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## Novel Fermented Marine-Based Products

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## Chapter 11

# Novel Fermented Marine-Based Products

Gaurav Rajauria, Samriti Sharma, Mila Emerald, and Amit K. Jaiswal

### 11.1 Introduction

Fermented food is currently experienced by every cultural society in the world according to the availability of the food substrate and their food consumption patterns. In many cases, such products play an important role in ethnic identity and culinary enjoyment. For instance, Europe produces the largest quantity of fermented dairy products while Africa is the largest producer of fermented starch crops and legumes based food products. Similarly, the fermented fish products are very common in south and south-east Asia whereas North America is presumably the biggest producer of fermented beverages and meat products (Khem, 2009). Over the centuries, fermentation techniques and procedure have evolved, refined and extended which helped some fermented products such as bread, cheese, butter and yoghurt to be produced all over the world.

Although, Asians were the pioneers in the development of fish-based fermentation food but marine organisms such as fish and shrimps have always been staple food for people in the coastal countries throughout the world. Because fishing is seasonal, there was a need to store and preserve fish through the winter months. Traditionally, drying and salting were the most prevalent preservation techniques

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that were in place not only to preserve food at home but also to trade food products, especially in the coastal regions (Kurlansky, 2003). However, in the case of fatty fish species (such as salmon, trout, charr and herring), the drying approach was not very effective tool for their preservations (Hagen & Vestad, 2012). In addition, the traditional preservation techniques weren't adding any nutritional value to the finished products which triggered the desire of exploring fermentation as an alternate tool for not only preservation but also for enhancing the nutritional value and flavours. Though, fermentation has always been an important part of human lives; it was not clearly understood of what actually happens during fermentation until the work of Pasteur in the latter part of the nineteenth century. Fermentation is the process by which a food can be spoiled or can be made by fermented microbes. Over the centuries, fermentation techniques have been refined and diversified for wine making, brewing, baking, preservation and dairy and non-dairy based product formation. (<http://www.accessexcellence.org>). However, probably the discovery of fermentation was a 'serendipity' phenomenon when friendly food microorganisms utilized the incorporated salt, and made food products more flavourous and nutritious.

Nowadays, consumer interest in fermented foods and the demand for naturally healthy and culturally embedded foods prepared with artisan technique is growing constantly. This consumer-driven trend explain the growing desire for exploring more marine-based fermented foods such as fish sauce and shrimp paste. The umami flavour, also known as the fifth taste, of these fermented products is seen as the key driver which is carving its route towards restaurants and consumer homes (<http://www.prnewswire.com>). Sushi and Sashimi are examples of ancient fermented Japanese fish foods that are prepared on the basis of ancient preservation methods which were the origin of such fermentation process (Skåra, Axelsson, Stefánsson, Ekstrand, & Hagen, 2015). In addition, apart from artisan taste and historically rich fermented foods, consumers are preferring foods that have beneficial components towards health and wellness. As an important aspect of this trend, probiotic fermented food are getting more attention because of their image as a gut health booster. The increased demand of traditional and/or novel value-added fermented marine products has brought new challenges to the market to develop new products to fulfil consumer demand (<http://www.prnewswire.com>). Therefore, this chapter focuses on various marine (animal/plant)-based fermented products currently consumed by various ethnic group worldwide. It also explores their usage through history along with current research trends and future challenges associated for their commercial production.

## 11.2 History of fermented food

The relation between fermented food and health dates back from Neolithic Chinese to ancient Roman era and the earliest evidence suggests that fermentation was an integral part of the old civilization. It is anticipated that it was Chinese and Georgian who prepared first fermented alcoholic beverages from fruit, rice and honey,

dates from 7000–6000 BCE. Evidence also suggests that people were fermenting beverages in Babylon, pre-Columbian Mexico and Sudan circa 3000 BC, 2000BC and 1500BC respectively (Sahrhage, 2008; Ray & Roy, 2014). The production of fermented dairy based products is well written in ancient Sanskrit and Christian scripts, while Romans were first who revealed the recipe of fermented milk preparation at around 1900 BP. In the same century, the preparation of marine based fermented foods (especially fish based) was also well practised in Europe and North Africa, but nowadays Southeast and East Asian countries are the frontrunner for its production (Khem, 2009).

