich et al. 2017).



**Figure 22.1** Classification of polymers, starring biodegradable polymers from agricultural waste.

## 22.3 Polyhydroxyalkanoates

PHAs are microbially-synthesized polyesters with comparable properties to petroleum-based plastics. They are simple and fully biodegradable to CO<sub>2</sub> and water in usual habitats. Many bacteria accumulate PHA as carbon and energy storage under nutrient-limiting or unbalanced growth conditions. Nitrogen and phosphate limitation are thereby the most commonly used triggers during PHA bioprocesses (Steinbüchel et al. 1992). *Ralstonia eutropha* is the model organism for PHA production, since it can store up to 90% of its cell dry weight (CDW) as PHA, can consume various carbon sources and is easily modifiable through metabolic engineering (Reinecke and Steinbüchel 2008). Recombinant strains are used to produce a wide range of different PHA polymers with varying properties. The characteristics of PHAs are highly influenced by the length of the polymer sidechains. Three classes of PHAs have been defined: Short chain length (*scl*) PHAs with side chains of 3–5 carbon atoms, medium chain length (*mcl*) PHAs with side chains of 6–13 carbon atoms and long chain length (*lcl*) PHAs, which are consisting of monomers with side chains of  $\geq 14$  carbon atoms (Rehm 2003). In heteropolymers, the molar concentrations of the different monomers can control the physical properties of the polymers (Noda et al. 2005). A combination of the choice of the carbon feedstock and a proper metabolic engineering strategy can be used to create tailor-made polymers with properties useful for specific applications (Riedel et al. 2014). Overall, *scl*-PHAs are more crystalline and brittle, whereas *mcl/lcl*-PHAs are more amorphous and flexible. Besides *Ralstonia* strains, strains of *Pseudomonas* and recombinant *Escherichia coli* are widely used for PHA production (Lee 1996). However, further screening and bioprospecting for wildtype bacterial strains, which naturally produce diverse types of PHA with unique monomers is done to avoid working in a genetically modified organism (GMO) facility, which could reduce operation costs of the plant (Koller et al. 2017). Regardless of the use of wild type or recombinant strains for PHA production, there are some ground rules that need to be followed when working on a bioprocess that is aimed to be industrially viable. At the end of the process, the production strain must accumulate a sufficient PHA content per CDW (>50%), which facilitates the downstream process. Furthermore, the polymer productivity and total production needs to be high with  $>1\text{ g (l h)}^{-1}$  and  $>100\text{ g l}^{-1}$ , respectively (Riedel et al. 2014).

PHA is a promising candidate for substitution of synthetic plastics. PHA can replace plastic material in common household and industry products (as bottles, bags, or foils), they are used in the cosmetic industry and are also playing a role in the pharma industry (Raza et al. 2018). Their biodegradability has not only environmental advantages, but also it can be targeted to medical or agricultural products (Ong et al. 2017; Lizarraga-Valderrama et al. 2016). Other specific PHA properties like high thermal stability, high ultraviolet light protection capability and a low gas permeability can further improve its application utility, e.g. in the food and packaging industry (Fabra et al. 2014; Koller 2014). PHA exhibits a better oxygen barrier performance than synthetic polymers, such as e.g. PET. Using packaging material made from PHA could allow for a better food conservation, since oxidation can affect color, flavor, and the microbial stability of many foods (Koller 2014). To further improve the hygiene conditions of PHA material, antimicrobial substances like zosteric acid or silver and zinc oxide nanoparticles can be integrated into the polymer (Hany et al. 2004; Castro-Mayorga et al. 2014; Diez-Pascual and

Diez-Vicente 2014). Overall migration behaviors (i.e. “leaching”) of PHA-based material have been reviewed by Scarfato et al. Not many studies have been done in this direction, however the ones that were performed showed that the migration stayed under a critical value (Scarfato et al. 2015). Overall migration of PHA food packing material has been tested with food simulant solvents alone, such as distilled water, 3% acetic acid, 15% ethanol (Bucci et al. 2007) and with a combination of simulant solvents (isooctane and 10% ethanol) together with zinc oxide nanoparticles (Diez-Pascual and Diez-Vicente 2014).

However, due to high production costs and inexpensive, readily available synthetic plastics, PHA has, up to now only played a minor role in the bioplastic market. The reasons for the comparable high market price of PHA are the costs of the carbon feedstock, the recovery costs and the low production volume. To accelerate industrial PHA production, low cost carbon feedstocks need to be applied in efficient bioprocesses. Therefore, many agricultural waste streams containing various sugars and lipids are investigated for their potential use as feedstocks for PHA production, as described below (Table 22.1).

### 22.3.1 PHA from Feedstocks Containing Sugars

Agricultural waste streams provide a lot of sugar rich feedstocks like molasses, starch, or lignocellulosic plant biomass. Molasses can be used directly as carbon feedstock, due to its composition of di- and oligosaccharides, such as sucrose and raffinose (Solaiman et al. 2006). Whereas, plant-based materials must be hydrolyzed to produce fermentable carbon feedstocks prior to cultivation (FitzPatrick et al. 2010). The efficient conversion of biomass to fermentable sugars is the main challenge for the production of value-added products like PHA (Lynd et al. 2008; Fava et al. 2015). The sugar concentration, composition, and concentrations of inhibitors in the feedstocks, such as lignin and organic acids, can influence the PHA productivity. For efficient PHA production, it is important to use bacterial strains that can utilize all the various kinds of carbohydrates present in the feedstocks, such as sucrose, fructose, glucose, galactose from molasses or xylose, glucose arabinose, mannose, and glucuronic acid from hydrolyzed plant based biomass (Solaiman et al. 2006; Salgaonkar and Bragança 2017). Several wild type and recombinant strains have been used to produce PHA from various sugar-containing feedstocks (Table 22.1). The highest productivity was reached using highly concentrated sucrose feedstocks with  $4\text{ g (l h)}^{-1}$  polyhydroxybutyrate (PHB) and a total PHB production of  $>100\text{ g l}^{-1}$  (Yamane et al. 1996). Recently, a membrane-based feeding system for a lab scale reactor has been described by Haas et al., which allows high-cell-density cultivations with a high productivity of  $>3\text{ g (l h)}^{-1}$  of PHB in cultures using dilute carbon feedstocks (Haas et al. 2017). In this membrane-based feeding system, the cells remain in the bioreactor, whereas the media with the carbon feed passes through. Thus, the cultivation volume can be maintained at a smaller level. However, the disadvantages to this process are that the membrane must be changed during cultivation because of clogging. The use of non-sterile replacement membranes showed the robustness of the process. Since this process was developed with artificial sugar waste streams, a confirming study with real wastewater feedstocks is desirable. The Italy-based company Bio-on, which is producing 5000–10 000 tons of PHA (tradename Minerv<sup>®</sup>) annually from renewable sugar feedstocks, will open a special PHA production plant in the summer of 2018, focusing on the use of agricultural waste streams. Among others, byproducts of the sugar beet, sugar

**Table 22.1** PHA and lactic acid production by different strains from selected carbon sources.

Carbon source	Strain	Product	Production/Yield			PHA/CDW [wt%]	Reference
			[g l <sup>-1</sup> ]	[g (l h) <sup>-1</sup> ]	[g g <sup>-1</sup> ]		
Sucrose	<i>Alcaligenes latus</i>	PHB	068	4.0		50	Yamane et al. 1996
Glucose	<i>Ralstonia eutropha</i> H1	PHB	112	3.1	0.3	76	Haas et al. 2017
Whey	<i>Haloferax mediterranei</i>	P(HB- <i>co</i> -8 mol%HV)	006	0.05	0.3	50	Koller et al. 2007
Whey	<i>recom. Escherichia coli</i>	PHB	168	4.6		87	Ahn et al. 2001
Activated sludge + acetate	Mixed culture	PHB				70	Serafim et al. 2004
Palm oil mill effluent	Mixed culture	P(HB- <i>co</i> -23 mol%HV)			0.6	64	Lee et al. 2015
acetic, propionic, butyric acid	<i>R. eutropha</i> H16	P(HB- <i>co</i> -6 mol%HV)	094	2.1		83	Huschner et al. 2015
Waste glycerol	<i>R. eutropha</i> H1	PHB	038	1.1		50	Cavalheiro et al. 2009
Waste rapeseed oil + propanol	<i>R. eutropha</i> H16	P(HB- <i>co</i> -8 mol%HV)	138	1.5	0.8	76	Obruca et al. 2010
Coffee oil extracted from SCG	<i>R. eutropha</i> H16	PHB	049	1.3	0.8	89	Obruca et al. 2014
Plant oil	recom. <i>R. eutropha</i>	P(HB- <i>co</i> -19 mol%HHx)	103	1.1	0.6–0.8	74	Riedel et al. 2012
Tallow	<i>R. eutropha</i> H16	PHB	024	0.3	0.4	63	Riedel et al. 2015
Waste animal fats	recom. <i>R. eutropha</i>	P(HB- <i>co</i> -19 mol%HHx)	027	0.4	0.5	60	Riedel et al. 2015
Coffee pulp hydrolysate	<i>Bacillus coagulans</i>	LA		4.0	0.8	–	Pleissner et al. 2016
Waste bread	<i>B. coagulans</i>	LA	100	2.5		–	Venus 2014
Mixed food waste	<i>Streptococcus sp</i>	LA		2.2	0.8		Pleissner et al. 2017
Chicory flour	<i>Lactobacillus paracasei</i>	LA	134	1.1		–	Petrova et al. 2015

cane, potato, and fruit industry will be tested as carbon feedstocks for PHA production with a capacity of 1000–2000 tons per year ([www.bio-on.it](http://www.bio-on.it)).

### 22.3.2 PHA from Whey

Whey, a byproduct from the dairy industry, contains 4–5% lactose, a disaccharide consisting of glucose and galactose. The worldwide whey production of around  $10^8$  t per year exceeds the demand on whey powder production. This makes whey an interesting feedstock for PHA production, since the current surplus is waste (Koller et al. 2013). Whey could be used directly as a carbon source, or after hydrolysis, depending on the lactose consumption capability of the strain of PHA producing bacterium used. Koller et al. compared several wild type strains for their ability to produce PHA from whey. Using wild type strains has the advantages that no special GMO facility is needed for the production process. Only *Hydrogenophaga pseudoflava* could use lactose directly as a carbon source. However, the PHA accumulation with 12% per CDW was insufficient. For the other wild type strains, the lactose from the whey permeate was hydrolyzed enzymatically. Also, a cheaper chemical hydrolyzation of lactose is possible in this process (Koller et al. 2017; Pais et al. 2016). *Haloflexax mediterranei*, was selected as most promising strain, because it accumulated 50% PHA per CDW from whey hydrolysate. Another benefit was seen by the production strain's high tolerance of salts, which on the one hand allows for growth and synthesis in semi-sterile conditions, saving energy costs from sterilization, and on the other hand allows a simplified recovery in distilled water as a result of the high inner salt content of the cells (Fernandez-Castillo et al. 1986; Koller et al. 2007). However, cultivation in this manner also creates large amounts of wastewater with high salt concentration, which must be recycled at the end of the process. A life cycle analysis (LCA) study about using *H. mediterranei* for industrial PHA production indicated that there are still several issues to overcome. The long cultivation time of over  $>100$  h and the very low conversion rate of whey to PHA from only 0.8% at pilot plant scale are the two fundamental issues (Koller et al. 2013). Therefore, bioprocesses using recombinant *E. coli* are still preferable for PHA production from whey. Hydrolysis of lactose is not necessary, since *E. coli* contains genes for  $\beta$ -galactosidase. Ahn et al. demonstrated a high PHB productivity of  $2.6\text{--}4.6\text{ g (l h)}^{-1}$  from concentrated whey solutions, using recombinant *E. coli*, which harbored the PHA production genes from *Alcaligenes latus* using different high cell density (up to  $200\text{ g l}^{-1}$ ) fed batch cultivation systems. The PHA content per CDW of 87% was also very high, which can easily facilitate the downstream processing (Ahn et al. 2001; Ahn et al. 2000) (Table 22.1).

### 22.3.3 PHA from Anaerobic Treated Wastewater

Several studies have shown a relatively simple method of PHA production from wastewater, after its anaerobic treatment, using microbial mixed cultures (MMCs) (Morgan-Sagastume 2016; Dias et al. 2006). Among others, sugar cane molasses (Albuquerque et al. 2007), brewery wastewater (Ben et al. 2016), dairy manure (Coats et al. 2016), cheese whey (Colombo et al. 2016), olive oil mill effluent (Dionisi et al. 2005) and palm oil mill effluent (Lee et al. 2015) have been used as carbon feedstocks. Usually this process is performed in multiple steps. In the first step, an acidogenesis occurs, allowing for the conversion of insoluble polymers, like carbohydrates, to volatile fatty acids (VFAs).

During the second step, the VFA-containing supernatant is used to feed active sludge for the selection and enrichment of biomass having high potential of PHA accumulation using feast and famine conditions (Salehizadeh and Van Loosdrecht 2004). This biomass is then transferred to another reactor where the PHA is produced under batch conditions. Thereby, the VFA-containing supernatant from the acidogenesis step, serves again as carbon feedstock. The second and third step can also be performed under aerobic conditions to enhance PHA accumulation, depending on constituents of the MMC, since only the acidogenesis requires anaerobic conditions (Beccari et al. 2009). Also, this process can be combined with a fully anaerobic digestion for biogas production (Campanari et al. 2017; Ntaikou et al. 2009). Besides all the advantages of a non-sterile production process as a cost-saving operation cost or the ability of running open cultivation in sewage treatment plants, disadvantages are present, including a relatively low productivity mainly due to the low carbon content in the wastewater and an undefined culture, which leads to a variation of the quality and composition of the produced PHA. Different microorganisms are producing diverse types of PHAs. A constantly changing PHA mixture is challenging for processing of the polymers (Koller et al. 2017). To avoid producing a PHA mixture with MMC, Serafim et al. only fed acetate under ammonia limitation conditions to activated sludge for PHB production. Under these controlled conditions, biomass with high (>70%) PHB per CDW could be produced continuously over a time frame from two years (Serafim et al. 2004)! (Table 22.1). However, this strategy requires a separation of acetic acid from VFA mixtures. Another way to overcome the issue of inhomogeneous PHA accumulation is by using pure cultures following acidogenesis. The VFA supernatant can be used directly or after concentration to feed pure cultures for PHA production (Cerrone et al. 2014). Using concentrated VFA can markedly increase the productivity (>2 g (1 h)<sup>-1</sup> PHA). However, due to the high toxicity of VFA, a sensitive feeding strategy must be applied (Huschner et al. 2015) (Table 22.1).

### 22.3.4 PHA from Waste Lipids

Lipids as fatty acids, oils, and fats, whether plant- or animal-based, are favorable feedstocks for PHA production. They have a high carbon content and can be used as “100%” feedstock during the fed-batch production process, which allows the achievement of high cell density cultivations. Conversely, sugars have a lower molar carbon content and can only be fed as a maximum of an 85% solution, in the case of glucose. Also, lipids are favorable carbon sources for the synthesis of *mcl*-PHA, since the *mcl*-HA precursor molecules can be generated more efficiently from  $\beta$ -oxidation intermediates, as compared to synthesis from fatty acid biosynthesis pathway in PHA production strains. (Riedel et al. 2014). However, cultivations with lipids are much more challenging than cultivations with sugars, since the PHA production organisms are cultivated in an aqueous medium. The usage of the hydrophobic lipids in these media creates a 3-phase system between the air, the lipids, and the aqueous media. The hydrophobic characteristics, especially from fats and oils, make them difficult to consume by most microorganisms. *R. eutropha* can consume oils and fats by secreting a lipase enzyme, which cleaves the fatty acids from the glycerol backbone of the fat/oil molecules (Lu et al. 2013). The cleavage products are then creating an emulsion, which increases the surface area of the lipid droplets and therefore increases their bioavailability in the aqueous media.

Direct use of plant oils like soy bean, rape seed or palm oil has been widely studied as carbon sources for PHA production (Riedel et al. 2012; Fadzil and Tsuge 2017). In terms of agricultural waste streams, cooking, waste vegetable oil or waste frying oils has been used as feedstocks (Obruca et al. 2010; Verlinden et al. 2011; Kourmentza et al. 2018). However, the feedstock prices of these used cooking oils are not much below the prices of the abundant available plant oils, such as palm or soy bean oil. This is due to the fact that these used oils can be used as starting material in the biodiesel industry.

Spent coffee grounds are byproducts of the coffee industry and contain around 15% coffee oil. Obruca et al. reviewed the PHA production from this waste lipid feedstock (Obruca et al. 2015). PHB was produced from extracted coffee oil by using the wild type *R. eutropha* strain H16. A biomass of  $50\text{ g l}^{-1}$  (CDW) with a high PHA content of 89%, a productivity of  $1.33\text{ g PHB (l h)}^{-1}$  and a yield of  $0.82\text{ g PHB}$  from  $1\text{ g}$  coffee oil were reached during this process. (Obruca et al. 2014) (Table 22.1). Accumulation of the *scl*-PHA co-polymer P(HB-*co*-HV) from spent coffee ground hydrolysate was achieved by the cultivation of *Burkholderia cepacia* (Obruca et al. 2014). Recently, Bhatia et al. were able to engineer *R. eutropha* to produce the *mcl*-PHA P(HB-*co*-HHx) from coffee waste oil. The recombinant strain Re2133 accumulated 69% PHA with a high molar content of 22 mol% HHx.

Byproducts of the biodiesel industry are also in focus as PHA production feedstock. There are two main streams of the biodiesel industry that are suitable as PHA production feedstocks: fatty acid ethyl esters/fatty acid methyl esters (FAEEs/FAMEs) of poor quality and raw glycerol, both direct products of the fat/oil conversion to biodiesel (Koller et al. 2017; Titz et al. 2012; Muhr et al. 2013; Hermann-Krauss et al. 2013; Cavalheiro et al. 2012). Basnett et al. produced a novel multi *mcl*-PHA copolymer from biodiesel byproducts with *Pseudomonas mendocina* CH50 and showed its potential use as new material for soft tissue engineering (Basnett et al. 2017).

Riedel et al. investigated the usage of waste animal fats (WAFs) as direct feedstock for PHA production with *R. eutropha* (Riedel et al. 2015). Annually around 10 million tons of animal byproducts are accumulated by the agricultural industry in Europe alone. The WAF are categorized in three classes by their risk potential (high, medium, and low) (Eu 2009). High risk, class 1, material needs to be destroyed by burning, whereas class 2 and class 3 material are promising candidates for biotechnological feedstocks. Especially material with high contents ( $>30\%$ ) of free fatty acid (FFA), since these materials are not suitable for an efficient biodiesel production. Because of a higher content of saturated fatty acids, as compared to plant oils, WAFs have high melting temperatures from  $34$  to  $>55\text{ }^{\circ}\text{C}$ . The used WAF by Riedel et al. were hardly consumable for *R. eutropha* at the cultivation temperature of  $30\text{ }^{\circ}\text{C}$ . Through optimized pre-culture conditions and a mixed feeding strategy of liquid waste frying oil and solid WAF, a cultivation in bioreactors without emulsifying agents was possible. Using a recombinant *R. eutropha* strain, around  $50\text{ g l}^{-1}$  biomass (CDW) with a content of 75% of the co-polymer P(HB-*co*-HHx) could be produced from WAF in fed-batch cultivation (Riedel et al. 2015) (Table 22.1). The process was scaled up to a pilot scale of  $150\text{ l}$  (data not published). The developed feeding strategy can also be used for other solid fat containing waste lipid feedstocks.

Animal byproducts can be separated into a fat, a water/protein and a bone sediment phase (Stanley 2014). However, between the fat and water phase an interphase occurs which consist of proteins and fat, which can be described as fat emulgate. The production

of value-added PHA biopolymers from fat emulgates is a very promising approach, since alternatively fat emulgates can only be used for lower value products like biogas, or it is burned directly for thermal usage. Riedel and co-worker could show in preliminary experiments the suitability of waste fat emulgate as carbon feedstock for PHA production. From 1 g fat in the fat emulgate, 0.6 g of the PHA copolymer P(HB-*co*-HHx) could be produced with recombinant *R. eutropha* strains (data not published), which is the same yield as reached from plant oil (Riedel et al. 2012) (Table 22.1). As an alternative to commonly used nitrogen sources, chicken feather hydrolysate was used to produce PHA with *R. eutropha* from waste vegetable oils (Benesova et al. 2017).

## 22.4 Polylactic Acids

PLAs are biodegradable polyesters, which have similar thermoplastic properties as petroleum-based plastic. Unlike PHA, only the monomers of PLA, the lactic acid, can be produced biotechnologically. In contrast, the final synthesis takes place chemically. Synthesis, properties, and applications of various PLA polymers have been described previously (Belgacem and Gandini 2008; Johnston and Adhikari 2017; Pang et al. 2010). Lactobacilli have been used to produce lactic acid from a wide range of renewable carbon sources including sugars, starch, or lignocellulosic feedstocks as molasses, corn or corn stover, grains, and green biomass (Ghaffar and Fan 2014; Venus 2006). Worldwide lactic acid production is expected to reach 1.3 million tons in 2020 (Venus et al. 2018). Pleissner et al. produced lactic acid at pilot plant scale with *Bacillus coagulans* from coffee pulp hydrolysate. Prior fermentation they could convert 70–80% of the coffee pulp into fermentable sugars. The lactic acid production at 50 l scale was high with  $4.0 \text{ g (l h)}^{-1}$ . They reached a yield of 0.78 g lactic acid per gram sugar (Pleissner et al. 2016) (Table 22.1). Using waste bread from the bakery industry, which is rich in starch,  $100 \text{ g l}^{-1}$  lactic acid could be produced during fermentation with strains of *B. coagulans* (Venus 2014) (Table 22.1). Since annually over 1 billion tons of food is wasted worldwide (Gustavsson et al. 2013), waste food mixtures are in the focus for lactic acid production. Venus et al. compared available one- and two-step lactic acid processes regarding titer, yield, technical complexity and economy (Venus et al. 2018). In the classical two-step lactic acid process, the potential carbon feedstock is pre-treated enzymatically, thermally, or chemically for the conversion of feedstock compounds, like polysaccharides, to fermentable sugars. The resulting hydrolysate is then used directly or after concentration as fermentation feedstock. In a one-step process, the feedstock is used directly in the fermentation process. The release of sugars takes place through enzyme secretion by the production strains. The process is called SSF – simultaneous saccharification and fermentation. The SSF approach saves time and simplifies the bioprocess control, since the sugars are consumed as they appear. The highest reported total production was reached with *Lactobacillus paracasei* from chicory flour with a final lactic acid titer of  $124 \text{ g l}^{-1}$  and a yield of  $0.9 \text{ g g}^{-1}$ . The strain produced natural inulinase to saccharify the starch. (Petrova et al. 2015). Pleissner et al. developed a SSF lactic acid production process using simultaneous saccharification during the lactic acid fermentation with *Lactobacillus* sp. or *Streptococcus* sp. (Pleissner et al. 2017). The waste had a

composition of 34% starch, 15% proteins, 13% fat and 9% free sugars. A productivity of 0.3–0.5 g (l h)<sup>-1</sup> lactic acid was reached with *Lactobacillus* sp. strains, whereas *Streptococcus* sp. produced lactic acid at a maximum rate of 2.2 g (l h)<sup>-1</sup> with a yield of 0.8 g g<sup>-1</sup> (Table 22.1).

However, after the fermentative production of lactic acid, a complex recovery process, depending on the used feedstock, is necessary to purify the lactic acid before the chemical synthesis of PLA (Abdel-Rahman et al. 2010). The chemical synthesis of PLA also hinders its biodegradation. Two steps play a key role in the biodegradation of PLA. The first step hydrolyses at high temperatures (55–70 °C) and high humidity takes between one and two weeks. During this period, the polymer is broken down into smaller fragments of ~10 000 Da. In the second step, microorganisms are adsorbing and degrading these smaller fragments to CO<sub>2</sub> and water (Emadian et al. 2017). The initial need of high temperature for degradation made common PLA poorly biodegradable in natural environments. Blending PLA with other better biodegradable polymers, as, e.g. PHA, enhances its biodegradation, since the initial hydrolysis is accelerated (Rasal et al. 2010).

## 22.5 Chitin and Chitosan

Chitin, a natural polysaccharide based on *N*-acetylglucosamine monomers, is the basic structural polymer of fungi, insects, and crustaceans. Its structure is similar to cellulose, but where the hydroxyl at position C2 is substituted by an amino group. Chitin is one of the most common biopolymers on earth with an annual turnover of 10<sup>10</sup>–10<sup>11</sup> t. It is a hard and inelastic, nitrogen-rich polymer (Ravi Kumar 2000). Chitosan is similar to chitin, although the monomers in chitosan have been significantly deacetylated. Chitin and chitosan are used for a wide range of applications. The high nitrogen content of chitin (up to 7%) makes it suitable as chelating agent. Chitosan and chitin are used in the field of biotechnology as immobilizer, in the field of agriculture as fertilizer and antifungal agents, in biomedicine they play a role in cancer treatment, in wastewater treatment they serve as natural adsorbents, in the cosmetic industry they are used for hair and skin care and in the food industry they serve as nutrition or coating material (Philibert et al. 2017). Chitin can be produced from crustaceans, insects, and microorganisms. However, their main production source comes from byproducts of the shellfish processing industry as shells from shrimps, crabs, lobsters, and krill. Chitin can be recovered chemically with acids and bases or microbiologically with lactic acid bacteria and protease-producing bacteria. The added acids or the produced lactic acid are converting the main inorganic component of the shells, calcium carbonate, to calcium lactate or other calcium salts, which allows the recovery of the chitin (Arbia et al. 2013). Chitin production by *Lactobacillus plantarum* fermentation of shrimp biowaste resulted in a chitin yield from up to 13% from shrimp shells. The crude chitin was converted to chitosan using a strong 13 M NaOH (Rao and Stevens, 2005). Enzymatic chitin recovery has been reported as a cleaner alternative, if water recovery is employed (Lopes et al. 2016). The current status of chitin and chitosan production and the potential extraction from fungal and insect sources as well as their application as functional biopolymers is described by Philibert et al. (Philibert et al. 2017).

## 22.6 Other Polymers

Further polymers which can be recovered directly from waste biomass with components belonging to the groups of polysaccharides, proteins, and lipids. The main polysaccharides include starch, lignin, cellulose, and pectin. Casein, whey, collagen and gelatin belong to the group of animal proteins, whereas zein, soya, and gluten can be recovered from plant biomass. Cellulose can also be produced, without lignin and hemicellulose, by microorganism from renewable carbon sources (Reiniati et al. 2017).

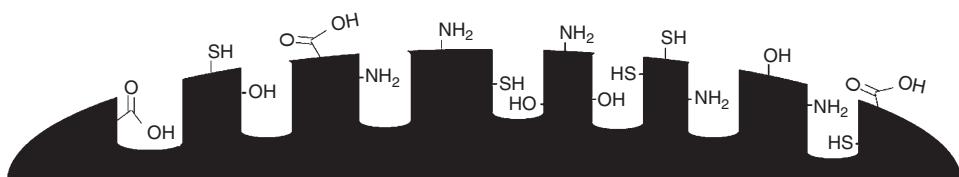
## 22.7 Adsorption

Adsorption can perform many separations that are considered inefficient or impossible using conventional separation methods. The process of adsorption is commonly used in wastewater treatment, and a key separation that is often performed in this way is the removal of heavy metals. The ingestion of heavy metals in contaminated water can result in very detrimental effects to the organism's health. For example, while zinc and copper are essential to human health in small quantities, ingestion of excessive amounts of both metals leads to toxic effects that can be as fatal as death to the organism. Nickel at critical levels can cause lung and kidney problems. Lead causes central nervous system damage, among other things, while cadmium is thought to be a human carcinogen (Fu and Wang 2011). Also, with adsorption, trace compounds that are toxic or impart unpleasant odors, etc., can be removed from waste streams. Beyond waste treatment, there are numerous applications for adsorbents of varying shape, size, and chemical makeup, with different properties being required for different processes. There are several attributes of adsorbents that are critical for any application: capacity, regenerability, kinetics, compatibility, and cost (Table 22.2).

Inorganic materials, like metal chlorides or oxides, have been used as adsorbents. These compounds are inexpensive, and many, including  $\text{CaCl}_2$ ,  $\text{SiO}_2$ ,  $\text{CaO}$ ,  $\text{MgO}$ ,  $\text{ZnO}$ , and zeolites, are very effective adsorbents for specific purposes (Fu and Wang 2011). Hydroxide precipitation is a very common method for removing heavy metals from wastewater, mainly due to its simplicity and low cost. However, the large volumes of sludge produced is one drawback to the method. Sulfide precipitation can also be used to remove heavy metals, but the potential of evolving toxic  $\text{H}_2\text{S}$  fumes and settling of

**Table 22.2** A list of key properties of adsorbents for any application and definition or example of these properties.

Adsorbent property	Definition
Capacity	The amount of adsorbate taken up per unit mass or volume
Regenerability	How easily the adsorbate can be removed from the surface of the adsorbent
Kinetics	Rates of adsorption by different adsorbates
Compatibility	Resistance to conditions that could decrease adsorbent life
Cost	The price per unit mass of adsorbent required for different applications



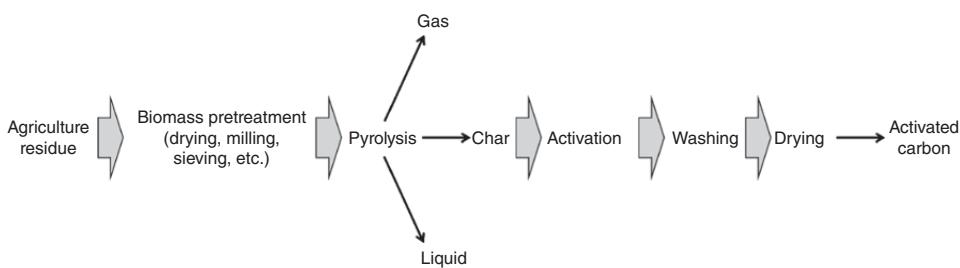
**Figure 22.2** Scheme of the surface of an activated carbon particle.

filtration issues that may occur with this process make it non-ideal (Fu and Wang 2011). Organic materials, specifically polymers and activated carbon, are also very effective as adsorbents. The utility of activated carbon as an adsorbent derives from its large micropore and mesopore volumes, which give activated carbon adsorbents high surface area. Given the amount of solid waste that is produced by many agricultural and industrial processes, organic adsorbents can be produced easily and relatively cheaply by repurposing of these waste streams. Indeed, much recent attention is being paid to conversion of waste carbon into effective adsorbents (Hao et al. 2017).

Bio-based adsorbents can be obtained from three main sources: (i) “non-living” biomass like tree bark, lignin, crustacean shells; (ii) algal biomass; and (iii) microbial-based biomass (bacteria and fungi/yeast). Use of agricultural waste materials as raw material for adsorbents can be economic and environmentally friendly due to the chemical composition of the waste stream, abundance, and efficiency (Sud et al. 2008). Agricultural waste materials are usually composed of biopolymers like lignin, cellulose, chitin, or other natural polysaccharides (Sud et al. 2008; Wan Ngah and Hanafiah 2008; Ahmad et al. 2017). The presence of other compounds in the waste biomass can provide a variety of functional groups that are useful in the binding of adsorbates. Some functional groups that are often present on activated carbon made from waste biomass include: carboxylic acids ( $-COOH$ ), amines ( $-NH_2$ ), hydroxides ( $-OH$ ), sulfhydryls ( $-SH$ ), and others (Sud et al. 2008). Figure 22.2 illustrates the different functional groups that could be present on the surface of an activated carbon adsorbent molecule.

## 22.8 Pretreatment of Agricultural Wastes

The pretreatment of plant-based agricultural wastes is a key step, which can help to extract soluble compounds present and enhance chelating efficiency. Commonly, pretreatment of plant biomass is pretreated with acids (e.g. HCl,  $HNO_3$ , citric acid) or bases (e.g. NaOH,  $Ca(OH)_2$ ). Seafood wastes, specifically chitin and chitosan from crab, shrimp and lobster shells, can be treated to produce an effective adsorbent. Chitosan, as a charged polymer, already exhibits some of the qualities necessary for a good adsorbent (Ahmad et al. 2017; Brigham 2017). Also, chitin without modifications has been shown to adsorb heavy metal ions like  $Fe^{2+}$ ,  $As^{5+}$ , and  $Cr^{6+}$  from wastewater, although efficiencies of these methods were not overly high (Anastopoulos et al. 2017). Chitin and chitosan can be pretreated to further increase their efficiency as adsorbents: e.g. more amine groups can be added by condensation or nucleophilic substitution reaction, which allow for more functional groups aiding in adsorption. Chitin and chitosan can be modified by other compounds like imidazoles, aminoaryl molecules, halogen-containing



**Figure 22.3** Process flowchart of the carbon activation process.

compounds, etc. (Ahmad et al. 2017). Cellulose is another polysaccharide that can be modified and used as an adsorbent. Functional groups with good adsorption properties, like carboxylic acid, amine, amidoxime, nitrile, and GMA-imidazole, are attached to cellulose to enhance adsorption capacities for different pollutants. Many different modified cellulose adsorbents have been studied for their effective removal of heavy metals and other toxins from wastewater. Dilute acids and chelating agents like ethylene diamine tetra-acetic acid (EDTA) have been shown to be effective in regenerating cellulose-based adsorbents. If locally-sourced cellulose (i.e. agricultural waste streams) is used, the price per kg of this adsorbent can be kept low, suggesting it can be an effective alternative to commercially-produced activated carbon adsorbents (Hokkanen et al. 2016).

Figure 22.3 shows a process flowchart of activated carbon preparation from agricultural and other bio-based (e.g. forestry, food processing) wastes. In general, there are two key steps involved in the preparation of such materials from agro-wastes: (i) carbonization; and (ii) activation (Canales-Flores & Prieto-García 2016). Carbonization involves pyrolysis of the biomass, specifically in the absence of O<sub>2</sub>, at temperatures <800 °C. For high yields and low volatilization of product, the heating rates of pyrolysis must be kept low (10 ~ 15 °C min<sup>-1</sup>). The goal of activation is to increase the surface area and optimize the pore volume for the activated carbon. There are three methods by which the activation process can be performed: physical, chemical, and physicochemical. Physical activation involves incubation of the carbonized waste at high temperatures (600–1000 °C) in the presence of oxidizing gases, like steam, air, or CO<sub>2</sub>. Chemical activation typically combines carbonization and activation into a single step. The waste biomass is mixed with chemical activating agents. These chemical agents function as oxidants or dehydrating agents and result in the development of better porous structures, which is the goal of activation. Some common compounds that are used for chemical activation include ZnCl<sub>2</sub>, H<sub>3</sub>PO<sub>4</sub>, KOH, and K<sub>2</sub>CO<sub>3</sub> (Ioannidou and Zabaniotou 2007). Physicochemical activation is another one-step method, like chemical activation. The biomass is heated to moderately high temperature (500–700 °C) under constant flow of pure steam (Canales-Flores and Prieto-García 2016).

## 22.9 Adsorption by Treated Agricultural Waste Biomass

The components of most agricultural waste, including cellulose, hemicellulose, lignin, lipids, chitin, proteins, etc., contain a variety of functional groups that could facilitate metal complexation (Sud et al. 2008). Consequently, these wastes make good

bioadsorbents. Coffee grounds, cashew nut shell and potato peels have been shown to adsorb Cd<sup>2+</sup> from contaminated water, while orange peels have been shown to assist in removal of Cu<sup>2+</sup>, Ni<sup>2+</sup>, and Zn<sup>2+</sup>. The adsorption effectiveness of waste biomass depends to a certain extent on the available surface functional groups. Desiccated coconut, with its free hydroxyl, carboxyl, and amine groups is a good adsorbent for Hg<sup>2+</sup>, while orange peels and olive pomace with surface carboxyl and phenolic groups are effective for adsorbing Pb<sup>2+</sup>, Cu<sup>2+</sup> and Cd<sup>2+</sup> (El-Sayed and El-Sayed 2014). To improve adsorbent properties, agriculture, and food processing wastes can be pretreated by a number of methods, including heat treatment, acid treatment or base treatment (Nguyen et al. 2013). Orange peels treated with 0.8 M NaOH and 0.8 M CaCl<sub>2</sub> showed improvement in adsorbing Cd<sup>2+</sup>, Cu<sup>2+</sup>, and Pb<sup>2+</sup> (Nguyen et al. 2013). Pineapple peel fibers treated with succinic anhydride showed improvement in adsorption of the same divalent metal ions (Nguyen et al. 2013). Many agricultural wastes, including oat biomass, beech sawdust, bagasse fly ash and coconut shell fibers were shown to be very effective in adsorbing Cr<sup>2+</sup>, with over 80% of the ion removed from wastewater in each case. Rice husk and coconut char have been shown to be very effective (100% adsorption) in removal of Pb<sup>2+</sup>. Waste tea leaves are effective in removal of many different metal ions, including Pb<sup>2+</sup>, Fe<sup>2+</sup>, Zn<sup>2+</sup>, and Ni<sup>2+</sup> (Sud et al. 2008). For any bio-waste being used as an adsorbent, one must be mindful of the optimal parameters for removal of metal ions from wastewater. Under acidic or neutral pH conditions, adsorption was found to be maximum, whereas under basic conditions, adsorption ability significantly decreased. As expected, a decrease in particle size increased overall efficiency of adsorption, because a decrease in size means an increase in surface area. The optimal adsorption temperature depends on whether the reaction is endothermic or exothermic (El-Sayed and El-Sayed 2014).

Agricultural and food processing wastes have been shown to be effective in adsorption and removal of more than just heavy metals. Grape stalks were used as a feedstock for adsorbents for caffeine removal. Grape stalks were ground to powder and left unmodified, or pretreated with phosphoric acid, or pretreated with acid and heat treated to produce activated carbon that was shown to be most effective in adsorption of caffeine (~20 mg caffeine adsorbed per gram of adsorbent). In such study, acid-pretreated grape stalk performed slightly better than untreated grape stalk (Portinho et al. 2017). Lignocellulosic waste (wood residues and coconut mesocarp) were shown to be effective in removing dyes from wastewater. Both were shown to remove over 60% of both Remazol Red and Remazol Brilliant Violet dyes at pH = 6.0 (Monteiro et al. 2017). Palm oil mill effluent sludge and sugarcane bagasse waste were shown to be able to remove Methylene Blue dye, with sugarcane bagasse having an adsorption capability of over 200 mg dye per g adsorbent (El-Sayed and El-Sayed 2014).

## 22.10 Bio-based Polymers as Adsorbents

Nature has designed many biomacromolecules that have efficacy as adsorbents. Many of these molecules are found in, or are made from, agricultural waste streams. Chitin derived from shrimp was used as an adsorbent to remove lead ions (Pb<sup>2+</sup>) from aqueous

solutions. In solutions of  $\sim 20 \text{ mg l}^{-1}$  lead, over 99% of the metal was removed at  $30^\circ\text{C}$ ,  $\text{pH} = 9.0$  (Foroutan et al. 2017). Unmodified chitin has also been used for adsorption of  $\text{Cd}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{As}^{5+}$ , and  $\text{As}^{3+}$ , with varying yields and efficiencies (Anastopoulos et al. 2017). Chitosan, a charged derivative of chitin, is a highly functional biopolymer that has been examined as an adsorbent. The chitosan polymer itself is highly functional, flexible, and hydrophilic. Chitosan's functional groups are highly reactive, and so the polymer is amenable to different types of modifications that allow the creation of adsorbents with high selectivity for specific heavy metal ions (Ahmad et al. 2017). Cellulose, like chitin, is a highly abundant natural polysaccharide often found in agricultural or food processing wastes. As with chitosan, many attempts have been made to chemically modify cellulose to confer higher selectivity of specific pollutants. Modified cellulose has been used to examine the adsorption of many different heavy metal ions, including  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$ , and  $\text{Ni}^{2+}$  (Hokkanen et al. 2016).

PHAs have been modified and used as adsorbents. As discussed above, microbial synthesis of PHA is possible using bioavailable carbon found in many different agricultural waste streams. The hydrophobic nature of PHA has been used in the adsorption of oils from human skin, in a potential cosmetic application. Two types of PHA copolymer, P(HB-*co*-HV) and P(HB-*co*-HHx) were shown to be able to absorb oils equally well, even in the absence of lipophilic additives. The oil retention of a film fabricated from P(HB-*co*-HHx) was demonstrated to be 80% (w/w) (Sudesh et al. 2007). Films of PHB were shown to remove dye compounds from wastewater from the batik industry. It was demonstrated that approximately 38% of the color was removed from the wastewater using unmodified PHB. Production of electrospun PHB films from chloroform/ dimethylformamide (DMF) solutions produced films that were able to adsorb about 80% color from dye wastewater. PHB was further modified by coating with  $\text{TiO}_2$ , and these modified films demonstrated complete removal of up to 74% of the chemical oxygen demand from batik dye wastewater (Sudesh et al. 2011).

## 22.11 Conclusion

Production of biodegradable polymers and adsorbents from agricultural waste streams have been reviewed in this chapter. Some of the polymers (e.g. Chitin and PHA) could be used as both, biopolymers and adsorbents. The usage of agricultural waste streams as inexpensive substrates or feedstocks has the potential to reduce the production costs of both adsorbents and biopolymers, since they are available in enormous quantities at low costs. Both substances have the demand to be produced at a very low price since they are in concurrence with cheaply available products or they are involved in processes where cheap items must be utilized, in areas such as wastewater treatment. Several promising approaches have been discussed in this chapter. Besides an industrial upscale of these processes, an efficient and economical recovery process is needed to generate value added products to a low price. Also, substrate-flexible, closed-carbon, processes should be developed, to prevent regional and seasonal shortages as well as price increases through rising competing processes.

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## 23

# Technologies for Compost Production from Plant Byproducts

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## 23.1 Introduction

### 23.1.1 Definition of Composting

*Composting* is commonly defined as the managed, microbial degradation of organic material under aerobic, thermophilic conditions (Haug 1993). In this chapter, composting is considered an aerobic process and therefore does not include anaerobic biodegradation. *Compost*, the material result of the process, is a stable, humus-like product, free of pathogenic organisms and viable weed seeds that is a valuable agronomic soil amendment.

Composting is gaining popularity in many economic sectors as an inexpensive method for the processing and valorization of organic residuals. It is practiced at various scales and with widely ranging levels of technical sophistication. On one hand, the underlying concept is simple enough that children can learn to build and manage a compost pile in their home garden. On the other hand, the many interacting physical, chemical, and biological processes at play make the optimization of large-scale composting a challenge. Composting therefore bridges rural and urban, agricultural and municipal, individual and community contexts. The recycling of organic residuals into a useful product can be a catalyst for broad public engagement and civic pride for municipalities, project a positive public image and turn a profit for businesses, and inspire a sense of accomplishment and satisfaction for individuals (Kury de Castillo 2017).

The groundswell of public support for and engagement in composting is reflected by a shift in terminology, one that is also gradually occurring in other aspects of waste management (Banna 2014). Organic byproducts are less often referred to as “garbage” or “waste” and more frequently as “residuals” or “resources”. In this chapter, the term “organic residuals” is used in acknowledgment of the growing concern for responsible environmental stewardship and the desire to valorize organic byproducts as part of the circular economy (Ellen MacArthur Foundation 2017). The term “organic waste” is also used on occasion in deference to the vast literature about waste management.

### 23.1.2 Agricultural Use of Compost

Composting has been practiced for millennia, probably since humans began to distinguish between fertile and unproductive soils and to develop practices to improve them for agriculture. The first scientific study of composting, however, is attributed to Lady Gabrielle and Sir Albert Howard. Sir Albert was employed during the early part of the twentieth century as an Imperial Economic Botanist in the erstwhile South Asian colonies of the British Empire (Howard 1953). During this time, the couple documented the agronomic benefits of composting as part of the enduring agricultural traditions in areas of present-day China and India. Following the endorsement of the Howards, composting was widely adopted throughout the British Empire (Haug 1993).

Composting in mainstream commercial agriculture was subsequently eclipsed by the invention and refinement of the Haber-Bosch process for fixing atmospheric nitrogen gas as ammonia (Smil 2001). The Haber-Bosch process ultimately made synthetic nitrogen fertilizer inexpensive and convenient. Agricultural scientists and engineers during the Green Revolution capitalized on the potential of abundant nitrogen fertilizer, in conjunction with improved hybrids, irrigation, and agricultural mechanization, to substantially increase crop yields (Evenson and Gollin 2003). Hence, despite the many agronomic benefits of compost, its use was relegated largely to backyards and gardens for many decades. Synthetic fertilizers still dominate nutrient management in commercial agriculture; however, as mentioned below, the rising popularity of organic produce, a renewed appreciation for the role of organic matter in maintaining good soil health, and other factors, are spurring renewed interest in the agricultural use of compost at all scales. In light of this resurgence, there is a persistent need for impartial research of the benefits and drawbacks of agricultural compost use (Martínez-Blanco et al. 2017).

### 23.1.3 Municipal Waste Management

In the 1960s, municipal waste management engineers identified composting as a method of stabilizing and sanitizing biosolids from wastewater treatment plants. The accelerating growth of urban populations during the twentieth century was worsening aquatic pollution by the discharge of untreated sewage into surface waters (Delleur 2003). Public outcry grew against this practice, leading to substantial investment in wastewater treatment infrastructure. The newer infrastructure included separated, instead of combined, stormwater and sewage networks and increased capacity for wastewater treatment. A resulting conundrum was the management of the large amounts of biosolids generated by the wastewater treatment plants. The Beltsville composting method was developed as an alternative to landfilling the biosolids; instead, they are composted in static, aerated piles (described below) to produce a hygienic soil amendment (Haug 1993).

The Beltsville process offered a disposal option for the biosolids generated by the growing number of increasingly efficient wastewater treatment plants. However, municipal engineers needed a market for the compost, a challenge still faced by many compost producers today. Despite the agronomic benefits of compost, as famously described by the Howards and further discussed below, there was and continues to be public unease about the agricultural use of compost that originates in part from human excrement and could conceivably contain pathogens and other harmful contaminants (Beecher et al. 2004).

Research is ongoing into the safe recycling of composted biosolids and many jurisdictions strictly regulate their quality and use to safeguard public health (CCME 2005).

At the close of the twentieth century, the inexorable rise of consumer capitalism and the convenience credo of the “throw-away” society began to develop into a crisis in solid municipal waste management, which continues in much of the world (Rodić and Wilson 2017). Existing landfills were reaching capacity and it became increasingly difficult to find sites for new ones. More and more, the public considered the practice of landfilling garbage as undesirable and unsustainable. This spurred the implementation of alternative waste management practices, including composting of the organic fraction of municipal solid waste. Municipal governments and regional waste management authorities began to construct large, increasingly sophisticated composting facilities. The compost produced by these facilities is used in municipal parks, gardens, and street tree pits, by the horticultural and agricultural sectors, and in numerous other applications. This trend continues in many areas at the time of writing, but operational challenges have emerged that have thwarted this growth in some areas (Wei et al. 2017).

The composting of organic residuals from wastewater treatment and municipal solid waste has begun to redress, in a modest way, the wholesale export of nutrients and organic matter from agricultural soils to urban centers and then into waterways and the oceans. The recycling of composted municipal organic residues directs the organic material and the nutrients that it contains back to productive soils. These organic amendments can satisfy, at least in part, the nutritional needs of crops, offsetting some the need for energetically expensive synthetic or nonrenewable mineral fertilizers while also imparting other agronomic benefits (Martínez-Blanco et al. 2017).

### 23.1.4 Organic Agriculture, Agroecology, and Soil Conservation

A complementary trend to the increasing supply of compost is the demand by consumers for “organic” produce, grown without the use of synthetic fertilizers or chemical pesticides. Since its emergence in the mid-1900s, organic agriculture has grown to account for a substantial component of agricultural production in developed economies and is an important receiver of compost (Brzezina et al. 2017).

Composting is also an important tool in agroecology, which is a growing movement reactionary to the dominance of multinational agribusiness interests and the industrialization of agriculture. Agroecology strives to combine modern science with traditional agricultural practices, establishing sustainable agricultural landscapes that emphasize the natural cycling of nutrients and minimize the need for industrial inputs (Gliessman 2017). In this context, composting is a way of enhancing local nutrients cycles by returning long-lasting, healthful organic carbon to the soil.

Many conventional agricultural producers are also adopting a more progressive stance toward soil management. North American soil conservation initiatives began in response to the Dustbowl of the 1930s, which confronted producers and agronomists with the destructive effects of intensive soil tillage, burning of crop residues, and letting fields lie fallow. Efforts in conservation research and education led to current best farming practices, which include minimum or zero tillage, reincorporation of crop residues, the use of cover crops, and crop rotations that include legumes (Govers et al., 2017). The consensus regarding soil management is now transitioning beyond conservation to the proactive regeneration of soil organic matter (van Es 2016). The addition of

compost, with its complement of rich, stable organic compounds, is an excellent way to further this goal.

### 23.1.5 Circular Economy

The *circular economy*, a term coined by Pearce and Turner (1989), refers to an economic system in which products, components, and materials are intentionally designed and managed to retain their highest utility at all times. The circular economy emphasizes minimal consumption of new resources, the reuse of products where possible, and recycling and reclamation of materials at the end of their useful life. The two complementary resource cycles in the circular economy are the technological and biological, with composting as a prominent part of the biological cycle (Ellen MacArthur Foundation 2017).

Governments at the supranational, national, and subnational levels are now adopting the idea of a circular economy and the attendant objective of recycling organic residuals by composting (e.g. European Union 2014; Province of Ontario 2017). Public concern is mounting about the sustainability of our civilization in the face of destabilizing trends such as unchecked resource consumption, global climate change, the loss of biodiversity, and the eutrophication of the oceans. Citizens are forcing their governments to consider these phenomena and to devise possible mitigation strategies. The circular economy is a convenient conceptual framework for sustainable resource management policy and responsible environmental stewardship (Stahel 2016). Programs are being rolled out to operationalize such policies but, in many cases, local governments have already built organic waste management infrastructure and implemented appropriate services for the reasons described previously (e.g. Gamble 2005; Kury de Castillo 2017).

It seems that the impetus toward a circular economy will continue to drive the widespread adoption of composting as a key method for recycling organic residuals. The harmonization and integration of municipal and agricultural strategies for the management of organic residuals could rationalize regional nutrient flows by returning these valuable resources to the soil in a circular flow reminiscent of nutrient cycles in wild ecosystems.

## 23.2 Benefits and Challenges

### 23.2.1 Agronomic

Compost is a good source of macronutrients and micronutrients, although not as rich in readily bioavailable nitrogen as synthetic fertilizers (Hepperly et al. 2009). The nutrients that it does contain, however, are in relatively stable forms and so release gradually into the soil as the compost degrades. This prevents quick spikes of excess nutrients that could move into surface water or leach downward out of the root zone into groundwater.

In addition to the systemic benefits of recycling nutrients and organic matter, compost directly benefits other aspects of soil health. *Soil health*, as defined by the United States Department of Agriculture (USDA-NRCS 2012), is “the continued capacity of the soil to function as a vital living ecosystem that sustains plants, animals, and humans.” Important characteristics of soil health include: good tilth or physical character; sufficient depth to allow good root development; good water storage and drainage capacity; sufficient but not excessive nutrient supply; small populations of pathogens, pests, and weeds;

abundant beneficial organisms; absence of potentially harmful chemicals and toxins; and resistance to degradation by potentially damaging influences such as adverse weather events (van Es 2016). Compost enhances all these characteristics. Its addition increases the volume of soil. The recalcitrant, humic organic compounds that it contains help to improve the soil's structure, water holding capacity, and resilience. The high cation exchange capacity of these compounds allows them to adsorb and gradually release nutrients ions, as well as immobilize potentially toxic compounds so that they can be degraded. The thermophilic, microbial process of composting inactivates pathogens and weed seeds and populates the compost with abundant beneficial microorganisms that bolster the soil microbiome.

### 23.2.2 Municipal

Municipalities can benefit in several ways from composting. Composting is a management option that aligns with the social imperative of sustainability by diverting organic materials from landfill or incineration, and reduces the risk of environmental impacts such as the emission of greenhouse gases and the pollution of surface and groundwater (Peigné and Girardin 2004). Composting valorizes materials that might otherwise be considered as waste, turning them into products that are useful to the municipality in its own greening operations, to its citizens, and to local agricultural and horticultural industries. Well-managed municipal composting generates civic pride among residents and political capital for the administration (Pollans 2017).

### 23.2.3 Circular Economy

In accordance with the emerging perspective of environmental stewardship and holistic resource management, composting fits well into the rubric of the circular economy (Ellen MacArthur Foundation 2017). Composting is an important technology for the stabilization, sanitization, and recycling of organic material. The use of compost reduces the need for inputs of energetically intensive and nonrenewable mineral nutrient supplements and hence reduces the release of greenhouse gases that would otherwise be associated with the production of those fertilizers. Beyond conserving and recycling organic matter and nutrients, compost is a stable amendment that increases the amount of recalcitrant organic carbon in the soil (Peltre et al. 2017).

### 23.2.4 Challenges

Composting is not a panacea and is not suitable for every scenario. Public health and safety challenges must be considered when evaluating composting as an option for valorizing organic residuals. Most jurisdictions have regulations or guidelines in place to safeguard the public by either governing the process or the product quality, or both (Bernal et al. 2017). A principle concern is the possible survival of human pathogens in compost if the process does not meet safety standards. Potentially toxic materials such as trace metals could be present, especially in municipal or industrial compost, as could persistent organic pollutants such as antibiotics, hormones, and endocrine disruptors. Other possible hazardous contaminants are sharp foreign (inorganic) objects and materials that do not degrade, or degrade very slowly, in the environment, such as some plastics.

Poor-quality compost can also pose a threat to the health of crops (Bernal et al. 2017). Some of the same or similar hazards as those mentioned with respect to humans can damage plants. Plant pathogens or weed seeds might survive an improperly managed composting process, and plants are also susceptible to inorganic and organic toxicants. Excess application of compost, especially if it is not fully mature, can deplete oxygen as the compost continues to decompose in the soil. Any non-degradable fragments that it contains could accumulate in the soil over time, which would be esthetically unpleasing and might present a threat to wildlife.

## 23.3 Composting Process

The requirements for successful composting relate to the conditions for healthy microbial activity and, whimsically, correspond to four elements of Western alchemy: earth (feedstock), air, fire (heat), and water. These requirements are interrelated and make the management of the process complex and challenging.

### 23.3.1 Feedstock

The compost substrate must contain adequate nutrients, or additional ingredients will be required for effective co-composting. Importantly, the feedstock must provide carbon and nitrogen in a mass ratio (C : N ratio) of about 25 : 1 (Haug 1993). This ratio is habitually estimated based on total carbon and total nitrogen, but the full amounts of these elements are not necessarily readily bioavailable; for instance, materials such as lignin, cellulose, and hemicellulose are recalcitrant (Anwar et al. 2017). In feedstock containing woody materials, on one hand, the C : N ratio might need to be adjusted upward to maintain an appropriate nutrient balance. Green plant material, on the other hand, usually has a lower C : N ratio, but both elements are more bioavailable. Micronutrients are normally present in adequate quantities in most organic materials, but in exceptional cases might need to be supplemented (Sánchez et al. 2017).

The feedstock must also have adequate physical structure so that airflow and drainage are unimpeded, and so the substrate does not compact excessively during the composting process (Haug 1993). The addition of a suitable bulking agent such as woody residuals can redress an otherwise amorphous substrate. Even synthetic material, such as shredded tires, can be used if it can be screened out easily for reuse at the end of the process.

### 23.3.2 Aeration

Related to the physical structure of the substrate, it is important to maintain an aerobic environment in the compost bed. An oxygen concentration of 5% or more ensures aerobic conditions (Haug 1993). Adequate airflow is necessary for this purpose and to remove carbon dioxide and other volatile metabolites, as well as any excess moisture or heat released during the metabolism of the substrate. The maintenance of the required airflow depends on convection through the porous substrate, possibly assisted by periodic turning or agitation of the compost or, in some systems, by mechanical ventilation.

Excessive aeration, however, can cool or dry the compost, thereby slowing the process. High aeration rates can also strip nitrogen from the substrate in the form of ammonia, a nuisance gas, the loss of which lowers the fertilizer value of the finished product.

### 23.3.3 Temperature

Composting, as explicitly defined in this chapter, is a thermophilic process. A well-managed process usually attains thermophilic temperatures in excess of 45 °C after a short adaptation period of a few days, depending on the mass of the compost pile, its composition, and the type of facility (Haug 1993). The exothermic reactions of a well-managed composting system of appreciable mass will maintain such temperatures during a “high-rate” period of several weeks. A graduate decrease in temperature will follow, as easily degradable compounds are exhausted. Composting activity then trails into an extended, mesophilic curing period during which the substrate finally stabilizes.

Thermophilic temperatures, being substantially above human body temperature, will destroy pathogenic organisms after an exposure time dependent on the temperature, specific organism, and the substrate (Bernal et al. 2017; Germer et al. 2010). Insect larvae and weed seeds are also killed or inactivated at thermophilic temperatures, which is important if the product is for agricultural or horticultural use. The compost must be carefully turned, however, or insulated in a manner that ensures all the substrate attains thermophilic temperatures and so there are no pockets or exterior layers that are inadequately heated.

Temperatures above about 70 °C tend to slow the composting process, as the heat begins to denature proteins in the cytoplasm of the microbes and impair their metabolism. Excessive heat can be relieved by turning the compost or by increased aeration. Hyperthermophilic composting processes have been developed, however, which incorporate microorganisms isolated from thermal springs and that are adapted to temperatures above 80 °C (Kanazawa et al. 2006). Since biological processes tend to operate faster at higher temperatures, hyperthermophilic composting can process feedstock more rapidly than conventional, thermophilic composting and therefore requires a smaller footprint to process equivalent amounts of substrate.

### 23.3.4 Moisture

Effective composting requires careful attention to the moisture content of the substrate. The optimal moisture content for composting is approximately 60% of total wet mass (Haug 1993). On one hand, excess moisture can block the pore space in the substrate and hinder aeration. The microbial population will lack oxygen and the process will become anaerobic, resulting in a much slower, cooler, and malodorous process that does not produce satisfactory compost. Moreover, water is likely to drain from overly moist compost as leachate, creating challenges related to its collection, storage, and treatment or recycling.

Inadequate water content, on the other hand, also depresses microbial activity due to a lack of moisture for dissolution and transport of nutrients. Drying can also lead to the development of cracks in the substrate and preferential airflow through the cracks, which in turn exaggerates local drying, creating a vicious cycle. The increased airflow through the dry compost causes inadequate aeration and uneven composting in other parts of the

compost bed (Gilley and Van Durme 2002). In some circumstances, a drying front can advance through the compost from the air inlet. The relatively cool ambient air has a low moisture holding capacity. When heated by the compost, the air's moisture holding capacity increases and moisture evaporates into it from the surrounding substrate. Microbial activity is thereby depressed as the compost cools and dries. Incoming air then travels ever further into the compost bed before being warmed and humidified. Mixing can remedy heterogeneous moisture content in a compost bed. Irrigation of the compost or humidification of the inlet air can prevent drying in the first place.

### 23.3.5 Management and Quality Assurance

Capable management of the composting process is at least as important as the physical process parameters mentioned previously or, better, are prerequisite to them (Bernal et al. 2017). Management expertise requires both education and experience, because each composting operation is unique. This is truer of composting than of many other processes because composting substrates are very often extremely heterogeneous and variable. Effective control of any process requires adequate observation and, again, this is especially so for composting. Monitoring and record keeping are indispensable for process control and for quality assurance or continuous quality improvement. Depending on the jurisdiction, in fact, large-scale composting will probably be subject to obligatory permitting and reporting requirements.

One aspect of compost management is to optimize the transformation and valorization of organic residuals. This requires an understanding of the available feedstock in terms of its nutrient and moisture content, physical structure, and degradability, as discussed previously. These characteristics may well vary by source and by season or, in some situations, even by week or time of day. To compensate for variation, it might be necessary to stockpile and blend the feedstock, or to mix and co-compost several feedstocks, amendments, or bulking agents in varying proportions. Apart from changes in feedstock, other external factors such as seasonal climatic changes or weather events can influence the composting process and necessitate compensating management strategies (Fuchs and Cuijpers 2016).

Another aspect of management is that of risk, or the assurance of the health and safety of workers and of the public. Risk results from the existence of a hazard and of exposure to that hazard. Mitigation of risk involves, therefore, either elimination of the hazard or prevention of exposure. Risks to workers could stem from hazards associated with material properties of the feedstock or compost, the presence of pathogens, the generation on site of potentially toxic gases such as hydrogen sulfide, or the machinery or structures used on the site (Brown 2016). Occupational safety and health guidelines are meant to protect workers by eliminating such hazards so far as possible. Where elimination of a hazard is impossible, appropriate measures should be put in place or personal protective equipment made available to prevent exposure of the workers to the hazard.

When there is a potential risk to the public then the preferable option is, as with worker safety, to eliminate the hazard. Guidelines exist in many jurisdictions for process control that ensures safe compost, and operators must be familiar with any applicable regulations (Bernal et al. 2017; CCME 2005). One goal of such guidelines is the elimination of human pathogens by maintenance of temperatures above a certain level for a specified period. Turning or mixing of the compost might also be prescribed to ensure adequate

heat treatment of all the material. In addition to characterization of the process, there also exist guidelines for the quality of the resulting product. Product quality guidelines usually specify the presence of maximum numbers of potentially pathogenic organisms or indicators organisms, such as fecal coliform bacteria. Guidelines might also specify the number and size of foreign objects allowed in a sample of finished compost; the presence of organic contaminants, such as pesticides; the concentrations of potentially toxic metals; and the maturity or stability of the compost. Guidelines also generally include, or refer to, specific testing protocols for each relevant measure. Finally, where a hazard cannot be eliminated from a product, then there might be restrictions on where and how that product can be used.

## 23.4 Compost Facilities

A variety of different styles of composting exist, each of which is appropriate to a different scale and context (Haug 2013; Haug 1993). The kind of process that is most appropriate depends on many factors, including the location, surroundings, feedstock, market conditions, social and regulatory context, and the availability of labor. The cost of capital, operation, and maintenance varies according to the type of process, and so financial resources also dictate what is feasible. In capital-intensive and technologically sophisticated operations, the durability, maintenance, repair, and ultimate end-of-life replacement of the capital plant must be carefully considered. Machinery and metallic structures are vulnerable to corrosion, for instance, which can be accelerated by exposure to ammonia emissions from active composting of nitrogen-rich feedstock.

### 23.4.1 Windrow

The simplest form of large-scale composting is the turned windrow. The compost ingredients are mixed to ensure appropriate starting conditions, as described previously. The mixed feedstock is then formed into windrows, which are long, narrow piles with a triangular or trapezoidal cross-section, usually less than 2 m high and several meters across. The windrows are monitored for temperature, oxygen concentration, and moisture content. They are turned regularly using machinery such as a tractor with a front-end loader or more specialized equipment, such as a windrow turner. The windrows should be turned about once a week during the high-rate, thermophilic phase to control temperature, promote aeration, and homogenize the substrate. It might be necessary to add water during turning to maintain a proper moisture content. Turning can be less frequent as composting progresses and the substrate cools. Finally, once the temperature has neared ambient, the compost cures for as long as several months and might then remain stockpiled until sold or used.

### 23.4.2 Aerated Static Pile

In an aerated static pile system, the mixed compost usually is formed into short, broad piles. A method of pulling or blowing air through the compost bed is installed when the piles are built, through perforated pipes in the bottom of the pile, a porous sublayer, or

an aerated floor. The Beltsville system, mentioned previously, comprises an aerated static pile built on a layer of porous substrate (Haug 1993). A mechanical blower draws air through perforated pipes laid in the porous substrate, and exhausts the air through a pile of wet woodchips or similar material that acts as a biofilter to remove odorous gases. As the name implies, the compost pile is not mixed, but instead remains static throughout the process. A layer of finished compost covers and insulates the active compost in the pile to ensure that the whole mass of feedstock reaches thermophilic temperatures. The layer of finished compost also acts as a biofilter to reduce odors emitted from the pile.

Contrary to the Beltsville system, which operates under negative pressure, commercial systems are available, in which a static aerated pile operates under positive pressure (Kasinski et al. 2016). A semipermeable, synthetic textile covers the pile, allowing the passage of moist air but restricting its flow to distribute it evenly throughout the pile. This kind of system is advantageous in that, as compared to the Beltsville system, it requires a smaller blower and less energy to distribute airflow evenly throughout the compost bed. The initial and replacement costs of the textile cover are substantial, however. Apparatus is also required to facilitate the deployment of the cover over the pile and its subsequent stowage at the end of the active composting phase.

### 23.4.3 Channel Composting

Composting in channels or bunkers is more capital-intensive than windrow or static pile systems, but the footprint for such systems is smaller because the cross section of the compost pile is constrained by walls and is therefore rectangular rather than trapezoidal (Diaz et al. 2011). Channel compost beds can also be deeper than windrows, for instance, since aeration is mechanically assisted. Channels and bunkers offer convenient control over process conditions. As with a static pile, the facility must provide adequate aeration of the compost, usually through an aerated floor on which machinery drive for loading and unloading. Such systems run as all-in, all-out batch operations. The process conditions for the entire batch can be adjusted as the compost matures, requiring changing rates of aeration and irrigation.

More sophisticated channels may be constructed using specialized turners with wheels that roll along the top of the channel walls. A table pivots from the turner frame downward into the channel, supporting rotating chains or augers that mix the compost and move it a few meters along the channel with each pass. As the compost matures, repeated passes of the turner gradually move it from one end of the channel to other. New feedstock can be loaded from one end and finished compost removed from the other in a flow-through fashion. In this kind of configuration, variable control of conditions along the length of the channel can optimize the process.

### 23.4.4 In-vessel Composting

Composting vessels are more capital-intensive than open systems (Diaz et al. 2011). However, they offer greater control over the process and can therefore be better optimized for faster processing, resulting in a more compact footprint. Their smaller relative size and the containment of odors make them better suited to locations that are more public. Composting vessels can have a wide variety of configurations, but a common type

is a horizontal cylinder with a slight incline. The vessel is loaded through an access door at the elevated end and either an internal auger or the entire cylinder rotates occasionally around its longitudinal axis, slowly moving the compost down the incline toward the unloading port. Moisture and aeration can be precisely controlled and, because the vessel is closed, the internal temperature is uniform so that the entire mass of the compost is thoroughly heated. Any unprocessed aggregates that reach the outlet of the container can be retained inside the vessel by a screen or else recycled to the inlet.

#### 23.4.5 Agitated Beds

Very large-scale composting operations are often configured as agitated beds. Such facilities share the advantages of channel composting for good control of process conditions and are often housed inside a large building (Gamble 2005; Haug 1993). Different zones of the bed can be controlled to provide conditions appropriate to the stage of composting, thus optimizing the process. An aeration floor provides airflow, usually through a negative pressure system that pulls air down through the compost so that it can be captured and treated to remove odors and nuisance gases. A specialized apparatus, such as an overhead bridge crane, supports equipment for agitation and irrigation of the compost. When the compost is mixed, it can also be shifted in one direction so that it eventually reaches the edge of the bed, where it is removed. The compost bed can be replenished with fresh feedstock from the opposite side to implement a flow-through system.

### 23.5 Siting Considerations

When planning a composting facility, it is wise to consider first the larger context in which it will operate (de Bertoldi-Schnappinger 2011). An initial focus on the narrow, technical considerations of the actual process is likely to put the viability of the whole project at risk, because planners might easily neglect larger concerns that determine the long-term sustainability of the operation.

#### 23.5.1 Regulations

Apart from developing a sound business plan, familiarization with the regulatory environment is perhaps the most important aspect of starting a new composting operation (Bernal et al. 2017; CCME 2005). On one hand, there may be critical restrictions and substantial permitting costs. On the other hand, regulatory personnel and published guidelines can be invaluable sources of advice and information about the business and social environment, appropriate technologies, and management strategies. Local, regional (state, provincial, or departmental), and national legislation could all be relevant, and all levels of government should therefore be consulted. When planning an operation of appreciable scale, therefore, a professional consultant is indispensable to effectively navigate regulatory complexities.

### 23.5.2 Space

Numerous physical considerations come into play when the focus shifts from the regulatory environment to the selection of the location. Again, those concerns that are perhaps most important for the long-term success of the project are those of the largest scale and which are therefore likely to impinge on neighboring residents or businesses.

The amount of feedstock to be composted, and any requirements for co-composting ingredients, bulking agents, curing, and stockpiling of the finished product will dictate the overall space requirements. The required footprint for the overall operation will be a primary driver in site selection and, depending on the scale of the operation, municipal zoning restrictions could come into play.

### 23.5.3 Traffic

Associated with the scale of the operation is the amount of traffic required to bring feedstock and other materials to the site and to remove the finished product. The increased traffic raises concerns about the safety of drivers and pedestrians. Frequent passage of potentially heavy vehicles might inconvenience other drivers, lead to congestion, and even increase road maintenance requirements. Increased traffic noise, dust, and light pollution could certainly be detrimental in a residential or commercial neighborhood.

### 23.5.4 Odor

Odor reduction and control is a consideration that is frequently overlooked and often leads to the demise of poorly planned composting facilities. Although finished compost has an inoffensive odor, the feedstock and the process itself are potential sources of nuisance odors. Odor control is therefore imperative and must be incorporated into the earliest design stages of the site and process (Bidlingmaier and Müsken 2011).

First, cleanliness and conscientious management of a site will greatly reduce the potential for odors and other nuisances, as well as improve overall safety and productivity. Prevailing winds and the local topography must also be considered when planning the location of operations on the site. In terms of operations, the stockpiling, grinding and mixing of feedstock and other ingredients should be done in an area that is remote from neighboring homes or commercial buildings. It is wise to use trees or fences to screen potentially odorous operations from public view and roadways. Such screens will also serve as windbreaks and reduce odor dispersion. It might even be necessary to house odorous operations inside a closed structure from which the exhaust air can be contained and treated before release.

If well managed, the composting process itself will likely be less odorous than the handling of putrescible feedstock. Even the best operations are never ideal, however, and there is potential for anaerobic pockets within the compost pile that produce malodors. The type of composting process and equipment should therefore be chosen carefully to ensure that odors are not a problem either on or offsite. Proximity to a residential neighborhood for instance, could elevate the risk of nuisance odors enough to warrant investment in a negative-pressure system such as aerated static piles, an enclosed process such as a channel, bunker, or agitated bed, or even in-vessel composting. Enclosed or in-vessel systems offer the possibility of complete containment to treat all process air.

Finally, an important part of odor management is to implement a proactive, in-house monitoring program (Laor et al. 2014). The public should also be actively involved by setting up a well-advertised, highly visible, and easily accessible reporting system. A rapid and effective response protocol is required to deal with any incidents or complaints.

### 23.5.5 Pests

Animal and insect control are crucial to safeguard the health and safety of workers, guarantee public health and hygiene, and maintain good community relations (Haug 1993). The control of rodents should be considered, as they are drawn to ready food sources and nesting sites in warm compost and can carry disease. Insects can be problematic in stockpiles of putrescible feedstock, poorly managed compost beds, or untidy yards. Birds also pose potential problems in open facilities, especially those located near airports, because they can interfere with air traffic. Maintaining a clean, tidy yard, reducing or eliminating stockpiles of feedstock, and covering or enclosing any operations that might attract animals can reduce the incidence of problems. Constant vigilance is required, however, together with a readiness and ability to respond quickly when problems arise.

### 23.5.6 Water and Leachate

The management of surface water and leachate is an important aspect of site design that is often tightly regulated (Ontario Ministry of the Environment 2012). Consideration of run-on water is potentially important if the site is located downslope from poorly drained or poorly managed property. It might be advisable to construct diversion or drainage infrastructure such as berms, ditches, or swales at the upper edge of the property to prevent inundation of the site or erosion of any outdoor stockpiles or windrows, for instance. Similarly, run-off from the site should be appropriately prevented or controlled. Any leachate from the composting process itself should be collected and possibly recycled into the process or impounded for storage and treatment. It may be necessary to pave the composting pad with an impermeable surface such as asphalt, concrete, or a compacted clay liner. If circumstances and the scale of the operations allow, the composting site could be roofed to shed precipitation or covered by a completely closed structure.

## 23.6 Products from Composting

### 23.6.1 Compost

Several products can be produced by composting, the most obvious being the compost itself. Compost is a beneficial soil amendment with numerous agronomic benefits, as outlined previously, and there may be local markets for bulk sales of compost for agricultural and horticultural use (Eggerth et al. 2011). Alternative markets may also exist in applications such as construction, to stabilize exposed soil surfaces on construction sites and promote the establishment of vegetation. Compost can help in other applications to establish or improve plant growth, as on landfill covers or for mine site reclamation, for

instance, or stimulate microbial activity in the soil, as in brownfield reclamation or soil decontamination. Dry, finished compost is also good animal bedding, so livestock producers might prove to be alternative clients. Moist compost, on the other hand, is a good biofiltration medium and either can be applied in a layer over an extensive odor source, or used in a pile or large container to filter an air stream from a point source. Retail sales of bulk or packaged compost for home gardening is a possibility, although this market is often highly competitive with slim profit margins. Composts from specialty substrates can be produced to exploit niche markets; for instance, composted poultry manure is suitable as a specialty fertilizer because of its high nitrogen content. Compost can also be blended into potting media, or pelletized for ease of transport, handling, and application.

### 23.6.2 Compost Tea

Compost tea is a water extract of finished compost. It can be prepared aerobically by bubbling air through the water as the compost soaks, or anaerobically in sealed containers (Scheuerell and Mahaffee 2006). Compost tea can be applied to the soil or directly to plants as a foliar spray. It contains soluble organic compounds, suspended particulates, and microbes from the compost, the exact nature of which will vary depending on the compost and the mode of preparation. There are certainly some nutrients in compost tea and there are claims that it suppresses plant pathogens, promotes growth, and enhances the soil microbiota. There is much anecdotal literature about these supposed benefits, however the burgeoning scientific literature about them is currently inconclusive.

### 23.6.3 Heat

An emerging trend in some larger composting operations is the capture of heat for use in adjacent facilities (Walther et al. 2017). The airflow during the active phase of composting removes sensible and latent heat and, in fact, the removal of excess heat is required to optimize the process. The resulting thermal energy is low-grade because the process temperature during composting does not normally exceed 70 °C. However, the process air can directly warm a building, for instance, or a heat exchanger could capture thermal energy either from the process air or from the compost bed and then warm air or water. Thus, compost heat can partly or completely offset the use of more expensive energy sources such as electricity or gas.

### 23.6.4 Gases

Composting liberates biogenic, or “short-cycle” carbon dioxide, which has value in some contexts. For instance, greenhouse operators commonly use carbon dioxide enrichment to promote plant growth (Poudel and Dunn 2017). Process air from composting often is saturated with humidity and can contain some trace gases, such as methane and ammonia, so it might need to be washed and dehumidified before use, depending on the application.

## 23.7 Conclusion

The use of compost in agriculture and horticulture, on one hand, is increasing due to the growing popularity of organic produce and the growing recognition of the capacity of compost to increase soil organic matter and improve soil health. Municipalities, on the other hand, view composting as a useful technology to process organic residuals into value-added products that offset some of the costs of waste management. These two complementary trends bode well for the continued growth of the composting industry as a means of integrating and harmonizing regional management of organic residuals and the nutrients that they contain.

In the larger social sphere, the return of organic residuals to productive soils is an important part of the circular economy, touted as the underpinning of a more sustainable society. Furthermore, some consider the intentional, large-scale increase of soil organic matter as an important means to reverse the global increase in atmospheric carbon dioxide concentrations by sequestering large amounts of carbon (Minasny et al. 2017). Such an initiative would have the added and perhaps equally important benefit of reclaiming soils degraded by desertification, salinization, and erosion.

Market development and diversification are currently barriers to more widespread adoption of composting. An appropriate regulatory framework is required to facilitate industry growth and assure consumers of the safety and quality of the industry and its products. Investment in research is required to continue the improvement of process technology, adapt it to new contexts such as large-scale processing of municipal residuals, and identify and mitigate associated risks. Standards and guidelines need to evolve to suit the changing industry and to allow it to thrive and grow. Equally important is research about the social aspects of the composting industry and its acceptance and support by the public. Continuing education initiatives are required to sensitize the public to the importance of investing in technologies, like composting, that promise to enhance the sustainability of our cultures in the face of growing population and affluence, and increasing resource constraints.

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## 24

# Utilization of Selected Tropical Crops (Cocoa, Kola Nuts, Sorghum, Millet, and Shea Butter)

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## 24.1 Introduction

The daily dependence on plant products of tropical origin by human beings is amazing. The lives of humans are enriched by beautiful hardwoods, spices, essential oils, and fruits. Tropical countries export many fibers, gums, resins, dyes, and plant essences that may never be seen directly, but which are widely used in medicine and industry. Latin America and Africa are major producers of coffee and cacao while Asia produces most of the rice and natural rubber that are utilized daily by human beings worldwide. The crops that produce these plant products are mostly housed by the tropical region of the world. Some of these important crops include maize, cassava, potato, sugar cane, rice, sorghum millet, cocoa, coffee, shear butter, cola nuts, and many others. These crops play important roles in the development of the lives and the economy of the people and countries of the tropics respectively.

Unfortunately, the processing of these food crops mostly targets the most widely used products to the detriment of other byproducts that could be of commercial value. For example, cocoa is processed mainly for chocolate with little importance to the pods and other parts of the fruit which could be processed for other commercial products. This chapter focuses on all viable products that could be processed from Cocoa, Cola nuts, Shea butter, Millet, and Sorghum for better utilization of the crops.

The chapter is compiled to serve as standard text/reference material for teachers, students, researchers, and other professionals in disciplines involved in the utilization and/or management of agricultural harvesting, food processing, and related discards. The chapter is especially useful for Faculties of Agricultural and Environmental Sciences offering undergraduate and graduate programs in animal science, plant science, food science and technology, postharvest management, fermentation technology, waste management, and agricultural economics; and the libraries that serve them. It is also useful to entrepreneurs, companies, managers, and policy makers working in the agricultural, bio-energy, and allied industries.

## 24.2 Cocoa

### 24.2.1 Description of the Cocoa Plant

Cocoa, *Theobroma cacao*, is an evergreen tree in the family Malvaceae, native to the deep tropical regions of Central and South America to the east of Andes, grown for its seeds (beans) which are used primarily in the manufacture of chocolate. The cocoa plant is a branching tree with simple, pointed (lanceolate) leaves which can measure up to 61 cm (24 in.) long and 10 cm (4 in.) wide. The tree produces clusters of pale yellow flowers each with five petals and sepals. The cocoa pods (drupes) can be green-white, yellow, purplish, or red in color each of which contains 20–50 seeds, usually arranged in five distinct rows. The cocoa tree can reach 4–20 m (13–66 ft) in height and can live for up to 40 years, although the commercial life of a cocoa tree is usually about 25 years. Cocoa is produced in countries in a belt between 10°N and 10°S of the Equator, where the climate is appropriate for growing cocoa trees. The current largest producing countries are Côte d'Ivoire, Ghana, and Indonesia. Cocoa may also be referred to as cacao, koko or Kacao and originates from upper Amazon region of South America.

The natural habitat of the cocoa tree is in the lower level of the evergreen rainforest, and climatic factors, particularly temperature and rainfall, are important in encouraging optimum growth. Cocoa plants respond well to relatively high temperatures, with a maximum annual average of 30–32 °C and a minimum average of 18–21 °C. Cocoa needs a soil containing coarse particles and with a reasonable quantity of nutrients, to a depth of 1.5 m to allow the development of a good root system. Below that level it is desirable not to have impermeable material, so that excess water can drain away. Cocoa will withstand waterlogging for short periods, but excess water should not linger. The cocoa tree is sensitive to a lack of water, so the soil must have both water retention properties and good drainage.

### 24.2.2 Cocoa Cultivation and Harvesting

Cocoa trees grow best in humid conditions at temperatures between 18 and 32 °C (65–90 °F). It is typically grown in regions where daytime humidity reaches up to 100% and night time humidity is between 70% and 80%. The plants require a deep, fertile, and well-draining soil with a pH of 5.0–7.5 for optimum development. Cocoa is usually grown in tropical lowland areas and it is sensitive to drought. It should typically not be grown in regions which experience less than 1 cm of rainfall for periods in excess of three months.

Cocoa seeds from healthy, ripe pods remain viable for three weeks and are usually planted straight after harvest to produce new seedlings. Seeds are planted in a fiber basket or plastic nursery bag filled with clean soil and placed in a shaded place protected from the sun to prevent scorching. Seedlings grow quickly and are ready to be transplanted after four to six months. Seedlings are usually planted in the ground when they are four to six months old. The young trees are delicate and require some protection from strong sunlight and wind damage. Protection is usually provided by planting seedlings next to mother trees. This shading also helps to prevent the trees from growing too tall, keeping them at a manageable size for maintenance and harvest. Shade trees are usually other crops such as banana, plantain coconut, or rubber. Cocoa seedlings should be

planted 3–4 m (10–13 ft) apart and 3–6 m (10–20 ft) from the shade trees. The shading can be reduced once the cocoa trees have formed a closed canopy, but some should be retained to reduce water stress.

Cocoa can also be vegetatively propagated via cuttings, marcotting, and budding. Cuttings should have two to five leaves and one or two buds. Leaves should be cut in half before placing the cutting in a pot and covering with polyethylene to allow roots to develop. Marcotting or air layering is achieved by removing a strip of bark from a tree branch and covering the area with a layer of sawdust before covering it with polyethylene. The covered area will develop roots and can then be removed and planted. The final method, called budding, can be used to rejuvenate older plantings and involves excising a bud and positioning it under a flap of bark on another tree. The join is then sealed with raffia and waxed tape. Once the bud begins to grow the tree above the new growth should be removed.

The cocoa nursery should be kept weed free while the seedlings are established but generally do not require weeding after the trees have formed a closed canopy as the lack of light under the trees prevent any further growth. Cocoa should be supplied with additional nutrients by fertilizing, particularly when trees are grown on poor soils or without shade. Organic fertilizers are generally preferable to inorganic ones as they do not deplete the soil organic content and conserve soil structure. The amount of fertilizer required is dependent on many factors, such as the age of the tree and the amount of shading but mature cocoa generally requires at least 50–100 kg ha<sup>-1</sup> of nitrogen, 25 kg ha<sup>-1</sup> of phosphorus, 75 kg ha<sup>-1</sup> of potassium and 15 kg ha<sup>-1</sup> of magnesium each year.

Cocoa trees take three to five years to yield a crop, with hybrid varieties providing crops earlier. The crop has a peak growing period of 10 years, but can extend for decades and the trees should be productive for about 25 years. The age at which a tree is first harvested does not influence production during the life of the tree. Many other factors such as maintenance, variety of cocoa tree, weather, etc. have more effect on production during the life of the tree than the time of first harvest.

Cocoa harvest is spread over several months, and in some regions there may be pods available for harvest throughout the year. Typically, there are one or two peak harvest periods influenced by flowering in response to rainfall and humidity. However, local climate and the crop already on the tree will also influence flowering so that the yearly-cropping pattern can vary in areas with a relatively uniform climate. On ripening, pods turn from green or deep red to yellow or orange and only ripe, colored pods are harvested. However, the timing is not critical since under-ripe pods will ferment satisfactorily and ripe pods can be left on the tree for two to three weeks. Ripened pods are suitable for harvest three to four weeks, after which time the beans begin to germinate and may rot. Harvesting is done manually by making a clean cut through the stalk with a well sharpened blade with machetes or knives to cut pods from the tree since pulling the pods from the tree can damage the flower cushion and tear the bark. For pods high on the tree, a pruning hook type of tool (*go-to-hell*) can be used with a handle on the end of a long pole. By pushing or pulling according to the position of the fruit, the upper and lower blades of the tool enable the stalk to be cut cleanly without damaging the branch which bears it. It is necessary to harvest at regular intervals as the pods do not all ripen at the same time. The frequency of harvesting can have an effect on yield. During harvesting it is important not to damage the flower cushion which will produce the flowers and fruits of subsequent harvests, and care must be taken not to damage the

tree, which would make it easy for parasitic fungi to penetrate the tissues of the tree. In general the harvested pods are grouped together and split either in or at the edge of the plantation.

### 24.2.3 Cocoa Processing

After harvest, cocoa pods are opened to extract the wet beans a week to 10 days after harvesting. This is a manual operation; usually the pod is cut open and the beans are scooped out by hand. The placenta, which joins the beans inside the pod, is preferably separated from the wet beans prior to fermentation. If the pods are opened in the planting areas the discarded husks can be distributed throughout the fields to return nutrients to the soil. The best way of opening the pods is to use a wooden club which, if it strikes the central area of the pod, causes it to split into two halves; it is then easy to remove by hand the beans. A cutting tool, such as a machete, is often used to split the pod though this can damage the beans. Some machinery has been developed for pod opening but small-holders in general carry out the process manually. After extraction from the pod, the beans undergo a fermentation and drying process before being bagged for delivery.

Fermentation and drying are the last operations carried out on-farm before trading the dried beans. Fermentation is essential for the development of chocolate flavor (further developed during roasting). After extraction, the wet beans are bulked together and gradually heat up as a result of exothermic chemical reactions in the pulp caused by the activity of microorganisms (yeasts and acetic and lactic acid bacteria). Initially, the mucilage is broken down and drains off as "sweatings." After 36–72 hours the beans are killed and a series of chemical changes take place inside the bean, some of which continue during drying. Although chemically complex, fermentation methods are simple. Fermentation is carried out in specially constructed wooden boxes, in heaps covered by banana leaves or in baskets. Much of the heat generated is retained by insulation, but this is more difficult with small quantities of beans and a minimum of about 90 kg is required when using traditional heap or box methods. The process usually takes from five to seven days to complete depending on the type of cocoa being grown and local practice. The mass of beans is turned or stirred at least once for aeration.

Fermented beans are then dried in the sun or artificially until suitably dry (6–7% moisture content dry basis) for storing and transporting. Artificial drying can cause beans to be very acidic if they are dried too quickly. Dried beans are hand sorted or mechanically sieved and winnowed to remove defective beans and debris. The "pod index" expresses the number of pods required to produce 1 kg of dried beans. A low pod index usually means good bean size and a saving in harvesting costs since the weight of beans per pod is high. The "recovery" is the proportion of dry fermented beans to wet unfermented beans expressed as a percentage. It ranges from about 40% for under-ripe pods to 45% for over-ripe pods, but is also affected by variety and season.

Manufacturing cocoa for the principal chocolate ingredients and byproducts is generally an industrial process requiring expertise and specialized equipment. Physical characteristics assessed by manufacturers to determine the quality of cocoa beans (in addition to flavor attributes) are of relevance to growers. Increasingly at the higher end of the market, there is a move to artisan production or so-called "bean to bar" manufacturers. These boutique chocolate producers make and market high quality chocolate.

#### 24.2.4 Uses of Cocoa

The principal product from cocoa is chocolate. There are other products from cocoa which may be of commercial importance. Products that can be made from the cocoa beans include Moisturizing Cocoa Butter Soap, Body Pomade, Lotion and cream, Cocoa Liquor, and Pastries. The butter from discarded beans (salty, germinated, diseased, and small size beans) has been systematically researched into and formulated with other vegetable oils to produce cocoa butter based soap of acceptable quality. Body pomade has been carefully formulated with butter that has been extracted from roasted cocoa beans. Cocoa butter extract from discarded cocoa beans, has been used to formulate lotions and creams as a way of maximizing the usage of the discarded beans. Defatted cocoa powder has been used for the production of wine called cocoa liquor. This wine is noted for its tremendous antioxidant properties. Pastries can also be made from defatted cocoa powder in combination with cereals such as maize and rice.

Cocoa pod husk (CPH) which is normally discarded on the farm after breaking up the pod to extract the beans could also be processed commercially to serve as animal feed, potash, Medicinal soft soap (Dark soap) and potash fertilizer. CPHs have been processed into animal feed by processes of slicing, partial drying, and pelletizing into granules. The dried pellets are used in animal feed formulations for sheep, pigs, and poultry. Nutrient composition of CPH feed on dry basis is as follows: Crude protein – 6.5%, Crude fiber – 27%, Ash – 8.0%, Ether extract – 4.4%, Nitrogen – 43.0%, and others – 11.1%. CPH that has been dried and burnt can produce potash that can serve as potash fertilizer or used by local soap industry as source of lime for local soft soap (Alata Samina) production. Liquid soap can also be made from this potash. Medicinal soft soap (Dark soap) can also be made from combination of potash produced from CPH and cocoa butter. This soap which derived its name from its color, does not only have a moisturizing effect on the skin but also has curative powers against all manner of shin diseases.

Cocoa products that could be of commercial value from Cocoa Pulp Juice (Sweating) include the following uses.: Fresh cocoa drink can be made from pressing freshly harvested ripe cocoa pods to produce pulp juice. The juice is then flashed pasteurized and contains all its natural flavors. It is served best when chilled. Pectin can also be obtained from fresh cocoa juice (sweatings). It is used in confectionery, food and pharmaceutical industries as a setting agent. Cocoa pulp juice in its natural state contains some amount of sugar and pectin. Jams or jellies of acceptable quality and consistency are produced from the pulp juice. Cocoa pulp juice (sweatings) contains some fermentable sugars. Cocoa wine is produced from the pulp wine juice when pasteurized and fermented with appropriate wine yeast. Vinegar has been produced from cocoa pulp juice (sweatings). The pulp juice is pasteurized and fermented with wine yeast to produced cocoa wine. Further fermentation of cocoa wine with appropriate acetic acid bacteria produced vinegar.

Cocoa pulp juice is also rich in fermentable sugars. Alcohol is produced from the pulp juice after fermentation and distillation. The alcohol produced is then blended to the acceptable concentration as gin or brandy.

#### 24.2.5 Global Cocoa Production Data

While cocoa originated in Central America over 5000 years ago, it's popularity and production has spread globally. The top ten cocoa-producing countries come from warm,

**Table 24.1** Global cocoa bean production from 2012/2013 to 2015/2016 by country (in 1000 metric tonnes).

Year	Country							
	Cote D'Ivoire	Ghana	Indonesia	Ecuador	Cameroon	Brazil	Nigeria	Papua new guinea
2012/2013	1449	835	410	192	225	185	238	41
2013/2014	1746	897	375	234	211	228	248	36
2014/2015	1796	740	325	250	232	230	195	36
2015/2016	1690	840	300	230	230	210	200	36

Source: Statista, The statistics portal.

wet climates similar to where the bean originated. However, nations across four continents make the top ten, and the largest contingent does not come from the Americas, with four of the top five nations found in Africa. The top ten countries are; 10 – Peru, 9 – Dominican Republic, 8 – Mexico, 7 – Nigeria, 6 – Brazil, 5 – Cameroon, 4 – Ecuador, 3 – Indonesia, 2 – Ghana, and 1 – Cote d'Ivoire. The Ivory Coast supplies 30% of the world's total cocoa, leading the rest of the world by over half a million metric tonnes (Table 24.1). Companies like Nestle and Cadbury receive much of their cocoa from the Ivory Coast, and cocoa alone is responsible for almost two-thirds of the trade revenue coming into the nation.

#### 24.2.6 Global Cocoa Market Trends

Africa is the largest producer of cocoa, accounting for 68% of the production followed by Asia Pacific (17%) and Latin America (15%). Europe is the largest consumer and importer followed by North America. Major cocoa bean exporting countries include Ivory Coast, Ghana, Nigeria, Cameroon, Brazil, Ecuador, Colombia, Indonesia, and Malaysia among others. Although the production of beans is dominated in Africa, Latin America and Asia Pacific; the major grinding facilities are placed in the Americas and Europe.

The global cocoa beans market was USD 13.38 billion in 2015. Cocoa beans are primarily used as raw material for chocolate and 90% of the global cocoa beans produced are consumed for chocolate production. On an average, around 4 million metric tons of cocoa beans are produced each year. Global production of cocoa beans during 2015 was 4.36 million metric tons. The international market prices of cocoa are very volatile and changes variably with demand and supply. It is forecast that during 2016 the crop prices will go down due to expected surplus supplies. However, the low prices will drive the demand in the coming years with expected changes in product pipelines of major confectionery companies. The high prices in 2015 has led to lesser profits in grinding which in turn forced grinding companies to close their operations at various places.

The cocoa beans captured the attention of consumers from around the world, due to fast growth of chocolate confectionary market. The same is the major factor driving the market growth. Other than chocolate confectionary market factors stimulating the

market growth include increasing disposable income among middle class and increasing popularity of cocoa based products like cocoa beverages and cocoa powder. However, the commodity price fluctuation, pest and diseases, low productivity, high dependence on seasons and environmental conditions and high cost of farm inputs are restraining the market growth.

#### 24.2.7 Potential Markets for Cocoa Byproducts

The post-harvest processing of cocoa pods into cocoa beans generates a number of byproducts and wastes that are usually discarded, but which in fact could be processed into other economic products, using the appropriate technical know-how. The beans extracted from cocoa pods constitute only about 19% of the fresh weight of the pod and the remaining 81% in the form of fresh CPH and bean pulp juice are discarded as wastes. Equally, germinated beans, and other substandard beans that do not meet the quality requirements of chocolate manufacturers and thus have little market value are in many cases destroyed as wastes. It could, therefore, be rightly said that the economic potentials of cocoa production have not been fully exploited through the efficient use of all output from cocoa production.

Three distinct categories of wastes are derived from cocoa pods after removing cocoa beans used for the manufacturing of chocolate and other related products. These wastes are CPH, cocoa sweatings or pulp juice, and discarded cocoa beans. CPH are derived when cocoa pods are opened, and the beans still covered with mucilage or pulp are removed. Cocoa sweatings are derived during the fermentation process of the beans, when chemical changes cause the mucilage covering the beans to liquify and run off as "sweatings". Discarded cocoa beans are germinated beans and substandard beans that would not be accepted by chocolate manufacturers.

CPHs can be processed into animal feed, potash for soft soap manufacturing, compost, and organic fertilizer. CPHs can also be used to produce biogas. Cocoa sweatings or pulp juice can be processed into soft drinks, wine, vinegar, alcohol, and pectin, while discarded cocoa beans can be processed into cocoa butter soap and cocoa butter based cosmetics. The three categories of cocoa wastes and byproducts could therefore be used to develop a CPH-based industry, a cocoa sweatings-based industry and a discarded cocoa bean-based industry.

The possible byproducts that could be produced from cocoa therefore include; Potash for soap production, Fertilizer, Mulch, Animal feed, Cellulosic ethanol, Soft drinks and Alcohol, Wine and Vinegar, Pectin for jam and marmalade. The commercial production of these products could tap the full potential of the crop.

### 24.3 Kola Nuts

#### 24.3.1 Description of the Kola Nut Plant

Kola nut (*Cola nitida*) is the nut of the kola tree, a genus of trees native to West Africa and has been introduced throughout the forested areas of West and Central Africa, classified in the family Sterculiaceae. Kola is represented by over 40 species in West Africa alone, however, only two species *Cola acuminata* and *C. nitida* are of major economic

importance. Kola nut is evergreen tropical tree growing up to about 20 meters tall, with spreading open canopy. The leaves are oval with pointed ends, leathery, with a shiny upper surface borne alternately on the stem. The flowers are off-white to cream, star-shaped with five petals and a blotched red-purple center with a prominent stigma which are pollinated by flies. The fruits are large ( $13 \times 7$  cm), knobbly, green pods, splitting into two equal halves to reveal four to eight, smooth, red or white, seeds (kola nuts). Commercial crops are grown mainly in Nigeria, Ghana, Côte d'Ivoire and Sierra Leone and also to some extent in India, Brazil, and Jamaica. In Ghana, the crop is cultivated mainly in the Eastern, Volta, Ashanti, Brong Ahafo, and the Western regions, where it serves as the main livelihood of many farmers and traders.

#### 24.3.2 Cultivation and Harvesting of Kola

Kola tree requires a hot humid climate to grow but it can withstand a dry season on sites with a high ground water level. The crop may therefore be cultivated in drier areas where ground water is available. Although Kola is a lowland forest tree, it has also been found to do well at altitudes above 300 m on deep, rich soils under heavy and evenly distributed rainfall. Mean annual temperature requirement ranges between 26 and 35 °C and mean annual rainfall requirement also ranges from 1200 to 1800 mm.

Propagation of Kola can be undertaken by the sowing of fully ripened seeds or by cutting. When growing by seed, the seed is planted in a pot and later transplanted to plain field. To grow a kola nut, tree soil must be deeply rich and fertile and well drained. Accumulation of water might prove to be unsatisfactory for the plant except in dry weather conditions. Adequate drainage is important as *C. nitida* does not perform well in waterlogged soil. Kola can be grown in open or partially shaded sites. The plant can grow 40–60 ft and may take 7–10 years to bear fruits from seeds.

Good agronomic and cultural practices after planting will ensure good field establishment and yield. Early fertilizer application enhances growth. This is usually done six months after planting in the field. It is also advisable to plant food crops such as cocoyam, maize, and legumes as intercrops to maximize land use. It is appropriate to weed the field three to four times each year for juvenile farms and one to two times for matured farms. This practice will reduce the pressure on nutrients in the soil and also prevent the build-up of pest.

Kola nuts can be harvested by hand directly from the tree branch with a machete. At maturity, the fruit is inconspicuously brown and changes color from deep green to paler tint. Special equipment such as *go-to-hell* could be used to harvest kola pods that are high in the branches.

#### 24.3.3 Processing of Kola

Processing of Kola begins with careful examination and sorting out pods that are infested with weevils, diseases, and other deformities, from the healthy pods. The healthy pods are then broken up and the nuts removed. The nuts are then soaked in potable water for 24 hours to allow the softening of the testa or the seed coat. The testa is then removed and the nuts are rinsed in potable water. The rinsed nuts are collected in wide flat baskets through which excess water drains off for three days before to continue the curing process. After the third day, a new basket is lined with fresh leaves (mostly bana leaves) and

the nuts are transferred into it. The basket is then covered with leaves and stirred periodically to avoid excessive heat build-up during this curing process, which last for approximately three weeks.

Another curing method involves the removal of the testa around the nuts and subjecting the nuts to direct sun light for four weeks at ambient day temperature of 32 °C in wooden trays. Cured nuts could now be stored on mats or trays in a cool dry place.

Processing of kola could also be done using conventional methods. The conventional method involves the drying of peeled nuts in ovens at constant temperature of about 32 °C to remove moisture from the nuts. This method is not very common compared to the non-conventional method.

#### 24.3.4 Uses of Kola

Kola nut contains caffeine. Caffeine works by stimulating the central nervous system (CNS), heart, and muscles. Kola nut is therefore generally used for short-term relief of fatigue, depression, chronic fatigue syndrome, melancholy, lack of normal muscle tone (atony), exhaustion, dysentery, a type of diarrhea called atonic diarrhea, weight loss, and migraine headaches. Seeds from *C. nitida* and *Cyanea acuminata* have been used in western African and Anglo-American herbal medicine as an antidepressant. They have also been used to treat headaches, migraine, dysentery, and diarrhea. In Africa, *C. nitida* bark is used to treat wounds and swellings, roots to make teeth-cleaning sticks, and pod bark mixed with other ingredients to reduce labor pains. In the past, kola nuts have been given to troops on African battlefields, with the aim of enabling prolonged exertion without fatigue or thirst but also preventing dysentery and even supposedly giving rise to a feeling of bravery.