Undoubtedly, Asian civilizations in particular East Asians have developed a series of fermented food products such as Lao pa daek (fish sauce) by Chinese, Mám (seafood) by Vietnamese, Natto (soybeans) by Japanese and Banchan (vegetables) by Koreans for their everyday cuisine. The other fermented foods like pickles, vinegar, sauerkraut, butter, yogurt, cheeses, and a number of fermented milk and traditional alcoholic beverages products that were developed by Asians, are still popular globally. Furthermore, fermented food such as beer and wine were also used for medicinal purposes and played an extensive role in Asians food system. Though, LAB was quiet prevalent in making traditional fermented food across the world but it was Chinese who took this research further and produced salt-fermented soyfood products such as miso, soy sauce, soy nuggets, tofu, sake, shochu (spirits), and rice vinegar (yonezu) by using fungal enzymes (Ray & Roy, 2014). The wide application of fungus in Eastern fermented foods was because of the climate of this region as fungus (especially molds) can easily grow in humid and long rainy seasons during the warm months. Contrary to East, the application of mold in food fermentation was limited in West due to their strong flavors and aromas; however, some fermented alcoholic beverages were produced by using molds (Ray & Roy, 2014).

Despite the long history of fermented food preparation and consumption, the people were unaware about the role of microorganisms, microbial enzymes and their interaction during the process of fermentation. The first breakthrough in this area came when German scientist Korschelt unveiled the role of fungus *Aspergillus oryzae* in the preparation of koji in 1878. The discovery of the role of another fungus *Rhizopus oligosporus* in fermentation fuelled the research in this area which further triggered the work on bacterial led fermentation.

### **11.3 Role of Microorganisms in Marine Product Fermentation**

Fermented foods are foods modified by microorganisms or enzymes via desirable biochemical changes (Campbell-Platt, 1987). Though the earliest fermentation of food was carried out by resident microorganisms naturally presented on the food however, it is nowadays quite a controlled process wherein a starter culture is used to ensure a standard quality of the fermented products. Traditional fermented product is often possessing distinctive sensory qualities than the controlled process which is

believed to be due to the properties of the raw material and the traditional practices employed (Moretti et al., 2004; Ojha, Kerry, Duffy, Beresford, & Tiwari, 2015). However, such artisan fermentation can sometime deteriorate the quality and can compromise with the safety of food products. In addition, natural microflora led fermentation can also affect the uniformity of the products as the composition of so-called house-flora may vary with the manufacturing location and with the origin of raw foods. Therefore, in order to produce a more consistent and stable product with superior sensory quality and organoleptic features, a starter culture was recommended for the controlled fermentation process (Leroy, Verluyten, & De Vuyst, 2006; Ojha et al., 2015). The most auspicious microorganisms for starter culture are those isolated from native microflora of traditional fermented products. Because these microorganisms are well adapted to the selected food environment and offer a strong competition to the harmful undesirable microorganisms. The starter culture contains a mix of various type of microorganism wherein the selection of each microorganism is based on specific and required function as the functional characteristics of different bacterial strain even within the same species are always unique (Socol et al., 2010; Ojha et al., 2015). Commercially, this careful selection of wild microorganism from traditional food can also enhance the technological innovations for novel fermented products (Leroy et al., 2006; Kołożyn-Krajewska & Dolatowski, 2012). Apart from functional specificity, the selected strain should also contain bacteriocin production capability which is a key characteristic of a microorganism to act as a starter culture. Bacteriocins, cationic peptides with hydrophobic or amphilitic properties, are mainly produced by *Lactobacillus acidophilus*. They are divided into the following main classes: Lantibiotics which are small peptides, and small head proteins subclasses IIa (pediocin-like bacteriocins) and IIb (two peptide bacteriocins), and helveticin (Dobson, Sanozky-Dawes & Klaenhammer, 2007). Bacteriocins demonstrate significant inhibition of a number of spoilage and food-borne pathogens, including but not limited to *Staphylococcus aureus*, *Enterococcus faecalis*, *Clostridium botulinum*, and *Listeria monocytogenes*. They have a wide antibacterial spectrum with potential applications in fermented products and foods, such as meat and fish products, fruits and vegetables, cereals and beverages (Gürakan, 2007).