In foods and beverages, kola nut is used as a flavoring ingredient. Kola nuts contain caffeine, theobromine, tannins, fructose, and kolanin (a heart stimulant). They are chewed as a stimulant and are especially flavored as a snack by African Muslims when fasting in the month of Ramadan. Kola nuts are reported to suppress hunger and thirst and have been used in western and central Africa for thousands of years. Kola nut extract was reportedly used as a source of caffeine in pharmacist John Pemberton's "French Wine Coca," a forerunner of the soft drink Coca-Cola. Natural kola nut extract has now been replaced by synthetic citrate caffeine in many leading brands of cola drink, although some advertised as "natural cola" include kola nut in their ingredients.

Kola nut is used widely in Nigeria and many West African countries as part of traditional hospitality, cultural, and social ceremonies. Kola nuts are used in many African ceremonies, for example the welcoming ceremony of the Igbo culture of Nigeria. The seeds are passed among visitors to a village and then blessed by the village elder. Kola nuts are central to many other ceremonies in western and central Africa including marriage, child naming, investiture of tribal chiefs, funerals, and sacrifices to deities.

*C. nitida* seeds are also used for dyeing, water purification, and the production of liquid soap and fertilizers. Byproducts of kola nut processing are used as poultry feed. Its timber is used for furniture and as fuel wood.

#### 24.3.5 Global Kola Production Data

(Table 24.2)

**Table 24.2** Global Kola nut production for 2013 by country (in 1000 metric tonnes).

Year	Country					
	Nigeria	Cote d'Ivoire	Cameroon	Ghana	Sierra Leone	Benin
2013	132 000	82 000	46 500	24 000	8 645	600

Source: FAOSTAT: <http://faostat3.fao.org/home/index.html#DOWNLOAD>.

### 24.3.6 Global Kola Market Trends

Kola nuts are exported to Europe and North America for flavoring kola drinks and for use in the manufacture of pharmaceuticals. Industrial exploitation is mainly for the caffeine. Beverages like kola wine, kola cocoa, and kola-chocolates, and assorted medicinal products have been derived from kola nuts. At any rate, off-continent exports appear to absorb only a minor part of the world production estimated at 180 000 tonnes, of which 120 000 tonnes are produced by Nigeria and used either internally or in neighboring countries. *C. nitida* is preferred in international trade because of its high caffeine content and the white strain is most valued. A publication by United Nations' Food and Agriculture Organization estimated that from a total West and Central African production of kola nuts of 180 000 tons only 60 000 are exported; the rest are consumed internally. Thus, it is clear that the product remains virtually unknown in other part of the world.

### 24.3.7 Potential Markets for Kola Byproducts

Kola nuts are mainly used as flavorings for soft drinks. Other parts of the Kola plant could be exploited for use in the dyeing industry, water purification, in the production of liquid soap, and in the production of fertilizers. Byproducts of kola nut processing could also be used as poultry feed. The hard wood from the aged plants could also be used as timber for furniture and as fuel wood. Biogas could also be generated form the pods of the fruits after extracting the seeds.

## 24.4 Sorghum

### 24.4.1 Description of the Sorghum Plant

Grain Sorghum (*Sorghum bicolor* (L) Moench) originated in Africa and it is uniquely adapted to Africa's climate. Grain sorghum is the staple food for low income farmers in many developing countries in the semi-arid tropical areas of Africa and also used for forage and silage production. Grain sorghum in Africa is processed into a very wide variety of attractive and nutritious traditional foods such as semi-leavened bread, couscous, dumplings and fermented and non-fermented porridges, thick and thin porridges. It is the grain of choice for brewing traditional African beers. Sorghum grows in harsh environments where other crops do not grow well, just like other staple foods, such as cassava that are common in impoverished regions of the world. Sorghum is a drought tolerant crop but responds well if enough water is applied during its early stages to

prevent any sign of drought stress. It is usually grown without application of any fertilizers or other inputs by a multitude of smallholder farmers in many countries. The yield of sorghum varies depending on the variety and the environmental conditions pertaining in a growing region. Sorghum yield harvested from farmers in Ghana vary between 500 and 900 kg ha<sup>-1</sup> in Northern region and 700 kg ha<sup>-1</sup> on average in Upper East and West regions annually. According to 2009 world sorghum statistics, an average yield harvested annually by farmers in Ghana is 0.8 tonnes ha<sup>-1</sup> where as a developed country such as America produces over 33% of the world production from only 13% of total world area with yield productivity of 3 071 kg ha<sup>-1</sup>. Over the past 25 years sorghum production has increased steadily in Africa, from 11.6 million tonnes in 1976 to 20.9 million tonnes in 2001. However, increased in production has been as a result of increasing the land area under cultivation and there has been no overall improvement in yield.

#### 24.4.2 Cultivation and Harvesting of Sorghum

Sorghum is a warm-weather crop, which requires high temperatures for good germination and growth. The minimum temperature for germination varies from 7 to 10 °C. At a temperature of 15 °C, 80% of seeds germinate within 10–12 days. The best time to plant is when there is sufficient water in the soil and the soil temperature is 15 °C or higher at a depth of 10 cm. Temperature plays an important role in growth and development after germination. A temperature of 27–30 °C is required for optimum growth and development. The temperature can, however, be as low as 21 °C, without a dramatic effect on growth and yield. Exceptionally high temperatures cause a decrease in yield. Flower initiation and the development of flower primordia are delayed with increased day and night temperatures.

Sorghum is mainly grown on low-potential, shallow soils with a high clay content, which usually are not suitable for the production of maize. Sorghum usually grows poorly on sandy soils, except where heavy textured subsoil is present. Sorghum is more tolerant of alkaline salts than other grain crops and can therefore be cultivated successfully on soils with a pH between 5.5 and 8.5. Sorghum can better tolerate short periods of water-logging compared to maize. Soils with a clay percentage of between 10% and 30% are optimal for sorghum production. Sorghum is grown on well-prepared seedbed. The seedbed preparation should begin promptly after the previous crop is harvested to allow ample time for weed control, decay of crop residue, infiltration and storage of soil moisture, fertilizer application, and soil firming.

Grain sorghum is physiologically mature when moisture content drops to about 30%. At moisture content higher than 25%, however, the seeds are too soft to withstand adequate threshing action, leading to either unthreshed heads or cracked seeds. Sorghum dries rapidly in the Great Plains, often down to the 12% moisture level needed for safe storage. But, because of the danger of shatter loss and lodging from wind and rainstorms when moisture is under 20%, many western operators prefer to harvest early (20–25%) and dry artificially.

#### 24.4.3 Processing of Sorghum

Industrial methods of processing sorghum and millets are not as well developed. Sorghum is usually pounded followed by winnowing and sieving. The grains are then milled

into flour for various forms of meal. Flour made by grinding whole grain is occasionally used, particularly with the smaller sized varieties, but in most places where sorghum is consumed the grain is partially separated into its constituents before food is prepared from it.

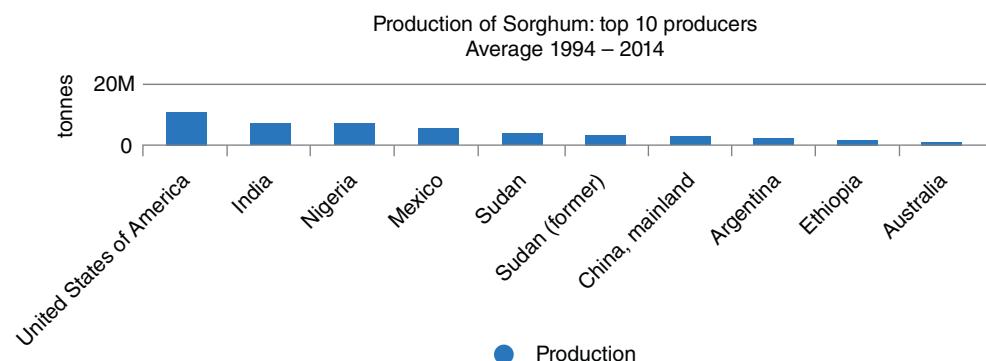
Malted sorghum has traditionally been used in several countries in Africa, but always after careful removal of the toxic parts. Hullu-murr is an important traditional food prepared from malted sorghum in the Sudan. Alcoholic beverages and dumplings are prepared in Kenya from germinated sorghum. Germination of grain is reported to change the amino acid composition, convert starch into sugars and improve the availability of fat, vitamins, and minerals. The use of only 5% malted sorghum or finger millet was found to reduce the viscosity of weaning foods.

#### 24.4.4 Uses of Sorghum

Sorghum is mainly used as a feed for livestock in the United States of America. It is, however, consumed as food in most developing countries, where it is milled into flour for preparing various forms of meals. Sorghum is used to brew a traditional drink, "pito," a traditional beer widely consumed in the Northern Regions of Ghana. According to the FAO (1995), the brewery industry had exhibited interest in sorghum in the past years when investigations were made into its possible use as a substitute for barley malt in the production of lager beer. This would have been an earmark for industrial breweries to save foreign exchange. Even though research experiments proved successful, inadequate local sorghum varieties suitable to local conditions in terms of grain quality resulted in industries losing interest in the local sorghum production and many others abandoning the idea.

#### 24.4.5 Global Sorghum Production Trends

The global harvest area for sorghum changed between 38 and 44 million hectares according to the data of United Nations Food and Agriculture Organization between 2003 and 2013, as can be seen in Figure 24.1. Regarding the harvest area, the 2012 harvest



**Figure 24.1** Source: FAO statistics 2017.

which is on 38 million hectares is the lowest of the last 10 years. Global sorghum harvest area reached 46.3 million hectares in 2005. This area became the highest rate in the last 10 years. Also in 2013, sorghum harvest area was around 42.2 million hectares.

America, Nigeria, Mexico, India, Sudan, Ethiopia, and Argentina are the most important production regions. Averagely 4–5 million tons of sorghum is produced yearly in each of these countries.

## 24.5 Millet

### 24.5.1 Description of the Millet Plant

Millets are a group of highly variable small-seeded grasses, widely grown around the world as cereal crops or grains for fodder and human food. Millets are important crops in the semiarid tropics of Asia and Africa (especially in India, Nigeria, and Niger), with 97% of millet production in developing countries. There are many millet varieties with the four major types being the Pearl millet (*Pennisetum glaucum*), which represents 40% of the world production, Foxtail millet (*Setaria italica*), Proso millet or white millet (*Panicum miliaceum*) and finger millet (*Eleusine coracana*). Although millet varieties with yellow, white, tan, red, brown, or violet color are available, only the red-colored ones are commonly cultivated worldwide. According to Ajiboye et al. (2014), incorporation of ancient based cereals such as sorghum and millet in our daily meals can reduce the risk of chronic disease, making them important crops. In Ghana, besides pito, millets are used for a wide variety of foods like porridge (commonly referred to as hausa koko), weaning foods, tuo zafi, and many other baked products. Millet provides a good source of polyphenol, calcium, and other essential minerals.

Cereals, in particular millet-based fermented foods and beverages, have been extensively studied and accounts for a major part of the diet in most African countries. Millet is a term used for a wide range of cereals. It is sometimes referred to as coarse cereals. They are a variety of small edible grasses belonging to the grass family (Gramineae/Paniceae), distributed into various genera and species. Appearance, morphological features, grain size, and maturity period are some of the characteristics by which the various species can be differentiated.

Millet possesses remarkable ability to survive under adverse conditions like poor soil fertility, limited/insufficient rainfall, and land terrain, thus making their growth in the Northern part of Ghana less challenging. India, Nigeria, Niger, China, Burkina Faso, Mali, and Sudan are some of the countries with millet as a major export crop.

### 24.5.2 Nutritional Content of Millet

Plant nutrients are of great essence in the food industry where grain cereals provide vital dietary nutrients across the world. Millet is composed of a total carbohydrate content approximately between 72% and 79.5% and proteins between 5.6% and 12.7%. Research reveals that finger millet is relatively better balanced in essential amino acids owing to its high lysine, threonine, and valine content. According to Ravindran (1991), lysine and methionine levels of the protein are inversely correlated with the protein content of the finger millet grain. Millets contain good amounts of magnesium and phosphorus.

In an interesting intervention carried out by Adebiyi et al. (2016), a spontaneously fermented millet-based product (koko) was naturally used as probiotic remedy for diarrhea in young children. Probiotics help the existing flora, or aid repopulate the colon when bacteria levels are diminished by antibiotics, chemotherapy, or disease. Malting stimulates important beneficial biochemical and functional changes in the millet grain. The millet grain is rich in phytochemicals, that include phytic acid, known to reduce cholesterol levels and phytate, believed to be effective in cancer risk reduction. These health benefits are due to the diversity of potential chemo preventive compounds defined as phytochemicals and include antioxidants that exist in remarkably high levels in foods such as millets.

#### 24.5.3 Processing of Millet

In general, industrial methods of processing sorghum and millets are not as well developed as the methods used for processing wheat and rice, which in most places are held in much higher regard than sorghum and millets. The first objective of processing is usually to remove some of the hull or bran – the fibrous outer layers of the grain. This is usually done by pounding followed by winnowing or sieving. When suitably prepared grain is pounded, the bran fraction contains most of the pericarp, along with some germ and endosperm. This traction is usually fed to domestic animals. The other fraction, containing most of the endosperm and much of the germ along with some pericarp, is retained for human consumption. Flour made by grinding whole grain is occasionally used, particularly with the smaller millets, but in most places where sorghum and millets are consumed the grain is partially separated into its constituents before food is prepared from it.

Malted millet has traditionally been used in several countries in Africa, but always after careful removal of the toxic parts. Alcoholic beverages and dumplings are prepared in Kenya from germinated millet. In India, malted finger millet is common and is considered to be superior to malted sorghum and malted maize. Germination of grain is reported to change the amino acid composition, convert starch into sugars and improve the availability of fat, vitamins, and minerals. The use of only 5% malted sorghum or finger millet was found to reduce the viscosity of weaning foods.

#### 24.5.4 Uses of Millet

Millet is an important grain product for the developing countries in Asia and Africa with semi-arid tropical climate. Millet flour is used to make bread and also to make *Boza* (malt drink) and alcohol after fermented. It is used as feed for birds and is one of the most important food stuffs in North African countries. In these regions, millet seeds are consumed as mash or flatbread after being boiled or milled. In addition, stems and seeds of all kinds of millets are used as animal feed.

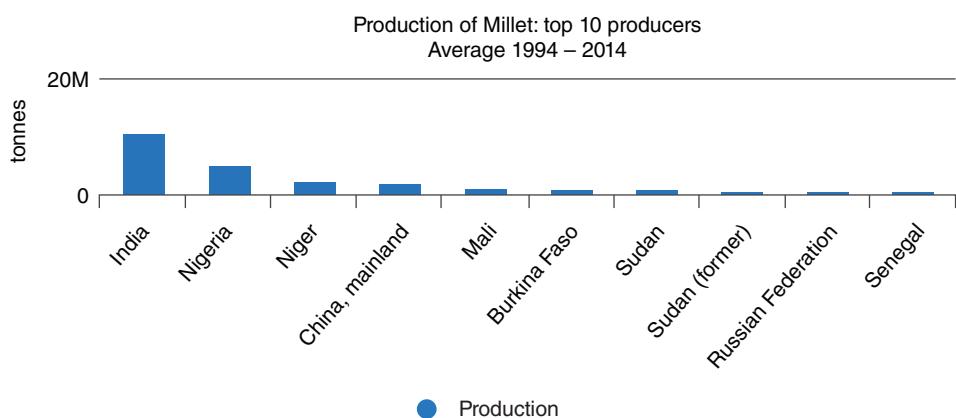
Though millets have higher nutrition than that of other known cereals, their utilization is not considerably developed. By mixing millet with wheat flour as composite flour, the nutritional, physical, chemical, as well as functional changes can be observed. Extruded products like spaghetti, and porridges made of millets and cowpea have higher nutrition. Millet along with buck wheat and amaranth are used in place of maize and wheat flour for extruded snacks manufacturing. Rheological, chemical, and baking property studies were

carried out in the past on millet and wheat composite flour. This type of replacement of wheat with millet in flour is 10–20% possible as this increases the ash content and also decreases the damaged protein and starch percentage. Thus, such a type of millet blending apparently shows that this is the easiest technique to get enhanced nutrition and functional levels of the food product for promoting its utilization. Millet may also be used in the processing and production of extruded products, flakes, and pops. The high fiber content is helpful for those people who are dealing with the problem of obesity and constipation. Pearl millet is a good source of fiber (1.2 g/100 g) and can be used to prepare healthy foods. Millet grains are rich in antioxidants and phenolics, so they can contribute to antioxidant activity important in health, aging, and metabolic syndrome.

#### 24.5.5 Global Millet Production Trends

According to the data of the FAO, the global harvest area for millet changed between 29 and 38 million hectares between 2003 and 2013. The most important producers are India, Nigeria, Niger, China, Mali, Sudan, and Burkina Faso. Solely India realizes most of the millet production with its production amount over 10 million tons. Annual millet production amount of the other countries changes between 1 and 5 million tons. Ranking second after India, Nigeria realized 4.8 million tons of millet production in 2014/15 season; while Niger realized 3.2 million tons of production.

The World's largest consumption in millet used mostly as human food and feed is India, that also ranks first in the production, as can be seen in Figure 24.2 India's millet consumption is slightly higher than the production. Despite the 10 million-ton production, 10.2 million tons of millet consumption was realized in 2014/15 season. Nigeria, Niger, China, Mali, and Sudan follow India in millet consumption. Nigeria realized 5 million tons of millet consumption, Niger realized 2.9 million tons, Mali realized 1.2 million tons, and Sudan realized 1 million tons in 2014/15 season.



**Figure 24.2** Source: FAO stats.

## 24.6 Shea Butter

### 24.6.1 Description of the Shea Butter Plant

The shea tree (*Butyrospermum parkii* or *Vitellaria paradoxa*) – commonly known as karité in the Wolof and French languages, grows wild in the equatorial belt of central Africa between Gambia and Sudan, and also in Uganda. The oil extracted has a relatively high melting point and is used in rural areas in the making of foods, soap manufacture, and cosmetics. Shea is mainly exported as kernels and can be used as an extender in chocolate as its properties are similar to cocoa butter. Women usually carry out small-scale processing on the kernels to extract the oil as an important source of income.

### 24.6.2 Shea Butter Cultivation

Shea trees are not cultivated but grow as wild plants. A shea-tree will bear fruit at between 8 and 15 years but reaches full capacity for several decades after this. A tree can yield of 15–20 kg of fresh fruit that will produce 3–4 kg of dry kernels. The kernels contain 42–48% oil (butter).

### 24.6.3 Harvesting of Shea Butter

Women and children collect the fallen fruit and take them back to their villages for processing into shea butter, an edible fat.

### 24.6.4 Shea Butter Processing

One method of processing shea nuts is to bury the fruit in the ground so that the pulp ferments and falls off. This takes about 12 days or more. The nuts are parboiled or sun dried and then dried by smoking over an open fire for three to four days. The dried nuts can then be stored for long periods without significant losses. Decortication is done by crushing the outer shell to remove kernels. Shea nuts are mainly exported as smoked kernels. The kernels will be further dried before any additional processing is carried out. Traditionally wet processing by hand is a slow and laborious process that uses large quantities of wood as fuel for roasting. Nuts are shelled by hand by being pounded individually using the end of a pestle. The resulting kernel particles are aggregated and roasted on a metal sheet over a fire. The kernels are then pounded in a mortar to produce a coarse paste and then ground between two stones to produce a smooth paste. A small amount of water is added to the paste and the mixture agitated by hand using a “paddling” motion. The mixture is continuously stirred for anything up to four hours. The length of time depends on the quality of the nuts. At the end of this time the mixture becomes lighter in color and more water is then added. The white shea butter then floats to the top of the mixture. At this point the stirring action is carried out much less vigorously. The resulting oil is decanted off the dark brown residue using a spoon and is washed repeatedly with warm water until clean. The remaining water is removed by heating. Impurities settle out and the butter can be left to cool and solidify. The butter is then boiled over an open fire until clear. The oil is left overnight and the next day is stirred with small sticks when it becomes solid.

The resulting shea butter is then ready to be used. Using this traditional technique, the fat obtained is between 25% and 40% of the dry kernel weight. The introduction of equipment may improve upon traditional methods of production by reducing the effort and time involved and by increasing the yield.

Attempts have been made to introduce small-scale technology to extract shea butter, especially the use of a bridge press with marginal yield increases over the manual method. The resulting press cake provides a useful fuelwood substitute. A fully-motorized method mimicking the steps involved in the manual rural butter extraction methods was developed by the Technology Consultancy Center of Kumasi University of Technology, Ghana, but the equipment is costly and, depending on the shea butter value, may not cover capital and operating costs. Commercial expellers are used to extract the butter from shea nuts due to economies of scale.

#### 24.6.5 Uses of Shea Butter

Shea butter is rich in vitamins and minerals. It is vital to the daily existence of the people in the Sahel region as it is used as a cooking medium. Shea butter is used as baking fat and to substitute cocoa butter in chocolate manufacture and chocolate confectionery products. In the cosmetics industry, shea butter is used as a base for cosmetics, including skincare products and moisturizing cream.

Soap manufacturers use shea butter typically in small amounts (5–7% of the oils in the recipe) as it has the property of leaving a small amount of oil in the soap. Other uses include as a waterproofing wax, for hairdressing, and for candle-making. Its use is also noted in traditional African percussion instruments to increase the durability of wood (such as carved djembe shells), dried calabash gourds, and leather tuning straps.

The bark of the shea tree is used to cure ailments in skin treatment in children and treat minor scratches and cuts. Shea unsaponifiables are used as anti-inflammatory treatment for arthritis and a topical treatment for eczema and other skin conditions including herpes lesions. A patented product “nutraceutical” is a shea product that has been developed for lowering cholesterol in humans. Its use as a base for medicinal ointments, has been claimed to have anti-inflammatory, emollient, and humectant properties.

#### 24.6.6 Global Shea Butter Production Trends

Africa produces about 1 760 000 tonnes of raw shea nuts annually from its wild trees, mainly in the Savannah and Sahel regions, but producers harvest and process only a fraction, about 35% (about 600 000 t), for exportation as butter or nuts. The FAO reports that Nigeria produces about 355 000 million tonnes of West Africa's crop (see Figure 24.3). In addition, producers export a large but unreported quantity of Nigerian shea nuts through neighboring Benin. Ghana produces about 55 000 tonnes of shea nuts and exports about 40 000 tonnes of nuts annually, making it the leading exporter in the sub-region. Four major players control the global refining of shea: Aarhus United in Denmark, Fuji Oils in Japan, Karlshamn in Sweden, and Lodders Croklaan in Holland, listed in the order of magnitude of size of operations in oils and fats.

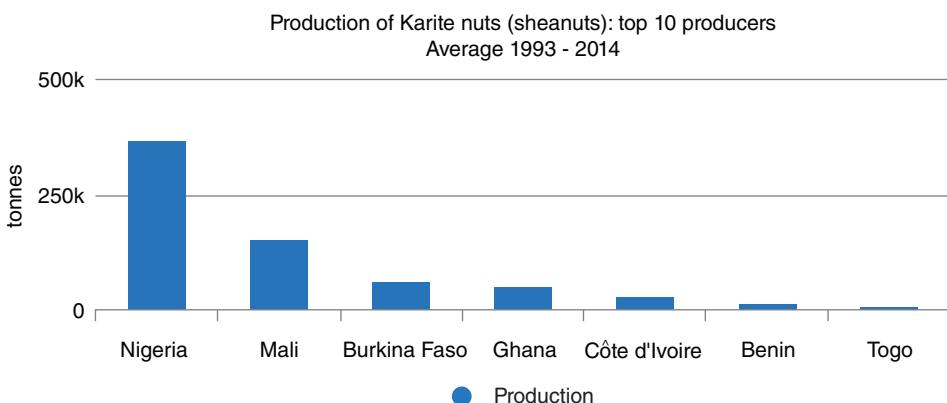


Figure 24.3 Top 10 Producers of Sheanuts. Source: FAO (2017).

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## 25

# Economic Value of Agro Waste in Developing Countries

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### 25.1 Introduction

Even with all the public policy programs in place, developing economies of the world are not close to achieving reasonable standards for food security, energy sufficiency, habitat preservation, and pollution-free environment. Exploring the scarcity concept may help us understand why this is the case. We, as a society, have unlimited wants and limited means. And, the limited means we have, are often dispensed to solve some issues, while ignoring other equally good ones. For example, what would a cash-strapped country primarily invest in? Would it: (i) address the food security problem by issuing vouchers to farmers that enable them to buy high-potency chemical fertilizers; (ii) address the energy sufficiency problem by building biorefineries that produce clean energy; (iii) address the habitat preservation problem by setting up monitoring stations that prevent incessant logging in protected rainforests; or (iv) simply do something else? How should we decide which mutually competing and equally pressing problems to solve? Obviously, we should select the most efficient solution. In other words, we must consider the program that justifies costs the most for the solutions it creates (Drummond et al. 1987). This type of process that compares one project's costs and consequences with another is what economists call the economic valuation.

In this chapter, we take up the economic valuation of agro-waste, with a special focus on developing economies. Here, we draw out the various possibilities and the limitations that an agro-waste reclamation program faces using the technique known as the cost-benefit analysis. This analysis technique was developed to assist governments and other not-for-profit agencies in making decisions on how to utilize public taxpayer monies in a way that maximizes public welfare.<sup>1</sup> The chapter will first outline a detailed summary of

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<sup>1</sup> Cost-benefit analysis is the most widely used economic valuation method in a public sector setting. Other methods more commonly used in health care sciences are cost-effectiveness analysis, cost-utility analysis, and cost-minimization analysis. See Evans and Hurley (1995) for more details. The cost-benefit analysis technique when applied to private sector decision-making is referred to as capital budgeting analysis.

the positive attributes of a project (called “benefits”) and negative attributes of a project (called “costs”). This will then be followed by the discussion of practical issues that might interfere with the outcome of the analysis. Finally, we provide an overview of the decision criteria by which a program’s relative merits are evaluated in a manner that fits a specific country setting.

## 25.2 Cost-Benefit Analysis: Benefits

Agro-waste recycling carries the following socio-economic benefits: energy production, greater reliability of reclaimed resources for agriculture and other activities, product development, employment, and the reduction in environmental and health costs.

### 25.2.1 Energy Production

For many of the countries in Asia and Africa, energy shortages are the principal deterrent in socio-economic development and political stability. Today, about 17% of the 6.2 billion people in low- and middle-income countries do not have access to electricity (World Bank 2017a).<sup>2</sup> Such a situation is further exacerbated by the World Bank’s decision to limit investments in coal-fired power stations in 2013 and in oil and gas exploration after 2019 as a part of a broader campaign to reduce greenhouse gas emissions. Considering these recent developments, one of the most promising ways to fill the gap in energy shortages is by drawing out a well-devised plan to use biofuels as replacements to fossil fuels. Within the use of biofuels as a fossil fuel substitute lies another interesting highlight – the local production of biofuels. Such production can not only reduce the country’s reliance on imported crude oil, but can also reduce trade deficits.

### 25.2.2 Greater Reliability of Reclaimed Resources for Agriculture and Other Activities

In low- and middle-income countries as a group, agriculture utilized about 38% of land and 79% of annual freshwater withdrawals in the year 2014.<sup>3</sup> Studies have also noted the pattern of increasing droughts in developing countries, further making land and freshwater a valuable agricultural commodity.<sup>4</sup> Bioremediation is a process of reviving both contaminated land and water using bioagents such as microorganisms, plants, and fungi. Treuer et al. (2018) notes one such example for the forest conservation efforts in Guanacaste Conservation Area, Costa Rica. In 1997, nearly 12 000 metric tons of agro-waste, mainly composed of orange peels and orange pulp from a local fruit juice company, was dumped in designated zones marked as “degraded.” After 15 years, ecologists found “richer soil, more tree biomass, greater tree-species richness and greater forest canopy closure” in the dumping region compared with a nearby area in which no dumping had

2 This includes 47% of Africa’s one billion people (Sekoai and Yoro 2016) and 21% of India’s 1.3 billion people (Sahni and Sinha 2018b).

3 Data obtained from World Bank (2017b) and World Bank (2017c).

4 For example, by studying the rainfall and human development data in India, Sahni and Sinha (2018a) show the impact of drought on violence in agricultural societies.

taken place. Therefore, reclaimed resources, to the extent that it is safe and socially acceptable for reuse, alleviates the pressure on virgin resources and reduces both environmental pollution and overall supply costs of the resource in question.

### 25.2.3 Product Development

Recent technological developments have used agro-waste innovatively in several profit-bearing initiatives.

#### 25.2.3.1 Soil Amendments

As per the International Fertilizer Development Center in the year 2009, between 50% and 77% of the world's production capacity of industrial fertilizers was controlled by a small group of firms in five fertilizer-producing countries (Hernandez and Torero 2013). Such high industry concentration puts enormous upward pressure in fertilizer prices. Also, the amount of industrial fertilizer consumption in low- and middle-income countries has increased from 97.2 kg ha<sup>-1</sup> of arable land in 2002 to 138.2 kg ha<sup>-1</sup> of arable land in 2014. In 2014, the lowest was in the Central African Republic at 0.3 kg ha<sup>-1</sup> of arable land and the highest was in Malaysia with 2063.9 kg ha<sup>-1</sup> of arable land.<sup>5</sup> And, with rising demand, costs also increase – industrial fertilizers are now far too expensive for farmers to afford without governmental assistance. This situation points to a growing need to use animal waste as an industrial fertilizer substitute. Potential benefits include the adequate disposal of animal manure, savings from foregone industrial fertilizer purchase, and prevented crop loss due to delays in fertilizer imports resulting from government inefficiencies.

#### 25.2.3.2 Building Materials

There is an increasing concern over the depletion of earth-based building materials such as clay and sand. Shortage in these materials poses a particular type of problem in developing countries, not seen in other parts of the developed world. In the southern Indian state of Tamil Nadu, there is rampant illegal sand and gravel mining from riverbeds and beaches that not only causes widespread riverbank erosion, but, has also drawn violence between environmentalists and illegal mining cartels.<sup>6</sup> Similar illegal riverbed and coastal sand mining has been noted in Pakistan, Nepal, Bangladesh, Tanzania, and Zanzibar. Most of these circumstances create immense political turmoil and enormous loss in terms of foregone taxes due to illegal sand sale. Therefore, there is a need to search and develop sustainable alternatives. The protracted use of plant-based residues, such as rice straw ash and wheat straw, to partially replace conventional building materials, is currently being tested.

### 25.2.4 Employment

While it is difficult to pinpoint the precise impact of waste reclamation on employment due to the lack of reliable statistics, there can be no doubt in designating employment as the main motivating factor to set up a comprehensive agro-waste policy in a developing

<sup>5</sup> Data obtained from World Bank (2017e).

<sup>6</sup> For details, see <https://ejatlas.org/conflict/illegal-sand-mining-in-tamil-nadu>.

country. A new facility may have job implications well beyond direct employment. Indirect employment arising from related industry services such as providers of materials and technology may also have a positive impact on employment numbers.

### 25.2.5 Environmental and Health Benefits

#### 25.2.5.1 Cost Savings from Reduced Carbon Dioxide Emissions

Atmospheric carbon dioxide ( $\text{CO}_2$ ) levels have been increasing consistently over the past few decades – from 1.1 metric tons per capita in 1960 to 3.5 metric tons per capita in 2014 in the low- and middle-income countries alone (World Bank 2017d). Biofuels are carbon-neutral, that is, the amount of  $\text{CO}_2$  consumed by the plant over its life is roughly equal to what the plant waste releases when burned as a fuel. Thus, the use of biofuels in place of fossil fuels can achieve sizable reductions in  $\text{CO}_2$  emission levels.

#### 25.2.5.2 Cost Savings from Reduced Crop Burning as a Means to Clear Land for Harvest

Stubble burning, a once popular way to clear the land for the next cycle, is now banned or closely regulated with permits in developed countries such as the UK and Canada. Still, the practice is fairly common in many developing countries in Asia and Africa.<sup>7</sup> Consider the case of India. Several million tons of post-paddy harvest crop is burned during the two weeks starting mid-October in the states of Punjab, Haryana, and western Uttar Pradesh. In this burning season, haze in the nation's capital, New Delhi, causes travel delays and restrictions, school closures, and increased hospital visits of mostly children and seniors for respiratory problems due to overexposure to particulate pollution. In 2010, about 42 000 deaths were attributed to stubble burning across India, a number that has increased to 65 000 in 2017 (Tallis et al. 2017). Therefore, an agro-waste reclamation policy that weakens stubble burning can lead to monetary savings and environmental protections.

#### 25.2.5.3 Cost Savings from Reduced Virgin Land and Water Use

Forests, pastures, rivers, and lakes provide unique habitats to numerous fish, animals, and plant life. Using treated land and water in place of virgin resources helps to conserve these habitats, producing substantial environmental gains.

#### 25.2.5.4 Cost Savings from Reduced Disposal in Landfills

Landfills and dump sites contain solid agro-wastes and plant residues. These sites, over time, become a breeding ground for a variety of diseases and emit flammable and odorous greenhouse gases that adversely impact the environment. In addition to the cost savings from reduced emissions, most of the landfill wastes are energy rich and can be a valuable commodity to produce biofuels (see Section 2.1) and soil amendments (see Section 2.3.1).

<sup>7</sup> See NASA's satellite imaging of stubble fires across India in 2016 at <https://www.nasa.gov/image-feature/goddard/2016/first-comes-fire-then-comes-crops-in-india> and central Africa in 2013 at [https://www.nasa.gov/sites/default/files/753040main\\_20130603-africa\\_946-710.jpg](https://www.nasa.gov/sites/default/files/753040main_20130603-africa_946-710.jpg).

## 25.3 Cost-Benefit Analysis: Costs

The costs associated with agro-waste recycling can be grouped in the following categories: cost of initial setup, cost of operations, cost due to rising food prices, cost of policy development, environmental and social costs, and health costs.

### 25.3.1 Cost of Initial Setup

We could hope for the picture to change in the coming years, but as it stands today, the cost of initially setting up an agro-waste processing unit is an overwhelming task for several developing countries. Let us consider the case of a commercial second-generation biofuel facility. The International Energy Agency's 2010 report estimates that financing and initial setup costs was between USD 125 million and USD 250 million. For countries such as Brazil, India, South Africa, Mexico, and Thailand, this does not pose a problem – a mix of foreign direct investment and domestic funding ensures the setting up of the biofuel facility. But in countries such as Cameroon and Tanzania, domestic funding sources are limited, and poor infrastructure and lack of skilled labor deter foreign companies from investing.

### 25.3.2 Cost of Operations

#### 25.3.2.1 Supply of Waste

Biomass is a seasonally varying raw material. During the season when it is relatively cheap, biomass energy facilities buy and store large quantities of biomass from decentralized small-scale waste producers, sometimes from much wider distances. Such procurement, transportation, and storage are associated with huge logistical costs. Storage also gives rise to another problem – biomass has a tendency to decay resulting in lower quality over time and depressing its effective usage as a fuel substitute.

#### 25.3.2.2 Supply of Skilled Labor

In general, studies show a net positive employment impact of starting a biomass energy facility in most developing countries. Jobs that are arduous and repetitive in nature such as gathering, collection, and transport of biomass are easily filled; however, those that require technical expertise go unfilled. Engineers are often in short supply except in countries that already have a long history of producing biofuels (e.g. Mexico, Brazil, India, and South Africa). Other developing countries find it hard to attract, train, and retain skilled workers and eventually lose them to other industries that offer more lucrative and stable employment.

### 25.3.3 The “Food Versus Fuel” Controversy

Both food and biofuel production entail the same set of land, water, labor, and other inputs, and any amount of diversion of these inputs away from food production into biofuel production might end up depressing an already growing population that are food insecure. Many premiere organizations including the UN, the IMF, the World Bank,

and the Organization for Economic Cooperation and Development (OECD) also warn that biofuel expansion may exert an upward pressure on food prices in the future.<sup>8</sup>

#### 25.3.4 Cost of Policy Development

Since the 1990s, several agro-waste reclamation projects have been carried out in developing countries in collaboration with external support agencies such as the World Bank. While some projects have succeeded, many have failed to support themselves over time or expand to accommodate the recipient country's growing waste production. One principal reason for this failure was found to be the lack of optimal institutional capacity, a governing agency that overlooks all aspects of the planned waste reclamation efforts. Governments seeking external support must identify subsequent resource requirements, understand socio-economic and political settings that might adversely influence waste processing efforts, and develop a comprehensive agro-waste policy that fully encompasses the short-term and long-term goals. While coming up with such a policy is feasible theoretically, in practice, it is a daunting task for many low- and middle-income countries.

#### 25.3.5 Environmental and Social Costs

Environmentalists have concern over the philosophy behind some resource reclamation efforts. Despite the legal framework within which they work, waste producers have a strong economic incentive to oppose any pollution control standards set. Adding to this fact, the success of a newly established waste reclamation facility heavily depends on the continuous supply of wastes. Collectively, this situation might give rise to a mentality that opposes all forms of resource conservation efforts and promote a heightened waste production paradigm.

In some countries, biofuel production results in significant social costs. In 2016, the palm oil industry, in partnership with the Colombian government, continued to expand palm oil plantations, despite deforestation concerns and displacement of farm workers.<sup>9</sup> A 2017 plan to build a biorefinery near the Kaziranga National Park, a world heritage site in the north-eastern state of Assam in India, has sparked concerns from those arguing that there will be a negative impact on the habitat of elephants, rhinos, and numerous other threatened and endangered wildlife.<sup>10</sup> Similar fears regarding habitat preservation, indigenous people's territory and culture protection, and the welfare of farmers have been raised by conservationists in Indonesia and Paraguay as well.

<sup>8</sup> See the following article: <https://www.theguardian.com/global-development/poverty-matters/2011/jun/01/biofuels-driving-food-prices-higher>.

<sup>9</sup> See the following article for details: <https://www.laborrights.org/blog/201605/displacement-death-and-worker-exploitation-corporate-crimes-colombia%E2%80%99s-palm-oil-industry>.

<sup>10</sup> See the following article: <https://news.mongabay.com/2017/11/biofuel-project-near-indias-rhino-heartland-sparks-protests>.

### 25.3.6 Health Costs

Gathering and transporting raw agro-waste, as well as handling treated waste, may have significant occupational health risks to workers engaging in these activities. For some developing countries, educating and monitoring workers against these health risks may be a cumbersome task.

## 25.4 Practical Issues in Conducting a Cost-Benefit Analysis

A cost-benefit analysis offers the most comprehensive method of economic evaluation. But in practice, there are many issues to be addressed before a decision can be made – whether to accept a project. Each of the following subsections discusses the challenges involved in a cost-benefit analysis.

### 25.4.1 Valuation of Costs and Benefits

It is important to establish a uniform measurement unit for the various cost and benefit items enumerated earlier, so that a valid evaluation can be made. On economic grounds, the most simplistic approach is to assign a monetary value to both costs and benefits, so that comparisons across a wide range of agro-waste reclamation projects, and/or between agro-waste reclamation projects and other public policy projects, can be made. The monetary value of project inputs is based on the “opportunity cost” concept – the cost that is foregone by not doing the next best activity as a consequence of doing the given activity. Other monetary values are assigned based on the observed and stated preferences (“willingness to pay”) of all the people in the society by determining how much they are collectively prepared to pay for an activity that benefits them or willing to get compensated for an inaction that causes harm. True value estimation of benefits and costs can also be distorted by the presence of mechanisms that cause deviations in free-market prices such as penalties (e.g. fines on stubble burning in India), incentives (e.g. voucher programs distributing affordable fertilizer to farmers in sub-Saharan African countries), quotas (e.g. a 5% mandatory biodiesel blending quota in Brazil), and other stakeholder monopolies along the supply chain of the agro-waste industry. Failing to correct for these distortions might lead to mistakenly allowing an ineffective project to commence into action (“Type I error”) or delaying, rejecting, and scaling back of a useful project (“Type II error”).

The tricky part is the valuation of activities that do not have a market, thereby making it extremely difficult to estimate prices to be used in the cost-benefit analysis. Generally speaking, most health-related activities fall into this category. For example, how does one measure the indirect and intangible costs associated with stubble burning? To do this exercise, we need to estimate the true value in a roundabout way – that is, by determining simultaneously the loss of life and life quality. Quality-adjusted life years (QALYs), a measure first introduced by Klarmann et al. (1968), provides a score of years lived, relative to those years spent in perfect health. QALY varies between 0 (death) and 1 (perfect health). If the calculated quality of health is only two-thirds of perfect health, then a year spent in such a diseased state equals 0.67 QALY. Years spent in disability or more commonly called disability-adjusted life years (DALYs), proposed by Murray

(1994), measures the burden of illness through the reduction in “human function” (Murray 1994, page 438). DALY varies between 0 (perfect health) and 1 (death). Hence, the health assessment objective is to either maximize QALYs or minimize DALYs.

### 25.4.2 Discount Rate

Next, we need to establish a common time reference to compare costs and benefits that happen at different dates. Based on natural human behavior, people have positive time preferences, and therefore all costs and benefits that are realized in the future must be “discounted” to what it is worth today. Now, we must determine by how much the future costs and benefits are to be reduced to reflect today’s value. This procedure has a two-pronged approach, illustrated below.

#### 25.4.2.1 Determination of the “Opportunity Costs of the Project’s Funds”

This cost is the market-driven risk-adjusted rate of return on the next best investment strategy to which funds employed might otherwise have been put. This return computation is tricky for many developing countries where capital markets are of limited sophistication as it is not always possible to calculate many of the variables that are known to be relevant.

#### 25.4.2.2 Determination of Society’s Collective Time Preference Discount

Governments’ taste for the consumption at present over the consumption in future dictates certain adjustment weights to the project’s otherwise market-based after-risk rate of return calculated previously. A “perfect” estimation of these weights is futile for developing countries; however, a valid approximation can be obtained based on the society’s mix (poor vs. rich, young vs. old), population growth rate, political motives, and measures aimed at correcting market irrationality. For more discussion on this, see OECD (2009).

Improper discount-rate estimation may lead to the same perils discussed in Section 4.1. Assume that projects are of the high capital investments – low running costs profile with benefits in the distant future. A discount-rate that is “too-low” inflates future benefits in present value terms, thereby allowing a bad investment to go ahead (Type I error). Likewise, a discount rate that is “too-high” depresses future benefits in present value terms, thereby rejecting a good investment (Type II error).

Typically, discount rates in developing countries are higher (8–15%) than in developed countries (3–7%) (ADB 2013).

### 25.4.3 Analysis Period

How can citizens that face immediate deteriorating health, poverty, and unemployment concerns approve of governments that require them to make sacrifices on their current needs in order to participate in projects that will not yield benefits in time to improve their welfare? This is the dilemma that many local governments in developing countries face when they have a public policy investment decision to make. A project, however good and environmentally-sensible, which takes a long time to show gains is unequivocally rejected. At the same time, a project that is assessed over a small horizon may not be economically prudent considering the large project expenditures that come with

the initial setup. Therefore, the selection of an appropriate analysis period becomes one of the key deciding factors to bring a project to its fruition.

The analysis period is also closely related to the choice of discount rate (see Section 4.2). A high discount rate compels a shorter analysis period because benefits that accrue over longer horizons become too insignificant to make a difference at the present time. The opposite is true when the discount rate is set low.

#### 25.4.4 Impact of Corruption

In a manner of speaking, most agriculture-based countries get low scores for governance (World Bank 2008, page 245). This paints a poor picture – the governments and related agencies implementing policies are riddled with inefficiencies, vested interests, and corruption, adding a strain on the project's operational costs. In fact, governance issues are the main reason why many of the 1982 World Development Report's recommendations on agriculture failed. Bribery is also the key reason why deviations from environmental regulations such as land, water, and air pollution often go unpunished. Corruption influences agricultural projects in many ways. They are outlined here.

##### 25.4.4.1 Improper Resource Administration

Land and water administration institutions are seldom independent from political pressure and are sometimes forced to authorize development in protected lands and water bodies. In Kenya, there was a systemic problem of forced eviction and land grabbing by public officials during 1980–2005. Conflicts over land displaced millions of farmers in Columbia since the late 1990s. Additionally, farmers' livelihood shrinks with the payment of bribes to public officials to obtain services such as land registration, access to irrigation systems and ground water, and access to electricity. In India, the collective annual worth of bribes given by users of land administration services is USD 700 million, almost equal to the public spending channeled toward a sustainable environment agenda.

##### 25.4.4.2 Informal Banking Services

Public- and private-sector commercial banks, village banks (rural banks), cooperative banks, and insurance companies form the basic network of formal financial institutions in most developing countries and are often not well-suited to serve the needs of poor farmers. Studies in Kenya show that poor farmers do not trust banks and are unwilling to incur the account opening and withdrawal charges.<sup>11</sup> Farmers also refrain from using bank loan services because they are unwilling to bear the risk of losing collateral, unable to meet the requirements of collateral, or do not tolerate the rigidity of loan options. Instead, the primary source of money access and lending for poor farmers comes from professional and nonprofessional money brokers (commonly referred to as "loan sharks") whose terms are based on familiarity (kin-, religion-, or ethnic-based memberships, and geographical location), dictating the cost of doing business, the size of the loan, and the rate of interest charged. The National Crime Records Bureau (2015) of India

11 See Dupas et al. (2012) for more details.

reports that debt burden from informally borrowed loans and the harassment that accompanies it are the main contributors to thousands of farmer suicides every year.<sup>12</sup>

Most of these costs are unnecessary and cannot be priced using conventional economic models. However, some of the costs can be branched out through investments in technology and innovations.

## 25.5 Decision Rule

On what basis is project selection made? The decision rule favored in most economic analysis is the net present value (NPV) criterion, which simply states that the discounted stream of net economic benefits (benefits minus costs) must at least exceed zero.

$$NPV = \sum_{t=1}^n \frac{B_t - C_t}{(1+r)^t}$$

where  $B_t$  is the aggregate benefits in year  $t$ ,  $C_t$  is the aggregate costs in year  $t$ ,  $n$  is the number of years used in calculations of benefits and costs (from 25.4.3), and  $r$  is the discount rate (from 25.4.2). When comparing more than one project, the rule of thumb is to always select the project with the highest NPV.

### 25.5.1 Special Cases

There are rare occasions when the project with the highest NPV is sidelined and other positive NPV projects (that is, projects with NPV greater than zero) are selected instead. Many other decision rules that complement the NPV criterion come into play in these types of situations.

- It is well established that the rate of return from public policy projects must be at least as high as the return from the next best alternative use of project funds. For example, how does the rate of return on a wastewater recycling facility fare against the annual return from a fixed term bank account or against the rate of return on a public education program? These comparisons can be made by the creation of a criterion that is based on a return measure instead of a monetary value. We compute what is known as the “internal rate of return” (IRR) of a project – that is, the annual rate that equates the stream of benefits to its cost counterpart.

$$\sum_{t=1}^n \frac{B_t}{(1+IRR)^t} = \sum_{t=1}^n \frac{C_t}{(1+IRR)^t}$$

When many positive NPV projects are available, it is also possible to rank and compare projects based on their respective IRR. The IRR criterion will dictate the selection of the project with the highest IRR.

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<sup>12</sup> The National Crime Records Bureau (2015) tracks suicides by category year-wise since the mid-1990s. Farmer suicide figures also showed an unexpected rise of about 42% between the 2014 (total suicides: 5650) and 2015 (total suicides: 8007).

- Governments facing hard budget constraints want a measure that delivers the maximum output for cost under the stringent economic conditions they face. In these situations, the criterion referred to as the benefits-to-costs ratio (BCR) is used. This criterion is presented as a ratio of discounted benefits to discounted costs, which must be at least greater than one.

$$BCR = \frac{\sum_{t=1}^n \frac{B_t}{(1+r)^t}}{\sum_{t=1}^n \frac{C_t}{(1+r)^t}}$$

The decision rule based on the NPV criterion and the BCR criterion provide the same result when analyzing one project facing a dichotomous choice – that is, either to “accept” or to “reject.” But, the economic interpretation of the BCR criterion – the benefits (present value) for every unit of investment capital spent, is particularly useful when more than one project needs to be ranked based on their relative merits. For example, a small project having a BCR of 4.0 would be ranked above a large project having a BCR of 2.0, even if the former has a smaller NPV than the latter. Therefore, governments can decide whether to implement one big project or a combination of several small- and medium-sized projects to achieve the same stated objectives.

- Sometimes, various levels of financing constraints force low-income countries to choose the “do-minimum” option in public policy projects. Under these circumstances, the group of positive NPV projects can be ranked by expenditure, and the selection criterion will dictate the nomination of the project that carries the lowest cost profile. This criterion is called the least-cost option.
- Financiers of public policy projects might be interested in knowing the quickest possible time frame to recover their investment capital. The payback period (a time-based measure) criterion is used for this purpose, which gives the number of years necessary to earn back the initial capital investments put into projects, forming a lower bound of the financiers’ investment horizons (in years) to make a positive return for the project.

### 25.5.2 Sensitivity Analysis

Is the decision rule robust or fragile to changes in parameters and inputs of the project? The answer to this question relies on conducting a sensitivity analysis – that is, the identification of various forms of uncertainties that might affect the economic valuation of the project. It involves the following steps: (i) identify the parameters that are vital to the decision rule; (ii) calculate “switching values” of those parameters; and (iii) gather as much information about related market uncertainties.