Yeast, mainly *Saccharomyces cerevisiae*, has a long history to ferment glucose while numerous other fungi or bread moulds are regularly used to produce various value added products like alcohol, enzymes, and sugars after aerobic and anaerobic fermentation. However, of all the genus of bacteria researched, *Lactobacillus*, *staphylococcus* and *Bifidobacteria* have received much attention in food research. According to the Inventory of MFC, 195 species of bacteria and 69 species of yeasts and moulds have been used in the food fermentation. Among them, the bacterial genus *Lactobacillus* (84 species), *Staphylococcus* (15 species), *Weissella* (9 species), *Acetobacter* (9 species), *Gluconacetobacter* (9 species) and *Bifidobacterium* (8 species) whereas fungal genus *Candida* (10 species) and *Penicillium* (7 species) provide the largest number of species for various food matrices fermentation (Bourdichon et al., 2012). In marine product fermentation, the most popular and widely used bacterial strains are represented by the genus *Lactobacillus*. They are Gram-positive, rods or coccus-shaped, non-flagellated, non-spore-forming,

aerotolerant or anaerobic bacteria. During fermentation, lactobacilli utilize carbon sources (such as glucose) and convert it into lactic acid, carbon dioxide and ethanol (and/or acetic acid) as by-products (Hammes & Vogel, 1995; Soccol et al., 2010). LAB-led fermentation inhibits the growth of spoilage bacteria and pathogens with the help of lactic acid which consequently extended the shelf-life and the safety of products (Hu, Xia, & Ge, 2008). These bacteria are natural resident and distributed throughout the gastrointestinal tract (GIT) of human and other higher animals and are rarely associated with any kind of GIT infection. Because of their friendly nature these microorganisms have the reputation of improving gut health (Soccol et al., 2010). The effects of *Lactobacillus* on quality attributes have been extensively studied in different fermented marine products. Lactic acid bacteria are found as the dominant microorganisms in many fermented fish products. The bacteria utilize carbohydrate and reduce the pH of fermented products by producing organic acids. The reduction in pH increases the texture firmness and mouthfeel and adds a unique lactic acid flavour to the fermented product (Gelman, Drabkin, and Glatman 2000). The other genus which is quite prevalent in marine product fermentation is *Staphylococcus* which is Gram-positive, round shape, grape-like clusters and is responsible to enhance the colour and flavour of the fermented products. The lipolytic and proteolytic characteristics of few staphylococci (for instance *Staphylococcus xylosus*) contribute to the aroma of fermented sausages by producing of esters and other aromatic compounds from amino acids, whereas catalase activity of such species prevents rancidity of the products. The esterase activity of *S. xylosus* is important antimicrobial as well as crucial for the proper fermented sausage aroma (Barriere et al., 2001; Mauriello, Casaburi, Blaiotta, & Villani, 2004). Thereby mixing culture in combination with LAB and *Staphylococcus* contribute to the control of microbial safety and quality of the products. In this fermentation, LAB produces lactic acid and kills the harmful bacteria thus ensures the microbial safety while *staphylococcus* influence other technological properties through nitrate reductase and flavour forming activities (Hu et al., 2008). Talon, Walter, Chartier, Barriere, and Montel (1999) suggest that it is *staphylococcus* which contributes in the aroma and flavour of fermented sausage products rather than LAB. However, low pH and organic acids are the main factors that help to preserve the fermented marine food. Additionally, high salt and spices (such as garlic, pepper or ginger) may also add to the safety and quality of fermented products (Paludan-Müller, Madsen, Sophanodora, Gram, & Møller, 2002).

#### 11.4 Current Research Trends in Fermented Marine Products

The current trends of fermented marine products are driven firstly by food authority's recommendations and secondly by the market demands. Furthermore, today's consumers are more inclined towards health and well-being and prefer mild taste and low salt. Because of these, the production and the intake of traditional fermented food are quite low as they are both salty as well as strong in taste. However,

the consumption of these type of foods among the adults (age >40 years) in northern Europe has slightly increased in recent years. The potential reason for this could be related to the scientific documents, awareness and health benefits associated with these products (Skåra et al., 2015). Furthermore, the efficient and rational use of microbes in the production of fermented food has opened up a new perspective and opportunities in this area. Advance scientific research has demonstrated the application of novel microbial cultures or revealed the greater role of our own microbiota which can not only enhance the nutritive value of fermented marine products but can also deliver various health benefits to the consumers (Bourdichon et al., 2012; Ojha et al., 2015). In addition, the application of industrial starter culture and genetically modified microorganisms can offer additional characteristics which can further enhance the functional, nutritional and health properties of the final product (Ojha et al., 2015). However, despite the major breakthrough in microbial research, the role of numerous species isolated from traditional fermented foods is still undefined (Bourdichon et al., 2012).

## **11.5 Marine Animal/Organism-Based Fermented Products**

### ***11.5.1 Fish-Based Fermented Products***

Despite the increased dependency on fish and fish-based products across the globe, several challenges like seasonal availability and easy susceptibility to contamination have resulted in the development of certain processing technologies to deal with them. Fish and fish-based products have been preserved for ages through the civilizations. One of the major preservation methods involved the use of salts. However, due to decreased imports across the countries, the use of salt remained restricted. Of all the preservation measures of freezing, canning, etc. fermentation was the most widely used method due to the distinctive and unique sensorial properties such as flavour, colour, texture, etc. Also, it is the cheapest of all the preservation methods.

Fermented fish products are highly popular in the Indian subcontinent, parts of Africa and Europe. Fermentation protects the outer surface of fish from microbial contamination and renders the enzymes present in the fish allowing fermented product to remain stable for a considerable amount of time (Clucas, 1982). It further breaks down the wet proteins present at the surface of the fish into substances that are simple and stable at room temperature. Depending upon the degree of this breakdown, salt is added in varying amounts to obtain the desired taste. Mainly, three different types of products are obtained using fermentation. These are (a) products in which whole or large pieces of fish are retained, (b) products where the fish is reduced to form a fine paste and (c) fish sauces where the flesh is reduced to a sauce. However, these types of products are mainly found in the Asia (Clucas, 1982). These fermented products are sold as whole fish or cut in pieces (Essuman, 1992). Following are some of the major fish-based fermented food products available in different parts of the world, most of which are confined to their specific regions are mainly known by their local names.



Herring (*Clupea harengus*) is a small salt water fish found in the North Atlantic Ocean, the North Sea and part of the Pacific Ocean. Owing to its availability, herring has been an essential part of the traditional diets in European countries like Norway, Sweden, Finland and Denmark. It is an oily fish with several essential nutrients and fatty acids. The fish is normally eaten salty. The fish is eaten as salted whole herring, herring fillets, spice-salted herring, etc. in different Nordic countries.

*Surströmming* is a fermented fish product staple of traditional Swedish cuisine since sixteenth century. It is made from herring caught from the northern region of the Baltic Sea thus locally known as the Baltic Herring. The fish is mainly caught in the months of May, June and early July when the fat content is low (Skåra et al., 2015). The fish is first pre-salted in a strong brine of saturated salt solution for 1–2 days with continuous stirring for the first 4 h to drain out all the blood. The fish is then beheaded and gutted and put in barrels containing 17% salt solution and stored at 15–18 °C for 3–4 weeks. The barrels are never filled to the top and some space is always left because the fermentation process releases certain gases over the storage period. The fermented product is then transferred to cans along with the brine. However, the fermentation process continues inside the cans and upon opening these gases escape out giving a characteristic smell.

*Rakfisk* is another fermented fish produced from salmonid freshwater fishes like trout and char. The fermented fish is a native food of Norway produced by salting and fermenting for 2–12 months. The gutted fish is rinsed and put in wooden barrels and covered with salt or preformed brine of salt concentration 4–6% w/w (Skåra et al., 2015). The lid of the barrels is placed in a way to provide pressure to the fish. The fish is stored in these barrels at 4–8 °C for 3–12 months.

*Hákarl* is a Greenland shark fermented product famous as the national dish of Iceland. These Greenland sharks are poisonous when consumed fresh due to the presence of high quantities of urea and trimethylamine oxide (Skåra et al., 2015). They are therefore processed to remove these toxic compounds. Owing to the poor production of salt in the country, fermentation and drying were the major traditional food preservation methods. Greenland sharks were cut into chunks and washed with seawater, followed by burying them into the pits prepared mainly close to the sea. This was done to make the seawater available to the pits at the time of high tides. These pits were then covered with sand and stones to compress the fish to extract all the fluids out of them. The sharks were left to ferment for 6–12 weeks depending upon the season of the year. The fermented sharks were taken out of the pits and cut into small pieces and then dried in the air for several weeks. It is this process of fermenting and drying, which makes this shark safe and edible.