Identifying the parameters that affect the cost- and benefit-streams the most is an important step in risk management. This is because the changes in the largest cost and benefit items are more likely to alter the findings of the decision rule. When tabulating the costs of a biofuel facility, the key contributing factor in many cases is the cost of ongoing operations. Like chemical facilities, biofuel facilities transport and store large amounts of combustible ingredients such as biodiesel, bioalcohol, biogas, feedstock, and wood. Given this fact, what happens when typical process hazards (fire, explosion, leak/uncontrolled reaction, and steam flashes) cause facility shutdowns?<sup>13</sup> How would

13 For a detailed list of biofuel project and plant hazards, see Nair (2011).

such an incident raise the operating costs and by how much? To accommodate such incidents, we come up with operating cost scenarios and recheck the decision rule calculations. Would the NPV still be positive if the operating costs go up by 10% and everything else remains the same? Now, by 20%, 30%, and so on. Such an analysis provides a cutoff amount (also referred to as “switching value”) for operating costs after which NPV turns from positive to negative and the project is no longer financially feasible. In the same way, the adjustment to the largest benefit-causing factor also needs to be assessed for its vulnerabilities.

Can we anticipate all the possible things that can go wrong with the project? In many cases, uncertainties related to specific market events (e.g. demonetization, wage law changes, labor strike, and technology change) may play a major role in determining whether a project is successful. For example, many Zimbabwean public policy projects that started in the 1980s with an analysis period of over 20 years faced the effects of unexpected hyperinflation.

Developing a conceptual estimate of switching values for each of the project parameters and market variables provide the decision maker with valuable priorities.

### 25.5.3 Typical Benchmarks for Key Financial Parameters

Table 25.1 provides the International Finance Corporation’s benchmarks for key financial parameters used in some of its public policy projects for developing countries. Actual estimates may vary depending on country-specific or project-specific conditions.

**Table 25.1** Typical benchmarks for key financial parameters.

Parameters	Biomass to energy Source: IFC (2017)	Hydroelectric power Source: IFC (2015)	Miscellaneous Source: ADB (2013)
NPV	>0, depending on project risk	≥25% of investment	
IRR	≥10%	≥10%	
Payback	<10 yr	<10 yr	
Discount rate	≥9%		
<i>By Institution</i>			
– World Bank			10 – 12%
– AfDB <sup>a</sup>			10 – 12%
– IADB <sup>b</sup>			12%
<i>By Country</i>			
– Philippines			15%
– India			12%
– Pakistan			12%

<sup>a</sup>African Development Bank.

<sup>b</sup>Inter-American Development Bank.

## 25.6 Summary

As developing countries move toward more agro-waste reclamation campaigns, questions about it still linger. What are the benefits of reuse? Is it worth the effort? At present, conservative estimates indicate 40% of the land area and 70% of the freshwater resources are utilized by various agricultural activities. Moreover, most of the rainforests located in developing countries are under a direct threat due to aggressive agricultural expansion. Therefore, waste reclamation efforts in general, and those pertaining to agro-waste in specific, play a significant role in reducing the use of virgin land and water. In addition to this, the production of biofuels from farm waste both improves a country's energy security and reduces the dependence on fossil fuels, which comes with its own benefits in the form of greenhouse gas emission reductions.

Last, but not least, the health gains that are associated with reduced stubble burning and landfill disposal are also substantial. But, waste reclamation efforts are costly. The initial setup costs, ongoing operations costs, environment-related costs, and social costs all add up to be a significant figure, especially for cash-strapped countries. Further, developing countries lack adequate institutional capacity and investor protections to govern public policy projects that make foreign investments hard to obtain.

The cost-benefit analysis equates these cost- and benefit-streams in present value terms. If the analysis results in positive net benefits, the project is green-flagged, otherwise, the project is dropped. Sufficient care must also be taken to understand the various project-specific and market-driven uncertainties that might affect the economic valuation of the project. A sensitivity analysis is performed, and switching values are tabulated for all influential factors.

Despite the hurdles, reuse of agro-waste is a beneficial economic activity. More and more countries must develop comprehensive waste reuse policies and address their growing food, energy, and pollution concerns. Such a plan must include a study on all aspects of the cost-benefit analysis discussed in this chapter. In particular, those that are related to the local socioeconomic attitudes toward agro-waste production and reuse, the effects of corruption on public policy, the measurement of non-monetary environmental and health gains and losses, society's time preferences, and waste management technical know-how would be particularly useful in providing us the tools required to assess project risks and perform a more robust cost-benefit analysis.

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## 26

# Energy and Environmental Mitigation Potential of Rice Byproducts

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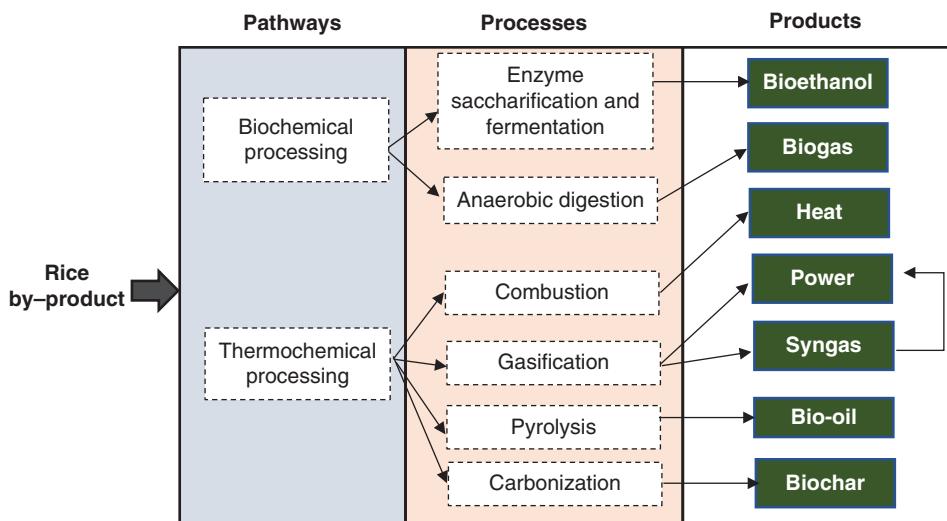
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## 26.1 Introduction

Global energy supply and use in last few decades have become critical to sustainability largely due to environmental concerns and availability. As at 2016, global energy consumption stood at 13 276 million tonnes oil equivalent representing an increase 17.8% over the last decade out of which 33.3% was from oil and 3.2% from other renewables (excluding hydro) (BP 2017). It is obvious that a very small percentage of global energy comes from biomass. However, there exists a huge amount of biomass resource particularly, agro waste, with the tremendous potential of meeting our energy demands and mitigating climate change but have not be harnessed fully.

Certainly, climate change is now broadly recognized as a threat to development, hence the present growing interest in finding alternative application for agro-industrial residues which are readily available and have the potential for energy applications. Within this context of agro waste-to-energy, rice husk presents an enormous possibility of meeting some demand if harnessed effectively and efficiently.

Rice is an important crop and the most rapidly growing food worldwide. Part of the challenge with it processing is the disposal of the byproducts mainly rice husk and straw. The straw, the main byproduct of harvest is too bulky to store or haul as fuel and so it is usually left on the paddy field to decay or burned. Sometimes it may be used to cook pottery by open-field firing. The husk is normally heaped in piles around rice mills at processing centers and set on fire to dispose of them without energy recovery. This uncontrollable combustion of rice husk in open fields as a result of variable winds, leads not only to the release of pollutants into the environment including carbon dioxide ( $\text{CO}_2$ ), carbon monoxide (CO), un-burnt carbon (with a trace amount of methane) as well as NO<sub>x</sub> and a trace amount of sulfur dioxide ( $\text{SO}_2$ ) (Lim et al. 2012) but also presents a health danger of skin, nose, and eye irritation due smoke and particulate matter (Burritt et al. 2009).



**Figure 26.1** Value addition pathways for rice husk.

Rice byproducts are potential alternatives for energy production for heating application as well as power generation both at the household level and on a commercial scale with an added advantage of reducing the negative environmental impact arising from current disposal techniques. There are several technological options for harnessing energy from byproducts broadly categorized as biochemical and thermochemical. The biochemical pathway involves the application of chemicals for material breakdown to release energy. These options include biogas and bioethanol. Thermochemical conversion techniques involve the thermal degradation of the material with or without oxygen to release solid, liquid, or gaseous product. The various techniques for harnessing energy from rice by product is shown in Figure 26.1. This chapter examines the potential of different technological options in harnessing rice byproduct energy and the degree to which it can mitigate environmental degradation.

## 26.2 Global Rice and Rice Byproduct Production

Rice is an important staple food around the world. More than 600 million tonnes of rice are produced globally with more than 90% of it produced in Asia. Table 26.1 shows the estimated contribution to rice production by the top 10 rice-producing countries. Rice byproducts can broadly be divided into starch-rich products and the lignocellulose substrate. The starch-rich byproducts, which include rice bran, broken, unripe, and discolored rice are usually valorized in feed formulations. The lignocellulose substrates, including rice husk and straw may undergo biological and thermal processing for energy recovery. The stem of the rice plant remaining as a residue after grain harvest is the rice straw while the outer layer of the grain produced during milling is referred to as rice husk. For every kilogram of rice produced, 290 and 200 g of straw and husk are respectively generated. This implies a significant amount of rice byproducts are produced annually,

**Table 26.1** Estimated contribution to rice production per annum by the top 10 producing countries averaged across 2010–2014.

Country	Percent (%)
China	28.0
India	21.3
Indonesia	9.4
Bangladesh	7.0
Viet Nam	5.9
Thailand	4.9
Myanmar	3.9
Philippines	2.4
Brazil	1.7
Japan	1.5
Others	14.0

thus presenting a huge opportunity for their conversion to useful energy products. Figure 26.1 shows the historical trend of the world rice production (FAOSTAT 2017) and the calculated world rice husk output.

## 26.3 Characteristics of Rice Byproducts

### 26.3.1 Chemical Characteristics

Rice husk and straw are non-starch fibrous lignocellulosic rice byproduct. Like most biomass, they are composed of extractives, cell wall components, and ash. The cell wall components which determined their suitability for fuel composed of cellulose, lignin, and hemicellulose. Rice straw is high in cellulose (32–47%), and hemicelluloses (19–27%) thus, showing a high potential for hydrolyzing into fermentable sugars. It also contains lignin (5–24%) (Garrote et al. 2002; Saha 2003; Zamora and Crispin 1995). The pentoses are dominant in hemicellulose, in which xylose is the most important sugar (14.8–20.2%) (Maiorella 1983; Roberto et al. 2003). Microfibrils are the basic structural unit in cell walls of the straw. The cellulose chains within the walls are bonded to form crystalline microfibrils through hydrogen bonding. The mechanical strength of the straw is provided by the microfibrils, which are bonded by a gel matrix composed of lignin, hemicellulose, and other carbohydrates polymers to form bio-composites (Singh et al. 2016).

Rice husk is also lignocellulosic in nature and composed of hemicellulose, cellulose, and lignin as well as extractive matter such as proteins, oil, sugar, etc. (Williams and Nugranad 2000). Their cellulose, hemicellulose, and lignin composition ranges from 17.7–28.0%, 28.7–35.6%, and 15.4–24.0%, respectively (Di Blasi et al. 1999). Though

the ultimate analysis does not give information about the suitability of a biomass fuel for combustion or gasification, the information about elemental composition for the biomass fuel is useful in the determination of stoichiometric formula, stoichiometric air-fuel ratio, gas composition, temperature limits and gas production rate through a mass and energy balance over the thermochemical conversion process (Jain 2013). The combustion efficiency of any biomass may be influenced by its chemical composition. For instance, the presence of phosphorus and the high alkali content reduces the melting temperature of rice husk during combustion. This reduction in melting point has been associated with fouling and corrosion during combustion or gasification as well as agglomeration in a fluidized bed. In a similar way, rich husk with high ash content ( $>16\%$ ) is classified as low fouling fuel (Skrifvars et al. 2005a,b) due to their low multi fuel fouling (MFF) index.

The ratio of cellulose to lignin is an important parameter in classifying biomass as a good combustible fuel. Considering rice byproducts as combustible for energy generation requires assessing their chemical characteristics and understanding the conversion potential based on the known chemical properties. The atomic ratio provides an understanding of the heating value of the fuel. Hence, the H/C and O/C ratios are important for validating their suitability as a fuel substitute. Biomass generally has H/C and OC ratios of 1.2–1.85 and 0.4–1.0, respectively (Basu 2010a). Rice husk has H/C and OC ratios of  $1.8 \pm 0.2$  and  $0.9 \pm 0.3$ , respectively, hence their lower heating value compared to most woody biomass.

Another important chemical characteristic of biomass fuel is the chemical composition ratio. The biomass behavior during combustion or pyrolysis can be predicted from these chemical composition ratios. The hemicellulose to lignin ratio of rice byproducts falls within the general biomass range of 0.5–2.7 (Basu 2010a), which is an indication that their combustion behavior will be similar to other biomass.

### 26.3.2 Thermophysical Characteristics of Rice Husk

Physical properties of biomass are important parameters in determining the suitability of any biomass material for fuel. Among these properties, biomass density, moisture content, and angle of repose are the key points necessary to characterize its use as fuel. Among the different measurable density data, the bulk density expressed as the ratio of the weight of bulk biomass to the volume occupied is the most widely used mainly due to the ease of its measurement. The bulk density of rice husk varies from 98 to  $106.3 \text{ kg m}^{-3}$  (Jha and Singh 2007).

Another important physical parameter is the moisture content. The most reported moisture content values usually represent inherent moisture in biomass capillary opening and surface moisture. Moisture content influences the overall amount of heat generated from the rice byproduct. Higher biomass moisture content will impair the heat budget of the thermochemical process reactions (Jain 2006). For most thermochemical conversion of rice biomass, moisture content above 15% will result in poor performance.

The angle of repose expressed in degrees is the angle made by biomass from the horizontal to the side of a pile under free falling conditions. It is useful in determining the angle for feeding hopper and ash hopper designs. Rice husk has an angle of repose range of 35–42 (Olivier 2010) degrees depending on the moisture content.

**Table 26.2** Thermophysical characteristics of rice byproducts (Jha and Singh 2007; Brand et al. 2017).

Characteristics	Unit	Rice straw	Rice husk
Moisture	%	19–24	7.9–12
Bulk density	$\text{kg m}^{-3}$	106	331
True density	$\text{kg m}^{-3}$	198–210	331–380
Angle of repose	°	—	35–42
Specific heat	$\text{kJ kg}^{-1} \text{K}^{-1}$	—	1.098–2.754
Thermal conductivity	$\text{J/s.m.K} (\text{Js}^{-1}\text{m}^{-1}\text{K}^{-1})$	—	0.024–0.099
Volatile matter	%	65.5–79.7	63.5–71.2
Fixed carbon	%	11.2–15.9	16.2–17.3
Ash Content	%	9.8–20.0	12.3–20.6
Higher heating value (HHV)	$\text{MJ kg}^{-1}$	12.4–17.7	14.9–16.3
Lower heating value (LHV)	$\text{MJ kg}^{-1}$	11.2–16.1	13.5–17.7

The most important parameter of any biomass fuel is the heating value, which determines the available energy of the fuel under ideal combustion. It may be expressed as a higher heating value (HHV) or lower heating value (LHV), depending on whether the latent heat of vaporization is considered. HHV considers latent heat of vaporization. Gasifier operations occur at constant pressure and the vapor leaves with flue gas without being condensed. Under such conditions, the heating represents the LHV and it is usually 10–15% of the HHV (FAOSTAT 2017). The heating value is dependent on the proximate composition of the fuel including moisture, volatile matter fixed carbon and ash. The volatile matter represents the condensable and non-condensable gases released when the fuel is heated. Amount of these gases may be used in estimating the thermodynamic properties of the fuel. Table 26.2 shows thermophysical characteristics of rice byproducts.

## 26.4 Biochemical Energy Potential of Rice Husk

### 26.4.1 Biogas Production

Anaerobic digestion involves the decomposition of organic material in the absence of free oxygen using microorganism. The products include methane, carbon dioxide, ammonia, and traces other gases. There may be also the presence of other organic acids with low molecular weight. Anaerobic digestion technique has primarily been used to as a pollution control measure and has been applied for waste water and industrial waste treatment, municipal solid waste treatment and the production of biofuel.

Rice byproducts conversion to biogas is considered one of the most environmentally benign pathways to energy production. It requires less energy input compared to thermochemical processes and the anaerobic digestion can accommodate either wet or dry biomass. Among rice byproducts, straw has been the most successful choice for biogas

production and has gained much research attention. This may be attributed partly to recent data on the significant contribution of straw to the atmospheric emission with current disposal methods and the recent global effort toward renewable resource utilization and greenhouse gas emission mitigation. 95–96% reduction of methane emission from rice soils have can be achieved when rice straw is removed from soil instead of returning to the rice fields (Koga and Tajima 2011). Mussoline et al. (2013) provides a comprehensive review on the contribution of straw to greenhouse gas emissions and the production of biogas from straw. The cellulose structure of rice straw leads to lower digestion and biogas production hence pre-treatments are used to increase accessibility and biodegradability hence higher yields. Milling, alkaline treatment, acid treatment or biodegradation are considered the most effective pretreatment methods. The pretreatment method, process condition including time and temperature significantly influences the biogas production. Depending on the pretreatment method and the conditions used, specific methane production from anaerobic digestion of rice straw could range from 92 to 280 l kg<sup>-1</sup> VS added. Table 26.3 shows the methane yield for different pretreatment and process condition. Typically, methane production begins in the first few days and after 15 days of anaerobic digestion, it will remain constant and vary between 50% and 65%. The variation is dependent on the process conditions. Wet digestion may result in up to 65%, 59%, and 62% of methane content for ambient temperature, mesophilic, and thermophilic conditions, respectively. Dry anaerobic digestion, on the other hand, produces methane up to 64%, 63%, and 59%, respectively for similar conditions. From Section 3.2 the concentration of nitrogen is generally low, therefore the use of inoculums such as cow dung, cattle, or pig manure could provide a buffering capacity

**Table 26.3** Methane yields (in terms of VS) from anaerobic digestion of rice straw (Mussoline et al. 2013).

Methane yield (l/kgVS added)	Type of pretreatment	Digestion temperature (°C)	Time period (days)
195	Cut (50–100 mm)	40	10
280	Cut (3–5 mm)	22	120
215	Pulverized	35	120
190	2% NH <sub>3</sub>	35	24
198	Cut (25 mm) + 2% NH <sub>3</sub>	35	24
245	Ground (25 mm), 2% NH <sub>3</sub> preheated to 110 °C	35	24
273	Cut/pre-digested with biogas sludge for 46 h	26–28	146
224	Cut/delignified	30	63
328	Cut/delignified/white rot fungi	30	63
296	Cut/delignified/brown rot fungi	30	63
240	Cut/white rot fungi	18–28	89
92	Milled/white rot fungi	25	59
120, 124	Milled/white rot fungi	35	59

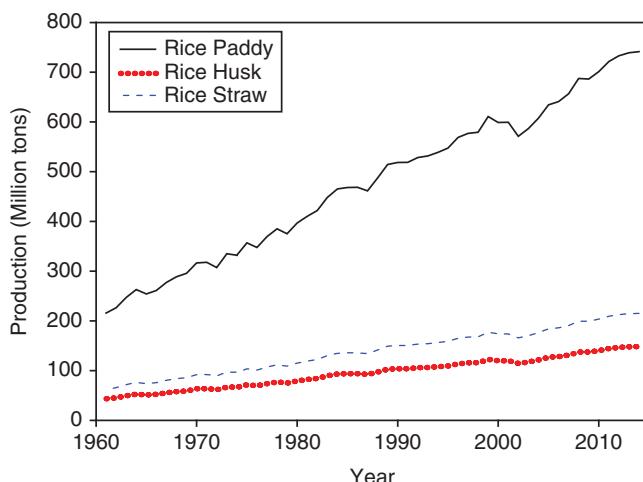
thus ensuring a suitable nutrient balance. As can be seen from Table 2.3, mesophilic temperatures (35–40 °C) are suitable for optimal methane production in comparison to ambient (20–30 °C) or thermophilic (50–55 °C).

Research on the conversion of rice husk into biogas has been very limited. However, there are reports of its usage as co-substrate to augment animal waste such as cow dung for biogas production. Utilization of rice husk as the substrate is, however, known to lower biogas production due to the lower nitrogen content of the husk. In many cases pretreatment of the biomass is required to increase biogas production. Commonly used pretreatment methods have been alkali pre-treatment, heat pre-treatment, and size reduction.

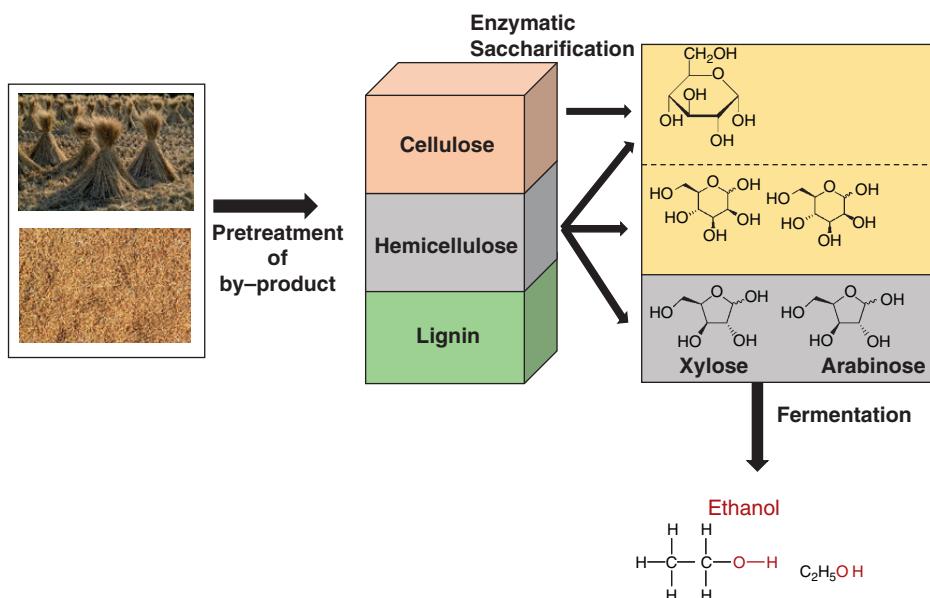
#### 26.4.2 Bioethanol Production

Second generation bioethanol is produced from biomass containing sugar or those that can be converted into sugar. Cellulose and hemicellulose from rice byproducts can be converted to glucose and several pentose and hexoses during enzyme hydrolysis. Lignin and ash components may be converted to energy through combustion. The glucose is finally fermented into ethanol by elected microorganism (Lim et al. 2012).

The conversion of rice husk to ethanol requires pretreatment to extract cellulose and hemicellulose leaving behind the non-biochemical conversion materials including ash and lignin. This process is then followed by the hydrolysis of cellulose into glucose and hemicellulose into xylose. The rate of conversion is dependent largely on the hydrolysis conditions. Acid hydrolysis has been the primary conversion technique. Depending on the nature of acid used, whether diluted or concentrated, the amount of sugar may vary. For every kilogram of rice husk, about 13 and 9 g of glucose and xylose, respectively, will be produced under dilute acid conditions. Concentrated acid hydrolysis, on the other hand, generates about 22.1 and 6.8 g of glucose and xylose, respectively. The sugar obtained undergo fermentation to produce bioethanol. Figure 26.2 provides a schematic diagram to produce bioethanol from rice husk.



**Figure 26.2** Historical trend of the world rice production and the corresponding byproduct output.



**Figure 26.3** Schematic diagram to produce bioethanol from rice husk. Source: Kwofie et al., 2017.

Following these conversion rates, the yield of ethanol from rice husk has been estimated to be 13.9–16.1%. (Abbas and Asumali 2010). Using the average global rice husk generation for the last five years (2011–2015) the potential bioethanol production can be estimated at 25.8 and 30.0 GL for diluted acid process and the concentrated acid process, respectively.

Pretreatment of rice husk to produce cellulose and hemicellulose is an essential and perhaps the most expensive step in the bioethanol production. This step must be undertaken carefully not only to remove lignin and produce monomeric sugars but must also limit cellulose crystallinity while increasing porosity. Several methods have been proposed for lignocellulose biomass conversion pretreatment with the aim of minimizing sugar loss and maximizing lignin removal as well as limiting the formation of inhibitors such as furfurals and acetic acid. These include alkaline, lime, microwave alkali, diluted and concentrated acid, hot compressed water, ultrasonic, and hydrogen peroxide, ionic liquid treatment, wet air oxidation, ammonium carbonate, and biological pretreatment. The fermentation stage of the bioethanol process may be standalone or combined with the hydrolysis stage. The three processes used include separate hydrolysis and fermentation (SHF) process, simultaneous saccharification and fermentation (SSF) process and the semi-simultaneous saccharification and fermentation (SSSF) process. Although issues of optimal temperature for enzyme action and microorganism growth have been found with the SSF process, it is known to have some benefits, including lower cost and higher ethanol yield, because of the feedback inhibition on enzymatic saccharification over the SHF approach.

## 26.5 Thermochemical Energy Potential of Rice Husk

Thermochemical conversion pathways have been the primary method for the disposal of rice biomass. The process involved the application of heat and catalysis for thermal decomposition of material in little to no oxygen or in the presence of excess oxygen resulting either in the release of energy or intermediate energy carrying products which may be combusted to produce clean energy or upgraded to transport fuels. Figure 26.1 also shows the different thermochemical process and their products. They are generally considered advantageous over biochemical processes. One important benefit of thermochemical conversion is the generation of fuels that are compatible with existing fuel infrastructure with little to no modification. Additionally, most thermochemical systems can handle a variety of biomass feedstocks including lignocellulosic material, liquid-rich biomass as well as waste materials from municipal sources. Table 26.4 summarizes the benefits of thermochemical conversion pathways over biochemical processes. This section reviews the common thermochemical processes used in harnessing rice biomass energy.

### 26.5.1 Combustion

Rice byproducts have primarily been disposed of by combustion. Straw is usually burnt on rice farms, whereas the husk is heaped in open spaces around rice mills. These combustion processes are not controlled and in most cases, have led to adverse

**Table 26.4** Advantages of thermochemical conversion of rice husk over biochemical processes.

	Thermochemical	Biochemical
Feedstock preparation	The feedstock can be used without any size reduction process.	Requires feedstock to be ground for effective utilization
Number of product	Multiple high-value products can be achieved with fractional separation of products	Products are limited to few options
Secondary waste generation	Ash produced as waste could be used for the production of useful products such as activated carbon, biochar, cement, etc.	Production of rice husk sludge (secondary waste)
Influence of climate condition	Thermal decomposition of rice husk occurs at higher temperatures and will be minimally affected by climate condition	Biogas production may be susceptible to ambient temperature
Level operation	Can be operated at very small scale with minimum expertise (e.g. Rural rice husk combustion/gasifier stove can be operated at household level with minimal skills)	Requires some significant level of expertise for operation
Productivity	Higher productivity per unit time can be achieved due to chemical nature of reactions	Reactions for conversion are usually biological hence limited. Expansion of reactor size may be capital intensive

environmental and health issues. Of the two main rice byproducts, rice husk has a higher potential of being converted to energy by combustion, because rice straw requires transportation from the field to the energy application facility, and it is usually wet, hence requires pre-drying prior to combustion. Effectively harnessing rice husk energy requires the use of specially made combustors which overcome the two primary challenges with rice biomass combustion – easy and continuous flow of the biomass and the removal of ash. Several combustion techniques have been developed for such processes. In these systems the rice husk biomass burns in excess oxygen supplied as forced air blown into the combustion area from a blower. Continuous combustion is achieved using an inclined static grate or moving grate. The systems are also equipped with units which allow the continuous removal of ash to avoid putting off the fire. The system's combustion efficiency can be improved by the introduction of secondary air to burn charred carbon and smoke. In most rice producing communities, rice husk combustors are used for boiler operations generate steam for parboiling or hot air for paddy drying. On a larger scale, combustion of rice husk has been used to generate electricity for rice mills using steam or internal combustion engines (Wibulswas et al. 1994). These processes have proven to be economically feasible (Sookkumnerd et al. 2005, 2007).

### 26.5.2 Briquette Production

One of the primary challenges with rice byproduct utilization is the issue of handling, storage, and transport, because of the low bulk density of the biomass. Densification of rice byproducts has been identified to increase energy density and lower transportation costs. To ease the labor and improve the energy density of rice husk, briquettes are produced and used. The bulk density of briquettes can be increased from 40 to 200 kg m<sup>-3</sup> to 600–800 kg m<sup>-3</sup> (Holley 1983; Mani et al. 2003; McMullen et al. 2005). Increasing the bulk density and energy density facilitates easy transportation, better handling, and storage as well as making the briquette comparable to wood in terms of energy content so that it could serve as an alternative. Miah et al. (1999) estimates that a kilogram of densified rice husk briquette could replace 1.67 kg of wood.

Production of briquettes from rice byproduct requires the application of physical force to bind the loose particles. The compaction mechanism is achieved through a combination of forces. Five types of binding forces include: solid bridges; attraction forces between solid particles; mechanical interlocking bonds; adhesion and cohesion forces; and interfacial forces and capillary pressure, all of which are known to have been associated with the briquette formation (Behnke 1994; Tabil 1996). At high temperature and pressure, solid bridges are developed through inter-particle molecular diffusion, crystallization of ingredients, chemical reaction, hardening of binders, and solidification of melted components (Kaliyan and Vance Morey 2009).

Compaction of the biomass into briquettes takes place in a piston or screw press. A screw extrusion, also known as the heated die screw press, is commonly used in small-scale briquette production in most developing countries (Ahiduzzaman & Islam 2013). There is evidence that briquettes produced with the screw press are much compact and combust better than those from a piston press (Moral and Rahman 1999). The compaction ratio for densification ranges from 2.5 : 1 to 8.25 : 1 (UNEP 2009; Ahiduzzaman 2007).

Production of briquettes requires a pretreatment step where rice biomass is milled to reduce the particle size and enhance the binding mechanism. Drying may also be required contingent on the moisture content of the biomass. Depending on the lignin fraction of the rice byproduct, the densification process may require binding materials to hold the particles together. Minimum lignin requirement for self-binding briquette is 34%. Rice byproducts with up to 42% of lignin and extractives require no binding materials (Lim et al. 2012). Starch and molasses have been used as external binders for straw and husk with lower lignin content. There are reports that even water could be a good a good binder for briquette production (Chin and Siddiqui 2000). The thermophysical properties of the briquettes may be improved by a combination of rice byproducts. For instance, the addition of up to 10% of rice husk ash could improve the physical compressive strength of briquette by up to 12% but reduces the heating value (Brand et al. 2017). Brand et al. (2017) have reported that optimal thermophysical properties could be achieved with a 30% rice husk, 60% rice straw and 10% rice ash. Although briquettes produced with such a combination has an HHV of  $16.9 \text{ MJ kg}^{-1}$  (<1% lower than a 100% husk briquette or 6% lower than a 100% straw briquette), its bulk density of  $1174 \text{ kg m}^{-3}$  is about 3% higher than 100% briquette and its compressive strength of 3.56 MPa is at least 19% higher than any of the 100% briquette.

Techno-economic assessment of briquettes made from rice byproducts has been reported to be profitable with benefit–cost ratio of 1.8 (Kamruzzaman 2001). However, the benefits of briquette production are optimal when they are produced in automated or semi-automated systems. In communities where manual presses are used, the production rate is very small beside the labor intensiveness of the process. These challenges could limit their utilization, especially in an area where wood is readily available. Besides the system, there are other challenges which make the operation difficult and sometimes unsustainable. These include excess energy consumption, lack of skilled operators and mechanic, frequent damage of screw and main bearing, unavailability of the heating coil, exhaust gas and damping of husk (Alam et al. 2002). On a laboratory scale, about 116 kWh is required to produce a tonne of rice husk briquette. Commercial, on the other hand, require up to 179–250 kWh to produce a similar quantity of briquettes (Hardman 2001). Pre-heating of biomass feedstock prior to densification has been found to reduce energy consumption by 10% (Bhattacharya et al. 2002a).

In comparison with direct combustion of rice biomass, briquetting seems to be a much better choice in terms of the overall energy density of fuel and efficiency of the combustion system. Typical rice product briquettes have up to  $3.3 \text{ MJ kg}^{-1}$  energy more than the raw rice biomass (Bhattacharya et al. 1985; Mai Thao et al. 2011). Additionally, briquette combustion systems have a higher efficiency of 17% compared with 12% for raw biomass combustor (Taho et al. 2011).

### 26.5.3 Gasification

Another thermochemical option for harnessing rice energy is gasification. This process is the combustion of rice biomass into low molecular weight combustible gases in a limited oxygen environment. The rice biomass undergoes four processes namely drying, pyrolysis, combustion, and gasification or reduction to generate syngas and char, the two primary products. The oxygen requirement is provided through a gasifying medium such as air, oxygen, or steam. The gasifying medium determines the quality of the syngas

produced (heating value). Air gasification has the least product heating value of 4–7 MJ Nm<sup>-3</sup> in comparison to steam (10–18 MJ Nm<sup>-3</sup>) or oxygen (12–28 MJ Nm<sup>-3</sup>) (Basu 2010b). However, from an economic perspective, air gasification of rice biomass presents the lowest operating cost and is most suitable for small-scale gasification of rice biomass. Rice biomass gasification overcomes the challenge of controlling the heating during combustion presented by direct combustion of the rice biomass. In addition, the combustion process produces higher flame temperature and little to no smoke.

During gasification, the rice biomass is dried with heat from hot zone downstream as it enters the gasifier. The heat dries the feed and releases water. This occurs at 100–200 °C with a reduction of moisture content of <5% (Puig-Arnavat et al. 2010). As the temperature increases the low-molecular weight extractives begin to volatilize. As the biomass proceeds in the gasifier, it enters a pyrolysis zone. In this zone, there is a thermal breakdown of larger hydrocarbon molecules of biomass into smaller gas molecules. The process does not involve any reaction with the gasifying medium. During pyrolysis, the dried feedstock with increased temperature is converted into char. The origin of the rice biomass determines the type of volatiles released during the process. This may include H<sub>2</sub>O, H<sub>2</sub>, N<sub>2</sub>, O<sub>2</sub>, CO<sub>2</sub>, CO, CH<sub>4</sub>, H<sub>2</sub>S, NH<sub>3</sub>, C<sub>2</sub>H<sub>6</sub>, and very low levels of unsaturated hydrocarbons such as acetylenes, olefins, and aromatics and tars (Bhavanam and Sastry 2013). The third zone after pyrolysis is combustion. The combustion process provides the necessary thermal energy required for the gasification reactions. Char or dry feedstock and sometimes volatiles from the pyrolysis reaction undergo oxidation and transfer the required heat for the endothermic gasification reactions. The last and most critical step of the thermochemical conversion process is the gasification or reduction stage. It involves the chemical reaction between the hydrocarbons in the fuel, steam, carbon dioxide, oxygen, and hydrogen usually in the temperature range of 800 – 1000 °C. Gasification of char is dependent on the reactivity of the char and the reaction potential of the gasifying medium. The extent and rate of char gasification determine the reactor size and gasification efficiency.

Gasifier classification is based on their gas-solid contacting mode and gasifying medium. There are three broad types of gasifiers based on the gas-solid contacting mode – fixed or moving bed, fluidized bed, and entrained bed. Due to the nature of rice biomass, a downdraft gasifier has been identified as the most suitable for its gasification and has been the most popular with rice husk gasification. It is a co-current reactor where both the product gas and the rice biomass flow downwards. The gasifying medium usually enters the gasifier at a height below the top. Downdraft gasifiers are known for their low production of tar therefore suitable for engine applications for power generation. This is because the acid and tar product in product gas leaves through a bed of hot ash which provides a favorable condition for tar cracking. This renders downdraft the lowest tar production gasifiers among all types of gasifiers. Downdraft gasifiers may either have throat or be throatless. The throated rice biomass gasifier has a flat-type or V-like constriction located at the oxidation or combustion zone. The essence of the constriction is to force all pyrolysis gas to pass through the hot and narrow constriction to achieve a uniform temperature distribution which will ensure cracking of most tars (Basu 2010c). For small-scale rice biomass gasification, a throatless design also known as open-core gasifier is used, where the air is added from the top and not from the middle as in other types of downdraft gasifiers. Air is drawn into the gasifier from the top by the suction created downstream of the gasifier.

Gasification of rice biomass has primarily been for heat generation for steam production or for power production. For steam generation, the syngas requires little to no cleaning and its combusted directly after production in specially designed boilers. For electrical power application, the syngas undergoes cooling and cleaning. Cooling is done to raise the energy density of the gas. Cooling equipment used is mostly air heat exchanger which employs free convection of air on the outside of heat exchanger. Cyclones, scrubbers, and dust filters are used to make gas suitable for use in gas engines for electricity generation. Yoon et al. (2012) reported that for a 40–45 kg h<sup>-1</sup> feed of rice biomass in a downdraft gasifier operated at an equivalence ratio of 0.4 and efficiency of 60%, 8–10 kW electricity can be stably generated. Modern milling requires 29.26 kWh/tonne electrical energy for milling which is usually provided by diesel generators. Electrical energy from rice husk gasification can be effectively harnessed to augment the total energy required for milling using dual fuel systems to reduce the cost of operation. In Cambodia, Batt Daeng Electricity Company, reduced its diesel cost from \$10 000 to 3500 per month by operating a 200 kW downdraft gasifier supported by a three diesel engine gensets to produce 1530 kWh of electricity daily using 60 tons of rice husk and 4000 l diesel fuel per month (Akgün and Luukkanen 2012).

#### 26.5.4 Pyrolysis

Pyrolysis of biomass in recent years has gained attention not only from the agriculture sector but also from forestry and municipalities primarily because of its potential to convert virtually all kinds of biomass. Unlike combustion and gasification, pyrolysis of rice husk involves thermal degradation in the absence of oxygen. Depending on the product sought after, the process can be fast or slow pyrolysis. Fast pyrolysis produces liquid fuel products known as bio-oil. This oil has the potential as a substitute fuel to petroleum for heat and power generation or extraction of bio-based chemicals. The primary constituents of the oil obtained from fast pyrolysis include phenols, cresols, benzenediols, guaiacol, and the alkylated derivatives (Lu et al. 2008).

The key parameters in evaluating the quality of bio-oil from pyrolysis include homogeneity, stability, heating value, pH, water, flash point, solids, ash, viscosity, and lubricity. Bio-oils are known not to be in thermodynamic equilibrium, hence their components are bound to partake polymerization and polycondensation reactions leading to gradual changes during storage. Variations in properties such as viscosity and moisture are determined to establish the aging of bio-oils. The rate of aging can be minimized by the addition of methanol, thus stabilizing the oil.

### 26.6 Environmental Mitigation Potential of Rice Byproducts

An important characteristic of rice biomass utilization is its potential for environmental mitigation. Rice biomass produces less emissions than conventional fuel and other biomass material such as fuelwood, hence it is considered environmentally benign with a higher mitigation potential. This section reviews the emission characteristics of rice biomass combustion, examines the mitigation potential as a replacement fuel for wood and compares the potential for power generation.

### 26.6.1 Emission Characteristics of Rice Straw

Rice fields are considered a major source of greenhouse emissions. Rice straw is primarily disposed of by either tilling it back into the soil and using as crop fertilizer or undertaking an open field combustion. When rice husk is tilled back, about 80–90% is decomposed during the first year (Glissmann and Conrad 2000). Research has shown that decomposition of rice straw, root exudates, and organic matter in the soil account for methane production through anaerobic degradation (Kimura et al. 1991). In China, a straw-ammonium sulfate mixture added to soil increased the methane production 25 times, but did not result in any increase in yield (Wang et al. 2002). Addition of rice straw to soil may be considered as a soil enhancement approach, as it improves atmospheric CH<sub>4</sub>, thus influencing source strength of rice field (Watanabe and Kimura 1998; Huang et al. 1998). Glissmann and Conrad 2000 found that irrespective of the fraction of rice straw added to the soil, there is an increase in the release of CH<sub>4</sub>, H<sub>2</sub>, and CO<sub>2</sub>. However, there is evidence of increase greenhouse gas emission with a detrimental effect on the environment when the straw is tilled back into the soil. For instance, in Northern Italy, a life cycle assessment of white milled rice production revealed that the field emission alone contributed 68% toward global warming (Blengini and Busto 2009). There is also evidence that this process creates a conducive atmosphere for crop diseases and reduces rice yield due to the negative effect of nitrogen immobilization in the short term (Gadde et al. 2009a).

Certainly, there seems to be a huge environmental gain through greenhouse gas emission reduction when rice straw is not tilled back to the soil but removed from the field. The alternative to tilling strategy available to rice farmers at relatively lower cost is to burn the straw in the open field. This thermochemical process is also known to release several gases and particles into the atmosphere. These gases include CO, CO<sub>2</sub>, CH<sub>4</sub>, SO<sub>2</sub> non-methane hydrocarbon, and nitrogen compounds. The two primary contributors out of these gases to global warming are the NO<sub>2</sub> and CH<sub>4</sub>. There is the release of harmful air pollutants, which are potential carcinogens with severe health implications including polycyclic aromatic hydrocarbons and dioxins (polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans) (Gadde et al. 2009a; Gullett and Touati 2003) with health consequences. Certainly, the process of disposal has not only health and environmental consequences but also indirect economic implications as well. Table 26.5 shows the emission factors from open field combustion of rice straw.

Clearly, neither tilling rice straw back into the soil nor open field burning seems to provide a better disposal pathway for rice straw. Harnessing energy from the rice straw would mean mitigating 68% of global emissions in addition to providing clean energy. This would represent a significant contribution of the rice industry toward global environmental impact.

### 26.6.2 Mitigation Potential of Rice Husk as Fuelwood Replacement

Most developing countries have an agricultural based economy and biomass energy represent a significant part of their daily energy source. This is primarily due to biomass availability and low cost. World Health Organization (WHO) reports have indicated, more than three billion people use fuelwood daily for domestic cooking and heating purposes in developing countries using wood, charcoal, or agricultural residue (WHO 2014).

**Table 26.5** Emission from open burning of rice straw (Gadde et al. 2009b).

Name of pollutant	Unit	Emission factors
CO <sub>2</sub>	g/kg <sub>dm</sub>	1460
CH <sub>4</sub>	g/kg <sub>dry fuel</sub>	1.2
N <sub>2</sub> O	g/kg <sub>dry fuel</sub>	0.07
CO	g/kg <sub>dm</sub>	34.70
NMHC	g/kg	4
NO <sub>x</sub>	g/kg <sub>dm</sub>	3.10
SO <sub>2</sub>	g/kg <sub>dm</sub>	2
Total particulate matter (TPM)	g/kg <sub>dm</sub>	13
Fine particulate matter (PM <sub>2.5</sub> )	g/kg <sub>dm</sub>	12.95
PM <sub>10</sub>	g/kg <sub>dm</sub>	3.7
Polycyclic aromatic hydrocarbon	g/kg <sub>dry fuel</sub>	18.62
Polychlorinated dioxins and furans (PCDD/F)	ng international toxic equivalency (I-TEQ)/kg	0.5

The bulk of the biomass is fuelwood and other agro-wastes, which are collected and transported over long distances by women and children. For commercial purposes, the wood may be purchased from bulk wood sellers (Kwofie and Ngadi 2016). The choice of fuelwood over agro-residue, such as rice husk may be due to the difficulty in their combustion in comparison to fuelwood and the consistency of their availability. The use of fuelwood has serious social and health implications besides environmental concerns such as deforestation and pollution. For instance, the wood collection process may not only be energy and time consuming but also contribute to children missing school sessions (WHO 2014). More important is the impact of fuelwood combustion. More than 4.3 million premature deaths are estimated from the use of fuelwood every year. These includes deaths from pneumonia (12%), stroke (34%), ischemic heart disease (26%), chronic obstructive pulmonary disease (COPD)(22%) and lung cancer (6%) (WHO 2014). In addition to these staggering statistics, the stoves used for combustion are very inefficient with efficiency up to 15% (Ayoub and Brunet 1996; Berrueta et al. 2008).

In rice producing communities, the availability of rice husk presents a health and environmental mitigation opportunity knowing that smokeless rice husk systems can be built to replace or augment the continuous use of fuelwood. These rice husk systems have characteristics of using forced air which allows better mixing of combustible gases and oxygen hence a significant decrease in incomplete combustion reactions. Table 26.6 shows emission factors of wood stoves in comparison to rice husk stoves. Evidently, the emission factors of rice husk combustion systems are significantly lower than fuelwood. The use of rice husk as domestic fuel could decrease CO and CO<sub>2</sub> emissions by up to 28.3 and 736 g kg<sup>-1</sup> of fuel, respectively. These represent a mitigation potential of 76% of CO emissions and 51% of CO<sub>2</sub>. Other benefits such as fuelwood savings, benefits from preservation of forest reserves, as well as income generated from saved time may all be accrued from the use of rice husk as an alternative to fuelwood.

**Table 26.6** Emission factors from rice husk combustion in comparison to fuelwood (Kwofie et al. 2017).

Stove type	Fuel	Emission factors ( $\text{g kg}^{-1}$ fuel)			Source
		CO	$\text{CO}_2$	$\text{C}_x\text{H}_y$	
RPS	Rice husk	$11.2 \pm 0.8$	$789 \pm 4$	$1.3 \pm 0.9$	(Kwofie et al. 2017)
RPS	Wood	$39.5 \pm 3.2$	$1525 \pm 6$	$0.9 \pm 0.5$	(Kwofie et al. 2017)
TSF	Wood	$56.0 \pm 3.0$	$1745 \pm 5$	—	(Preble et al. 2014)
BDS	Wood	$42.0 \pm 3.0$	$1767 \pm 5$	—	(Preble et al. 2014)
VIC	Wood	$47.1 \pm 4.5$	$1577 \pm 4$	$5.3 \pm 1.0$	(Bhattacharya et al. 2002b)
LIC	Wood	$51.9 \pm 2.5$	$1565 \pm 4$	$6.0 \pm 0.6$	(Bhattacharya et al. 2002b)

RPS is rice parboiling stove, TSF is the three-stone fire stove, BDS is Berkeley-Darfur stove, VIC is Vietnamese Improved stove and LIC is the Lao improved cookstove.

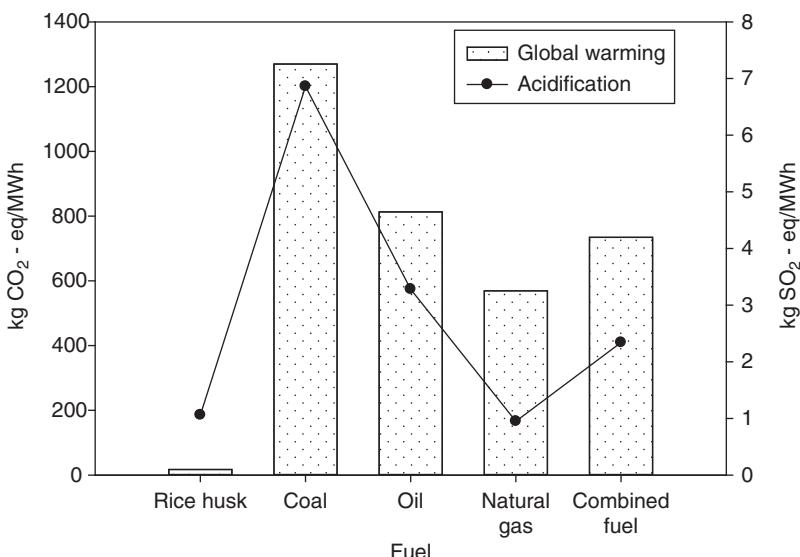
**Table 2.7** Characterized environmental impact of fuelwood stove and rice husk combustor during parboiling of 1 ton of paddy rice (Kwofie and Ngadi 2017).

Impact categories	Unit	Wood stove	Rice husk stove
Climate Change (CC)	kg $\text{CO}_2$ eq	2029	298
Terrestrial Acidification (TA)	kg $\text{SO}_2$ eq	5.22	0.35
Particulate Matter Formation (PMF)	kg $\text{PM}_{10}$ eq	6.09	1.93
Photochemical Oxidant Formation (POF)	kg NMVOC	11.92	0.88

A comparative life cycle assessment of rice husk combustion system and a fuelwood stove by Kwofie and Ngadi (2017) indicates that the Global Warming Index (GWI) and terrestrial acidification potential of a fuelwood system were 6.8 and 14.9 times more than a comparable rice husk system. Table 26.7 shows the characterized environmental impact of fuelwood stove and rice husk stove.

### 26.6.3 Mitigation Potential of Rice Husk as Fuel for Power Generation

Application of rice husk for power generation has shown great potential for mitigating environmental impact. For every megawatt of power generated with rice husk, about 1685 kg of  $\text{CO}_2$ , 8.58 kg of CO, 1.31 kg of  $\text{NO}_2$ , 0.39 kg of  $\text{SO}_2$ , 0.11 kg of TSP, 164 kg of fly ash, and 34 kg of bottom ash are released to the environment (Chungsangunsit et al. 2010). A comparative assessment of these emission data with current fuels for electricity generation shows that rice husk has the potential of significantly mitigating greenhouse gases when considered for power generation. Figure 26.4 shows the global warming and acidification potential of rice husk in comparison to other conventional fuels. The result reveals that using rice husk as an alternative fuel to coal, oil, natural gas, or combined fuel could decrease the GWI by up to 97%.



**Figure 26.4** Global warming and acidification potential of rice husk fuel in comparison to conventional fuels per MWh power generated. Source: Plotted with data from Chungsangut et al. 2010.

## 26.7 Conclusion

Rice straw and rice husk are rice byproducts generated after rice harvest and milling, respectively. These agro-residues are currently being disposed of through unsustainable practices with negative consequences on the environment and human health. Removing rice straw from the soil after harvest could reduce rice field emissions by 68%. However, the alternative of open field burning also poses a high release of greenhouse gases and carcinogens with health risks. Several biochemical and thermochemical conversion pathways for conversion of the straw have been discussed and have proven to be sustainable and environmental benign approaches to harness energy. Anaerobic digestion of the straw for biogas production and thermochemical conversion into syngas have proven to be the most popular and viable options which have gained attention in the research community. Similarly, the open field burning of rice husk also poses environmental dangers. Overall, thermochemical conversion pathways seem to favor rice husk energy. Rice biomass has the potential of providing energy for heating purposes as well as power generation. Following the continuous rise in global rice production rice byproducts will continue to rise. The implication of this rise would be a continuous increase in global warming if current processes of disposal are maintained. As the world works toward a clean global environment, it is important for steps to be taken toward harnessing rice energy.

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## 27

# Thermochemical Conversion of Lignocellulosic Biomass for the Production of Bioenergy

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## 27.1 Introduction

Fossil energy carriers are limited resources and its combustion produces high amounts of carbon dioxide, which is not compensated by growing of plants. The use of biomass as fuel directly has some main disadvantages:

- Even dry biomass has a high oxygen content. In a carbohydrate, the major component of biomass, every carbon atom is connected to one oxygen atom. This leads to a low heating value.
- As a consequence of the high oxygen content biomass is hydrophilic. The major amount of biomass has therefore a very high water content. A fresh, “green” biomass has, for example, a water content of around 80–90%. Such biomass cannot be used for combustion at all.
- Biomass production is widespread and the composition or other properties differ. This is especially in combination with the low heating value, an important disadvantage. For a cost effective production of energy, large facilities are necessary and the feedstock should be uniform. For such plants the feedstock should be uniform in composition and has to be transported via large distances. During transport, energy it is consumed.
- Biomass can be stored but, depending on its origin, losses by biological processes may occur.
- The volatiles formed from biomass during heating-up limits the application possibilities of biomass, e.g. by leading to lower combustion temperatures.
- The ash content of biomass can be rather high. In the ash, the potassium content is high. This leads to low ash melting temperature ranges. This is an important disadvantage if biomass combustion is used for electricity production. Here the combustion temperature should be as high as possible, to reach high electrical efficiencies. Therefore in the case of biomass with high ash content, usually combustion temperatures

below 800 °C are applied to avoid plugging by salt melts. This is too low for electricity production (Anthony 1995).

In general, thermochemical conversions have the goal to overcome two of these disadvantages: The oxygen content is decreased and therefore the heating value increased, the products are more uniform than the biomass feedstock. This means, they are better fuels. Usually other advantages occur in addition, but this depends on the type of thermochemical conversion.

Thermochemical means here that chemical reactions occur by means of heating. Here, the elimination of water and carbon dioxide is of special importance, because both increase the carbon and lower the oxygen content of the feedstock.

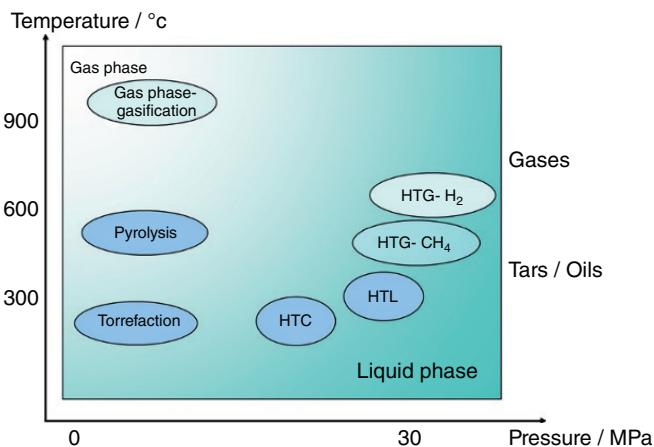
Fossil energy carriers are gases, tarry oils, or solids. These are also the products desired from thermochemical biomass conversion, as well. Gases and oils can be fed easily by pumps/compressors, which make them attractive as energy carriers for small engines like in cars or households. Fossil coal is mainly used in big plant to produce electricity. In such plant, chars from biomass can be used as an energy resource as well.

From the chemical point of view, thermochemical conversions should be classified in "dry" conversions for biomass with low water content like wood, and hydrothermal conversions, which are useful for biomass with high water content. In addition, the conversion technologies should be classified by the type of wished product into carbonization/charring, liquefaction, and gasification (Dahmen and Kruse 2012; Dinjus et al. 2009).

## 27.2 "Dry" Thermochemical Biomass Conversion

In dry biomass conversions, the biomass particles are heated up in a gaseous atmosphere. First the biomass is dried. As water has a high evaporation enthalpy, this is an energy consuming step. Therefore, in most cases, dry biomass conversions are restricted to biomass with a water content of below 10%. It is not a technical hurdle, but higher water contents lower the energy efficiencies more than usually accepted. Such low water contents can be found for wood, energy crops like miscanthus, or straw dried on the field. For the choice of the suitable reactor type, the scale and the feedstock are important. Most of the reactors can only be applied for wood, because the higher salt content of, e.g. straw, causes problems by the ash melting (Kan et al. 2016; Garcia-Nunez et al. 2017; Roy and Dias 2017).

After the physically bonded water has left the biomass particle, the chemical conversion of the material starts by the elimination of volatiles. This process is called "pyrolysis" and is usually done in the absence or with only low amounts of oxygen to avoid combustion. The volatiles are low molecular weight organic compounds like different acids, aldehydes, and phenols, etc., which condensate at lower temperature as tarry oil. In addition, permanent gases like carbon monoxide, carbon dioxide, and methane are formed. Pyrolysis therefore always leads to solid carbonized product, a liquid tar and gases. The ratio of these three product phases depend on the reaction conditions. At higher temperature of around 800–1000 °C, the solids start to be degraded further, which is called "gasification". Here, the atmosphere usually has a certain oxygen content;



**Figure 27.1** Overview of “dry” and “wet” biomass conversion technologies.

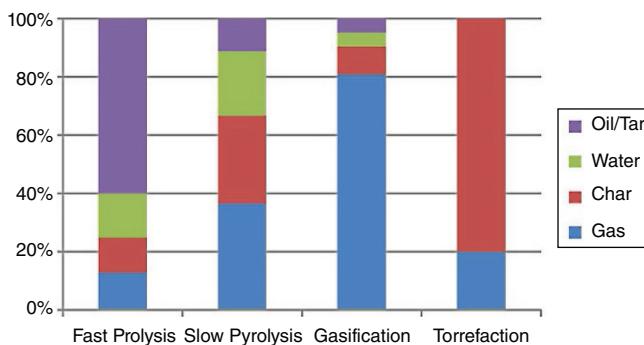
not enough for complete combustion, but enough to create enough heat for the gasification (autothermic process) (Dahmen et al. 2010).

The production of charcoal is a technique, which is thousands of years old. In fact, the use of iron as material was only possible with charcoal, not with wood. The classical charcoal production in kilns, covered, e.g. with ash or soil, leads to very high emissions. Therefore in Germany, they are not allowed in particular cases with a historical background (Demirbas et al. 2016; Weber and Quicker 2018). Other dry conversion technologies, like gasification, are developed from fossil coal processing. As consequence, dry conversion technologies are regarded to be well-developed compared to other technologies. On the other hand, most of the reactors can be applied with wood, not typical agricultural residues (Kan et al. 2016; Garcia-Nunez et al. 2017; Roy and Dias 2017) (Figure 27.1).

### 27.2.1 Torrefaction

Torrefaction is an incomplete conversion at rather low temperature between around 250 and 300 °C (van der Stelt et al. 2011; Chen et al. 2015). In the lower temperature range, volatiles are split from hemicellulose, at higher temperature also cellulose are partly carbonized. The conversion of lignin in the biomass is low. As rule of thumb, the solid product has 66–75% (g/g) of the original mass and contains around 90% of initial energy content. The volatiles are usually burned outside of the torrefaction reactor and the heat of the flue gas is used to run the process (van der Stelt et al. 2011; Chen et al. 2015).

Torrefaction is usually regarded as a pre-treatment step. The energy density is only slightly increased to around  $5 \text{ GJ m}^{-3}$ , but the grind-ability is much better. For consecutive processes, like gasification in an entrained flow gasifier, the feedstock has to be milled. This costs in the case of wood a lot of energy and 80–90% less energy is needed after torrefaction. In addition, torrefied biomasses are more uniform, less hydrophilic and biological stable than the original materials, which enable easier and cheaper storage (van der Stelt et al. 2011; Chen et al. 2015 Figure 27.2).



**Figure 27.2** Products of "dry" carbonization. Source: Data from Czajczynska et al. (2017).

### 27.2.2 Slow Pyrolysis

The goal of a slow pyrolysis is to produce a carbon-rich material (Weber and Quicker 2018), e.g. to produce activated coal (Bamdad and Hawboldt 2018). The yields depend on the temperature and reaction time. At a usual temperature of 600 °C, the char yields are around 25 wt%. After pyrolysis, the char is activated, e.g. by steam, to get activated coal. Commercially, activated coal from coconut shells is available. New applications are the use of direct carbon fuel cells (Cao et al. 2007; Giddey et al. 2012), which have – theoretically – a higher electrical efficiency than a process based on combustion in combination with a turbine.

### 27.2.3 Fast Pyrolysis

Here, very high heating of  $1000\text{ }^{\circ}\text{K s}^{-1}$  and short reaction time are necessary to get a liquid, called pyrolysis oil as main product (Meier et al. 2013; Vamvuka 2011; Venderbosch and Prins 2010; Mohan et al. 2006). In addition, the vapor forming the oil must be cooled down very fast as well, because pyrolysis oil is an intermediate product. Pyrolysis oil (tar) contains hundreds of different compounds, which further reacts to gases and chars. To avoid this, high heating rates and short reaction times are necessary. Pyrolysis oils include acids, which may lead to corrosion and up to 30 wt% water. The heating values of pyrolysis oil are around  $17\text{ MJ kg}^{-1}$ , which is not much different from the original biomass (for example birch wood with 7 wt% moisture:  $17.9\text{ MJ kg}^{-1}$ ). Pyrolysis oil can be used as fuel for large engines, e.g. in ships or for electricity production, but not for cars. Pyrolysis oil can be upgraded to be more suitable as car fuel by hydrogenation, but this needs a lot of hydrogen. Fast pyrolysis oils are used as "liquid smoke" to create a "barbecue" flavor, and can be used for the production of resins (Meier et al. 2013; Vamvuka 2011; Venderbosch and Prins 2010; Mohan et al. 2006).

### 27.2.4 Gasification

Gasification is usually carried out in the range of 800–1000 °C and leads to syngas, a mixture of hydrogen, carbon monoxide, and methane. "Tar-free syngas" can be produced above 1000 °C (Henrich 2003). Low tar contents are useful, if the syngas is used as

feedstock for a catalyzed reaction, like the formation of fuel, methanol, or for Oxo synthesis to produce aldehydes or ketones. Tars deactivate catalysts, especially of noble metals; therefore, syngas-cleaning is necessary in every case.

As a consequence of the high temperature, gasification processes are usually conducted with air or oxygen as the gasification agent. By combustion of a part of the products, the necessary heat is produced (Dahmen et al. 2010; Hofbauer 2009; Dahmen and Henrich 2007).

A general challenge is that such a gasifier should have a high throughput to get lower relative production costs. On the other hand, biomass is widespread and has than to be transported via long distances. In the Bioliq™ concept, straw is converted by fast pyrolysis as a first step in small plants. The products char and pyrolysis oil are mixed to slurry. This leads to an energy density increase of a factor of 10. The slurry is delivered to a larger plant, consisting of a gasifier, gas cleaning and a synthesis unit to produce, e.g. fuel. The concept includes an entrained flow gasifier with a cooled wall. The ash is condensed at the wall and protects it from corrosion. At the internal surface of the ash layer, an ash melt flows down and is collected at the bottom. Here, the syngas produced is converted to car fuels (Henrich 2004; Dahmen and Henrich 2007; Dahmen et al. 2012).

## 27.3 "Wet" Processes

Hydrothermal processes are thermochemical process in water. Therefore, they are very useful especially for wet biomass. Here, as in dry processes, the products are solids, liquids, or gases. In comparison to "dry" processes, the necessary temperatures are lower and the pressures higher (Figure 27.1) (Dahmen et al. 2010). The reason is that water takes part in the reaction by hydrolysis. The rather fast splitting of biomass polymers like cellulose and hemicellulose, and, at a higher temperature, also lignin, leads to a formation of intermediates which can be derived in water (Figure 27.3). Therefore, no heat and mass transfer limitations occurs, which leads to fast further reactions and lower necessary temperature to reach complete degradation. As water is needed as reaction partner and solvent, most of the hydrothermal conversions occur in liquid water at increased temperature. This means that high pressure is needed to avoid evaporation. For gasification, temperatures above the critical point of water ( $374^{\circ}\text{C}$ ) are applied, at which no liquid water can exist. Also, supercritical water high pressure levels of 25–35 MPa are used to get the wanted reactivity and solvent properties (Kruse and Dahmen 2014, 2017).

A disadvantage of hydrothermal processes is that organic compounds stay in the aqueous phase. This means that these processes need in addition a method for water cleaning or the use of derived organic compounds.

### 27.3.1 Hydrothermal Carbonization (HTC)

At a temperature around  $200^{\circ}\text{C}$  (Figures 27.1 and 27.3), carbohydrates are completely split by hydrolysis, with lignin almost not reacting. The sugars react to furfurals by water elimination or to smaller compounds like acids by aldol splitting (Figure 27.3). The furfurals polymerize to a product called "Hydrothermal carbonization (HTC)-coal" or "hydrochar." The hydrochar has a similar heating value as lignite, but a different structure. Recently,

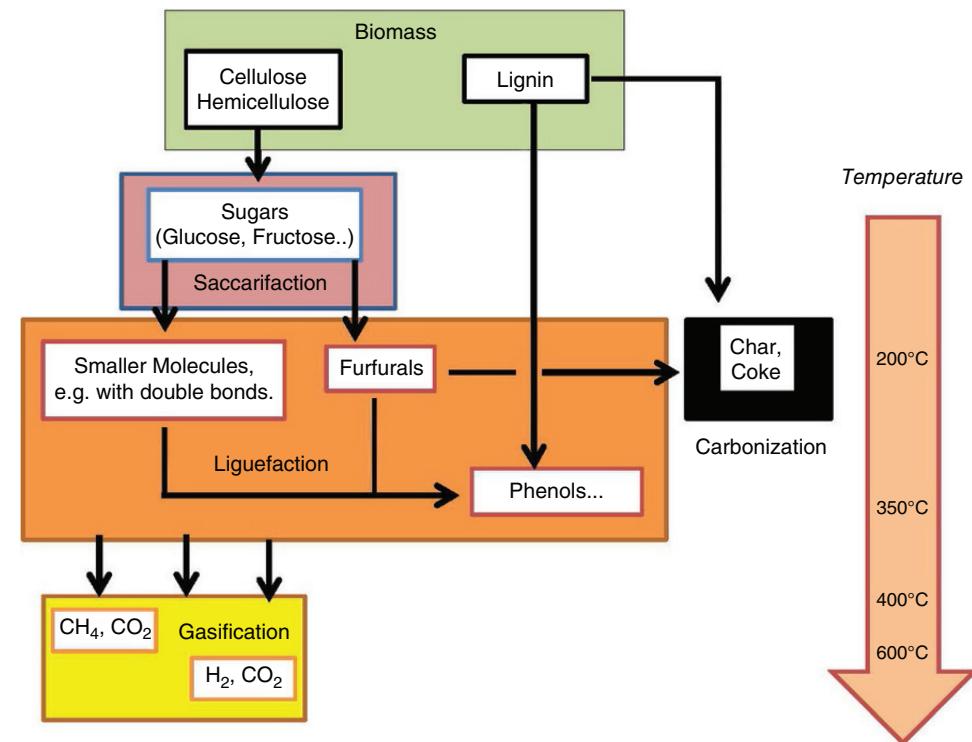


Figure 27.3 Reaction scheme of hydrothermal processes. Source: Kruse and Dahmen (2017).

hydrochar is under investigation as a starting material for the production of advanced carbon materials, e.g. electrodes of batteries or supercapacitors (Zhu et al. 2017), or in soil application (Kruse et al. 2013; Kruse and Dahmen 2017). By acidic leaching of hydrochar, phosphates can be first produced, and by pH-change precipitated, for the use as fertilizer. This is demonstrated, e.g. for digestate (Zhao et al. 2017).

### 27.3.2 Hydrothermal Liquefaction (HTL)

At a temperature of around 300–350 °C, biomass is converted to a tar and solved organic compounds. At this temperature also lignin is degraded, leading to a lot of different phenols (Figure 27.3). Hydrothermal Liquefaction (HTL) tar has a much higher heating value (HHV: 30–39 MJ kg<sup>-1</sup> (Gollakota et al. 2018)), very low water content, and higher viscosity than pyrolysis oil. High viscosity, in particular, is a disadvantage. For application as transportation fuel hydrogenation is necessary. Recently, the application of HTL of algae is studied by different research groups (Toor et al. 2011; Kruse and Dahmen 2017; Cheng et al. 2017; Elliott 2016; Kumar et al. 2016).

### 27.3.3 Hydrothermal Gasification (HTG) and Supercritical Gasification (SCWG)

At a rather low temperature of 200–250 °C, hydrogen can be produced from derived bio-based compounds. This process is called aqueous reforming. Because of thermodynamic reasons, hydrogen as the main burnable gas is only possible at a low concentration

(around 1 wt% dry mass). In addition, noble metals as catalysts are necessary. Aqueous reforming is suitable to “clean” the aqueous effluent of other biomass conversion processes (Coronado et al. 2016), like hydrothermal conversions or biodiesel production. As catalysts for hydrogen production are usually also hydrogenation catalysts, the produced hydrogen can be used to hydrogenate the feedstock. In this case hydrogen-rich compounds, e.g. alkanes are formed (Coronado et al. 2016).

For methane formation (Figure 27.3), the presence of a hydration catalyst, e.g. nickel or noble metals, is required. A combination of nickel with alkali salts has proved to be very useful. In gasification experiments, a nearly complete conversion into gases with a methane content of up to 50–60 vol% was reached. Hydrothermal Methane formation is usually performed near to the critical temperature (374 °C); just below or above. At this temperature, methane is the thermodynamic favorite burnable gas (Boukis et al. 2016; Elliott et al. 2012; Peng et al. 2017).

In the case of a dry matter content of around 10 wt%, which is near to value for a green plant (10–20 wt%), a temperature of around 600 °C is necessary to get hydrogen (Figure 27.3), not methane, as the main component together with carbon dioxide. A challenge is that at supercritical conditions, the solubility of salts is very low. Therefore, plugging by precipitated salts has to be avoided. On the other hand, this is also a chance for the recycling of salts as fertilizer (Rodriguez Correa and Kruse 2017; Vadillo et al. 2013; Matsumura et al. 2013). Recently, several studies concerning the hydrothermal gasification (HTG) of algae is studied. As a biomass with high water content, it is a biomass suitable for a hydrothermal process (Elliott et al. 2012; Peng et al. 2017).

### 27.3.4 Platform Chemicals

Not only energy carriers, but also platform chemicals can be produced by a hydrothermal route (Kruse and Dahmen 2017). As shown in Figure 27.3, carbohydrates are hydrolyzed as a first step of hydrothermal processes, introduced here. In demo and pilot-scale, the company Renmatix produces sugars and lignin from biomass in a stepwise process: First in subcritical conditions, hemicellulose is hydrolyzed and the first sugar solution is produced (Cantero et al. 2015). Then, in a supercritical hydrolysis cellulose is split into glucose and other hexoses. These sugars can be used as feedstock for biochemical conversions. Furfurals are regarded as intermediates for the formation of hydrochar (Figure 27.3). At modified conditions, from hexoses hydroxymethylfurfural (HMF) can be formed. HMF can be further converted to Nylon 6,6, Nylon 6, pulsed electric field (Polyethylene 2,5-furandicarboxylate) for bottles or other packing materials (Cantero et al. 2015; van Putten et al. 2013). The hexoses sources are cellulose, inulin from roots or others. Pentoses, e.g. as a side product of the pulp and paper industry, form furfural which may substitute formaldehyde in resins (Cantero et al. 2015).

## 27.4 Conclusion

Thermochemical conversions can be used to produce energy carriers, materials like activated carbons or platform chemicals. The choice of a suitable process depends on the feedstock and the desired product:

Wet biomass with high water content, like digestate or most of the agricultural wastes, should be converted by a hydrothermal process, occurring in water. Dry biomass, like

straw, is suitable for dry conversion process. In both cases, the product could be gaseous, liquid, or solid. Platform chemicals are a very attractive product, but should be produced from rather homogeneous waste. A very interesting concept is the cogen-  
eration of products and energy carrier.

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## 28

# Dioxins from Agro Waste Combustion: Evaluation and Management

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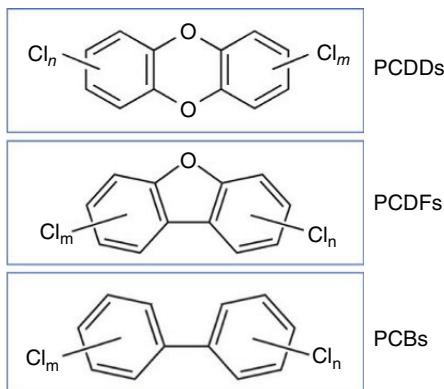
## 28.1 Introduction

The term “dioxins” is used for different species by different authors. It comprises up to three distinct groups of polychlorinated aromatic compounds, namely 75 polychlorinated dibenzo-p-dioxins (PCDDs), 135 polychlorinated dibenzofurans (PCDFs), and 209 polychlorinated biphenyls (PCBs) (see Figure 28.1).

Based on these structures, different congeners are possible by varying the number and position of chlorines, each one presenting a different toxicity. The most toxic compound is 2,3,7,8-tetrachlorodibenzo-p-dioxin or TCDD. The toxicity of other “dioxins” is measured in relation to TCDD. Two different standards are used nowadays: the first is I-TEQ toxicity (international toxic equivalent toxicity), used for measuring toxicity of PCDDs and PCDFs; and the second is WHO-TEQ (world-health-organization toxicity) also including “dioxin-like” PCBs (US EPA, OEI, OIAA, n.d.). To apply this concept, the equivalent factor of each congener present in a mixture is multiplied by its respective mass concentration, and the products are added to represent the mixture’s TCDD equivalent toxicity that can be expressed in weight, I-TEQ-weighted and WHO-TEQ weighted.

PCDD/Fs are one of the most toxic chemicals to be known, and are highly persistent in the environment, as they are non-biodegradable. Due to this, PCDD/Fs accumulate in the tissues of living creatures, mainly in fatty materials. This is why major concentration is found in fish fat, meat fat, or in milk (human or animal milk).

The US Environmental Protection Agency clearly describes dioxins as a serious public health threat. According to a EPA report (U.S. EPA 2013), levels of dioxins and dioxin-like chemicals have been found in the general US population. The EPA report confirmed that dioxin is a cancer hazard to people; that exposure to dioxins can also cause severe reproductive and developmental problems (at levels 100 times lower than those associated with its cancer causing effects); and that dioxins can cause immune system damage and interfere with regulatory hormones.



**Figure 28.1** Molecular structure of PCDDs, PCDFs, and PCBs.

Due to their very low concentration (values are close to picograms or femtograms), the analysis of PCDD/Fs involves a concentration previous to the determination by means of a very sensitive and specific analytical method (US EPA 1994).

### 28.1.1 Origin of PCDD/Fs

Various sources indicate that thermal processes are responsible for dioxins' major contribution to the environment. PCDD/Fs form an unintentional byproduct of many industrial processes such as waste incineration, chemical and pesticide manufacturing and pulp and paper bleaching. PCDD/Fs production is inherent to controlled and uncontrolled combustion, as will be explained below.

### 28.1.2 Biomass Thermal Decomposition

During biomass thermal decomposition, different steps can be distinguished (Elías Castells 2012). In the first temperature range, up to about 200 °C, water evaporates leading to a first stage of volatilization. In a second temperature range, from 300 to 600 °C, volatilization itself follows. This phase can be superimposed on the previous one and/or the subsequent phase depending on the nature of the waste/fuel. A higher temperature (600–1000 °C) corresponds to the oxidation of organic matter, with the consequent formation of SO<sub>x</sub> and CO<sub>2</sub>. At a higher temperature, and depending on the reducing nature of the medium, gasification reactions take place, the equilibrium moves, and CO as well as SO<sub>x</sub> are formed. Limestone decomposition also occurs in this range. As the temperature continues to increase, NO<sub>x</sub> of thermal origin begins to form, that is, from the nitrogen in the air. As a rule, many of the fuels used as waste contain nitrogen. In this case, the formed NO<sub>x</sub> comes from this nitrogen since the NO<sub>x</sub> of thermal origin requires a very high temperature to form to a significant degree.

Wood offers advantages over fossil fuels with regard to emissions: contents of sulfur and nitrogen are low in wood, thus SO<sub>x</sub> emissions are negligible, and, if temperature is controlled to reduce oxidation of nitrogen from the air, the overall NO<sub>x</sub> will also be low (Lavric et al. 2004; McIlveen-Wright et al. 2001).

In combustion or incineration processes, two important steps are always present in the solid phase (Conesa et al. 1998): (i) a pyrolysis stage, in which the solid feed

undergoes devolatilization reactions to yield volatiles (gases and tars) and a solid fraction (char); (ii) a combustion stage, in which the char undergoes heterogeneous reactions to yield gaseous products and an inert residue (ash). The pyrolysis and combustion stages may be sequential or concurrent, depending on the feature of the process considered (Conesa et al. 1998).

During the pyrolytic and oxidative thermal decomposition of solid biomass, an important quantity of aromatics can be generated, that later react and polymerize to cyclic compounds, i.e. aromatics, polycyclic aromatic hydrocarbons (PAHs), and soot. If there is insufficient oxygen, incomplete combustion will occur and the volatile matter will not be completely converted into their principal products of combustion, i.e. CO<sub>2</sub> and H<sub>2</sub>O.

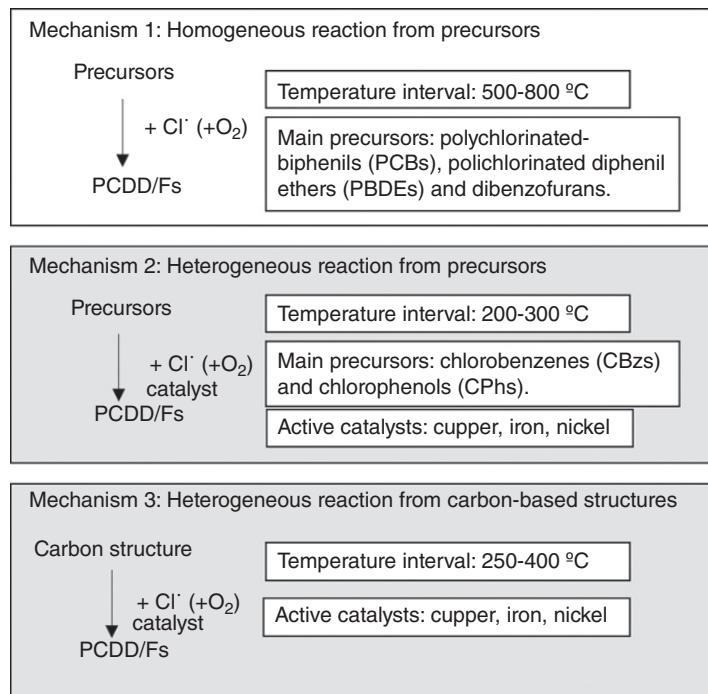
Previous studies have indicated that the evolution of pollutants is strongly dependent on the oxygen availability. In this way, Conesa et al. (2009) found a general behavior regarding the evolution of semi-volatile compounds with an increase of temperature, both in pyrolysis and combustion, when comparing different wastes. Results showed that emissions of many compounds decreased as oxygen ratio increased. Compounds with this behavior were mainly volatile hydrocarbons, which were consumed in the presence of oxygen. In addition, compounds with an oxidized structure presented a maximum at intermediate oxygen ratio. Intermediate compounds with very high resistance to oxygen under the working conditions saw their concentration increase continuously when the oxygen ratio increased. Paying particular attention to the evolution of PCDD/Fs, Conesa et al. (2007) showed that the rate of production of these pollutants was at its maximum when the presence of oxygen was at an intermediate level. Production was much lower when oxygen concentration was low or high.

## 28.2 PCDD/Fs Formation Mechanisms

During the combustion of agro-waste, two different processes can be related to the production of PCDD/Fs and similar structures (Figure 28.2). On the one hand, a "homogenous" mechanism of PCDD/Fs formation has been described in the range of 500–800 °C, while catalytic gas-solid reactions have been described as a "heterogeneous" formation between 200 and 400 °C (Lavric et al. 2004). Formation rates through these two mechanisms are quite different, the catalytic route being much more effective (Stanmore 2004).

Additionally, in the "heterogeneous" route, two different mechanisms can be observed: the "precursor" and "de-novo" pathways. The de-novo synthesis starts with molecular carbon or PAHs, that, in the presence of small amounts of chlorine and the appropriate catalyst can produce PCDD/Fs structures. The reagents in the "precursor" pathway are organic molecules similar to PCDD/Fs, including chlorophenols (CPhs), chlorobenzenes (CBzs), and poly-chlorophenyls (PCBs). Both pathways proceed at the surface of solid carbon (soot, charred materials), catalyzed by certain metal ions (e.g. copper, iron) that are usually present in agro-waste in small amounts (Addink and Olie 1995). The de-novo formation of PCDD/Fs has also been observed in the formation of the brominated congeners of dioxins and furans (PBDD/Fs) (Ortuño et al. 2014). These have been given more attention in recent years as they are considered at least as toxic as the PCDD/Fs.

Both de-novo synthesis and precursor routes have been studied in-depth (Huang and Buekens 1995; Huang and Buekens 1996; McKay 2002). Stieglitz et al. (1989) found



**Figure 28.2** Main mechanisms for PCDD/Fs formation during combustion.

that carbon degradation in the presence of such compounds can be described by a combination of two first order reactions. These reactions and the change of the organic chloride content correlated with the de-novo synthesis of organic chlorinated compounds.

Indicators of the prevailing pathway are the congener distribution in the emitted volatiles. In this way, an amount of dioxins higher than furans (PCDD > PCDF) indicates that the precursor pathway predominated, while in other cases, de-novo synthesis is supposed to be the dominant mechanism for the formation of dioxins (Huang and Buekens 1996; Conesa et al. 2002).

In this way, high temperature combustion of agro-waste decomposes pollutants present in the fuel, including PCDD/Fs, sufficiently, and new contaminants are formed. The formation of pollutants clearly depends on combustion conditions, entailing that there is a clear difference between combustion in industrial incinerators and open-fire conditions (Lavric et al. 2004; Skodras et al. 2002).

Industrial incineration is usually less problematic, as sources are equipped with flue gas cleaning systems. However, small scale stoves, fireplaces and house heating systems usually operate under conditions where PCDD/Fs are easily formed. Furthermore, they are rarely equipped with air pollution control devices. Open-fires are much more aggressive, as a considerable amount of products of incomplete combustions (PICs) are formed and emitted as a result of poor (low-oxygen and low-temperature), uncontrolled combustion conditions.

### 28.2.1 Influence of the Composition of Fuel on the Emission of PCDD/Fs

As mentioned, the presence of chlorine compounds and some metals can stimulate the formation of PCDD/Fs. Agro-waste is generally characterized by low ash content, high calorific values and large amounts of fixed carbon. Straw is the only material to present high chlorine content (Chagger et al. 1998) that can be a significant drawback. It has been proved to have low concentrations, if any, of iron, lead, zinc, and copper.

Iron and copper are also present in many plastic-contaminated wastes. The chlorides in these metals are involved in de-novo catalytic formation, as they are able to reduce the decomposition temperature of many materials. A strong accelerating effect of iron and copper chlorides has been reported (Blazsó 1999; Conesa et al. 2001) where the decomposition of tires, almond shells and sewage sludge were compared in the presence/absence of CuCl<sub>2</sub>.

### 28.2.2 The Influence of the Presence of Other Products in the Waste

Special attention has been paid to the combustion of agro-waste and wood, since not only natural biomass is being burnt but also materials coated and treated with organo-chlorines. Substances such as pesticides, wood preservatives and paint are frequently present and even mixed in biomass fuels. Metal-based solutions (copper arsenate, copper boron azole, ammoniacal copper quaternary, etc.) have been commonly used for preserving woods.

Native biomass generally contains only traces of amounts of chlorine (Tame et al. 2007). However, these contaminants are most likely to bring in more chlorine, metal catalysts, and even organic additives, which thermally decompose to provide precursors for dioxin synthesis, increasing the emission of PCDD/Fs. Another major source of dioxins can be salt-laden wood waste, burned in power boilers, at pulp and paper mills.

### 28.2.3 PCDD/Fs Formation from Biomass Constituents

Lignocellulosic materials are formed by three different fractions, mainly: hemicellulose, cellulose, and lignin. Thus, a critical question is whether the decomposition of any of these fractions separately produces more PCDD/Fs than others.

In this way, several authors have shown that lignin can be reagent of the so-called "precursor" pathway. Choudhry and Hutzinger (1983) postulated that the complex structure of lignin in wood fiber can be pyrolyzed to generate PCDD/Fs if a chlorine donor is present, as they detected benzene and phenol among the decomposition products. Other authors (Becker et al. 2001) confirmed that the formation of phenol or phenolic compounds originated from the lignin part of wood during the thermal degradation of natural and creosote-treated wood, and demonstrated that benzene could react with an inorganic chloride in the presence of heat to produce a variety of chlorinated aromatic compounds, including PCDD/Fs. Tame et al. (Tame et al. 2007) also found that lignin produced considerably more PCDD/Fs than cellulose and hemicellulose.

## 28.3 PCDD/Fs from Agro-Waste Emission Factors

Biomass burning is used in many rural areas as a way to dispose of residues such as firewood and animal manure. This practice is also attributed to agricultural and forestry purposes, such as converting natural land into arable land.

As mentioned before, incineration in a chamber or oven under controlled conditions can be considered as a favorable destruction procedure for many biomass wastes if it is combined with air pollution control devices (Lavric et al. 2004). Controlled incineration largely reduces the volume of waste and emits much less pollutants than uncontrolled combustion and open fires. Consequently, we will focus on these latter forms of combustion, as incineration of several wastes has nearly similar emissions in controlled conditions.

### 28.3.1 Emissions into the Air

There have been several studies on the emissions of PCDD/Fs from uncontrolled fires into the air. The formation of PCDD/Fs is intrinsic to conditions of low temperature found in uncontrolled fires, and different authors have concluded that smoke from domestic sources has a very high concentration of PCDD/Fs (Schatowitz et al. 1994).

Measurements of the emission factors for PCDD/Fs, PCBs, and hexachlorobenzene (HCBs) were first carried out by Gullett et al. (2003). These authors incinerated two natural wood types (oak and pine) and commercial fuels. Emission factors measured by these authors ranged from 0.25 to 1.4 ng I-TEQ/kg for natural fuels and 2.4 ng I-TEQ/kg for artificial woods, showing very similar levels.

Moltó et al. (2010) measured the emission factors of carbon oxides, light hydrocarbons, PAHs, polychlorodibenzodioxin/furans (PCDD/Fs), and dioxin-like PCBs in the combustion of a mixture of pine needles and cones in a residential stove. A value of 175.15 ng WHO-TEQ/kg was given. When comparing with runs performed in a laboratory furnace, the level of emissions of PCDD/Fs in the stove was intermediate between the emission found in combustion at 500 and 850 °C in the laboratory reactor. The authors indicated that there were cracking reactions inside the residential stove, causing a considerable decrease in PAH yield and a strong dechlorination of the PCDD/F isomers (Moltó et al. 2010). Conversely, an emission factor of only 0.22 ng WHO-TEQ/kg was measured by the same authors for the combustion of dried tomato plant (Molto et al. 2010) in a residential stove.

In another study undertaken by the Canadian government (Zhang et al. 2017), the emissions of two different fuels (hard maple and spruce) in a conventional wood stove and a certified non-catalytic, advanced technology wood stove were measured. The emission factors of PCDD/Fs from the certified stove (0.49–1.01 ng I-TEQ/kg of dry wood) were slightly higher than those from the conventional stove (0.20–0.33 ng I-TEQ/kg of dry wood), but no substantial differences of emission factors between the two types of wood was observed. The authors of the study estimated an overall emission factor of 0.5 ng I-TEQ/kg dry wood.

The aforementioned agro-waste often contains various types of contaminants (metal-based preservatives, creosote, etc.) that can stimulate the formation of PCDD/Fs. Lavric et al. (2004) showed that the combustion of uncontaminated woods leads

to much lower concentrations (0.001–7.2 ng I-TEQ/m<sup>3</sup>) than contaminated wood chips (44–58 ng I-TEQ/m<sup>3</sup>). Other authors (Becker et al. 2001) reported an emission factor of 22.4 ng I-TEQ/kg for non-treated wood, and a factor 76 times higher for treated woods.

Measures of open forest fires have equally been reported. Gullett and Touati (2003) burned different biomass in the EPA's Open Burning Test Facility, and provided an average emission factor of 19 ng I-TEQ/kg of fuel, using several samples of biomass from different countries.

Gullett et al. (2009) fired pine wastes in an open burning facility, to study the effect of moisture, the charge size, and chlorine concentration of the feed. Their results showed that the emission factor was independent of moisture content and charge size, but highlighted the importance of the presence of chlorine, as the increase of fuel chlorine from 0.04 to 0.8 wt% resulted in an approximately 100-fold increase of PCDD/Fs emissions.

Moreno et al. (2016) showed important differences between the emission of solid wood (2.5 ng WHO-TEQ/kg) and furniture wood waste (ca. 20 ng WHO-TEQ/kg) in an open stove. The authors attributed these differences to the presence of components containing chlorine or metal in the wood waste.

Ikeguchi (1999) tried to simulate open burning of wastes, by keeping inlet and outlet doors on their furnace open, letting combustion air flow freely. They found emission factors of 4.6 ng I-TEQ/kg using trees and leaves as fuel, increasing to 20.2 ng I-TEQ/kg when bundles of straw were used as fuels.

Gadde et al. (2009) calculated that the release of PCDD/Fs in India, Thailand, and the Philippines from open field burning of rice straw represented respectively 5.57, 4.18, and 4.06 g yr<sup>-1</sup>. These authors took into account an emission factor of 0.5 ng I-TEQ/kg, that was also used in other studies (Gullett and Touati 2003).

A few years ago, EPA (2013) summarized the emission factors for PCDD/Fs and selected 3 ng I-TEQ/kg as a representative value, based on field tests and chamber tests.

Hedman et al. (2005) mentioned factors of emission into the air of ca. 20–100 ng WHO-TEQ/kg for the combustion of garden waste in uncontrolled backyard burning conditions, and wheat straw in the same device produced 28 ng WHO-TEQ/kg.

Table 28.1 summarizes other data found on the emission of PCDD/Fs into the air from the combustion of biomass in devices with no air pollution control equipment. Data has been included from cases where biomass mixed with other wastes is used as fuel, to highlight the importance of the presence of waste other than biomass in pollutant emissions.

### 28.3.2 Emissions into the Land

Soot and ashes from agro-waste and biomass combustion can also be contaminated with PCDD/Fs. Indeed, the biggest amount of these compounds is usually found in the solid phase.

Nestrick and Lamparski (1983) evaluated the presence of dioxins in soot scrapings from chimneys of wood-burning stoves. The average total of PCDD/F levels in the chimney deposits were 8.3 ng kg<sup>-1</sup> in the eastern region of the USA, 42.1 ng kg<sup>-1</sup> in the central region, and 10 ng kg<sup>-1</sup> in the west. Differences were attributed to the design of the different units, which affected the sampling point and/or the conditions at the sampling point.

Molto et al. (2010) showed an emission factor of 33.24 ng WHO-TEQ/kg in a residential stove using tomato plant as feedstock, and ca. 7 ng WHO-TEQ/kg in open fires of the

**Table 28.1** Some emission factors of PCDD/Fs from biomass burning sources with no air pollution control devices, in ng I-TEQ/kg feedstock.

Type of combustor	Nature of fuel	Emission factor	Reference
Open fire	Natural wood chips	0.25–1.4	(Gullett et al. 2003)
Open fire	Commercial fuels (biomassic)	2.4	(Gullett et al. 2003)
Domestic stove	Pine needles and cones	175.25	(Moltó et al. 2010)
Domestic stove	Tomato plant	0.22	(Molto et al. 2010)
Conventional stove	Maple, spruce	0.20–0.33	(Zhang et al. 2017)
Certified stove	Maple, spruce	0.49–1.01	(Zhang et al. 2017)
Open burning facility	Pine wastes	0.75–3.00	(Gullett et al. 2009)
Open stove	Solid wood	2.5	(Moreno et al. 2016)
Open stove	Furniture wood waste	20.0	(Moreno et al. 2016)
Open stove	Tree and leaves	4.6	(Ikeguchi 1999)
Open stove	Straw	20.2	(Ikeguchi 1999)
Household stoves, open	Natural beech wood sticks	0.077	(Schatowitz et al. 1994)
Household stoves, closed	Natural beech wood sticks	1.25	(Schatowitz et al. 1994)
Batchwise operated masonry heater	Untreated beech logs	0.626	(Pfeiffer et al. 2000)
Conventional wood stove	Maple	0.27–0.30	(Launhardt and Thoma 2000)
Conventional wood stove	Spruce	0.20–0.40	(Launhardt and Thoma 2000)
5 kW stove	Birch wood	0.61–5.1	(Lavric et al. 2004)
Automatic chip furnace (150 kW)	Waste wood chips from demolition of buildings	170.7	(Schatowitz et al. 1994)
Household stove (6 kW)	Charcoal used for grillins meat	0.04	(Schatowitz et al. 1994)
Household stove (6 kW)	2/3 paper +1/3 plastics	3230	(Schatowitz et al. 1994)
Laboratory furnace	Crushed wood pellets	2.6–6.1	(Soler et al. 2018)
Laboratory furnace	Crushed wood pellets with 20% of waste circuit boards	0.55–9.0	(Soler et al. 2018)
Laboratory furnace	Crushed wood pellets with 20% of halogen-free electric wires	0.12–20.0	(Soler et al. 2018)
Woodstove	Oak	0.25	(Gullett et al. 2003)
Fireplace	Oak	0.35	(Gullett et al. 2003)
Fireplace	Pine	1.4	(Gullett et al. 2003)
Chamber tests simulating forest fire	Forest biomass from France	10.5	(Gullett and Touati 2003)
EPA's open burning test facility	Forest biomass from Oregon	15	(Gullett and Touati 2003)
Field test	Duke forest	0.52	(Gullett and Touati 2003)
Lab burn tunnel	Agro-waste from Australia	35	(Meyer et al. 2004)
Lab burn tunnel	Wheat straw from Australia	17	(Meyer et al. 2004)
EPA's open burning test facility	Rice straw from USA	0.73	(Meyer et al. 2004)
EPA's open burning test facility	Wheat straw from USA	0.47	(Meyer et al. 2004)
EPA's open burning test facility	Sugarcane from USA	11	(Gullett et al. 2006)
Field	Sugarcane from USA	1.39	(Gullett et al. 2006)

same material. When treating pine needles and cones, the ash presented a reduced 1.31 ng WHO-TEQ/kg content.

Other studies (Moreno et al. 2016) reported PCDD/Fs content in ash from biomass waste between 10 and 78 ng WHO-TEQ/kg ash, although the presence of chlorine was a determining factor. Bacher et al. (1992) found concentrations of 2,3,7,8-substituted PCDD/Fs in soot from wood burning ranging from 40 to 930 ng PCDFs/kg and from 30 to 150 ng PCDDs/kg. In this way, the PCDFs dominated the PCDDs by a factor of 5–10.

Differences have also been found with respect to the presence of salt in the fireplace (Van Oostdam and Ward 1995). In this sense, soot from two stoves –one of them a salt-laden wood burning stove from a coastal area– was analyzed. Soot from salted-wood combustion presented concentrations 20–90 times greater than the non-salted material.

## 28.4 Remediation

It is difficult to establish methods for the prevention of the emission of PCDD/Fs during the uncontrolled burning of waste. The elimination in industrial facilities (incinerators, furnaces) in which the combustion conditions are controlled is always recommended. In addition, these installations can incorporate gas treatment systems, which, if properly designed and operated, can be very effective in the elimination of such pollutants. However, some advice can be given.

As mentioned before, burning other residues (especially plastics) together with agricultural waste should be avoided. In addition, it would be advisable to avoid burning wastes that have recently been impregnated with pesticides or halogenated compounds. It would be recommended to allow sufficient time to elapse (weeks or months) between the impregnation and the burning of the waste.

Another important point of PCDD/F production is in the ashes, as shown above. It is important to collect these ashes for further treatment, in order to avoid the dissolution of contaminants in rainwater or rivers.

Different authors indicate that mixing biomass with calcium-containing reagents, as limestone, effectively decreases the dioxin emission factor. In this way, Si et al. (2017) observed a decrease of ca. 60% in PCDD/F emissions when adding limestone in a proportion of Ca/S = 2.

In recent years there has been intense research into the ability of certain N and S containing compounds to prevent the formation of PCDD/Fs during the thermal destruction of wastes. Thiourea, Ammonium Thiosulphate, and sulfamic acid have been extensively studied. Samaras et al. (2000, 2001) mixed several S and N containing compounds with wastes in a 10% weight proportion, decreasing the emission of PCDD/Fs in a 98%. Wu et al. (2012) used  $(\text{NH}_4)_2\text{SO}_4$  and pyrite to suppress the PCDD/F emissions.

Other authors (Åmand and Kassman 2013; Moreno et al. 2016) show the possibility of using mixtures of wastes to decrease the emissions. The effect of sewage sludge addition has been specially studied, as it generally contains a high percentage of nitrogenated compounds. Chen et al. (2015) measured a decrease of 98% in dioxin emissions by decomposing wastes using gases from the pyrolysis of sewage sludge. Moreno et al. (2016) mixed waste wood with 10% of polyurethane foam (rich in nitrogen), observing a decrease in the PCDD/F emissions.

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## Techno-economic Assessment of Bioenergy from Manure

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### Abbreviations

ABPs	Animal Byproducts
AD	Anaerobic Digestion
BMP	Biomethane Potential
CAPEX	Capital Expenditure
C/N	Carbon to Nitrogen Ratio
CHP	Combined Heat and Power
EEAG	Energy and Environmental State Aid Guidelines
FiP	Feed-in Premium
FiPC	Feed-in Premium operating aid Contract
FiT	Feed-in Tariff
GPR	Gas Production Rate
HRT	Hydraulic Retention Time
IRR	Internal Rate of Return
NPV	Net Present Value
OLR	Organic Loading Rate
OPEX	Operational income and expenses
RT	Reference Tariff
RES	Renewable energy sources
SRT	Solid Retention Time
SGP	Specific Gas Production
TS	Total Solids
VS	Volatile Solids
VFAs	Volatile fatty acids

## 29.1 Introduction

Undoubtedly, anaerobic digestion (AD) is a technological approach, during which waste management and production of green energy can be coupled. More specifically, the changes made in the European legislative framework during the last decades sparked a significant increase in the number of biogas plant installations (Stürmer 2017). Thus, the fact is not surprising that by the end of 2015 there were 17 376 biogas plants and 459 biomethane plants operating across Europe (European Biogas Association (EBA), 2016). The advantages of this process lie in feedstock diversity (Zhang et al. 2016), energy yield and, also, its several economic benefits in the form of reduced waste management costs due to biogas production, electricity, and heat recovery, its revenues from selling surplus power to local utilities, and reduced expenses for use of synthetic fertilizers (Stürmer 2017; Vasco-Correa et al. 2018). These are some of the reasons why the number of biogas plants has evolved from 6227 to 17 376 from 2009 until 2015 (EBA 2016).

Feedstocks suitable for anaerobic digestion can be found in animal byproducts (ABPs) which according to Regulation 1069/2009 (ABP Regulation) are defined as "*the entire bodies or parts of animals, products of animal origin or other products obtained from animals, which are not intended for human consumption, including oocytes, embryos and semen.*" Specific methods must be used for the treatment of the ABPs depending on their category, as stated in the ABP Regulation, to ensure the application of hygienic measures and thus, a high level of protection of public and animal health (Valta et al. 2015). Anaerobic treatment is a popular method for coupling animal manure treatment and biogas production, which has been extensively examined by many researchers. Manure, which according to ABP Regulation (Article 3) is "*any excrement and/or urine of farmed animals other than farmed fish, with or without litter*" has been categorized as medium risk ABP (i.e. Category 2) (Wellinger et al. 2013). According to the ABP Regulation such products can, inter alia, be treated aerobically (composted) or transformed into biogas in order to prevent and minimize risks to public and animal health arising from those products, and more specifically, to protect the safety of the food and feed chain. The most common are: swine manure, cattle manure and poultry manure. Generally, swine manure has higher water content than other types of animal manure, such as poultry and cattle (Pang 2008).