In African continent, fermentation is one of the main food processing techniques due to its ease of use, cost efficient and ability to increase the storage of meat products and reduction in spoilage over considerable time period. Of all the fermented products, fish-based fermented products are increasingly popular across the continent and one of the main source of animal protein in the diet. These fermented fish products are produced in three main forms as (a) fermentation with salting and drying, (b) fermentation with drying without salting and (c) fermentation with drying without salting (Anihouvi, Kindossi, & Hounhouigan, 2012).

Based on these three basic approaches of fermentation, some of the most famous fermented fish products in Africa are *Lanhoun*, *Momone* and *Guedj* (Anihouvi et al., 2012). *Lanhoun* is fermented fish product widely used in urban rural areas in Southern Benin, Togo and Ghana. It is mainly prepared from cassava fish (*Pseudolithus* sp.) or Spanish mackerel (*Scomberomorus tritor*) by spontaneous and uncontrolled fermentation that consists of dressing of fresh fish followed by 10–15 h of ripening (Anihouvi, Ayernor, Hounhouigan, & Sakyi-Dawson, 2006). Later, the fish is salted and allowed to ferment. The fish is wrapped properly and buried in 2 m deep pit and left to ferment for 3–8 days. The fermented fish is then washed to remove excess salt and sundried for 2–4 days.

*Momoni* is prepared from fresh water fish African Jack Mackerel (*Caranx hippos*) by scaling, gutting and washing with tap water. The fish is then salted with 294–310 g/kg. The gill and gut region is highly salted and the kept in baskets covered with aluminium trays and allowed to ferment for 1–5 days. The fermented product is then washed with brine and cut in pieces, salted and sun-dried for few hours (Sanni, Asiedu, & Ayernor, 2002).

The fermented fish products found in Asia differ from that of the rest of the world. Fish sauce is a fermented brown liquid seasoning known by different local names in different parts of Asia. Some of the well-known examples include *shottsuru* in Japan, *budu* in Malaysia, *patis* in Philippines, *nuoc-mam* in Vietnam, *yu-lu* in China, *nampla* in Thailand and *bakasang* in Indonesia (Kilinc, Cakli, Tolasa, & Dincer, 2006). Fish sauces are mainly salt-soluble proteins prepared with the help of halophilic bacteria (Lopetcharat, Choi, Park, & Daeschel, 2001). These sauces are known for their characteristic taste and nutritive value. With about 20 g/L of nitrogen fish sauces form a major part of the protein diet in various regions in Southeast Asia. All these and many more fish-based products are specific to the regions and thus the production method might vary depending upon the traditions of that region. Owing to this wide diversity across the continent and their individual geographical needs, the Asian food market is flooded with different types of fermented fish products ranging from whole fish, sauces, pastes to products containing mixture of fish, salt, rice, spices, etc. For instance, Thai fermented fish product *pla-som* consists of fresh-water fish, salt, boiled rice and garlic (Paludan-Müller et al., 2002).

### 11.5.2 Other Marine Animal-Based Fermented Products

Shrimps are other marine organisms that are extremely popular in Asian countries where they are consumed as fermented paste. *Kapi*, a traditional Thai fermented shrimp paste prepared from small shrimp (*Acetes vulgaris*) or krill (*Mesopodopsis orientalis*) with solar salt in the ratio 5:1 is widely used as a condiment across the Southeast Asia (Faithong & Benjakul, 2014). The sun-dried and grounded mixture is allowed to ferment in an earthen jar for 3–6 months. Based upon the source of the raw material, *Kapi* is classified as *Kapi Ta Dam* (Black paste) and *Kapi Ta Deang* (red paste) obtained from mangrove canals and seagrass beds. *Kapi Ta Dam* is

mainly prepared from *M. orientalis*, whereas *Kapi Ta Deang* is produced from *Acetes indicus*, *Acetes japonicus* and *Acetes erythraeus*. Both these products contain high salt concentration of about 13–17 g/100 g sample (Kleekayai et al., 2015). Besides their nutritive importance, these products are also very well known for their antimicrobial, antioxidative and ACE inhibitory properties (Kleekayai et al., 2015; Peralta et al., 2008).

Oyster sauce is another marine-fermented product that is highly popular in Korea. Oyster sauce is prepared from fresh oyster (*Crassostrea gigas*) by mixing with approximately 25 % (w/w) salt and fermented at 25 °C for about 6 months. These sauce have been found to contain free amino acids like glutamic acid, glycine, lysine and alanine (Je, Park, Jung, & Kim, 2005). Fermented oyster sauce is not only known for their nutritional and taste attributes but they also contain ACE inhibitory peptides that offer functional properties to the product (Je, Park, Jung, Park, & Kim, 2005). Table 11.1 lists some of the most common fermented fish and other marine products along with the region where they are mainly consumed.