This chapter is organized as follows. Section 29.2 describes basic information related to the anaerobic digestion process. In particular, the following issues are described: stages of the anaerobic digestion process, feedstocks and their characteristics, operational parameters and the potential uses of biogas. Following in Section 29.3 the methodology for the assessment of the financial viability of the manure treatment biogas plant is introduced. The methodology consists of six steps, i.e. feedstock availability and characteristics, energy analysis, technical calculations, operational income and expenses, investment analysis indices, and investment cashflows. Finally, in Section 29.4 the techno-economic assessment of two different scenarios (500 kWe capacity biogas plants) in Greece is conducted; the first one includes exclusively the mono-digestion of cattle manure, while the other focuses on the co-digestion of cattle manure with a byproduct from the cheese manufacturing process (cheese whey) and energy crops (maize silage). Finally, conclusions are driven.

## 29.2 Basics in Anaerobic Digestion

Anaerobic degradation or digestion is a biochemical process that occurs naturally whenever high concentrations of wet organic matter are under the absence of dissolved oxygen (Neshat et al. 2017). During this multistep process, organic carbon is converted by subsequent oxidations and reductions to simple gaseous forms, mainly consisting of methane ( $\text{CH}_4$ : most reduced state) and carbon dioxide ( $\text{CO}_2$ : most oxidized state), in the absence of oxygen, from various anaerobic microorganisms (Peiris 2016; Wellinger et al. 2013). Moreover, small amounts of nitrogen, hydrogen, ammonia, and hydrogen sulphide are also produced. The mixture of gaseous products is termed as biogas and the anaerobic degradation process is often termed as the biogas process (Angelidaki et al. 2003).

### 29.2.1 Anaerobic Digestion Process

According to Pavlostathis and Girardo-Gomez (1991), based on work previously contacted by Gujer and Zehnder (1983), four basic stages can be distinguished in the process of the anaerobic digestion. Information on the microorganisms involved and the main products of each AD process stage are presented as follows:

#### 1) Hydrolysis

The first step of the anaerobic digestion is hydrolysis, relying on microorganisms, known as facultative anaerobes (Merlin Christy et al. 2014). During this stage, the microorganisms produce extracellular enzymes that decompose complex polymeric materials, such as polysaccharides, proteins and lipids (fat and grease) into mono and oligomers, soluble products, such as amino acids, long-chain fatty acids, and sugars. These products are small enough molecules that allow their transport across the cell membrane (Pavlostathis and Girardo-Gomez 1991; California Environmental Protection Agency 2008). The decomposition rate depends on the characteristics of the substrate (Adekunle and Okolie 2015). The transformation of cellulose and hemicellulose generally takes more time in comparison to the decomposition of proteins (Schnürer and Jarvis 2010; Al Seadi et al. 2008).

#### 2) Acidogenesis

The relatively simple, soluble compounds are then fermented or anaerobically oxidized during acidogenesis to form three, four, and five-carbon volatile fatty acids (VFAs), alcohols, carbon dioxide, hydrogen, and ammonia (Pavlostathis and Girardo-Gomez 1991; California Environment Protection Agency 2008). Fatty acid concentrations within the digester is a very important parameter for the proper system functioning since large concentrations of fatty acids can cause inhibition of biological processes. During this stage, the main products are organic acids, e.g. acetic acid, butyric acid and propionic acid, alcohols, carbon dioxide, and hydrogen (Ali Shah et al. 2014; Schnürer and Jarvis 2010). Different species of bacteria carry out the various fermentation activities, i.e. *Saccharomyces* (alcohol fermentation), *Butyribacterium* and *Clostridium* (butyrate fermentation), *Lactobacillus* and *Streptococcus* (lactate fermentation), and *Clostridium* (propionate fermentation) (Kang and Yuan 2017). Another group of bacteria called acetate forming fermentative bacteria include the species of *Acetobacterium*, *Eubacterium*, and *Sporomusa* that are responsible for the production

of acetate in this stage (Amani et al. 2010; Li et al. 2011). Other products of acidogenesis are ammonia and hydrogen sulphide, which give an intense unpleasant odor to this phase of the process (Claassen et al. 1999; Conrad 1999; Ntaikou et al. 2010).

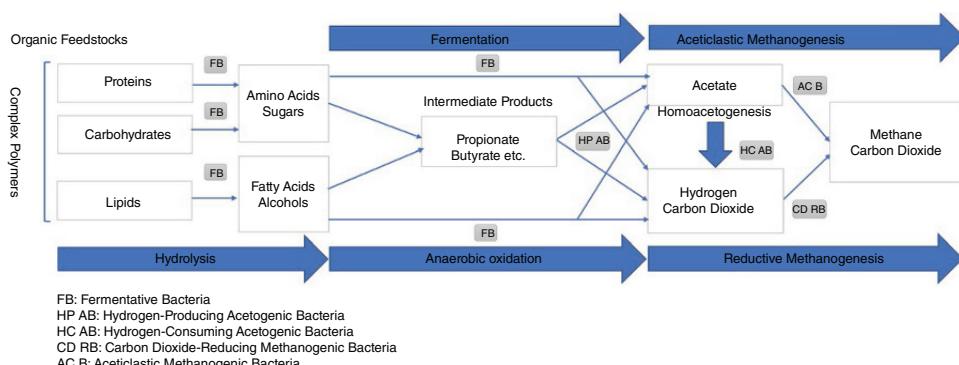
### 3) Acetogenesis

In this stage of the AD process, acetogenic microorganisms transform large compounds of the previous step, such as VFAs and alcohols in acetic acid, carbon dioxide, and hydrogen. Acetogens, such as *Syntrophobacter wolinii* and *Smithella propionicica* have been identified as the responsible bacteria that consume butyrate and propionate to form acetate, respectively (Kang and Yuan 2017). Other bacterial groups (*Syntrophobacter fumaroxidans*, *Syntrophomonas wolfei*, *Pelotomaculum thermopropionicum*, and *Pelotomaculum schinkii*) transform VFAs to into hydrogen and carbon dioxide, while *Clostridium aceticum* produces acetate from H<sub>2</sub> and CO<sub>2</sub> (Amani et al. 2010).

### 4) Methanogenesis

Methanogenesis is the principal methane formation stage of the AD process. At this stage, the dominant microorganisms are the methanogenic bacteria which transform the acetic acid into methane and carbon dioxide, and the hydrogen and carbon dioxide into methane and water (Aslanzadeh 2014). Specifically, 70% of the methane produced originates from the aceticlastic methanogenesis (i.e. the conversion of acetate to methane), while the rest 30% is produced due to the reduction of carbon dioxide by hydrogen (Wellinger et al. 2013). The bacterial groups involved that have been identified as methanogenic are: (i) *Methanotherrix soehngenii* and *Methanosaeta concilii* (conversion of acetate to methane and carbon dioxide – aceticlastic methanogenic pathway); (ii) *Methanobacterium bryantii*, *Methanobacterium thermoautotrophicum*, and *Methanobrevibacter arboriphilus* (production of methane from hydrogen and carbon dioxide – hydrogenotrophic methanogenic pathway); and (iii) *Methanobacterium formicum*, *Methanobrevibacter smithii*, and *Methanococcus voltae* (transformation of formate, hydrogen, and carbon dioxide into methane) (Amani et al. 2010).

In Figure 29.1 the steps of the anaerobic digestion process are depicted, as described by Pavlostathis and Giraldo-Gomez (1991) and INTERWASTE (2013).



**Figure 29.1** Conversion pathways of anaerobic digestion process. Source: Own elaboration based on Pavlostathis and Giraldo-Gomez (1991) and INTERWASTE (2013).

## 29.2.2 Potential Feedstocks and their Characteristics

Various feedstocks have historically been used as substrates for the production of biogas through anaerobic digestion. Generally, AD substrates can be categorized based on different criteria, i.e. origin, Total Solid (TS) content, Volatile Solid (VS) content, methane yield per VS or TS, etc. Their physicochemical characteristics play an important role in the process yield and thus in determining the design calculations of a biogas plant. In particular, the main feedstock characteristics which are principally considered are TS and VS.

More particularly, Total Solids content of the raw material is related to its dry mass. The initial concentration of TS of a raw material has a critical role in determining the wet mass required to prepare the suitable mixture to be used as feedstock in the AD process. It also determines the type of digestion which will be applied in each case and the type of mechanical equipment needed. In cases where the TS content is less than 15% the system is considered as "wet" while for substrates exhibiting TS higher than 15 and up to 35% the system is called "dry." Moreover, TS monitoring is important, because it prevents any mechanical damage to the feeding system as well as to the anaerobic digester, since the TS content is a key value from the conceptual design of the AD plant project, and thus the dimensioning of the plant is always performed for feedstock with a given TS content (INTER-WASTE 2010).

Volatile Solids content of the raw material is an indicator of the solids which are readily available to be biodegraded. Feedstock or Organic Loading Rate (OLR) of the anaerobic digesters are usually expressed in terms of volatile solids (% of dry mass of raw material) (Steffen et al. 1998).

Moreover, the ratio carbon to nitrogen (C/N) is examined. This parameter represents the relationship between the amount of carbon and nitrogen present in organic materials. The optimum range of C/N ratios for the AD process lies from 20 to 30 (Verma, 2002). Higher values of C/N ratios may lead to lower gas production due to the rapid consumption of nitrogen by methanogenic bacteria. On the contrary, if lower C/N ratios prevail, the activities of methanogenic bacteria are inhibited due to the accumulation of ammonia. The C/N parameter has a great influence on the performance of the AD process, and thus on the biogas production.

Suitable AD substrates are those that possess relatively low moisture levels but are high enough so as to be pumpable; high VS content; high methane yield per VS content; and absence of possible AD inhibitors. It is highly important to conduct sampling and laboratory measurements on the feedstocks to be used prior to the implementation of a biogas project so as to minimize any possible technical and financial risk that may occur.

Manure has been extensively studied as a potential substrate for AD process through mono or combined digestion. The selection of co-digestion for the treatment of manure comes mainly because of its relatively low biogas yield compared to that of other potential organic feedstock which can be introduced in a biogas plant. Considering this, the need to further improve the digesters' methane production have raised interest over biodegradable industrial wastes and other substrates rich in biodegradable organic matter (Mata-Alvarez et al. 2014).

An overview of the characteristics of various AD substrates is presented in Table 29.1.

**Table 29.1** Typical characteristics of organic substrates (Al Seadi et al. 2008).

Type of feedstock	Organic content	C:N ratio	TS <sup>a</sup> (%)	VS <sup>b</sup> (%TS)	Biogas yield (m <sup>3</sup> kg <sup>-1</sup> VS)	Unwanted physical impurities	Other unwanted matters
Pig slurry	Carbohydrates, proteins, lipids	3–10	3–8	70–80	0,25–0,50	Wood shavings, bristles, water, sand, cords, straw	Antibiotics, disinfectants
Cattle slurry	Carbohydrates, proteins, lipids	6–20	5–12	80	0,20–0,30	Bristles, soil, water, straw, wood	Antibiotics, disinfectants, NH <sub>4</sub> <sup>+</sup>
Poultry slurry	Carbohydrates, proteins, lipids	3–10	10–30	80	0,35–0,60	Grit, sand, feathers	Antibiotics, Disinfectants, NH <sub>4</sub> <sup>+</sup> ,
Stomach/ intestine content	Carbohydrates, proteins, lipids	3–5	15	80	0,40–0,68	Animal tissues	Antibiotics, disinfectants
Whey	75–80% lactose 20–25% protein	n.a.*	8–12	90	0,35–0,80	Transportation impurities	n.a.
Concentrated whey	75–80% lactose, 20–25% protein	n.a.	20–25	90	0,80–0,95	Transportation impurities	n.a.
Flotation sludge	65–70% proteins 30–35% lipids	n.a.	n.a.	n.a.	n.a.	Animal tissues	Heavy metals, disinfectants, organic pollutants
Ferment. slops	Carbohydrates	4–10	1–5	80–95	0,35–0,78	Non-degradable fruit remains	n.a.
Straw	Carbohydrates, lipids	80–100	70–90	80–90	0,15–0,35	Sand, grit	n.a.
Garden wastes	n.a.	100–150	60–70	90	0,2–0,5	Soil, cellulosic components	Pesticides
Grass	n.a.	12–25	20–25	90	0,55	Grit	Pesticides
Grass silage	n.a.	10–25	15–25	90	0,56	Grit	n.a.
Fruit wastes	n.a.	35	15–20	75	0,25–0,50	n.a.	n.a.
Fish oil	30–50% lipids	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Soya oil/margarine	90% vegetable oil	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Alcohol	40% alcohol	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Food remains	n.a.	n.a.	10	80	0,50–0,60	Bones, plastic	Disinfectants
Organic household waste	n.a.	n.a.	n.a.	n.a.	n.a.	Plastic, metal, stones, wood, glass	Heavy metals, organic pollutants
Sewage sludge	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Heavy metals, organic pollutants

n.a.: not available information

<sup>a</sup> TS: Total Solids<sup>b</sup> VS: Volatile Solids

### 29.2.3 Parameters Affecting the Operation and the Stability of the Process

Parameters affecting the AD process progress include the following: hydraulic retention time (HRT), solid retention time (SRT), OLR, specific gas production (SGP) and gas production rate (GPR) (Cecchi et al. 2003; INTERWASTE 2013). More specifically:

- *Hydraulic Retention Time (HRT)*. This parameter is very important for the design and dimensioning of the anaerobic digester. It shows the average time interval that the substrate spends inside the digester tank. HRT is correlated to the reactor volume and the volume of feeding per time unit. An efficient substrate flow rate is provided with a short HRT, but lower biogas yield is achieved. Therefore, it is critical to consider the specific degradation rate of the substrates employed. With known values the required HRT, daily feedstock input and degradation rate of the substrate, the required digested volume can be calculated.
- *Solid Retention Time (SRT)*. The average residence time of solids into the reactor is the ratio between the content of total solids in the reactor and the solids flow rate extracted from the reactor. If the quantity of biomass extracted from the reactor is equal to the biomass produced in the reactor then the solids concentration in the reactor will be constant and it can be said that the reactor is operating in steady-state conditions.
- *Organic Loading Rate (OLR)*. OLR is the substrate quantity introduced into the reactor volume in a given time. OLR is a critical parameter that indicates the quantity of the organic matter that can be fed into the reactor, expressed per volume and time unit.
- *Specific gas production (SGP)*. SGP shows how much biogas is produced from the substrate per mass unit, in terms of VS, as  $\text{m}^3 \text{biogas}/\text{kg substrate fed}$ . This indicator is correlated with the degradation rate of the feedstock and the performance of the process. It is a value often used in comparative assessments among different anaerobic processes.
- *Gas production rate (GPR)*. GPR indicates the produced biogas per reactor volume ratio in a given time (Cecchi et al. 2003).
- *Temperature*. Temperature is an important operating parameter which determines the performance of the AD process as it is greatly related to the survival and growth of the microorganisms. Moreover, temperature regulates the kinetics of the process. There are two ranges of temperature that provide optimum operating conditions for the production of biogas, namely are: the mesophilic (30–40 °C) and thermophilic (50–60 °C) ranges. Mesophilic temperatures are often preferred over thermophilic temperatures in the AD process since the microorganisms are more robust and not that sensitive to changes in their environment (INTER-WASTE 2010). Equations describing the operational parameters are given in Table 29.2.

The stability of the AD process is also significantly affected by the following parameters:

- *pH*. The pH is an indicator of the acid or alkaline nature of the raw material. When treating mixtures of different waste streams, the initial pH value of the feedstock is determined by the quantity of each raw material used. Monitoring and controlling of the pH in the anaerobic digester in the required level is important, since this parameter affects the various bio-conversion reactions that take place during the AD process. Failure may occur in case that acid is accumulated. Low pH values can inhibit acidogenesis stage and be harmful for the existence of methanogens. On the contrary, if the

**Table 29.2** Equations for the calculation of operational parameters for the AD process (Cecchi et al. 2003; INTERWASTE 2013).

Parameter	Equation	Symbols and units
Hydraulic Retention Time (HRT)	$HRT = \frac{V}{Q}$ , [days]	V = reactor volume ( $m^3$ ) Q = substrate flow rate ( $m^3 d^{-1}$ )
Solid Retention Time (SRT)	$SRT = \frac{V*X}{W}$ , [days]	V = reactor volume ( $m^3$ ) X = VS content in the reactor ( $kg VS m^{-3}$ ) W=VS content of effluent from the reactor ( $kg VS d^{-1}$ )
Organic Loading Rate (OLR)	$OLR = \frac{Q*S}{V}, \left[ \frac{kg\ substrate}{m^3_{reactor} - day} \right]$	Q = substrate flow rate ( $m^3 d^{-1}$ ) S = substrate concentration in the inflow ( $kg m^{-3}$ ) V = reactor volume ( $m^3$ )
Specific gas production (SGP)	$SGP = \frac{Q_{biogas}}{Q*S}, \left[ \frac{m^3_{biogas}}{kg\ substrate} \right]$	$Q_{biogas}$ = biogas flow rate ( $m^3 d^{-1}$ ) S = substrate concentration in the inflow ( $kg m^{-3}$ ) V = reactor volume ( $m^3$ )
Gas production rate (GPR)	$GPR = \frac{Q_{biogas}}{V}, \left[ \frac{m^3_{biogas}}{m^3_{reactor} - day} \right]$	$Q_{biogas}$ = biogas flow rate ( $m^3 d^{-1}$ ) V = reactor volume ( $m^3$ )

pH is not controlled, increased concentrations of ammonia may rise pH in values above 8, where acidogenesis may be hindered. An optimal pH operational range for the digester ranges between 6.4 and 7.2 (Östrem 2004).

- *Volatile Fatty Acids (VFAs)*. The VFAs are an indicator of the stability of the AD process. Usually, VFAs accumulation may cause process instability, which in turn will decrease the pH and possibly terminate the process.
- *Ammonia ( $NH_3$ )*. Ammonia levels is an important parameter that requires continuous monitoring and control, because increased concentrations may inhibit the process progress. Process inhibition is a common phenomenon when dealing with animal slurries, due to their high  $NH_3$  nature, resulting from urine. The limit value for ammonia concentration, in order to avoid process inhibition is below  $80\text{ mg l}^{-1}$ . In the case of thermophilic processes, ammonia inhibition may occur since free ammonia concentration is related directly to temperature (Al Seadi et al. 2008).

#### 29.2.4 Potential Uses of Biogas

Biogas energy potential is determined by its methane content which is highly affected by the type of feedstock, the operational parameters and the process progress. Considering biogas with methane content of 50%, the heating value is of  $21\text{ MJ Nm}^{-3}$ , the density is of  $1.22\text{ kg Nm}^{-3}$  and the mass is similar to air ( $1.29\text{ kg Nm}^{-3}$ ) (Al Seadi et al. 2008). Typical values of biogas composition are shown in Table 29.3.

In general, biogas is a versatile source of energy and can be utilized for heat production through direct combustion, electricity generation by fuel cells; gas engines or gas turbines, combined heat and power (CHP) generation or as vehicle fuel (Al Seadi et al. 2008; FNR 2009).

**Table 29.3** Typical composition of biogas (Al Seadi et al. 2008).

Compound	Formula	Content (Vol. %)
Methane	CH <sub>4</sub>	50–75
Carbon Dioxide	CO <sub>2</sub>	25–45
Water Vapor	H <sub>2</sub> O	2 (20 °C)–7 (40 °C)
Oxygen	O <sub>2</sub>	<2
Nitrogen	N <sub>2</sub>	<2
Ammonia	NH <sub>3</sub>	<1
Hydrogen	H <sub>2</sub>	<1
Hydrogen sulphide	H <sub>2</sub> S	<1

Biogas can be also upgraded to supply the gas grid. For biogas to meet the quality standards of the grid which usually require 97% methane, a number of undesirable substances has to be removed (Braber 1995; IEA BIOENERGY Task 37 2007). The presence of specific contaminants is usually related to the origin of feedstock. For instance, biogas generated in landfills may contain more than 500 contaminants, such as halogenated compounds, higher hydrocarbons and aromatic compounds. Moreover, landfill gas and biogas produced from the anaerobic digestion of sewage sludge might also contain siloxanes, which are widely used for a variety of consumer products and do not decompose during the activated sludge process. Finally, ammonia and sulphur compounds are two potential impurities in biogas which can be harmful for the mechanical equipment due to their corrosive nature. As stated above ammonia is a common inhibition factor of the AD process of manure. For instance, poultry manure leads to the formation crystalline ammonia in the excrements which might result in high ammonia levels, which in turn can lead to inhibitory effects during digestion and can even lead to the failure of the process (Steffen et al. 1998).

## 29.3 Methodology for the Techno-Economic Assessment

### 29.3.1 Overview of the Methodology

The methodology that has been applied for the execution of techno-economic analysis of a biogas plant for treating cattle manure is comprised of six steps. Each step serves a special purpose. In Step 1 of the methodology, feedstock availability in terms of quantity and quality is investigated so as to identify and estimate the manure potential for biogas production. In more detail, issues related to available feedstocks for the AD plant and recording of quantities as they relate directly to the biogas production as well as physico-chemical characteristics of organic substrates that affect the progress of the AD process, are studied. Following, Step 2 of the methodology involves the energy analysis by taking into account the energy content of the selected organic feedstocks in order to estimate the annual biogas production and consequently the potential annual energy

production that could be utilized for the substitution of fossil fuels for heat production or as electric power supply for on-site electricity needs or connection with the public grid. Step 3 involves technical calculations related to the dimensioning of the equipment needed and the determination of suitable operating conditions so as to obtain process efficiency. Step 4 includes considerations of all the cost categories resulting as operational incomes and expenses of the biogas plant, while under Step 5 the main economic indices are presented, such as Payback Period, Net Present Value, internal Rate of Return, in order to identify the feasibility and economic viability of investing in the AD plant. Finally, under Step 6 the investment cashflows are determined, based on which the overall assessment of the investment is executed.

### 29.3.2 Step 1: Feedstock Availability and Characteristics

As already mentioned, feedstock characteristics and availability are crucial parameters for a biogas project. Initially, the crucial step for the development of a biogas project is to investigate the existence, availability, and seasonality of feedstocks in the targeted region, to ensure a continuous feedstock supply.

During that stage, there are two main problems that arise, depending on the type of investor who will implement the project. In particular, a farmer or a group of farmers have usually a wrong impression on the adequacy in terms of the quantity of the manure that they create. On the other hand, there are cases that individual investors or funding groups are willing to invest in manure AD projects. In order to maximize their income and improve their cashflows they target in taking advantage of the scale economy, i.e. as the capacity of the project increases the cost per unit decreases. This kind of philosophy often creates the opposite misleading assumption than in the farmer-case resulting in the preparation of unrealistic business plan. For example, the assumption of treating 200 tonnes per day of animal manure in reality is equal to more than 5000 cows, a very large livestock capital which is very hard to find in just one farm.

To this end, during this step the investor has to reply to the following questions:

- Is the amount of manure to which I have access enough in order to make this investment?
- Is it possible that I might overestimate the amount of manure that can be found in the region?
- Should I consider the potential of mixing various types of manure and other biomass in a combined anaerobic digestion plant?
- What is the maximum distance at which is feasible to look for potential feedstock?

The last two questions are critical as their answer will strongly affect the investment feasibility.

For instance potential candidates include:

- i) animal waste: cow, veal, poultry, pig, sheep/goat manure;
- ii) plant biomass: grass, maize, sorghum, green wheat silage;
- iii) industrial wastewater: cheese whey, olive mill wastewater, juice industry wastewater, crude glycerol; and
- iv) solid waste: bone meat meal, animal fat, food waste.

For each raw material category, the annual generation quantities (tn/y) must be calculated, considering the relevant production sources, e.g. number of heads for animal waste, hectares for plant biomass, or annual amounts of food products processed (tn/y), etc.

Either in the case of a monodigestion or when examining the feasibility of a combined anaerobic digestion plant, it should always be firstly clarified the exact region of the project. By definition, the region of a biogas project is not just the field where the storage tanks and the digesters will be installed, but a whole area inside which the feedstock materials can be found and used in the plant. The area radius is a very crucial parameter; if not taken into account it might lead to increased transportation costs, affecting negatively the investment cashflows.

However, even though various maximum radius distances have been proposed ranging from 10 to 100 km from the biogas AD facility; there is not a standard radius in which it is viable to collect manure or biomass. The main decisive parameter is the energy content of the feedstock which is directly proportional to its moisture content, its TS and VS content. As the feedstock moisture content increases the potential distance (radius) at which it is cost-effective to transport the waste decreases (Kumar et al. 2006; Badger 2003); slurries and similar materials of high moisture content (above 95%) are only worth collecting for a biogas plant for just a few kilometers. On the other hand, it might be cost-effective, to boost biogas production and optimize investment indices to receive feedstock with high energy content, even if it must be transported to more than 50 km (Höhn et al. 2014; Einarsson and Persson 2017).

Therefore, the physicochemical characteristics of targeted feedstocks are taken into account for the AD plant design and more specifically during the calculation of the solid mass balances and potential biogas yield, as part of the assessment of the investment viability. The characterization of feedstocks is examined or estimated in terms of moisture content, Total Solids (TS) (%), Volatile Solids (VS) (% TS), Nitrogen content (% on a dry matter basis) and carbon to nitrogen (C/N) ratio. Based on these parameters, solid mass balances are made per category of raw material, calculating the annual inputs of TS (tn/y) and VS (tn/y), so as to estimate the biogas yield ( $\text{m}^3/\text{tn VS}$ ), according to the available feedstocks. At this step, fixed values are given for biogas yields usually based on relevant references or based on the execution of Biomethane Potential (BMP) trials in the laboratory.

### 29.3.3 Step 2: Energy Analysis

During this step, the biogas plant energy analysis is conducted. Taking into account the fixed values on biogas yield ( $\text{m}^3/\text{tn VS}$ ) set in the previous step and the daily VS input (tn/d), the daily biogas production ( $\text{m}^3/\text{d}$ ) is determined per raw material category. Consequently, the biogas production is calculated on an annualized basis ( $\text{m}^3/\text{y}$ ). Finally, assuming that the energy content of the biogas produced is  $6 \text{ kWh m}^{-3}$ , considering  $21.5 \text{ MJ m}^{-3}$  biogas (1 kWh = 3.6 MJ) (Abdeshahian et al. 2016), the annual energy production (kWh/y) is derived.

Furthermore, the electrical and thermal outputs for a CHP engine are determined. Assuming fixed design values regarding the electric efficiency (e.g. 40%) and the thermal efficiency (e.g. 45%) of the CHP engine, the following parameters are calculated: The Annual Electric Energy Production (kWhel./y) results from the Annual Energy

Production (kWh/y) multiplied by the chosen factor of Electrical Efficiency (%). Based on the CHP Engine specifications, with an annual availability of 7884 h y<sup>-1</sup> (or 90% availability), the Installed Electric Capacity (kwel.) is calculated. Moreover, the annual Thermal Energy Production (kWhth./y) results from the Annual Energy Production (kWh/y) multiplied by the pre-determined factor of Thermal Efficiency (%). Then, the Installed Thermal Capacity (kWth) is calculated by dividing the Annual Thermal Energy Production (kWhth./y) by the annual availability of 7884 h y<sup>-1</sup>.

Both parameters of installed thermal and electric capacity are a critical part of the economic viability assessment and can be translated into thermal energy adequate to replace the use of fossil fuels or electric power enough to meet the needs of a specific number of residences.

#### 29.3.4 Step 3: Technical Calculations

The third step includes the core technical calculations that are required to determine the suitable biogas plant size. Design parameters, related to the necessary equipment components and the technology to be applied are calculated. At the same time, critical operational parameters related to the AD process and efficiency rates are taken into account. An overview of the aforementioned parameters is given in Table 29.4.

#### 29.3.5 Step 4: Operational Income and Expenses

In step 4, calculations concerning the income and the operational expenses of the investment are determined. The operation of the biogas plant will create an income mainly due to the production of energy from the anaerobic treatment process. In particular, the

**Table 29.4** Technical specifications and operating parameters of AD plant.

Parameters related to equipment	Parameters related to operational conditions
Substrate Reception Tanks Volumes (m <sup>3</sup> )	Substrates Retention Time (days)
Required Digestate Storage Volume (m <sup>3</sup> )	Anaerobic Digesters Temperature (°C)
Storage Duration (months)	Hydraulic Retention Time (d)
Required Digestion Volume (m <sup>3</sup> )	Organic Loading Rate (kg VS/m <sup>3</sup> ·d)
Number of required digesters	Annual Digestate Production (tn/y)
Volume of Each Digester (m <sup>3</sup> )	VS Decomposition Percentage (%)
Silage Bunker Maximum Capacity (m <sup>3</sup> )	
Bunker Required Area (m <sup>2</sup> )	
Silage Bunker Number	
Required Area (m <sup>2</sup> )	
Decanter Solid Fraction (%)	
Amount of Solid Decanter Fraction (tn/y)	
Decanter Liquid Fraction (%)	
Amount of Liquid Decanter Fraction (tn/y)	

**Table 29.5** Sub-costs and incomes of the anaerobic digestion plant.

Plant Operational Income in €/y	Plant Operational Costs in €/y
Electric Energy Sale to the Public Grid	Personnel Cost
Thermal Energy Sale	Energy Cost
Solid Digestate Sale	Fuel Cost
Waste Management Gate Fees	Industrial Water Cost
	Raw Material Cost
	Maintenance Cost
	Insurance Cost

income can be generated from the supply of the electric energy to the public grid, the sale or on-site use of the thermal energy, the sale of the stabilized digestate produced and the gate fees related to waste management. The total income generated by the plant can be determined by considering all the above sources. The economic viability of a biogas project is also affected by the operating costs of the plant. Total operating costs are calculated taking into consideration the personnel that is needed to operate the plant, the energy and water consumption, the use of fuels, the utilization of raw material and the cost of maintenance of the equipment of the anaerobic digestion unit. In Table 29.5, the main categories of costs contributing to income and operational expenses are summarized.

In more detail, the electric energy sale to the public grid is determined on the basis of annual electric energy production (kWhel./y), after considering the electric energy feed-in tariff (€/kWh). In accordance, thermal energy sale is calculated by taking into account the annual thermal energy production (kWhth./y) and the selling price of heat (€/kWh). Incomes generated by solid digestate (€/tn) can vary depending on the quality and on the water content of the product. Earnings can be also generated from gate fees (€/tn). After having determined the incomes, evaluation of costs follows. The first cost to be determined is the personnel costs after considering the technical qualifications and personnel expertise needed for the safe and smooth operation of the plant. Typically, a plant manager, engineers, technical workers and accountants must be considered. Energy and water costs, associated with the needs of the processes involved, are calculated. For the determination of fuel consumption, the utilization of different fuel sources, such as fuel oil or natural gas that might be alternative or used in combination can be taken into account. Then, the costs are calculated by using the prices per unit. Regarding the raw materials costs, the composition of the feeding of the digester plays an important role. Depending on its type, the input can be provided either for free (e.g. if it is waste or manure) or after payment. For determining maintenance costs, the following categories must be taken into count: civil works maintenance cost; mechanical equipment maintenance cost; and CHP engine maintenance cost. These costs are usually calculated using standard coefficients, i.e. 0.5% upon the investment cost for civil works maintenance costs, 3.5% upon the investment cost for mechanical equipment maintenance cost, and 2.5% upon the cost of purchasing the CHP for the CHP engine maintenance cost. Finally, insurance cost is usually calculated by an appropriated percentage (1%), based on the equipment cost.

### 29.3.6 Step 5: Investment Analysis Indices

Apart from setting up a technically viable process, with high energy efficiency in accordance with the local environmental protection regulations, reassurance is crucial that the whole project is also economically feasible. Only in very special cases does this parameter not affect the decision of implementing a biogas plant project; as a general rule the financial analysis of the project should be addressed first, when examining such investments. Since bioenergy projects may have significant differences between each other, it is necessary to apply certain economic indices, so as to be possible to compare their financial performance.

To this direction, under Step 5, the investment analysis of the biogas plant is conducted, including data about the financing plan to be followed, which can be relied on own capital and/or foreign resources, e.g. bank loan schemes. The economic indicators used to examine and evaluate the financing scheme followed in the present work are summarized in Table 29.6.

The main investment analysis indices applied in the AD plant project are;

- Payback Period: is defined as the required period (in years) to break even on the initial investment. In case when a part of the initial investment comes from bank loan, then the payback period refers to break even on the own capital (but the bank loan capital expenditure should be also taken into account). By definition the smaller the payback periods the most financially attractive is the investment
- Net Present Value (NPV): is defined as the present value of all the annual payments. Because of the fact that it takes into account the current interest of the discount rate it considers also the time value of money, making NPV a highly popular index to compare different projects.

By definition the larger the NPV the most financially attractive is the investment. In case that the NPV is equal to zero, that means that the sum of all annual payments is equal to the return that the discount rate would provide.

- Internal Rate of Return (IRR): is defined as the discount rate that makes the NPV of the annual cashflows equal to zero. That means, that IRR is somehow the opposite

**Table 29.6** Indicators for investment analysis.

Financing parameters	Bank loan terms
Total Investment (€)	Initial Capital (€)
Public Subsidy (€)	Interest (%)
Own Capital (€)	Payback (y)
Own Capital in Cash (€)	
Own Capital in Bank Loan (€)	
<i>Investment Depreciation</i>	<i>Investment Evaluation</i>
Duration (years)	Own Capital Payback Period (y)
Amount (€/y)	Internal Rate of Return (%)
Net Present Value (€/y)	

index of NPV. IRR has the benefit that is not necessary to make a successful guess of the discount rate in order to complete the calculation (like in NPV calculation case).

By definition a project should be considered viable for implementation only if its IRR value is higher than the current bank discount rate. Among a series of projects, the most attractive is the one with the higher IRR value (Mackevičius and Tomašević, 2010).

Here, a financing scheme with the combination of own capital in cash (€), an amount of public subsidy (€) and a bank loan has been selected to be examined. The cashflow analysis is realized throughout the project lifetime (€), given a 20-year period for investment depreciation. The evaluation of depreciation of the fixed capital and operation costs are also included, considering civil works, electro-mechanical equipment and engineering costs for the same time period. The Payback Period, NPV and IRR are extracted through the above calculations.

### 29.3.7 Step 6: Investment Cashflows

The sixth and final step of the methodology followed comprises of the overall consideration of all the above-mentioned steps, namely are:

- the project mass and energy balance;
- calculation of basic technical and operational parameters;
- analysis of the operational income and expenses (OPEX);
- estimation the Capital Expenditure (CAPEX) of the project;
- estimation of the equipment, items, and services depreciation; and
- calculation of the annual bank payments depending on the bank loan interest and the loan payback period.

When these steps have been implemented, the annual investment cashflows calculation is simplified;

- calculation of the annual operational income;
- calculation of the annual operational expenses; and
- calculation of the annual bank loan payback (by adding the capital cost with the interest cost)

By subtracting operational expenses and bank expenses from the operational income, the project operational result is estimated. From the operational result, the annual depreciation must be also subtracted and from the last result the taxes. Finally, the net annual project cashflows are estimated. All the investment analysis indices are calculated on the net annual project cashflows.

## 29.4 Case Studies for Greece

In order to determine the economic viability of manure biogas plants, two scenarios were developed and compared. Those scenarios are set for the case of Greece in order to also show the importance of the policy context affecting the energy market. In particular, the projects analysis involves two different cases of 500 kW<sub>e</sub> capacity biogas plants; the first

one includes exclusively the mono-digestion of cattle manure, while the other focuses on the co-digestion of cattle manure with a byproduct from the cheese manufacturing process (cheese whey) and energy crops (maize silage).

#### 29.4.1 The Greek Policy Context

In line with the European Commission's Energy and Environmental State Aid Guidelines (EEAG) adopted in 2016, the Greek government adopted in 2016 the Law 4414/2016 related to the new support scheme for renewable energy sources (RES) and CHP generation. This law constitutes the main framework for the implementation of a new support structure for RES and CHP projects in Greece compatible with the EU Guidelines on State aid for environmental protection and energy 2014–2020. At the same time, it sets the roadmap for a gradual integration and participation of RES and CHP projects in the electricity market on an optimal cost-benefit basis for society and investors.

According to Law 4414/2016, for new (from 1 January 2016) RES projects in the Greek interconnected system or mainland grid the following shall apply:

- RES projects will participate in the electricity market and be subject to clearing and settlement procedures.
- Operating aid shall be granted in the form of a Feed-in Premium (FiP) (essentially a contract for difference) or, exceptionally, Feed-in Tariff (FiT) only for smaller RES installations (see below).
- The FiP, which is calculated as the difference between the Special Market Price and the applicable Reference Tariff (RT), will be paid monthly on the basis of a Feed-in Premium operating aid Contract (FiPC) signed between the producer and the Hellenic Electricity Market Operator.
- Any investment aid granted to a project to cover capital expenses will be taken into account to reduce the operating aid for that project. The reduction shall be made on the basis of the investment aid paid to the project company (Law 4414/2016, 2016; Watson Farley and Williams LLP 2016).

Wind farms with an installed or maximum output capacity below 3 MW; all other RES technologies with an installed or maximum output capacity below 500 kW; and demonstration projects which have started their operation after 1 January 2016 are exempted from the FiP scheme and the obligation to participate in the electricity market (Law 4414/2016, 2016; Watson Farley and Williams LLP, 2016).

Under the new support scheme RTs are set for each type of energy source and technology, on the basis of which the operating aid in the form of FiP or FiT is calculated; for example RT for biogas plants below 3 MW is equal to 220 €/MWh, for biomass gasification below 1 MW is equal to 194 €/MWh, and for biomass combustion plants below 1 MW is equal to €183/MWh. These RTs do not apply to projects which have entered into a FiPC or FiTC on the basis of a competitive bidding process; the RT for such projects is that derived from the competitive bidding process on a per-project basis, on the basis of which their FiP or FiT is determined (Law 4414/2016, 2016; Watson Farley and Williams LLP, 2016).

From 1 January 2017, operating aid will be granted to RES projects on the basis of competitive bidding processes. The projects exempted from FiP scheme and the obligation to

participate in the electricity market do not take part in the competitive bidding processes (Law 4414/2016, 2016; Watson Farley and Williams LLP, 2016).

Considering the above, based on the current legislative framework for developing biogas projects in the Greek energy market, the selection of projects with maximum capacity of 500 kWe is more favorable both in terms of the licensing procedure and the financial outputs.

#### Case Study #1: 500 kWe Biogas Plant with Cattle Manure Mono-Digestion

In the first assessment, the mono-digestion of cattle manure is examined. Based on the methodology analyzed in Section 29.2, the required amount of manure is calculated at 160 tonnes per day which is equal to 58 400 tonnes per annum. It is estimated that such an amount can be generated by 3500–4500 cattle. For cattle manure it is assumed that Total Solids (TS) content is 10%, Volatile Solids (VS) content is 80% (TS%), while biogas yield is 350 m<sup>3</sup>/tn VS. Such parameters are crucial for the precise estimations of the economic viability of the plant; that is why laboratory analysis must always be performed prior to the design of any biogas plant.

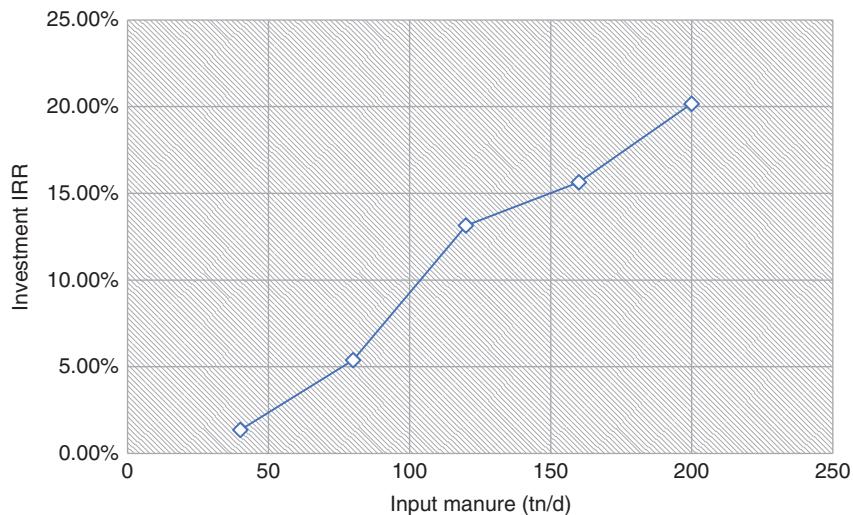
The determination of other design values is given in Table 29.7.

The anaerobic digestion process is performed under mesophilic conditions (temperature 35–38 °C) with HRT of 30 days. The plant will be equipped with two cylindrical primary digesters of 2 400 m<sup>3</sup> each and a secondary digestion tank of 5 000 m<sup>3</sup> volume. The OLR of the substrate in the digesters will be 2.7 kg VS/m<sup>3</sup>\*d.

It is assumed that manure cost is zero. Moreover, digestate produced from the digestion process is expected to be equal to 56 000 tonnes and can be applied as a biofertilizer in the fields.

**Table 29.7** Assumptions for the assessment of economic viability of Case Study 1.

Parameter	Values
CHP biogas engine electric efficiency (%)	40
CHP biogas engine thermal efficiency (%)	45
Biogas plant annual availability %	90
Biogas plant annual availability (hours per year)	7884
Electric consumption (% of the gross produced electric energy)	15
Mean plant distance from the manure production facilities (km)	40
Electric energy cost (€/kWh)	0.01
Process water consumption (m <sup>3</sup> /y)	1.500
Process water cost (€/m <sup>3</sup> )	1.1
Personnel required for plant operation	7 (2 engineers, 2 technicians and 3 for logistic purposes)
Feed-in-Tariff (€/MWh)	220



**Figure 29.2** IRR vs Input Manure for Case Study 1.

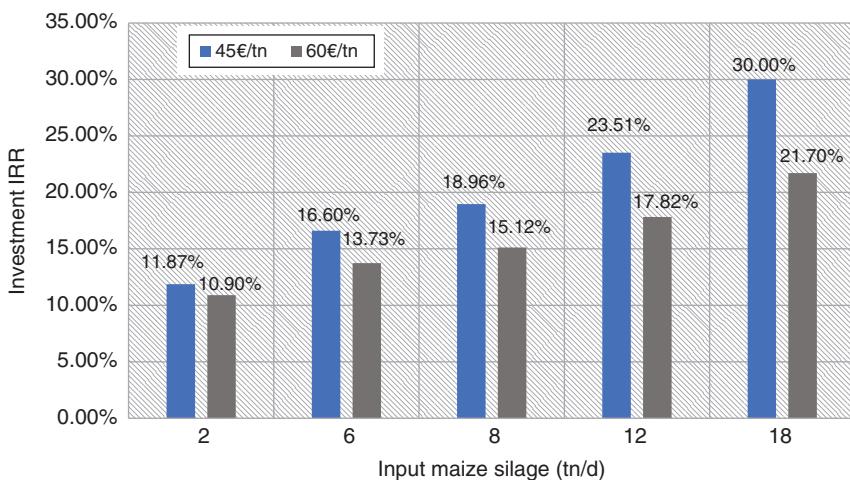
Considering that the investment operational income derives only from the sale of green energy at a price equal to the Feed-In-Tariff and that no extra state subsidies are provided to the plant, the main investment analysis parameters of the plant are: (i) the payback period: 8.5 years, (ii) IRR: 15.5%, and (iii) NPV: 970.000 € (7% discount rate).

Being so, the investment can be considered as quite attractive, as it takes advantage of the scale economies. However, there is a significant obstacle to its implementation which is related to the particular characteristics of Greece as in the Greek territory the possibility of having access to such large amounts of manure at a distance of 40 km is quite low. Considering this, when input is getting smaller, the effect of scale economy is eliminated to such an extent that the project turns into non-feasible. These conclusions are presented in Figure 29.2 in which IRR values as function of daily input manure is presented.

#### Case Study #2: 500 kWe Biogas Plant with Combined Digestion of Cattle Manure and Biomass

In the second assessment, the process of combined anaerobic digestion is examined. As it has already been stated, co-digestion process offers significant benefits in technical and management issues. In this case cattle manure is one of the three substrates while the other two are cheese whey and maize silage. Cheese whey is an effluent that is produced during the cheese manufacturing process. Maize silage is a plant raw material of high moisture content which is normally used as an animal feed, but during the last 15 years is very popular among biogas plant operators.

Based on the methodology analyzed in Section 29.2, the required amount of animal manure is calculated at 68 tonnes per day (annually equal to 24 820 tonnes). This amount is derived from 1600–1700 cattle. The biogas plant is fed also with 40 tonnes per day (14,600 tonnes per year) of cheese whey along with 6 tonnes per day (2190 tonnes per year) of maize silage. The required amount of maize silage derives from the cultivation of approximately 35–37 hectares.



**Figure 29.3** Effect of the price of maize silage to the investment for Case Study 2.

It is also assumed that: (i) cattle manure has 10% TS; 80% VS (TS%); and biogas yield 350 m<sup>3</sup>/tn.VS, (ii) cheese whey has 5% TS; 90% VS(TS%); and biogas yield 750 m<sup>3</sup>/tn.VS, and (iii) 35% TS, 95% VS(TS%) and biogas yield 700 m<sup>3</sup>/tn VS. As it can be easily noticed high VS content along with its significant biogas yield make maize silage as an ideal feedstock material for biogas production. However, unlike animal manure, maize is not a waste material; it is actually a valuable feedstock for the animal breeding industry, so for its acquisition the relevant cost must be calculated. Because of the fact that maize silage has other competitive uses its price is not stable and it is safer to utilize it as a complementary feedstock and not as the main substrate. This volatile trend in the input material purchase price has a significant effect on project viability, as it is obvious from Figure 29.3.

The effect of the input amount of maize silage to the project installed capacity is presented in Figure 29.4.

Values for CHP biogas engine electric efficiency and thermal efficiency, biogas plant annual availability, electric consumption, mean plant distance from the manure production facilities, electric energy cost, process water consumption, process water cost, personnel required for plant operation, and Feed-in-Tariff are as defined in Table 29.7 above.

The anaerobic digestion process is also performed in mesophilic conditions with HRT equal to 30 days and OLR equal to 2.7 kg VS/m<sup>3</sup>\*d. To this end, the plant will be equipped with two cylindrical primary digesters of 1700 m<sup>3</sup> each and a secondary digestion tank of 5000 m<sup>3</sup> volume.

Manure and cheese whey will be acquired for free, while maize silage will be purchased at the cost of 45 €/ton. From the digestion, there will be produced annually around 40.000 tonnes of digestate, which can be applied as a biofertilizer in the fields.

Considering that the investment operational income derives only from the sale of green energy at a price equal to the Feed-In-Tariff and that no extra state subsidies are provided to the plant, the main investment analysis parameters of the plant are: (i) the payback period: 7.4 years, (ii) IRR: 16.7%, and (iii) NPV: 992.000 € (7% discount rate).

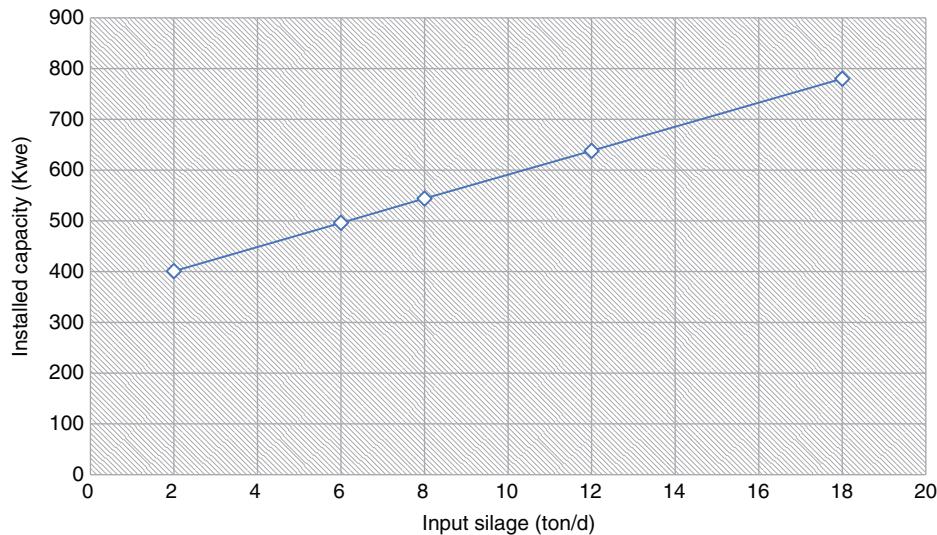


Figure 29.4 Effect of the input maize silage to the project installed capacity for Case Study 2.

Being so, the second case study is more attractive than the mono-digestion in the first case study. In addition, mixed feedstock (manure, cheese whey, and silage) is more balanced than in the mono-digestion where large amount of cattle livestock should be accessible in the limited area of 40 km. Thus, the second scenario, apart from being more viable as an investment, can be more easily implemented at the Greek territory. However, even here, if the input amounts become less, then the effect of scale economy is eliminated to such a level that the project turns into non-feasible.

## 29.5 Conclusions

Biogas is a promising renewable energy source which can be produced through the anaerobic digestion process of various substrates including manure; however, in order to proceed to an investment, a techno-economic assessment study has to be initially implemented. Therefore, the assessment must take into account feedstock availability in terms of quantity and physicochemical characteristics. Based on these characteristics and other technical and financial parameters, the annual biogas production and the needed equipment must be determined. Accordingly, all the cost categories including operational incomes and expenses of the biogas plant must be calculated. Finally, the main economic indices such as payback period, net present value, and internal rate of return must be estimated in order to define the economic viability of the AD plant.

Based on the case studies examined, for the production process to be economically viable various parameters must be considered including the policy and legislative framework affecting energy market, the types and the characteristics of feedstock and

feedstock availability. In Greece the regulatory framework for developing biogas projects promotes those with maximum capacity of 500 kW<sub>e</sub>, both in terms of the licensing procedure and the financial outputs. The study revealed that co-digestion of manure, cheese whey, and maize silage is a more attractive option given the availability of the feedstock and the economic viability of the investment (NPV: 992.000 € (7% discount rate), IRR: 16.7%, payback period: 7.4 years). The mono-digestion process of high volumes of manure (>50 000 tn/y) can also be a viable solution (NPV: 970.000 € (7% discount rate), IRR: 15.5%, payback period: 8.5 years), taking advantage of the economies of scale, especially when considering that its purchase is without cost and the relevant logistics are limited. In view of the above, the investment in anaerobic digestion process of manure can be a sustainable and economically viable solution for achieving environmental protection and profit from renewable energy production.

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## 30

# Fruit, Nut, Cereal, and Vegetable Waste Valorization to Produce Biofuel

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*Any vegetable matter capable of fermentation, crop residues, grasses, farm waste, and city garbage is a potential feedstock for the production of ethanol*

Alexander Graham Bell (1917)

## 30.1 Introduction

Reducing food waste is one of the strategies which the Food and Agricultural Organization (FAO) is implementing to achieve its specific target in the Sustainable Development Goals (SDGs), designed to guarantee food security for the rapidly growing global population. It was estimated that between 44% and 47% of the total food waste generated by households is linked to the consumption of fresh fruits and vegetables (Group et al. 2015). However, a sizable portion of waste generated by households (23–28%) originates from inedible parts of fresh fruits and vegetables such as the skin, peelings, and trimming (De Laurentiis et al. 2018). These are considered inevitable waste that will be generated, no matter what preventive measure is put in place, unless consumption patterns change. It is, therefore, imperative to consider processing and treatment techniques to manage and leverage the potential of these resources. This may include their valorization to value-added products including biofuel.

### 30.1.1 Types of Food Waste

The FAO, as well as other agencies recommend sufficient intake of fruits, vegetable, nuts, and cereals as an integral part of our daily meal. It is recommended that adults consume at least five servings of fruits and vegetables per day, excluding starchy vegetables, to

optimize the potential benefits and reduced risks of chronic diseases and for body weight management. In general, dietary recommendations are based on the associations between food groups and health outcomes. Table 30.1 below shows a typical classification of some fresh fruits, cereals/grains, vegetables, and nuts of commercial significance. A sizable portion of these is lost during processing, transportation, and consumption.

**Table 30.1** Classifications of fresh fruits, vegetables, nuts, and cereals from plant origin.

Class	Type	Group	Food
Plant origin	Cereal grains and legumes/pulses	Cereal grains	Rice (brown rice), wheat, barley, rye, Corn (maize, including popcorn and sweet corn), buckwheat, other cereal grains
		Legumes/pulses	Soybeans, dry, Beans, dry (including butter beans, cowbeans (red beans), lentil, lima beans, pegia, sultani, sultapya, and white beans), peas, broad beans, peanuts, dry, Other legumes/pulses
	Vegetables	Potatoes	Potato, taro, sweet potato, yam, konjac, other
		Sugar sources	Sugar beet, sugarcane
		Cruciferous vegetables	Japanese radish, roots (including radish), turnip, roots (including rutabaga), horseradish, watercress, Chinese cabbage, cabbage, brussels sprouts, kale, <i>Komatsuna</i> (Japanese mustard spinach), <i>Kyona</i> , <i>Qing-geng-cai</i> , cauliflower, broccoli, other cruciferous vegetables
		Composite vegetables	Burdock, salsify, artichoke, chicory, endive, <i>Shungiku</i> , lettuce (including cos lettuce and leaf lettuce), Other composite vegetables
		Liliaceous vegetables	Onion, Welsh (including leek), <i>Nira</i> , garlic, asparagus, multiplying onion (including shallot), Other liliaceous vegetables
		Umbelliferous vegetables	Carrot, celery, <i>mitsuba</i> , parsnip parsley, other umbelliferous vegetables
		Solanceous vegetables	Pimiento (sweet pepper), eggplant, tomato, other solanceous vegetables
		Cucurbitaceous vegetables	Cucumber (including gherkin), pumpkin (including squash), oriental pickling melon (vegetable) water melon, melons, <i>makuwauri</i> melon, other cucurbitaceous vegetables
		Legumes vegetables	Peas, immature (with pods), kidney beans, immature (with pods), green soybeans
		Mushrooms	Button mushroom, <i>Shiitake</i> mushroom, Other mushrooms
		Miscellaneous vegetables	Spinach, okra, ginger, bamboo shoots

**Table 30.1** (Continued)

Class	Type	Group	Food
		Vegetables not categorized in the above-listed vegetable groups.	Other vegetables
Fruits	Citrus fruits		<i>Unshu</i> orange, pulp, Citrus <i>natsudaidai</i> , pulp, Citrus <i>natsudaidai</i> , peels, Citrus <i>natsudaidai</i> , whole, lemon, orange (including navel orange), grapefruit, lime, other citrus fruits
	Pome fruits		Apple, Japanese pear, pear, quince, loquat
	Stone fruits		Peach, nectarine, apricot Japanese plum (including prune), mume plum, cherry
	Berries		Strawberry, raspberry, blackberry, blueberry, cranberry, huckleberry, other berries
	Grape		Grape
	Assorted tropical and subtropical fruits		Japanese persimmon, banana, kiwifruit, papaya, avocado, pineapple, guava, mango, passion fruit, date
	Fruits not categorized in the above-listed fruit groups.		Other fruits
Nuts and seeds	Oil seeds		Sunflower seeds, sesame seeds, safflower seeds, cotton seeds, rapeseeds, other oil seeds, ginkgo nut, chestnut, pecan, almond, walnut, other nuts
	Seed for beverages		Coffee beans, cacao beans
Spices and herbs	Spices		Sansho (Japanese pepper), Other spices (refer to all spices, except horseradish, wasabi (Japanese horseradish) rhizomes, garlic, peppers chili, paprika, ginger, lemon peels, orange peels (including navel orange), yuzu (Chinese citron) peels and sesame seeds)
	Herbs		Spearmint, peppermint, other herbs (refer to all herbs, except watercress, nira, parsley stems and leaves, celery stems and leaves)
Tea			Tea (green, black, oolong, and wulung tea), tea (black, oolong, and wulung tea) (except unfermented tea)
Hop			Hop

In general, food waste is defined as food originally produced for human consumption that leaves the food supply chain. In the study conducted by De Laurentiis et al. (2018), with considered food waste generated from the manufacturing stage up to consumption, it was estimated that approximately 180 kg per person per year of food is wasted annually in the European Union, of which 101 kg per person per year is generated after the food

reaches the consumer. This was further broken down to 76 kg for households and 25 kg for the food service industry. It is thus obvious that households are the highest contributor to food waste. In China, the high ratio of organic waste is attributed to the consumption of fruits and vegetables. This suggests that as industry grows, and wealth increases and living standards improve, fruit and vegetable wastes will continue to occupy a high ratio of the waste in China (Zhang et al. 2010). In Australia, over-supply of fruits and vegetables was reported as one of the reasons for wastage. Losses as high as 286 tons per year were reported (Ghosh et al. 2017). Majority of fruit and vegetable loss occur at the market and consumer levels.

Waste has been categorized in two groups, namely: unavoidable and avoidable waste. Unavoidable waste is often assessed at product level by considering the inedible fraction vis-à-vis the total amount of that product purchased in a given period. The generation of unavoidable waste is directly linked to inedible physical components, such as orange or banana peel. Unavoidable waste arises from food preparation or consumption, that is not and has never been edible under normal circumstances. Avoidable waste on the other hand, is a consequence of the behavioral choices of consumers such as poor planning, storage period, package properties, cooking skills, household size, high availability of cheap food and hectic lifestyles (Ponis et al. 2017; Parfitt et al. 2010; Roodhuyzen et al. 2017; Thyberg and Tonjes 2016). These two categories of waste can be quantified using the unavoidable waste intensity (UWI) and avoidable waste intensity (AWI) of a product. The UWI or AWI is defined as the share in terms of mass of unavoidable/avoidable waste out of the total purchased amount of that product. These terms are illustrated by Eqs. (30.1) and (30.2) below:

$$UWI (\%) = \frac{\text{unavoidable waste [Mt]}}{\text{total purchases [Mt]}} \quad (30.1)$$

$$AW (\%) = \frac{\text{avoidable waste [Mt]}}{\text{total purchases [Mt]}} \quad (30.2)$$

where, the numerator of Eqs. (30.1) and (30.2) is the total mass of that product which is wasted unavoidably or avoidably (e.g. an uneaten apple simply thrown away), respectively. The denominator in both equations is the amount of that product purchased in a given period. The UWI of fresh fruits and vegetable products is considered equal to the inedible fraction of that product as provided by food composition databases (Marletta et al. 2000; Public Health England 2015). These values are presented in Table 30.2 for fresh fruits and vegetables. Several factors were identified for deviations in the two sets of databases. For instance, in the case of asparagus, Public Health England states that the base is removed before measuring the edible fraction and thus, its value of inedible fraction will be quite a lot lower compared to the other study. Contributions of diverse types of fruits and vegetables to unavoidable and avoidable wastes are shown in Figure 30.1a,b, respectively.

There are some connections between some product characteristics and the generation of avoidable waste. The perishability of a product and its average price are expected to influence AWI. This is based on the fact consumers might be inclined to buy more than necessary cheaper commodities and as consequence, leave to spoil when compared to more expensive products. In the same vein, highly perishable products are inclined to be left as waste if consumers store them for too long or under suboptimal conditions.

**Table 30.2** Inedible fraction of fruits and vegetables (%) based upon literature values (Marletta et al. 2000; Public Health England 2015).

Commodity	Inedible fraction (Marletta et al. 2000)	Inedible fraction (Public Health England 2015)
Apples	15	9
Apricots	6	7
Avocados	24	26
Bananas	35	34
Blueberries	0	0
Cherries	14	14
Cranberries		2
Currants	2	0
Figs	25	
Gooseberries		2
Grapefruit	30	50
Grapes	6	4
Kiwis	13	26
Lemons and limes	36	37
Oranges	20	22
Peaches and nectarines	9	7
Pears	16	10
Persimmons	3	16
Pineapples	43	49
Plums and sloes	10	6
Quinces	21	
Raspberries	0	0
Sour cherries	15	14
Strawberries	6	2
Tangerines, mandarins, clementines, satsumas	17	23
Artichokes	66	57
Asparagus	43	47
Cabbages and other brassicas	16	22
Carrots and turnips	18	17
Cauliflowers and broccoli	42	20
Chillies and peppers, green	18	15
Cucumbers and gherkins	23	3
Eggplants (aubergines)	8	19

(Continued)

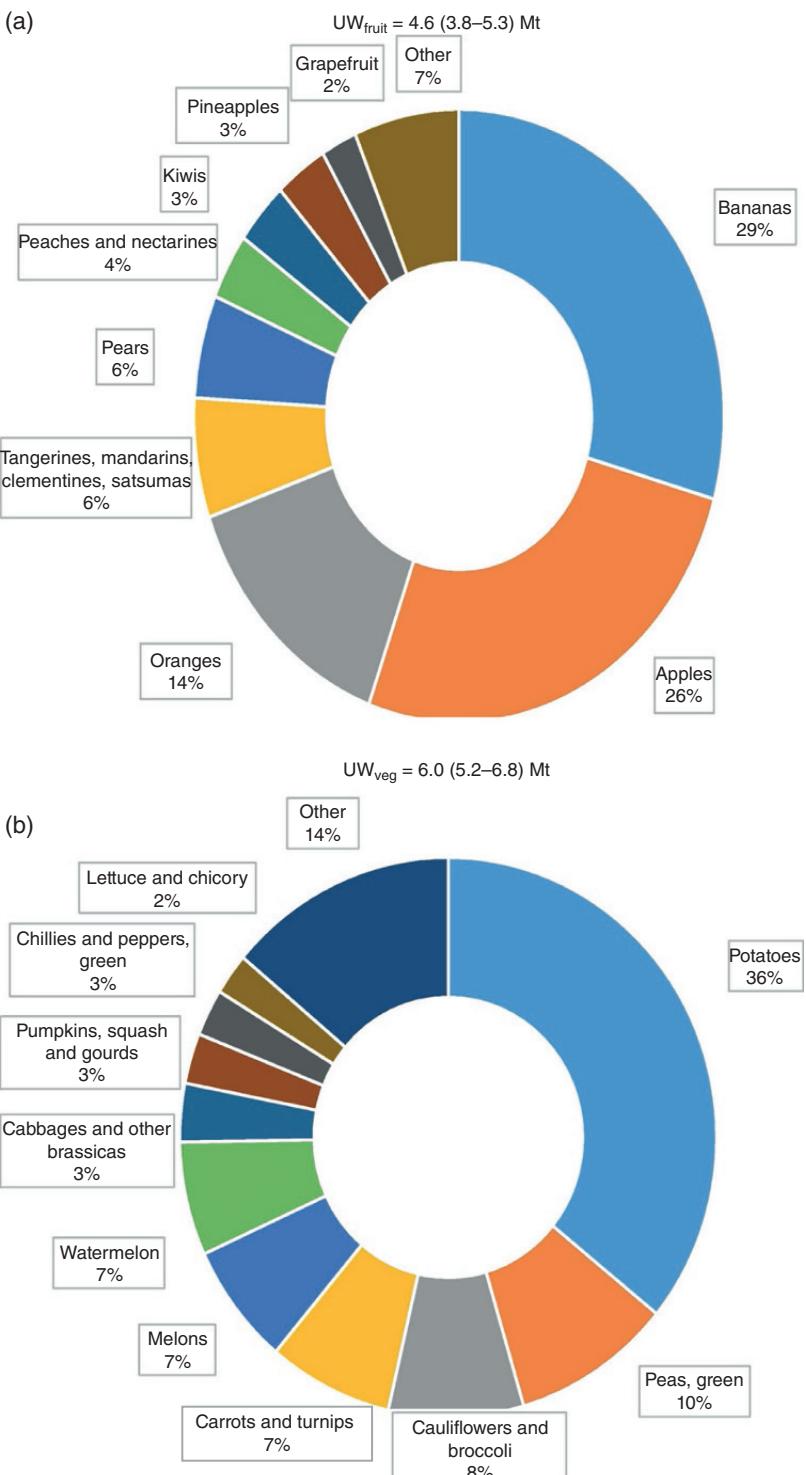
**Table 30.2** (Continued)

Commodity	Inedible fraction (Marletta et al. 2000)	Inedible fraction (Public Health England 2015)
Garlic	25	21
Leeks, other alliaceous vegetables	23	17
Lettuce and chicory	8	5
Maize, green		44
Melons	53	46
Mushrooms and truffles	4	11
Okra		
Onions, dry	17	7
Onions, shallots, green	7	4
Peas, green	69	
Potatoes	17	17
Pumpkins, squash, and gourds	16	21
Spinach	17	30
String beans	5	0
Sweet potatoes		28
Tomatoes	0	0
Watermelon	48	54

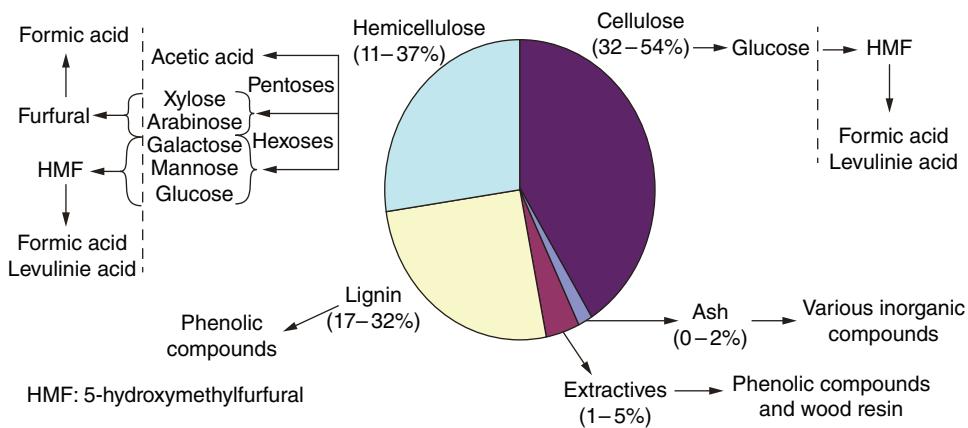
Product's perishability is generally described by its shelf life, that is, the time until a perishable product becomes unacceptable to consumers under given storage conditions. Fruits, vegetables, nuts, and cereals are living tissues up to the point of consumption and their shelf life are dependent on such factors as storage conditions, stage of ripening, harvesting time, growing conditions, and packaging type (Organization 2015). Classification of waste flow as unavoidable and avoidable waste present a better understanding of the drivers of food waste which will ultimately make it possible to create a suitable model, especially for prevention and accounting of the avoidable component of waste flow.

### 30.1.2 Current Use and Disposal of Fruit, Vegetable, Nut, and Cereal Waste

The production and transformation of fruits, vegetables, nuts, and cereals generate huge volumes of solid waste and wastewater (Valta et al. 2017). Solid waste includes peel waste, seeds, membrane residues, and inedible final products, while wastewater typically contains several types of liquid waste including condensate, press liquor, wash water, and other process solutions. Most often, the solid waste components are treated via composting, whereas the liquid waste is pretreated, and eventually treated in conventional ponds in combination with municipal wastewater (Amor et al. 2012; Nasr et al. 2014; Katerina Valta et al. 2015). Composting is presently explored for disposal of solid waste arising



**Figure 30.1** Contribution of different types of fruits and vegetables to the total unavoidable wastes of fresh fruits and vegetables De Laurentiis et al. (2018).



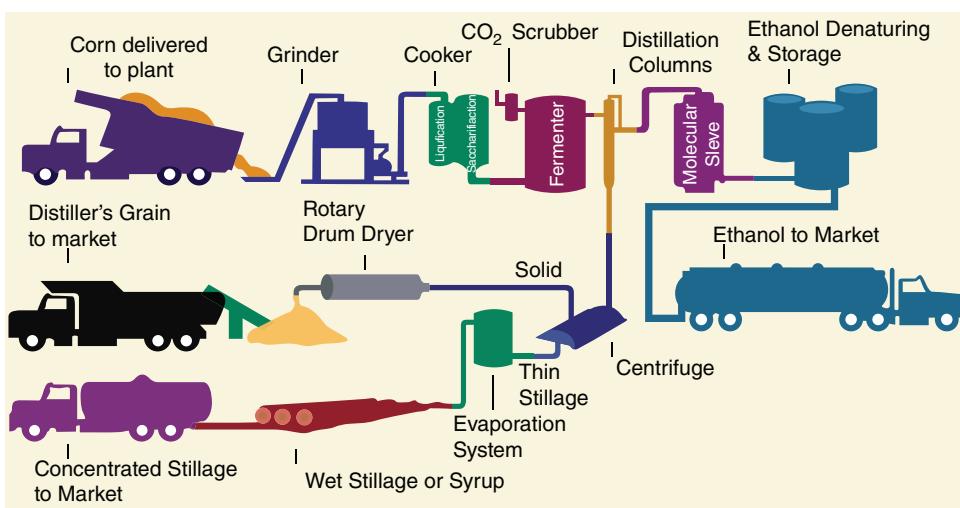
**Figure 30.2** Major components of lignocellulosic plant materials and their potential hydrolysis products and further degradation compounds (compounds behind the dashed lines) Guo et al. (2015).

from fruits, vegetables, nuts, and cereals. It involves spreading the compost on fields, and the conversion of all the carbon contents into  $\text{CO}_2$ , with a yearly emission of over 54.2 t. On the other hand, liquid wastes are primarily pretreated with hydrogen peroxide to oxidize sulfite and the pH value adjusted to neutrality via the use of sodium hydroxide. Following the pretreatment procedure, the organic content in the solid wastewater is oxidized using conventional biological process. About 90% of solid contained in the waste water are solids and monosaccharides (glucose). This approach leads to generation of  $\text{CO}_2$  emission of well over 380 t per year in a single plant.

Present waste treatment of fruit, vegetable, nuts, and cereals via composting and landfills or dumpsites for solid waste component and pretreatment/biological treatment of liquid wastes are not only costly but involve the introduction of toxic chemicals for wastewater treatment ( $\text{H}_2\text{O}_2$  and  $\text{NaOH}$ ) and net  $\text{CO}_2$  emissions into the environment. The valorization of these resources into biofuel and other co-products may be both economically and environmentally sound strategies. The benefits of using feedstocks from waste generated from fruits, vegetables, nuts, and cereal is huge in terms of savings from landfill diversion, electricity production, and substantial savings in the cost of raw materials for biofuel feedstock.

## 30.2 Biofuel

Biofuel or in general, bioenergy, is the energy generated in stored biomass. Biofuel is classified into three main groups, namely: solid, liquid, and gaseous forms. These include; bioethanol, biodiesel, etc. In fact, biofuel has been utilized for cooking, heating, and lighting since the dawn of humans. About 22 billion gallons of bioethanol is produced annually from food crops whilst biodiesel from oil seeds has reached production capacity of 5670 million gallons per year (Guo et al. 2015). It is expected that global development and utilization of biofuels will be ~30% of the world's energy demand by 2050. Fossil fuels are still the dominant energy sources today, meeting over 80% of the world's energy



**Figure 30.3** A technical flow chart of bioethanol production from corn (vegetable/cereal grain)  
Renewable Fuels Association (2013).

demand (Guo et al. 2015; IEA 2013). However, fossil fuels are limited in reserve and are non-renewable in addition to the enormous amounts of greenhouse gases released from their utilization. Thus, the most countries and jurisdictions are investing in the development and utilization of renewable energy such as biofuels in order to mitigate disastrous climate change effects (IPCC – Intergovernmental Panel on Climate Change 2013). Bioethanol generated from agricultural wastes can be used as a gasoline substitute to power petrol engine automobiles (Figure 30.3).

There are over 600 million passenger cars traveling the roads around the world and consuming approximately 930 million gallons of gasoline daily (EIA (U.S. Energy Information Administration) and U.S. Energy Information Administration 2013). This consumption pattern has generated serious environmental and socioeconomic impact. At the current consumption pattern, the supply of crude oil will only be able to last for another 45 years, and thus the need to develop renewable sources of fossil fuel substitutes. Appropriate adjustments of existing fermentation technology will generate adequate bioethanol from lignocellulosic bio-waste as an alternative from bioethanol from human food and animal feeds. As researchers continued to explore bioethanol production from lignocellulosic biomass. Thermostable cellulases that retain high cellulose-degrading activity at  $>70^{\circ}\text{C}$  were identified. These enzymes can be conveniently applied in cellulosic bioethanol production, thereby reducing the costs for preparing cellulosic enzymes. In the same vein, new yeast strains for more effective ethanol fermentation have been developed to further pave the way for effective commercialization of this process. Thermo-chemical transformation is another emerging process technology for bioethanol production from lignocellulosic agricultural wastes. The waste from fruits, vegetables, nuts, and cereals is first gasified to produce syngas (a mixture of H<sub>2</sub> and CO) which is subjected to some microorganisms in a specially-designed fermenter to generate bioethanol. Ethanol yields of up to 50% of the syngas mass have been reported (Badger 2002).

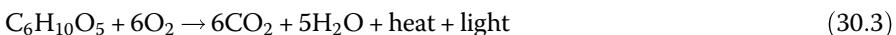
**Table 30.3** Biofuel from fruits, vegetables, nuts, and cereals (del Campo et al. 2006; Alshammari et al. 2011; Esteban et al. 2007).

Plant	Kind	Part used	Method	Properties of biofuel/ General comments
<i>Fruit</i>				
Pine apple	Waste	Peels	Acid hydrolysis/ fermentation	Bioethanol
Banana	Waste	Whole	Acid hydrolysis/ fermentation	Bioethanol
Butter fruit	Waste	Whole	Acid hydrolysis/ fermentation	Bioethanol
Jack fruits	Waste	Whole	Acid hydrolysis/ fermentation	Bioethanol
Cashew fruit	Waste	Whole	Acid hydrolysis/ fermentation	Bioethanol
<i>Vegetable</i>				
Potato	Waste	Whole/ Peel	Enzymatic hydrolysis	Bioethanol
Sweet potato	Waste	Hole/ Peel	Enzymatic hydrolysis	Bioethanol
Yam	Waste	Whole/ Peel	Enzymatic hydrolysis	Bioethanol
Cabbage	Waste	Whole	Enzymatic hydrolysis	Bioethanol
Cauliflower	Waste	Whole	Enzymatic hydrolysis	Bioethanol
<i>Nuts</i>				
Wheat grains	Waste	Whole	Acid hydrolysis/ fermentation	Bioethanol
Wheat-Distiller's dried grains with solubles (DDGS)	Waste	Whole	Acid hydrolysis/ fermentation	Bioethanol
Maize	Waste	Whole	Acid hydrolysis/ fermentation	Bioethanol
Maize DDGS	Waste	Whole	Acid hydrolysis/ fermentation	Bioethanol
Rapeseed	Waste	Whole	Acid hydrolysis/ fermentation	Bioethanol

### 30.2.1 Solid Biofuel

Solid biofuel includes: firewood, wood chips, wood pellets, charcoal, and other plant materials. Before the advent of fossil fuels, firewood was the predominant fuel for domestic cooking, heating, and lighting. Fire is generated through the combustion of bio-carbon

compounds at elevated temperatures ( $\sim 260^\circ\text{C}$ ) in the presence of oxygen as depicted in Eq. (30.3) below.



The energy content of firewood is approximately  $15 \text{ MJ kg}^{-1}$  which is almost half the energy of fossil fuels (Guo et al. 2015; IPCC – Intergovernmental Panel on Climate Change 2013). Wood chips from tree trunks and branches have been increasingly used for heating (bio-heat) and electricity generation (bio-power). A typical woodchip boiler installed in Colgate University (Hamilton, NY) consumes 20 000 t of locally produced wood chips per year and providing  $\sim 75\%$  of the heat and hot water demand of the campus (Guo et al. 2015). Wood pellets, a processed form of wood chips provides  $\sim 18 \text{ MJ m}^{-3}$  of energy. Interestingly, fruits, vegetables, grasses, crop residues, nutshells, and cereals can also be efficiently processed into wood pellets. For instance, mango tree has been used to produce biofuel for electrochemical renewable energy in fuel cell (Paul and Upendra 2016). Apple, pear, and plum trees have been used as solid biofuels. The woods obtained from these fruit trees could generate energy potential of 191 000 MJ/hectare (Winzer et al. 2017). Also, oil palm frond and the woody biomass of palm tree have been reported to be useful bioenergy feedstock (Matali et al. 2016). Automatically-fed wood pellet stoves are used for space heating with energy efficiency of nearly 80% (US Department of Energy 2013). The energy yield of charcoal is  $\sim 35\%$  but with an energy content of  $28\text{--}33 \text{ MJ kg}^{-1}$ . Charcoal is renowned to burn without flame and smoke, capable of achieving a temperature as high as  $2700^\circ\text{C}$  (Antal Jr and Grønli 2003). World annual production and utilization of charcoal remains  $\sim 51$  million tons since 2012 (Van Gerpen 2005).

### 30.2.2 Liquid Biofuel

Liquid biofuels include ethanol derived from fermenting plant biomass. The abundance of potential feedstock for production of ethanol was highlighted by Alexander Graham Bell in 1917: "any vegetable matter capable of fermentation, crop residues, grasses, farm waste, and city garbage." In 1913 ethanol was tested as an engine fuel well before the commercial production of gasoline from fossil. The American inventor, Samuel Morey designed an internal combustion engine in 1826 that was fueled by ethanol. Global bioethanol production as at 2013 was 23.4 billion gallons, with 56.8%, 26.7%, 5.9%, 3.0% and 2.1% contribution from US, Brazil, Europe, China, and Canada, respectively. The U.S. invested 42% (114 million tons/year) of its harvested corn grains in bioethanol production to replace 10% of its gasoline demand (Energy Information Administration and U.S. Energy Information Agency 2013).

Biodiesel is another important liquid biofuel. Diesel, in general, is a  $\text{C}_8\text{--C}_{25}$  hydrocarbon derived from fossil fuel by fractional distillation at  $200\text{--}300^\circ\text{C}$ . Diesel has an energy content of  $38.3 \text{ MJ l}^{-1}$  which is slightly higher than that of gasoline ( $34.7 \text{ MJ l}^{-1}$ ). It is an essential fuel for diesel engine and heavy-duty vehicles such as tractors, trucks, military carriers, construction machines, and mining equipment. Diesel is also used for home heating (U.S. Department of Energy 2015). Biodiesel is the alternative response and a petro-diesel substitute arising from the world's dwindling petroleum reserves. Biodiesel is a yellowish liquid biofuel derived from vegetable oil, animal fats, waste grease, or algae

(Van Gerpen 2005). The chemical structure of biodiesel can be described as a mono-alkyl ester of “trans-esterified” fats, oils, and greases (FOG) in the presence of alcohol and catalyst. The physical-chemical properties of biodiesel vary with the feedstock, type of alcohol and catalyst used. In general, biodiesel demonstrate a specific gravity of 0.873–0.884, cloud point of −4 to 14 °C, flash point of 110–190 °C, kinematic viscosity of 3.8–4.8 mm s<sup>−2</sup>, centane number of 50–62 and energy density (heating value) of 38–45 MJ kg<sup>−1</sup> (~90% of heating value of petro-diesel) (Hoekman et al. 2012).

Another important liquid biofuel is pyrolysis bio-oil which is obtained from elevated heating (300–900 °C) of plant biomass in the absence of air. Typically, pyrolysis of plant materials results in three products including biochar (the black solid residue), bio-oil (the brown vapor condensate), and syngas (the uncondensable vapor). Almost all organic residues can be employed produce pyrolysis bio-oil, such as wood, crop straw, sugar cane bagasse, switchgrass, peanuts hulls, and poultry litter. Crude pyrolysis bio-oil contains more than 300 compounds including colloidal char particles and water. Some components of crude pyrolysis bio-oil include alcohols, acids, aldehydes, ketones, esters, sugars, phenols, furans, alkenes, syringols, guaiacols, nitrogen containing compounds, and aromatics. Crude pyrolysis bio-oil is quite unstable, corrosive, viscous, immiscible with hydrocarbon fuels, low in energy density and difficult to ignite arising from its high moisture content (Vamvuka 2011; Ringer et al. 2006; Czernik and Bridgwater 2004; Junming et al. 2008). Moderately upgraded bio-oil can substitute heavy fuel oils as diesel and No. 2 heating oil to power static appliances including, furnaces, boilers, engines, and electrical generators. Crude bio-oil can be directly burned industrially using atomization techniques. Pyrolysis bio-oil is also an important feedstock for valuable chemicals, lubricants, preservatives, paints, stabilizers, binders, and thickeners.

Drop-in biofuels are another type of liquid biofuel that is presently in the research and development stage, intended to meet the existing petrol distillate fuel specifications without damaging the elastomer and metallic parts of engines at higher blending rates (Guo et al. 2015). Bioethanol and biodiesel have higher oxygen contents and greater dissolution capabilities than petrol fuels and at blending rates >20%, biofuel blends can damage the infrastructural components of engines including the elastomer and metallic parts. Suitable candidates for drop-in biofuel include butanol, liquefied biomass, syngas complex, and sugar hydrocarbons. Lignocellulosic sugars are easily transformed into petrol fuel-like hydrocarbon fuels via ruthenium diphosphine catalyst dehydrogenation and cyclization reactions (Duan et al. 2013; Dowson et al. 2013). Possible commercialization of this technique is in progress for probable modification of bioethanol plant to produce biobutanol.

### 30.2.3 Gaseous Biofuel

Gaseous biofuels are a renewable gaseous fuel alternative to natural gas. The energy content of natural gas is 53.3 MJ kg<sup>−1</sup> and consists of methane (95%), ethane (5%), and propane, butane, nitrogen, and carbon dioxide in trace amounts. Natural gas is used extensively for cooking, heating, transportation, electricity generation, and industrial energy. Biogas is generated by anaerobic digestion of organic waste including fruits, vegetables, nuts, and cereal wastes. Crude biogas consists of 60–65% methane, 30–35% CO<sub>2</sub> and a trace amount of water vapor, H<sub>2</sub> and H<sub>2</sub>S and others. CO<sub>2</sub>, H<sub>2</sub>S and other impurities are purified to produce biogas (biomethane) as an alternative to natural gas

(Niesner et al. 2013; Radu et al. 2017). Synthesis gas (syngas) is another important gaseous biofuel made up of a mixture of carbon monoxide, hydrogen, and CO<sub>2</sub>, derived from gasification or pyrolysis of plant materials (wood). Crude syngas contains 48% N<sub>2</sub>, tar and H, S in tiny amounts. Syngas is used to generate electricity and in its purified form it is a feedstock for synthesizing transportation fuel, methanol, ethanol, methane, dimethyl ether, and diesel (Fischer-Tropsch process). There are many syngas commercial gasification plants around the world. As at 2010, US, China, and Europe had 17, 56, and 42 plants respectively generating 71 205 MW<sub>th</sub> (megawatt, thermal). However, only 0.5% of the production was from biomass; the rest was from coal, petroleum petcoke, and natural gas (del Alamo et al. 2012; Wang et al. 2009). Renewable bioenergy has been recovered from organic wastes (including fruits, vegetables, nuts, and cereals wastes) by anaerobic digestion for biogas production. Biogas may displace about 25% of the current global natural gas consumption in the future if present technological development is effectively deployed.

### 30.2.4 Biofuel Feedstock

The U.S. Energy Independence and Security Act of 2007 mandates an increase annual biofuel addition to gasoline from 34 billion liters in 2008 to 136 billion liters by 2022, with 60 billion liters of the biofuel from lignocellulosic plant materials. In the same vein, the world population is growing steadily with significant increase in urbanization and municipal waste. These trends are creating serious economic, environmental, and social challenges, especially in developing countries (Ismail and Nizami 2016). With limited recycling capability especially in developing countries, most of the municipal waste generation end up in landfills or dump sites. This creates significant and socioeconomic impacts on soil on soil, water, land, food, and civil development.

To reduce all types of waste and produce environmental friendly biofuel, policy emphasis around the world should focus on producing biofuel from waste generated from fruits, vegetables, nuts, and cereals which are found in markets, households, and food processing industries. There is high potential of converting these types of wastes into biofuels including bioethanol, biodiesel, biogas, pyrolytic bio-oil, and syngas for electricity, heating, and cooking. For instance, sorghum bran and sorghum distiller's grains and soluble (cereal wastes) were found to contain ~10% oil. Sorghum bran oil was successfully converted to biodiesel with 98% yield using in-situ transesterification method (Wyatt and Haas 2009).

Production of bioethanol is currently carried out commercially using starch and sugar-based crops. These include, sugar cane, sugar beet, wheat, corn, potato, sweet sorghum, barley, yam, cassava, and millet (Zabed et al. 2014). In Brazil, the predominant feedstock is sugar cane while the European countries use primarily wheat and sugar beet. The principal feedstocks for bioethanol production in China are wheat, cassava, and corn, while Canada primarily use wheat and corn. Bioethanol made from food crops is often regarded as "first-generation" biofuel, which is seen to compete with animal feed and human food for the source of materials. To alleviate the adverse impacts on human food and animal feed, manufacturing "second generation" bioethanol from non-food lignocellulosic plant materials such as crop residues, food processing waste, forest slashes, yard trimmings, and municipal organic wastes have been explored. Some crops have been designated as "biomass crops" such as switchgrass, Miscanthus, giant reed, energy,

Napier grass, hybrid poplar, shrub willow, and grain sorghum to minimize competing with human food crops and animal feed. Ideally all plant materials can be used to generate bioethanol since they are composed of cellulose, hemicellulose, and lignin. The focus of intensive research is developing effective pretreatment methods to unbraid these three lignocellulosic fibrils and depolymerize cellulose and hemicellulose into simple sugar. These three major compounds (cellulose, hemicellulose, and lignin) and their potential hydrolysis products (simple sugar) are shown on Figure 30.3 below. Biodiesel is produced commercially today using vegetable oil and animal fats. Heavy reliance on edible seed oil such as soybean, rapeseed, sunflower, and palm oil for biodiesel is diminishing food availability for human consumption and animal feeds (Alptekin et al. 2014).

The current feedstocks of pyrolysis bio-oil and syngas are plant materials such as wood, switchgrass, crop straw sugar cane bagasse peanuts hulls, sawdust, and poultry litter (Mohan et al. 2006). However, wood remains the common feedstock of choice for quality bio-oil (Vamvuka 2011). Biogas production is presently carried out via anaerobic digestion of organic wastes. Most plant residues are gradually decomposed by microorganisms to smaller molecules in the natural environment. Microbial decomposition of organic waste (biomass) in the absence of oxygen is referred to as anaerobic digestion. This anaerobic condition is often encountered in landfill sites and manure lagoons where little oxygen is available. Certain microorganisms (e.g. Bacteriocides, Clostridia, or Bifidobacteria) break down organic residues to methane ( $\text{CH}_4$ ) and carbon dioxide ( $\text{CO}_2$ ).

### 30.3 Pre-Processing/Pre-Treatment Methods for Fruit, Vegetable, Nut, and Cereal Waste

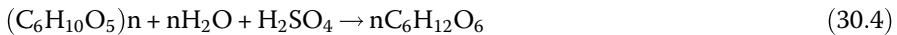
The first pretreatment step employed with these types of organic or agricultural waste is the separation of the material from environmental impurities such as sands, bottles, plastics, and other particulate objects. This may be followed by repeated steeping/washing to totally separate organic wastes and all solid particles by gravity. Clean-up organic waste can now be transferred to miller/grinder for particle size reduction. The two major pre-treatment processes often explored include; acids and enzymatic hydrolysis.

#### 30.3.1 Acid Hydrolysis

This involves the treatment of pulverized plant mass with an acidic solution (e.g. sulfuric acid) to facilitate sugar release. A two-stage acid hydrolysis process (slow process) is preferred to the rapid one-stage process under elevated temperature ( $237^\circ\text{C}$ ) and high pressure (e.g. 13 atm). The two-stage process has better sugar yield considering that 5-carbon sugars (pentoses) are degraded more rapidly than 6-carbon sugars (hexoses). Also, the bioethanol yield of two-stage process is quite a lot higher than the one stage rapid process. The two-stage acid hydrolysis processing involves subjecting the cleaned and pulverized plant biomass to mild temperature dilute acid hydrolysis ( $135^\circ\text{C}$  and 1–10%  $\text{H}_2\text{SO}_4$ ) to recover 5-carbon sugars initially before subjecting the biomass to harsher conditions of higher temperature and or higher acid concentration to recover the 6-carbon sugar (hexoses) (Lenihan et al. 2010). The reactor content is then filtered to remove lignin and may be recycled to the dilute acid hydrolysis reactor to maximize sugar recovery

and reuse the acid. Sugar recovery of this two-stage acid hydrolysis is up to 80%. However, the major bottleneck is separation of the sugar solution (Badger 2002). To this end, lime solution is employed to neutralize the sugar solution prior to fermentation.

Bioethanol is produced from vegetative biomass (fruits, vegetables, nuts, and cereal wastes) through fermentation, in which the following biochemical reactions shown in Eqs. (30.4)–(30.7) are involved:



Typical treatment and processing of bioethanol from lignocellulosic waste material can be summarized into several steps outlined below and depicted in Figure 30.3; washing, milling, liquefaction, saccharification, fermentation, or acid hydrolysis, distillation, drying, and denaturing.

### 30.3.2 Enzymatic Hydrolysis

In enzymatic hydrolysis, enzymes, instead of acids are added to decompose lignocellulosic materials into simple sugars. Alpha-amylase and glucoamylase are added during the liquefaction (80–90 °C) and saccharification (30 °C) stages, respectively. The saccharified mash is then fermented at 32 °C for 50 hours with the addition of yeast. Bioethanol is recovered from the slurry by distillation, de-watered by passing through molecular sieves, and denatured by mixing with 2–5% gasoline. Additional pretreatment, such as freezing, radiation, steam explosion, or auto-hydrolytic hydrothermal deconstruction may be necessary to ease enzymatic attack on the well-organized crystalline structure of cellulose. The pretreated (washed) organic waste comprising of fruits, vegetables, nuts, and cereal grains serve as feedstock which are milled using a grinder to 3–4 mm size particles, wetted with water into slurry and cooked (heated by steam at 110–120 °C for 2 hours). Commercial production of bioethanol from lignocellulosic organic waste (e.g. fruits, vegetables, nuts, and cereal waste) has not started in the US, largely due to the low profitability with the current conversion technology and feedstock supply system. However, gasoline blended with 10% bioethanol (E10) from corn feedstock is now available everywhere on the U.S. fuel market. With the advent of the Energy Independence and Security Act 2007 which regulates that the U.S. reduces its gasoline consumption by 20% within 10 years and increases biofuel addition to gasoline from 4.7 billion gallons in 2007 to 36 billion gallons in 2022, with 21 billion gallons of biofuels (bioethanol 18.15 billion gallons) from non-corn products, commercial production of bioethanol from lignocellulosic feedstock is gradually taking off with targets of 0.5 billion gallons in 2012 and 1.7 billion gallons in 2013. A few commercial-scale cellulosic bioethanol plants are under construction in the U.S. including Abengoa Cellulosic Ethanol Bio-refinery, Biofire Bio-refinery and Poet Liberty. Worldwide, the first commercial-scale cellulosic bioethanol plant is the Crescentino Bio-refinery, Vercelli, Italy which entered full operation in 2013. In China, Beta Renewables is planning to build another 80-million-gallon cellulosic ethanol plant in Fuyang, Anhui province (Institute for Energy Research (IER) 2015)’ (International Rice Research Institute 2007).

## 30.4 Other Value-Added Products from Fruit, Vegetable, Nut, and Cereal Waste Valorization

### 30.4.1 Biorefinery

Fruits, vegetables, nuts, and cereals are rich sources of value-added chemicals such as pectin, limonene, ethanol, 2,3-butanediol (BDO) and d-galacturonic acid (Rivas-Cantu et al. 2013; Kim et al. 2016). A new concept of enzymatic conversion of citrus waste in a citrus waste bio-refinery has generated multiple chemicals from citrus waste such as galacturonic acid from pectin via saccharification. The simple enzymatic process is advantageous over chemical hydrolysis from the perspective of cost effectiveness and simple reaction. Application of waste from fruits, vegetables, nuts, and cereals for coproduction of enzymes in solid state fermentation (SSF) further reduces the cost of enzyme production and purification process. This offers several advantages over submerged fermentation, including cost-effective, simple equipment requirement, reduced production cost and high rate of enzyme production (Wolski et al. 2009). Several fruits, vegetables, nuts, and cereal waste including; banana peel, lemon waste, papaya peel, orange peel, and apple pomace are utilized as suitable carbon sources for SSF enzyme production (Wolski et al. 2009).

Fruits and vegetables are also highly rich in various value-added components including polyphenols and pectin (bioactive compounds) which could be extracted prior to fermentation for ultimate development of industrial chemicals. Production of 2,3-butanediol (BDO) using agricultural wastes via fermentation could lead to the production of an important industrial chemical for sustainable chemical production, such as 1,3-butadiene and methyl ethyl ketone, or production of important end products including perfumes, plasticizers, printing inks, fumigants, moistening and softening agents, explosives and important food additives (Rivas-Cantu et al. 2013; Zeng and Sabra 2011). The use of low cost fermentation feedstocks (fruits and vegetable waste) could lead to significantly reduced production costs of BDO, other industrial chemicals and important end products.

### 30.4.2 Environmental Implication and Beyond

Diversion of waste stream from landfills and dumpsites for biofuel and bio-refinery activities does not only generate substantial amount of financial benefits but also saves huge expanse of land, further reduces environmental pollution and improve public health in general. Some far-reaching environmental impacts of converting agricultural food wastes to the production of conversion include land rights, food security, reduced greenhouse emissions, biodiversity, soil conservation, and water resources. It also enhances energy security and independence, job creation, increase in farm incomes, promotes research and development and local prosperity (Majdalawi et al. 2014; Fink and Medved 2013). Biofuel or bioenergy from lignocellulosic biomass wastes is obviously the most prosperous bioenergy with highest potential in the future. This will not only facilitate achievement in reaching pre-set goals of carbon emission reductions in the energy sector, but huge environmental implications as there are strong indications that bioenergy will meet 30% of the whole world's energy demand by 2050 as the global consumption of

bioenergy continue to increase under the present climate change policies (Guo et al. 2015; World Energy Council 2013).

## 30.5 Conclusion

This chapter explored the status of agricultural and food wastes mainly from fruits, vegetables, nut, and cereal origins, pretreatment/preprocessing methods for effective conversion to biofuels and anticipated valorization potentials. An overview of diverse types of biofuels, present disposal methods and actual processing technology for biomass conversion were explored vis-a-vis utilization, value addition and environmental implications of the present system. Reduction in greenhouse emission attained by this strategy, conversion of waste to important gasoline alternative (biofuel) for powering millions of automobile engines and production of BDO and other industrial chemicals from bio-refinery were extensively discussed as important value-additions. Preprocessing treatments of the food wastes together with the optimization of process conditions are of immense importance to optimize the bioenergy conversion efficiency, project viability, and commercialization of proposed process. Continuous research and enhancement of knowledge in the bioconversion of waste to important end products and industrial chemicals is an important way of addressing the environmental and sustainability questions facing the increasing growing world population.

A considerable number of valorization or value-added strategies are available for substituting present feedstocks for biofuel production with organic wastes from fruits, vegetables, nuts, and cereals. These does not only ensure adequate food for human and animal feed, but further reduce deleterious landfilling and open dumps considered in existing waste disposal methods. This approach is more environmentally friendly, sustainable, and profitable.

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## 31

# Economic Impacts of Value Addition to Agricultural Byproducts

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## 31.1 Introduction

The agricultural sector plays an important role in the economies of many countries by providing food for human consumption, raw materials for agro-industries, and generating income and employment. In developing countries, the sector is critical in efforts to reduce and alleviate poverty given that a significant share of the population is rural and depends on the sector for its livelihoods. To enhance the contribution of the sector, many countries have implemented policies aimed at increasing the sector's productivity. These policies include the use of improved technologies, the use of genetically improved crops and animal varieties, increased use of output-enhancing inputs such as fertilizers, pesticides and herbicides, and more efficient utilization of water resources. Given the many changes that have occurred in international agricultural markets in recent decades, agribusinesses need to adapt their strategies to not only respond to the greater competition that they face, but also to take advantage of new and emerging opportunities. To increase their income and market share, agribusinesses need to reduce their costs and harness the opportunities of value addition (Rosentrater, 2006). To some extent, some value addition has been undertaken in terms of processing agricultural commodities into qualities that are desired by consumers, command higher prices, and are less perishable with longer shelf lives. Value addition is also manifested in marketing functions such as storage and transportation that ensure that various products are available to consumers when and where they desire them. Some examples of value-addition include the processing of canola, corn, and sunflower seeds into cooking vegetable oils, the milling of wheat into wheat flour for use in bakeries, the milling of coffee beans into coffee and the various processing activities that are involved in the preservation of fruits and vegetables.

Value addition can also be captured from the use of byproducts generated from harvesting of crops, rearing of animals, and processing of agricultural commodities. Crop residues such as straw, usually represent relatively large amounts of cellulosic material that could be returned to the soil for its future enrichment in carbon and nutrients or could be made available for further conversion to biofuels and other products (Wadhwa and Bakshi, 2013; Sarkar et al., 2012). Similarly, animal wastes or byproducts

are high in cellulose content and can also be converted into liquid biofuels (Blaschek et al., 2010). Such agricultural byproducts can play an important role in triggering the transition to sustainable energy. The processing of agricultural commodities is also invariably associated with the production of byproducts such as molasses from sugar processing, whey from milk processing, and husks from coffee processing, distillers dried grain (DDG) from production of ethanol from corn. These byproducts present a significant challenge, given the limited disposal capacity in landfills and also their deleterious effects on the environment (Jayathilakan et al., 2012; Marcelo et al., 2017; Salihoglu et al., 2018; Shamsi et al., 2012; Wadhwa and Bakshi, 2013). However, they also present an important opportunity because they can be used as raw materials to produce new products of commercial value that can increase the competitiveness and sustainability of the agricultural and agri-food sector (Narayanan et al., 2017; Flores and Shanklin, 1998; Rosentrater, 2006).

This chapter examines the prospects for value addition to agricultural byproducts and identifies the diverse economic impacts of such value addition. The central argument advanced in this chapter is that value addition to agricultural byproducts is not only economically viable but also provides an opportunity that needs to be exploited to ensure total resource use efficiency (Prinyawiwatkul and Moody, 2002). Several examples are presented to illustrate instances where value addition has been undertaken and the benefits that can result from such initiatives. The chapter also discusses some policy measures that can be taken to promote value addition to agricultural byproducts as part of a broad strategy to increase the incomes of agribusiness operators and enhance their competitiveness and overall economic sustainability.

## 31.2 Why Value Addition to Agricultural By-Products?

The increased production and processing of agricultural goods has been accompanied by increased production of agricultural byproducts (Ayala-Zavala et al., 2011). Examples of these byproducts include straw, peels, and hulls. In many cases limited use has been made of these agricultural byproducts. This represents a lost opportunity given that considerable value can be derived from them. The disposal of agricultural byproducts, through burning or by being left on the ground as has commonly been the case is deleterious to the environment and can contaminate and pollute the soil and water systems. The burning of agricultural byproducts is also harmful to the soil and emits carbon dioxide and particulate matter into the atmosphere. These environmentally deleterious impacts can be ameliorated through the utilization of these byproducts in ways that generate additional economic value (Blaschek et al., 2010; Singh and Prabha, 2017).