### 11.5.3 Associated Changes After Fermentation of Marine Animals/Organisms

Preservation of fish products mainly involves the use of salt. However, the amount of salt used varies depending upon their availability, region of use and duration of fermentation. In certain cases, it is accompanied by other food processing methods of drying depending upon the kind of flavour and aroma required. Addition of salt provide the initial dehydration of the product to extract all the poisonous compounds, regulates the moisture content and also reduces the chances of contamination due to its intrinsic antibacterial nature (Beddows, 1997). Besides, salt also helps activate certain intrinsic enzymes present in the fish flesh or in the microorganisms that aid in the fermentation of the fish products. The duration of the fermentation process defines the aroma, taste and type of the final product.

#### 11.5.3.1 Microbial Changes

Studies have identified microorganisms ranging from aerobic, anaerobic, microaerophiles, thermophiles, halophiles, etc. involved in the fermentation of fish-based products. However, the amount of these microorganisms might vary depending upon the type of fish, concentration of salt and stage of fermentation (Table 11.1). Bacterial count of  $10^8$  cells/g is achieved at the end of fermentation in *Hákarl* (a type of fermented shark as discussed in the previous section). Some of the predominant bacterial species include those from *Acinetobacter* group along with *Lactobacillus*. The urease enzyme present in these bacteria converts urea present in *Hákarl* into ammonia, resulting in an increase in pH from 6 to 9 in the final product (Skåra et al., 2015). During this process, TMA is also formed from trimethylamine

**Table 11.1** List of fermented marine animal-based products and microorganism involved in the fermentation

Fermented products	Region	Microorganisms	Raw material	References
<i>Suströmming</i>	Sweden	<i>Halanaerobium</i>	Freshly caught Baltic herring ( <i>Clupea harengus</i> var. <i>membras</i> ), salt	Skåra et al. (2015)
<i>Rakfisk</i>	Norway	<i>Lactobacilli</i> ( <i>Lactobacillus sakei</i> )	Salmonid freshwater fish, salt	Skåra et al. (2015)
<i>Hákarl</i>	Iceland	<i>Moraxella</i> / <i>Acinetobacter</i> , <i>Lactobacillus</i> sp.	Greenland Shark ( <i>Somniosus microcephalus</i> ), salt	Skåra et al. (2015)
<i>Lanhouin</i>	Africa (Benin, Togo, Ghana)	<i>Bacillus</i> ( <i>B. subtilis</i> , <i>B. licheniformis</i> ), <i>Staphylococcus</i> ( <i>S. lentus</i> , <i>S. xylosus</i> ), <i>Corynebacterium</i> , <i>Pseudomonas</i> , <i>Micrococcus</i> ( <i>M. luteus</i> ), <i>Sreptococcus</i> , <i>Achromobacter</i> , <i>Alcaligenes</i>	Cassava fish ( <i>Pseudotolithus</i> sp.), Spanish mackerel ( <i>Scomberomorus tritor</i> ), salt	Anihouvi et al. (2006) and Anihouvi, Sakyi-Dawson, Ayemor, & Hounhouigan (2007)
<i>Momone</i>	Ghana	<i>Micrococcus</i> , <i>Staphylococcus aureus</i> , <i>Staphylococcus</i> sp., <i>Bacillus</i> sp., <i>Lactobacillus</i> sp., <i>Pseudomonas</i> , <i>Pediococcus</i> , <i>Klebsiella</i> , <i>Debaryomyces</i> , <i>Hansenula</i> , <i>Aspergillus</i> .	African jack mackerel ( <i>Caranx hippos</i> ), salt	Nerquaye-Tetteh, Eyleson, and Tete-Marmon (1978) & Sami et al. (2002)
<i>Pla-som</i>	Thailand	<i>Pediococcus pentosaceus</i> , <i>Lactobacillus alimentarius</i> / <i>fariciminis</i> , <i>Weissella confusa</i> , <i>L. planetarium</i> , <i>Lactococcus garvieae</i> , <i>Zygosaccharomyces rouxii</i>	Snakehead fish, salt, palm syrup, roasted rice	Paludan-Müller et al. (2002)
<i>Ngari</i>	India	<i>Lactococcus plantarum</i>	Fish ( <i>Puntius sophore</i> ) and salt	Thapa, Pal, & Tamang (2004)
<i>Hentak</i>	India	<i>Lactobacillus fructosus</i> , <i>Lactobacillus amylophilus</i> , <i>Enterococcus faecium</i>	Mixture of sundried fish ( <i>Esomus danricus</i> ) powder and petioles of aroid plants ( <i>Alocasia macrorrhiza</i> )	Thapa et al. (2004)
<i>Tungtap</i>	India	<i>Lactobacillus coryniformis</i> subsp. <i>Torquens</i> , <i>Lactococcus lactis</i> subsp. <i>Cremoris</i> , <i>Lactobacillus fructosus</i>	Dry fish ( <i>Danio</i> spp.) and salt	Thapa et al. (2004)
<i>Budu</i>	Malaysia	<i>Micrococcus luteus</i> , <i>Staphylococcus arlettae</i>	Raw anchovies ( <i>Stolephorus</i> spp.) and salt	Sim, Chye, & Anton (2015)
<i>Yu lu</i>	Southern and Eastern parts of China	Halotolerant and halophile groups (LAB and Yeast)	Anchovies ( <i>Engraulis japonicus</i> ) and snakehead fish ( <i>Channa asiatica</i> )	Jiang, Zeng, Zhu, and Zhang (2007) & Jiang, Zeng, and Zhu (2011)
<i>Kapi</i>	Thailand, Cambodia	<i>Staphylococcus</i> , <i>Tetragenococcus</i>	Shrimp ( <i>Acetes vulgaris</i> ) or krill ( <i>Mesopodopsis orientalis</i> ) and salt	Chun et al. (2014) and Faithong & Benjakul (2014)
<i>Prahok</i>	Cambodia	<i>Staphylococcus</i> , <i>Tetragenococcus</i> , <i>Rhodotorula</i> , <i>Candida</i>	Freshwater fish ( <i>Channa striata</i> ) and salt	Chun et al. (2014)