There are several ways of utilizing agricultural byproducts and enhancing their economic value. In the biofuels industry, this is possible for instance by using agricultural byproducts or biomass to produce energy instead of burning it (Blaschek et al., 2010; Bradburn, 2014). In many countries, agricultural and industrial byproducts such as oat hulls, sunflower hulls, straw, and molasses have been successfully harnessed to produce biofuels for use in the transportation sector. They have also been used to manufacture diverse bio-products for use in construction. These examples demonstrate that agricultural byproducts can underpin economic production and create employment

opportunities. They can thus facilitate the process of economic diversification by opening up new agricultural markets and providing alternatives to low-cost commodity production, by offering new perspectives for the management of resources, and by providing economic opportunities and environmental benefits based on decreasing use of finite petroleum-based resources.

The utilization of agricultural byproducts has the potential to support entire industries, increase incomes and generate valuable employment opportunities (Ayala-Zavala et al., 2011). The transformation of agricultural byproducts into high valued products is preferred to their disposal as wastes into the environment (Shamsi et al., 2012; Vuong, 2017; Wadhwa and Bakshi, 2013). For example, the use of byproducts such as straw and molasses to feed animals not only provides valuable sources of low-cost fiber and protein in animal feed, but can also contribute significantly toward the lowering of the cost of animal production and thus enhance its profitability (Schieber et al., 2001; Wadhwa and Bakshi, 2013; Joanitti and Silva, 2014). The utilization of agricultural byproducts is also valuable for the protection of the environment. In many parts of the world, agricultural byproducts have been regarded as wastes, that in many cases are often disposed of through burning. This practice is harmful to the soil and results in air pollution through the emission of carbon-dioxide and the release of particulate matter into the atmosphere.

### 31.3 Transformation of Agricultural By-Products

In several countries, innovative methods have been developed not only for extracting useful products from nutrient-rich agricultural byproducts, but also for using the byproducts to manufacture other goods for which considerable market demand exists (Das and Singh, 2004; Jayathilakan et al., 2012). Examples include the use of straw from corn, wheat, and rice as a substrate for growing mushrooms in areas where the soil is not suitable due to pest infestation and salinity; the use of poultry and cattle manure as fertilizer; the composting of crop residues and the subsequent utilization of the compost to improve the soil structure and as a bio-fertilizer; the use of agricultural byproducts as animal bedding materials and for building and heating; the transformation of agricultural byproducts into feedstuffs for livestock to supplement foraging and commercial feeds; the use of the byproducts from the processing of agricultural products to extract chemicals, vitamins, enzymes, and pharmaceuticals; and, the use of agricultural byproducts such as bagasse for the manufacture of boards, composites, textiles, and paper (da Silva, 2016; Salihoglu et al., 2018). These examples demonstrate that value-addition to agricultural byproducts holds considerable promise in ensuring the profitable utilization of products that would otherwise be discarded (Vuong, 2017). Given the great variety of agricultural byproducts that are available in abundance in different regions, and the research that is currently being undertaken to explore ways of utilizing these products, it is reasonable to expect the continued discovery of additional newer uses which represent value-addition and which can be profitably scaled up.

An area in which the use of agricultural byproducts has been finding increased emphasis is the production of biofuels and bioenergy (Sarker et al., 2012). This process entails using feedstocks from corn, rice, wheat, and other crop residues and industrial byproducts to produce ethanol which is then used to manufacture transportation fuels such

as gasohol.<sup>1</sup> (Swain, 2017). According to Bradburn (2014), Marcelo et al. (2017), Portugal-Pereira et al. (2015) and Sarker et al. (2012), these crop residues are also being increasingly used to produce heat, biogas, and electricity. Bentsen et al. (2016) argue that agricultural crop residues make up a significantly part of unused biomass potential and estimated that Canada's annual potential of agricultural crop residues was about 55 million tonnes and that between 2001 and 2010, about 13 billion liters of ethanol were produced annually from crop residues. They also noted that Ontario, Manitoba, Saskatchewan, and Quebec had enough agricultural residues to support the setting up of ethanol plants that utilized these crop residues as the primary feedstock. Bensten et al (2016) also estimated that the U.S. annual potential of agricultural crop residues was 125 million tonnes.

## 31.4 Economic Impacts of Value Addition in Agricultural By-Products

As explained above, agricultural byproducts are a valuable resource that can be productively harnessed and utilized either directly or indirectly as inputs into other production processes (Prinyawiwatkul and Moody, 2002; Salihoglu et al., 2018). The increased awareness of the diverse ways in which agricultural byproducts can be used has culminated in several initiatives that add value to these resources to generate income and improve living standards. In what follows we explore some of the economic impacts of value-addition to agricultural byproducts.

### 31.4.1 Revenue Generation from Value Addition

No direct estimates of revenue are available for value added products produced from agricultural byproducts. However, several studies and surveys have identified the contribution of bio-products in general to the economy of different countries.<sup>2</sup> In an economic impact analysis of the U.S. biobased products industry, Golden et al. (2015) concluded that the biobased industry in the U.S. contributed a total value added of US\$369 to the U.S. economy in 2013. This included US\$126 billion in direct sales, another US\$126 billion in indirect sales and US\$117 billion in induced sales. According to estimates by Rancourt et al. (2017), the 190 bioproduct establishments in Canada earned total revenues of CDN \$2.72 billion from ethanol and \$2.04 billion from biodiesel in 2015 from the use of biomass.<sup>3</sup> They also found that Canada had a vibrant and rapidly growing market for biomass for use in manufacturing other diverse bio-products that generated revenues of about \$4.53 billion in 2014 and \$4.27 billion in 2015.<sup>4</sup> Furthermore, they uncovered a trend in which establishments were increasingly being set up to develop and produce

<sup>1</sup> A mixture of gasoline and ethyl alcohol used as fuel in internal combustion engines.

<sup>2</sup> It must be noted that different countries define bio-products and the bioeconomy differently and bioproducts include products produced from different feedstocks including primary agricultural products, agricultural byproducts and residues and forestry residues.

<sup>3</sup> This includes both agricultural and forestry biomass.

<sup>4</sup> The sales reported here cover only the primary level of the bioproduct value chain. It also does not include any indirect or induced revenues.

bio-products. The principal drivers underlying this trend were the existence of market opportunity, the profitability of enterprises that transformed agricultural byproducts into value-added goods, the need to reduce the adverse environmental impacts of agricultural waste disposal, and the need to meet customer requirements.

Imerman (2017) examined the issue of value addition to agricultural byproducts in the United States with a specific focus on the economic impacts of using corn stover to replace coal in the generation of electricity. He established that this could increase economic activity significantly through activities such as the gathering of corn stover in the fields, the transporting of the gathered stover to processing facilities, the processing of stover as fuel for use in coal-fired electricity power plants, and the transporting of processed stover fuel to power plants. Imerman (2017) estimated that corn stover pellets could generate US\$105 per tonne in revenues; that over the 2017–2030 period, setting up a stover pellet plant in Iowa could generate US\$43 million in new labor income, a US \$2 billion addition to the GDP, and a US\$4 billion increase in the economic output. He also estimated that for Nebraska, the setting up of a pellet plant could generate US\$840 million in labor income and contribute US\$1.5 billion to the state GDP over the same period.

In Canada, it is estimated that at least 9 million dry tonnes of crop residue is available annually (Mabee et al. 2006) and as high as 56 million oven-dry tonnes of straw can be produced from crops annually (Wood and Layzell 2003). According to the 2015 Canadian Bioproduct Development Survey, only about 78 000 tonnes of agricultural residues and 624 000 tonnes of food processing byproducts were utilized for bioproduct production (Agriculture and Agri-food Canada 2017). The U.S. Department of Energy (2011a,b) projected primary agricultural residue supplies that can profitably be collected at farm gate to increase from 111 million dry tonnes in 2011 to between 180 and 320 million dry tonnes by 2030. Processing and other waste resources are also estimated to range between 20 and 26 million dry tonnes and collectible animal manure between 30 and 60 million dry tonnes in 2030. These can be sold at a price of between US\$40 and US \$60 per dry tonne. There are therefore significant opportunities for value addition in agricultural byproducts.

### 31.4.2 Employment

The diverse range of activities that are associated with the value-added utilization of agricultural byproducts are playing an increasingly important role in providing valuable employment opportunities both directly and indirectly (Cowan, 2002; Herren et al., 2011). The enterprises involved have a significant potential to stimulate rural economic activity such as the collection, aggregation, drying, refining, and packaging of the agricultural byproducts to make them usable (Cowan, 2002). It is difficult to establish with precision the exact number of jobs that are created through value addition to agricultural byproducts. Golden et al. (2015), estimates that the U.S. biobased products industry, as a whole, directly employed 1.5 million people in 2013. It also generated additional indirect and induced jobs of 2.5 million in other sectors of the economy. Rancourt et al. (2017) also estimated that in 2015, Canada had about 5618 people directly employed in the bio-product industry with total wages and salaries of about CDN\$ 1.05 billion in 2014 and CDN\$ 1.04 billion in 2015. The labor-intensive nature of the tasks involved in the utilization of agricultural byproducts and the high value of the products derived from these

processes implies that this is a promising approach to not only create green jobs, but also of increasing farm productivity and profitability and ensuring that agricultural byproducts are used more efficiently. It can be expected that the green jobs created through the value-addition will result in an increase in incomes and thus stimulate the demand for other goods and services that will in turn result in the creation of additional jobs in the rest of the economy.

### 31.4.3 Trade

The demand for value-added products manufactured using agricultural byproducts is strong and growing not only within domestic economies but also internationally. The global market for bioproducts is expected to reach over US\$700 billion in 2018 from \$388 billion in 2013 (BCC Research 2014). This implies that countries that have technologies that can be applied to efficiently transform agricultural byproducts into value-added products that satisfy consumer preferences can derive significant economic benefits by exporting these products. The range of value-added products that can be profitably exported is diverse and includes furniture, building materials, arts and crafts, paper, biochemical, biomaterials, bioenergy, pharmaceuticals, and cosmetics (Prinyawiwatkul and Moody 2002). The revenues earned from such exports can in turn be invested to expand value-added capabilities, promote economic diversification, and improve overall economic well-being. The potential that countries have for earning revenues from exports of value-added products derived from agricultural byproducts is evident in the case of Canada that earned \$1.28 billion in 2014 and \$1.42 billion in 2015 from the exports of these products (Rancourt et al. 2017). In many export markets, consumer preferences are changing and shifting toward renewable and environmental-friendly products. This is an opportunity that can be effectively harnessed by developing value-added products that are in demand in these markets.

As the outputs of agricultural products have increased over time so has the quantity of crop residues and agricultural byproducts (Jayathilakan et al., 2012; Singh and Prabha, 2017). A strong demand exists for these byproducts given the many possibilities for using them as inputs into other production processes or of recovering valuable extracts from them. Trade in these byproducts is an opportunity that the respective sellers and buyers can and have exploited to earn income, reduce their costs of production, and increase the profitability of their enterprises. Such trade occurs, for instance, when the byproducts from fruit and vegetable processing (e.g. pineapple juice waste, mango peels, wheat straw, and banana peels) are sold to livestock farmers who use them as feed or to enterprises that use them to extract value-added products such as edible oils, essential oils, pigments, food additives, dietary fiber, enzymes, and citric acid (Schieber et al., 2001; Martins et al., 2017; Salihoglu et al., 2018). Other examples of trade of agricultural byproducts include the sale of manure by livestock farmers to enterprises that produce compost, or to bio-energy firms that utilize the manure to produce biogas and electricity. Strengthening trade in agricultural byproducts is significant and can contribute toward developing an efficient and robust supply chain for the sustainable production of value-added products. For example, as middle-income population grows in many developing countries, there is a corresponding increase in demand for animal proteins. This has led to the need to increase the supply of such products. The demand for DDGs, which is used for animal feed, is therefore increasing. This provides significant opportunities to export DDGs. In

2015, a total of 16.7 million tonnes of DDGs were exported around the world. The U.S. accounted for 82.8% of these DDG exports. Canada was the second largest exporter of DDGs and accounted for 3.2% of the exports. China was the largest importer of DDGs, with the country's imports of DDGs increasing significantly from 1560 tonnes in 2000 to 7.52 million tonnes in 2015. This is due to China's increased demand for animal proteins which has subsequently led to the increase in demand for livestock feed products, including DDGs (DeOliveira et al. 2017).

### 31.4.4 Market Expansion

A major challenge that confronts producers of large quantities of agricultural byproducts is how to dispose them off in a way that is both environmentally and financially sustainable. In Maryland, for instance, the enforcement of nutrient management regulations has forced several small to medium sized farms to close down (Bogardus 2016). This challenge has resulted in the emergence of innovative businesses that focus on adding value to the agricultural byproducts to create products that can be marketed profitably as is the case with the composting of manure. The large quantities of equine and poultry manure that are available in Maryland are being composted and profitably marketed as mulch and to soil specialty companies, landscaping companies, and greenhouse growers (Bogardus 2016). The success of these enterprises and the growth and expansion of this market have been possible due, in part, to the provision of subsidies and grants from the government. Through these financial incentives, these businesses have been able to overcome the initial barrier of the prohibitive capital cost of the technology needed for large-scale composting.

Some of the measures that can be taken to expand this market are strengthening the network of farmers who supply raw manure to the composting companies, broadening the ways in which the manure can be used, e.g. in burned energy programs, marketing the compost more widely to clients in other regions, and producing more output to reduce operational costs, and thus realize economies of scale and increase profitability. The fact that the compost is in high demand in Maryland and sells for between \$30 and \$45/yard coupled with the fact that farms could save between \$100 and \$140/day from reduced disposal fees, reinforces the central argument that profitable opportunities exist for deriving multiple economic benefits from the transformation of agricultural byproducts into value added products. Furthermore, Maryland can derive additional economic benefits from this process through savings from reduced imports of peat moss – the state currently imports about 80% of its peat moss from Canada and the compost made from the manure is a good substitute for the peat moss (Bogardus 2016).

### 31.4.5 Rural Economy

Since a greater number of farms are located in rural areas, sustainability of many rural economies depends, in part, on the productivity, profitability, and sustainability of farms. Agricultural byproducts are a valuable resource that can be utilized in the rural areas to stimulate economic production, support rural livelihoods, and increase household incomes (Prinyawiwatkul and Moody, 2002; Singh and Prabha, 2017). The byproducts can be utilized as food for humans, a source of fuel, feed for livestock, and as inputs into agricultural production and rural industries. Several concrete examples can be cited to

demonstrate the various ways in which value addition to agricultural byproducts can enhance rural economies. In areas where crops are harvested, the residues such as corn stover can be gathered, sold, and used for the production of cellulosic ethanol, for the generation of energy, building materials, and for crafts (Swain, 2017). As has already been explained above, manure is not only valuable for improving soil structure, and as a source of vital plant nutrients, it can also be composted and sold as mulch and to greenhouse growers and soil specialty companies. Furthermore, the industries that are set up to use agricultural byproducts as an input also generate jobs and increase rural incomes. This is an important economic contribution particularly in areas where unemployment is high.

With appropriate investments in rural infrastructure and the provision of favorable economic incentives, the abundant agricultural byproducts in rural areas can also be utilized to manufacture novel products that can be exported. In developing countries, the manufacture of such novel products can, in addition to creating much needed jobs and being a source of foreign exchange earnings, also contribute to the diversification of rural economies.

## 31.5 Economic Policies to Promote Value Addition

There are many economic benefits that can be obtained through value-addition to agricultural byproducts. These benefits include enhancing the resource use efficiency of agricultural production, increasing farm incomes and reducing the costs of production and thus increasing the profitability of farming, producing novel products, creating jobs, reducing the disposal of the byproducts into the environment to ensure improvement in environmental quality (Prinyawiwatkul and Moody, 2002; Schieber et al., 2001; Singh and Prabha, 2017). Realizing these benefits however, requires overcoming the various barriers or challenges to value addition. Some of these barriers are the prohibitive costs of capital, the low profit margins of some value-added activities, uncertain supplies of agricultural byproducts, inadequate rural infrastructure for hauling these byproducts, lack of standards, and weak markets for agricultural byproducts and the products made from such byproducts (Portugal-Pereira, 2015). Several proactive government measures and actions are needed to overcome these barriers and promote value addition. Some of these government measures are discussed below.

### 31.5.1 Reducing Costs

Entrepreneurs will take advantage of the abundance of agricultural byproducts and undertake value addition if these activities generate reasonable profits. The profits from such enterprises have however tended to be low due, in part, to the high costs that are often involved in the collection, cleaning, aggregating, transporting, and processing of the byproducts (Derr and Dhillon, 1997). The implementation of measures that reduce these costs can therefore enhance the profitability and attractiveness of value addition to agricultural byproducts. Some ways of reducing these costs include setting up centrally and strategically located collection centers, supporting innovation, locating the processing plants closer to the destination where the final product will be marketed, becoming more efficient through technology adoption, and expanding the capacity of

value added operations to realize the benefits of economies of scale (Boland 2009). Governments can also aid in lowering business costs of these enterprises and enhancing their competitiveness through tax concessions.

### 31.5.2 Provision of Subsidies

Value addition to agricultural byproducts can be facilitated by providing the industry with initial economic and technological support to overcome barriers of high entry costs and make the operation of such activities profitable. The need for such support stems from the fact that value-added activities are often capital intensive with a high initial fix costs and in many cases only become profitable over time. Providing well-designed and targeted subsidies can aid the acquisition of the required capital and financial resources and incentivize enterprises to add value in cases where they would otherwise not be undertaken. It is important, however, that such subsidies be limited in scope and duration because they have the potential to generate significant deadweight losses and cause distortions to the economy. A good example of the role that subsidies can play is provided by DeVuyst et al. (2011), who discuss value addition to agricultural byproducts and note that synergies may exist between ethanol, methane and cattle production when a cattle feedlot is co-located with an ethanol plant. In this arrangement, the byproduct from the ethanol plant (i.e. DDG) can be fed to feeder cattle; the manure from the cattle can be used in methane production; and the methane can be used as an energy source in the ethanol plant. The methane can alternatively be used to produce electricity. However, on the basis of the cost estimates for constructing and operating a feedlot and anaerobic digester for methane production, DeVuyst et al. (2011) found that the investment could not be justified on economic grounds. They estimated that a renewable fuel/electricity subsidy of at least \$0.053 per kWh was required to break even when the methane was used to produce electricity. If, however, the methane was burned for thermal energy, breaking even required a subsidy of about \$72 per 1000 m<sup>3</sup> of methane. Burnes et al. (2004) came to a similar conclusion in their analysis of the economic feasibility of ethanol production in California using agricultural byproducts. They found that the cost of producing ethanol using agricultural byproducts in California was higher than the prevailing price of ethanol and that a public subsidy was required to encourage ethanol production. As noted above such subsidies need to be temporary with the expectation that value-addition would become economical and profitable over time.

### 31.5.3 Standardization

One of the attributes of agricultural byproducts that has constrained their utilization and value addition is the great disparity in the qualities of the byproducts. For example, the manure from poultry vary widely in nutrient content; plant feedstocks vary widely in the ease of hauling and suitability for production of ethanol; and, the byproducts from food-processing plants vary widely in their chemical and biological characteristics and suitability for use as livestock feed. In other words, the great variability in the qualities of agricultural byproducts is an impediment to their marketing. The establishment of standards in the agricultural byproducts industry can facilitate market development, expansion, and competition by providing potential users of the byproducts with valuable

information about the qualities of the different byproducts. Standardization can also contribute to operational and pricing efficiencies as well as increased returns to producers.

### 31.5.4 Development of Infrastructure

It is often the case that agricultural byproducts are produced in quantities and qualities that vary greatly from farm to farm or from location to location. Thus, their use requires that they be collected, bulked, and hauled or transported to processing plants, where they can be processed to produce value added products. This is important to ensure that the processing plants have adequate quantities of the byproducts on a regular basis to enable them to operate sustainably and to realize economies of scale and scope. Furthermore, these steps are necessary to ensure that the processing of agricultural byproducts is financially viable and profitable. Developing infrastructure to aid the collection, sorting, aggregating, hauling or transportation and storage of agricultural byproducts is critical for value-addition. Such infrastructure is also needed to transport and deliver the products made from the byproducts to their markets. This can be achieved, for instance, by establishing collection centers where farmers can deliver their agricultural byproducts (alternatively, the byproducts can be collected from the farmers). At the collection centers, the byproducts can be sorted out into various grades according to established standards and then transported to other levels or locations in their supply chain. Developing such infrastructure also has the advantage of altering the views of various stakeholders who are then likely to regard agricultural byproducts as a valuable resource rather than as a waste.

Tucker (2015) provides a good example of the role that such infrastructure has played in effluent and manure utilization in Australia. There are several parallels between the Australian case and the pork industry in Canada and the United States. The pork industry in these countries generates significant amounts of liquid (effluent) and solid (manure) byproducts or waste materials. The effluent and manure have the potential to provide significant productivity and profitability opportunities for producers. They can be used as fertilizers and soil conditioners on-site and can generate alternative income when used as fertilizers off-site or to produce energy for biogas. Tucker (2015) notes that infrastructure is needed for the proper management and utilization of such effluent and manure to avoid environmental issues such as nutrient loading, runoff, and amenity concerns such as generation of odor. Tucker (2015) points out that to aid value-addition to effluent and manure as outlined above, the Australian Pork Limited invested in the management and reuse of effluent, manure, and sludge across the pork industry production system.

### 31.5.5 Research and Development

The awareness that agricultural byproducts are a valuable resource warrants that more extensive research and feasibility studies be conducted into ways of improving how they are used and to identify newer uses. Some specific areas in which further research is needed include how to enhance the nutritive value and digestibility of byproducts that are used as animal feed; how to improve the treatment of manure to make it a better fertilizer and soil conditioner; how to better extract and utilize nutrients and biochemicals from residues of processed fruits and vegetables; how to establish standards for the

agricultural byproduct industry; and, how to reduce the cost of value-addition processes to ensure financial viability. Chaudhary et al. (2012) assert that such research has the potential of enhancing the proper utilization of agricultural byproducts and improving the economy of farming communities. So far, promising research has been done in the production of energy from manure, production of fuel pellets from compressed biomass, reduction of waste in the food processing industry, and identification of the properties of lignocellulosic materials and how these properties can be harnessed, for instance, in producing bricks and other construction materials (Barbieri et al. 2013; Shakir et al. 2013). The implementation and commercialization of the research results can yield solutions to problems and challenges faced by farmers in utilizing agricultural byproducts to increase their incomes and create jobs.

## 31.6 Conclusions and Policy Implications

The utilization of agricultural byproducts through value-addition has been identified as an opportunity that can be harnessed to bring about diverse economic benefits for farmers and rural economies and to improve the quality of the environment. The byproducts are rich in nutrients and valuable bioactive compounds that can be profitably extracted, purified and used to, among others, manufacture edible oils, vitamins, enzymes, dyes, pigments, livestock feed, alcohols, and biofuels. The byproducts, therefore, need to be viewed as an economic resource with potential for use in value addition and not as waste. Their effective use can increase farm incomes, reduce the costs of production, increase farm profitability, generate jobs, provide novel products for domestic use and export, increase overall efficiency of farm operations, and stimulate rural economic growth.

Although considerable value can be derived from value addition to agricultural byproducts, in many areas the byproducts have continued to be discarded into the environment, where they have contributed to the pollution and contamination of air, soil, and water. This has been due to, among others, a lack of awareness about the values that can be derived from the utilization of these byproducts, the costs involved in mobilizing them from farms and in transporting them to plants where value can be added, the high costs of the technologies required for value addition, lack of appropriate standards, and in some cases, weak markets for the products derived from agricultural byproducts. These hurdles can be effectively addressed through public policies such as the creation of increased awareness about the opportunities for utilization of agricultural byproducts, the provision of financial and economic incentives to overcome the initial cost barriers to enterprises undertaking value addition, and the development of standards that will facilitate the growth and expansion of markets for products derived from agricultural byproducts.

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## 32

### Constraints to Value Addition to Agricultural Byproducts

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#### 32.1 Introduction

Agricultural byproducts are valuable resources that can be profitably harnessed to derive products of economic value to improve the productivity of farms, increase farm incomes, and stimulate rural economic development. The utilization of agricultural byproducts is important, given that in many countries their disposal is costly and can have deleterious environmental impacts. A considerable potential exists for the recovery of useful chemicals from the byproducts, such as energy. It will also avoid environmental damages through their disposal in landfill, through burning, or by being left on the ground to rot. The United Nations Environmental Program (UNEP) (2009) estimates that globally, about 140 billion metric tonnes of biomass are generated every year from agriculture. Chaudhary et al. (2012), Swidiq et al. (2012), Shabani et al. (2013), and Reddy and Yang (2005) note that although agricultural byproducts are abundant in supply, there have been few attempts to increase their utilization, and that this has limited the realization of the benefits that could be derived from their use. This is because there are challenges to gathering and hauling these byproducts for the purpose of adding value. However, for social, economic, and environmental reasons, innovative strategies need to be developed to address the challenges and increase the utilization of agricultural byproducts. Implementing these strategies can contribute positively toward, among others, reducing environmental pollution, preserving natural resources, increasing economic value for farmers and contributing to rural development.

This chapter presents the diverse benefits that can be derived from value addition to agricultural byproducts. The prospects of realizing these benefits demands a paradigm shift from treating the byproducts as a waste to treating them as a valuable economic resource. The chapter also identifies and explains the constraints to value addition to agricultural byproducts and proposes some policy measures that can be used to address the constraints to increase value addition to these byproducts.

## 32.2 The Benefits of Agricultural Byproducts

A broad range of benefits can be obtained through processing to agricultural byproducts. These benefits include the following:

- i) Enhancing the efficiency of agricultural production: Improving the aggregate resource use efficiency of farms is one of the challenges that needs to be addressed to increase profits, reduce waste, improve environmental quality, and economize on scarce natural resources. This broad goal can be achieved through the use of agricultural byproducts as inputs for other productive activities (Magnan et al. 2012). It is a common practice on farms with mixed crop-livestock production systems where cereal production provides not only grain, but also crop residues that can be used as fuel, livestock feed, and as bedding material. On such farms, the manure from livestock production is often used as fertilizer. The complementarities that are entailed in the use of byproducts not only enable economies of scope but also significantly reduce the cost of production. According to Magnan et al. (2012), although it is difficult to quantify the nonmarket values of these byproducts, the shadow values for small scale crop-livestock farmers are substantial.
- ii) Promoting rural economic development: The increased utilization of agricultural byproducts can provide a sustainable basis for small and medium industries in rural areas and stimulate rural economic development. Reddy and Yang (2005) have noted that byproducts from the cultivation of corn, wheat, rice, sorghum, barley, sugar-cane, pineapples, bananas, and coconuts are abundant and cheap sources of bio-fibers that are suitable for various industrial applications such as the manufacturing of composites, textiles, and pulp and paper. Establishing industries for such value addition to produce useful consumer products, to generate revenues, provide employment opportunities, and thus positively impact the revitalization of rural communities (Mertens et al. 2018).
- iii) Improving environmental quality: disposing of agricultural byproducts such as crop residues by burning releases carbon dioxide and other pollutants into the atmosphere. This not only aggravates the risk of climate change but also poses a significant threat to human health from pollution. Similarly, leaving crop residues and manure on the ground are harmful practices that contribute to the accumulation of pathogens in the soil and the contamination of groundwater. Harnessing crop residues as manure and bio-fertilizers, and as raw materials for producing energy and consumer products, can increase the profitability of agricultural enterprises, significantly improve the quality of the environment, increase the profitability of agricultural enterprises, and enhance energy security.

## 32.3 Constraints to Value Addition to Agricultural Byproducts

Several authors hold the view that although agricultural byproducts are available cheaply and in copious amounts, they have not been fully utilized despite the existence of countless opportunities for value addition to these byproducts (Chaudhary et al. 2012; Swidiq et al. 2012; Shabani et al. 2013; Reddy and Yang 2005). This represents a loss of economic

opportunity. There is thus the need to identify the reasons for non/underutilization of agricultural byproducts so that they can be addressed through appropriate strategies and policy interventions. In what follows we present some of the constraints to value addition to agricultural byproducts. These include physical and chemical characteristics of agricultural byproducts, unreliable supply and heterogeneity, high transportation cost, lack of awareness of value about the value of agricultural byproducts, lack of markets, lack of financing, regulations.

### 32.3.1 Physical and Chemical Characteristics of Agricultural Byproducts

Although several uses have been proposed for agricultural byproducts, questions remain about the suitability of agricultural byproducts for the various uses. Ezejiofor et al. (2014) cite the example of lignocellulosic crop residues that are commonly used as animal feed, but have a very low content of protein, vitamin, oil, and other nutrients. These crop residues also have limited digestibility and palatability to ruminants and need to be enriched with the necessary nutrients prior to their use as animal feed. Ezejiofor et al. (2014) also assert that although the industrial processing of agricultural products generates byproducts that are rich in nutrients, these byproducts often have a large amount of moisture that needs to be removed as a precondition for their effective utilization. The high moisture content of agricultural byproducts also causes their quality to deteriorate rapidly, thereby restricting their use in energy production. Ajila et al. (2012) strongly support the use of agricultural byproducts as non-conventional feed, but caution that some of these products have toxic and deleterious effects on animals. A similar position is maintained by Montagnac et al. (2009) who posit that cassava is a rich and useful source of dietary energy for humans and domestic animals, but whose use is limited by the fact that some varieties contain cyanogenic glucosides that are toxic and can lead to serious health problems such as acute poisoning and neuropathy. They also point out that cassava contains anti-nutrients such as phytate, polyphenols, oxalate, and saponins that can reduce nutrient bio-availability. Such toxicity is also exhibited by bamboo shoots. Demirbas et al. (2011) maintain that biowastes are great as a renewable energy source in both developed and developing countries, but they contend that their uses are limited due to their highly variable properties. In particular, biowastes have high moisture content and tend to have a high ash content that can cause ignition and combustion problems. They assert that when agricultural byproducts are used as fuel through direct combustion, only a small percentage of their potential energy is available due to inefficient burners that are commonly used. With regards to bio-oil, they cite their high viscosity and acidic nature, low heating value, high water and oxygen content and incompatibility with conventional fuels as drawbacks to their use as transportation fuel. It follows, therefore, that these physical and chemical characteristics are a significant constraint to the utilization of these and other byproducts with similar attributes. It is commendable that several research efforts are currently underway to develop methods for making these products safe for human and animal use and more suitable for energy production.

### 32.3.2 Unreliable Supply and Heterogeneous Quality

The value-addition of agricultural byproducts for purposes such as the feeding of livestock and energy production depends on both the quality of the byproducts and

the reliability of their supply. Shabani et al. (2013) point out that although forest biomass can be used to produce electricity, heat, and biofuels, the unreliable raw material quality is a major constraint to such use given that it makes forest biomass an expensive energy source. Swidiq et al. (2012) regard agro-industrial byproducts and crop residues as important feed resources on integrated crop-livestock systems in Uganda but claim that their poor quality and unreliable supply have limited their use.

### 32.3.3 Unfavorable Economics

Rational economic agents will undertake value addition to agricultural byproducts only if the returns from the value additions compare favorably with other investments. Pokharel et al. (2017) cite unfavorable and insufficient returns as an economic constraint that has led to a low interest in the utilization of logging residues for electricity purposes in several parts of southern United States. This is evident from the estimates of the costs of producing electricity from biomass as compared to other methods of producing electricity. They estimated the cost of producing 1 kWh of electricity using coal, natural gas, nuclear fuel, geothermal energy, and hydropower to be US\$ 0.01–0.15, US\$ 0.07–0.10, US\$ 0.01, US\$ 0.05, and US\$ 0.08, respectively, and that of producing a kWh of electricity from woody biomass to be about US\$0.1. They also estimated the initial capital cost of producing electricity from biomass to be about US\$140–4260/kWh and the operational costs to be US\$0.21–0.40/kWh. Given an average industrial electricity price of about US\$ 0.07/kWh, they concluded that for many mills the internal production of electricity was not competitive. It is a plausible conjecture that similar unfavorable economic situations underlie many instances where value addition to agricultural byproducts is not undertaken.

### 32.3.4 Transportation Costs

Value addition to agricultural byproducts often entails the collection and transportation of the byproducts to distantly located factories for further processing. Agricultural byproducts can be bulky, making them costly to transport to processing facilities. The high transportation costs associated with the delivery of byproducts to the factories reduces the economic benefits from value addition. In extreme cases, the high transportation costs can render the processing of the byproducts economically unviable. The significance of transportation costs is evident in Sarkar et al. (2012) who argue that bioethanol production can be increased by the effective utilization of agricultural wastes. They, however, identify cost as a major obstacle to such value addition. According to Mertens et al. (2018), agricultural byproducts generally have low bulk density and high moisture content that leads to high collection, handling, and transportation costs. Shabani et al. (2013) also asserts that the costly transportation of forest biomass is a constraint to its value addition in energy production because it makes forest biomass an expensive energy source compared to the cost of conventional fuels.

### 32.3.5 Lack of Awareness About the Value of Agricultural Byproducts

Part of the reason for the underutilization of agricultural byproducts is the widespread lack of awareness about their properties and potential economic benefits. Chitah (2017)

studied the cotton industry in Zambia and cited a lack of awareness as an impediment to the enhanced utilization of byproducts such as cotton seed that has vast potential for the production of cotton seed oil and cotton seed cake. The edible oil produced from cotton seed is used in many countries for cooking, frying, and baking. The cotton seed cake, that remains after the extraction of oil from the cotton seed, is rich in energy and protein and is valuable in the formulation of animal feed. Chitah (2017) further asserts that the increased utilization of cotton seed can serve as a basis for the growth of the edible oil industry and can be a substitute to the imports of vegetable oils that Zambia heavily relies on. He notes that the oil from cotton can also be used to manufacture soaps and cosmetics. An increased awareness of these uses of the byproducts from cotton thus offers a realizable and attractive proposition and opportunity to develop the value chain and increase economic benefits (Chitah 2017).

### 32.3.6 Lack of Markets for Agricultural Byproducts

Markets for agricultural byproducts are essential for their commercialization, value addition, and efficient utilization. Several agricultural byproducts are, however, characterized by missing markets and are rarely sold or traded (Magnan et al. 2012). The absence of markets for agricultural byproducts has obscured their economic value and caused them to be regarded as "free". It has also stifled the evolution of value-based supply chains for the byproducts and the consumer products that can be made from them. In the absence of complete and well-functioning markets for the byproducts, incentives to undertake investments in industries that use them are weak and not responsive to economic signals. The impact of lack of markets for byproducts is evident in the limited use of crop residues to manufacture biofuels. Although it is technologically feasible to produce biofuels from crop residues, ensuring the economic and financial feasibility of biofuel production from these raw materials require not only that such operations be undertaken on a large scale to ensure lower unit costs, but also that investors in such enterprises have adequate and reliable supplies of the feedstocks. Such enterprises would be greatly facilitated by the existence of robust markets where the raw materials could be sourced cheaply and efficiently. The existence of these markets would also incentivize farmers to regard them as a potential source of additional revenue. The lack of markets has also been identified as a major constraint to the efficient utilization of large quantities of manure that are a byproduct of large-scale livestock production. Page (2014) observes that manure is a valuable renewable resource for which there is a need to not only establish ready markets but also to keep the operational costs of its value addition low enough to encourage the production and utilization of manure products.

It is important to note that even when markets for agricultural byproducts exist, the economic viability of value addition still depends on the conditions in those markets. DeVuyst et al. (2011) examined the investments required to build a slatted-floor feedlot and concrete anaerobic digester, and found that the investment was not economically viable. They recommended the provision of subsidies for the production of renewable fuel/electricity to make these value-added activities economically attractive. One implication of this is that market conditions can sometimes constrain value addition to agricultural byproducts.

### 32.3.7 Inadequate Access to Finance

Value addition to agricultural byproducts is an expensive enterprise that can be facilitated by access to affordable financing. Financing is often required to cover the initial investment and operation costs of facilities and equipment needed for value addition. Adesida et al. (2016) contend that innovation is vital in enhancing value addition to agricultural byproducts and that entrepreneurs need to be supported financially to spur more innovation. A concrete example of this is the Innovation Financing Vehicle of the United States Agency for International Development, through which loans have been provided to entrepreneurs in Macedonia to enable them to undertake innovative value addition projects, such as setting up a processing line for drying almond shells to serve as fuel for the almond drying process.

### 32.3.8 Trade Barriers

International commerce is one of the ways of enhancing the utilization and value addition of agricultural byproducts. A good example of this is the export of distillers dried grains (DDG), a nutrient-rich byproduct from corn-based ethanol production, which is mainly used as an alternative feedstock in livestock rations. In the United States where ethanol production using corn has been increasing, there has been a substantial increase in the supply of DDG. According to DeOliveira et al. (2017), DDG production in the U.S. increased by over 37.75 million tonnes (or 1646%) between 2000 and 2015. Besides its consumption by the U.S. domestic livestock and poultry sectors, DDG is also exported to foreign markets where demand for DDG has been growing (Mason 2017). The export of DDG has also contributed significantly to the profitability of ethanol plants. Since September 2016, however, U.S. exports of DDG has been negatively impacted by the imposition of punitive trade barriers by China, which had hitherto been the top market for U.S. DDG. It is estimated that in 2015, China imported 6.5 million metric tonnes of DDG from the U.S. worth about \$1.6 billion, accounting for 51% of total U.S. DDG exports. These trade barriers were imposed on the premise that the U.S. was not only dumping the DDG into the Chinese market but also unfairly subsidizing its producers (Mason 2017; Lane 2017). Initially, China imposed an anti-dumping duty of 33.8% and a countervailing duty ranging from 10% to 10.7% on imported DDG from the U.S. In January 2017, China increased its anti-dumping duty to between 42.2% and 53.7% and its countervailing duty to between 11.2% and 12%. These measures have effectively shut out U.S. DDG from the Chinese market and significantly curtailed a growing source of demand for an agricultural byproduct. According to Mongelluzzo (2017), the restrictive trade measures implemented by China caused U.S. exports of DDG to decline by 89% from 538 522 metric tonnes per month before the duties were imposed to 61 575 tonnes.

### 32.3.9 Environmental and Health Regulations

In several countries, regulations exist to protect the environment and human health from the harmful effects of the production and use of agricultural byproducts. These regulations explicitly recognize the benefits of utilizing agricultural byproducts but are primarily intended to mitigate the probable harmful effects of such use. For example, the Agricultural Waste Control Regulations in British Columbia permit the general use of

agricultural byproducts as soil amendments and mulches but require that the associated risks to the environment and human health be assessed and appropriate measures taken to mitigate them. In the regulations, the use of hog fuel, mill ends, saw dust, shavings, wood chips and barks as soil amendments or mulches is restricted because the leachates from these byproducts can be toxic and impact negatively on the environment and human health. Processing these byproducts to make them safe is costly and to some extent constrains their use for agronomic purposes in regions where the environmental risks are high, particularly on lands that are near vulnerable ground and surface water resources.

Another example of how regulations can constrain value addition to agricultural byproducts is the Renewable Fuel Standard (RFS) that the U.S. Environmental Protection Agency (EPA) enacted to advance climate and energy security goals. The RFS requires that gasoline be blended with biofuels with the current allowable blends being E10 and E85 – meaning 10% ethanol and 85% ethanol. Given that ethanol can be produced from agricultural byproducts, this mandate is problematic for at least two reasons. First, the E10 blending limit imposes an upper bound on the amounts of ethanol that can be used in transportation fuels. Tyner and Viteri (2010) and Stock (2015) maintain that the amount of ethanol currently produced in the United States exceeds the amount required for blending to ensure compliance with the E10 mandate. They argue that if the blending limit of 10% is maintained, the ethanol industry cannot grow and to this extent it represents a constraint to value addition. They propose an increase in the blending limit from 10% to 15%. Second, although higher blends can, at least in theory, increase the demand for ethanol and the utilization of byproducts used in its production, they have been strongly opposed by car manufacturers and retailers. Furthermore, oil companies claim that the costs of complying with higher blending limits are likely to be high given that such limits will necessitate a drastic reduction in the sale of gasoline and dramatically increase the price of gasoline and diesel. Regarding E85, its consumption has been limited by the fact that currently there are few sellers of the fuel in the market and by the small number of fuel flex vehicles in the national vehicle fleet. E85 vehicles have also had a low appeal to customers due to its high price.

## 32.4 Policy Implications

In what follows, we propose some actions that can address the constraints to value addition to agricultural byproducts and enhance their utilization. The measures are context-specific and can mitigate huge economic losses and resource use inefficiencies that result from the underutilization of agricultural byproducts.

- i) Publicity and awareness campaigns: The benefits of value addition to agricultural byproducts can be realized more fully if there is greater awareness not only of these benefits but also of the opportunities. This can be achieved through publicity and awareness campaigns that highlight the diverse social, economic, and environmental benefits of value addition to agricultural byproducts. Some practical ways of creating such awareness include appropriate labeling of products to inform consumers that the products have been made in sustainable ways using agricultural byproducts; educating farmers on alternative uses of manure, such as to produce methane, which can

- be used for domestic heating and cooking; providing farmers with information on how to enhance the quality and digestibility of vegetative materials used to feed animals e.g. by mixing with molasses and urea; sensitizing food processors of the potential of using food waste streams to produce high value products such as polymers, antibiotics, enzymes, organic acids, feed, and food supplements; and, encouraging entrepreneurs to develop new uses of agricultural byproducts. These actions are likely to be adopted if it can be demonstrated that there is strong demand for these products and that these enterprises are profitable.
- ii) Improvements to the quality of agricultural byproducts: Developing techniques for improving the quality of agricultural byproducts is critical in improving their utilization. Such measures would not only enhance the marketability of the agricultural byproducts but would also improve their safety for livestock and human use. Some examples of quality improvements recommended by FAO (2014) are enhancing the nutrient content and palatability of crop residues and thus improve their fitness for feeding livestock; pre-treating cassava peels to reduce their content of glucosides and improve their safety as livestock feed; and, proper processing of sunflower, cotton seed, and groundnut cakes (these are byproducts from extraction of oil from these crops) to reduce their contamination with mycotoxins. However, improvements in the quality of agricultural byproducts is also vital in boosting their market appeal and fostering trade in the byproducts.
  - iii) Research into more efficient uses of agricultural byproducts: To facilitate value-addition to agricultural byproducts and harness their huge potential, more research is required to evaluate the technologies that currently exist for their utilization and to determine how the byproducts can be used in a sustainable and cost-effective way. According to Blaschek et al. (2010) some promising research areas include the possibilities of applying microbial engineering and biotechnology to increase efficiency and reduce the cost of bioconversion of agricultural byproducts; adapting existing technologies to ensure that they are scalable and can be widely applied; and, improving pre-treatment strategies to agricultural byproducts. Yazdani et al. (2010) assert that glycerol, which is a byproduct of biodiesel production, is abundant and inexpensive and can be used to produce ethanol. They suggest that more research needs to be done to identify pathways for converting glycerol into higher value products by either biological or chemical transformation. These are just a few research areas that can generate useful insights into how to enhance value addition to agricultural byproducts. Ensuring success in value-addition to agricultural byproducts, however, requires broader research into other critical dimensions such as the legal frameworks, and the economic, social, and institutional contexts in which value-addition is undertaken.
  - iv) Use of supportive policies and incentives: Policies such as tax exemptions, tax credits, and subsidies have successfully been used to promote value addition to agricultural byproducts. Sam et al. (2017), Key and Sneeringer (2012) and Cowley and Brorsen (2018) contend that the adoption of anaerobic digester systems in the United States to manage farm waste and serve as a source of crop fertilizer and clean renewable energy has to a significant extent been driven by government incentives that include construction cost grants, guaranteed loan financing, renewable electricity subsidies, carbon credits, and technical assistance on biogas recovery. In countries such as India and China, government incentives have also been instrumental

in the adoption of anaerobic digester systems. According to Cowley and Brorsen (2018), these systems have large capital costs and would be economically infeasible without government support. Pelaez-Samaniego et al. (2017) have advanced a similar view and noted that often the revenues from biogas plants are not adequate to supply a preferred return on investment. A further reason why government support to these systems is warranted is the positive externalities such as reductions in greenhouse gas emissions associated with these systems. The successful use of government support to incentivize the adoption of anaerobic digester systems indicates that scope exists for using similar measures in other areas to enhance value addition to agricultural byproducts.

According to Blades et al. (2017) it is possible to link incentives for promoting value addition to agricultural byproducts to climate change policy. One way of achieving this goal would be to grant appropriate credits to fuels made from wastes/residues. In general, the production and use of such fuels is associated with lower emissions of greenhouse gases compared to conventional fossil fuels. When such credits are available and tradeable, they can stimulate the use of agricultural byproducts to produce biofuels that have a lower carbon foot-print.

- v) Investments in supportive infrastructure: The development of appropriate infrastructure is a vital precondition for certain uses of agricultural byproducts. This is the case, for instance, in Europe, where the Renewable Energy Directive requires that at least 27% of the total energy consumption be from renewable sources with at least 10% of the energy consumption in the transportation sector I, being from renewable sources by 2030. According to Blades et al. (2017) this goal can be achieved if investments are made to expand the energy infrastructure to facilitate the injection of the electricity produced in biogas plants into the electricity grid. They also maintain that natural gas from biogas plants can replace conventional fossil fuels to a significant extent if the natural gas network is expanded – to enable both the injection of the natural gas produced into the network and to provide more stations where vehicles that use natural gas can fuel.

## 32.5 Case Studies

### 32.5.1 Dairy Farms in Umbria Region in Italy

Venanzi et al. (2018) present real-world examples of value addition to agricultural byproducts from the Umbria region in Italy, where dairy farms are equipped with biogas plants. This practice is geared toward capitalizing on the synergies between the agricultural enterprises on the farms and energy generation. The biogas plants on these farms use agricultural byproducts produced on the farms, e.g. slurry, cattle manure, poultry manure, whey, cereal processing byproducts, etc. to produce electricity and heat. The electricity from the biogas plants is sold to generate revenue and the digestate from the plants is used in composts to produce biofertilizers. Some lessons that can be learned from these farms are that with appropriate innovation and suitable technologies, considerable value (e.g. from the electricity that is produced for sale and the methane that can be used for cooking and heating) can be derived from agricultural byproducts that would otherwise be wasted; that improving the organization of enterprises that use agricultural

byproducts (e.g. locating biogas plants close to dairy farms and other sources of crop residues) can significantly reduce costs and enhance the profitability of value addition; and, that governmental support through, for example, public subsidies, can catalyze the widespread adoption of value addition to agricultural byproducts.

### 32.5.2 The Beef Industry in the United States

Marti et al. (2011) conducted a detailed study of the beef industry in the United States with a focus on value addition to beef and pork byproducts. These byproducts, which include edible offal, inedible offal, hides and skins, blood, fats, and tallow, provide the raw materials used in pharmaceutical, cosmetic, household, industrial, and medicinal products. Not only are these byproducts of tremendous value domestically within the United States, but they are also an important export that contribute to the value and profitability of the U.S. meat processing industry. Marti et al. (2011) estimate that these byproducts account for more than 23–35% of the volume of U.S. beef/veal and pork exports and about 14–19% of their value. The strong demand for the byproducts in export markets (the main export markets for these byproducts being Mexico, Hong Kong/China, Russia, Japan, and South Korea) has in turn resulted in higher prices for livestock producers. This example demonstrates the vast opportunities for the U.S. to add value to the byproducts of the meat industry. These opportunities can be more fully harnessed through efficient and robust marketing of these products, developing, and implementing appropriate quality standards, and lowering barriers to trade.

## 32.6 Summary and Conclusions

Agricultural byproducts such as manure, corn stalks and cobs, wheat straw, barley straw, palm oil wastes, sawdust, wood chips, leaves, grass clippings, vegetable and fruit wastes, bagasse, molasses, dried distiller's grains, etc. are an important resource whose utilization can provide several economic and environmental benefits. They can be productively harnessed to increase the revenues of farmers; provide feed for livestock; provide raw materials for manufacturing other consumer products; provide feedstock to produce renewable energy such as ethanol, butanol, and methane; and, provide rural employment opportunities and support economic growth. Given that the disposal of agricultural byproducts has in many cases been through environmentally harmful measures such as leaving them on the ground, burning them, or spreading on farmland in the case of manure, their utilization can contribute significantly to the improvement of environmental quality. The use of cottonseed and sunflower cakes to formulate livestock rations, the use of molasses to produce ethanol for blending gasoline, the use of manure in anaerobic digesters to produce ethanol and methane, the use of various crop residues to produce fiber, and the extraction of oils, vitamins and vitamins from vegetable and fruit wastes demonstrate the potential for value addition to agricultural byproducts.

Despite the varied benefits of value addition to agricultural byproducts, progress in value addition has been slow due to legal, technical, economic, and institutional constraints. These constraints include regulatory requirements that restrict the use of meat byproducts such as blood and brains for reasons of food safety and quality; the low

protein content of crop straws and the presence of potentially harmful glucosides in cassava that affects their suitability as animal feed; the high costs of collecting and transporting agricultural byproducts; and the lack of organized markets for some agricultural byproducts.

Effective and well-thought out policy measures can ameliorate the diverse constraints to value addition to agricultural byproducts. Some measures that have been successfully implemented to promote value addition to agricultural byproducts include research into technologies for improving fermentation of crop residues; provision of incentives such as subsidies and tax exemptions where capital constraints would hinder value addition; and, the development of quality standards to stimulate the marketing of the byproducts.

As the global population continues to increase and as resource constraints become more severe, agricultural byproducts will play an increasingly important role given their wide availability and the many possibilities that exist for their utilization.

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