<i>Toeuk trey</i>	Cambodia	<i>Bacillus Virgibacillus, Lentibacillus, Lysinibacillus, Staphylococcus, Micrococcus, Kocuria</i>	Small freshwater fish	Chun et al. (2014)
<i>Bakasang</i>	Indonesia	<i>Micrococcus, Streptococcus, Pediococcus</i> sp.	Small fish like sardines ( <i>Engraulis japonicus</i> ) and salt	Ijong & Ohta (1996)
<i>Som-fug</i>	Thailand, Southeast Asia	LAB	Fish ( <i>Priacanthus tavenus</i> , <i>Nemipterus japonicus</i> , <i>Sphyræna langsar</i> , <i>Sphyræna obusata obtusata</i> , <i>Saurida tumbil</i> , <i>Trichiurus lepturus</i> )	Riebrov et al. (2007a, 2007b)
<i>Sausage</i>	Asia (specially in China)	LAB ( <i>Lactobacillus plantarum</i> , <i>Staphylococcus xylosum</i> <i>Pediococcus pentosaceus</i> <i>Lactobacillus casei subs casei</i> )	Fish ( <i>Macrurus novaezealandiae</i> , <i>Arripis trutta</i> , <i>Pseudocaranx dentex</i> , <i>Hypophthalmichthys molitrix</i> )	Hu et al. (2008) & Khem, Young, Robertson, & Brooks (2013)
<i>Miso</i>	Japan	<i>Aspergillus oryzae</i>	Fish meat paste ( <i>Scomber australasicus</i> and <i>Trachurus japonicus</i> )	Giri, Osako, & Ohshima (2010)
<i>Pla-ra</i>	Thailand	<i>Pediococcus</i> sp.	Freshwater fish	Lee (1997)
<i>Sikhae</i>	Korea	<i>L. mesenteroides</i> , <i>L. plantarum</i>	Seawater fish	Lee (1997)
<i>Narezushi</i>	Japan	<i>L. mesenteroides</i> , <i>L. plantarum</i>	Seawater fish	Lee (1997)
<i>Burong-ida</i>	Philippines	<i>L. brevis</i> , <i>Streptococcus</i> sp.	Freshwater fish	Lee (1997)
<i>Gravlaks</i>	Northern Europe	<i>Pediococcus</i> , <i>Lactobacillus</i> , <i>Leuconostoc</i> , <i>Micrococcus</i> and <i>Staphylococcus</i> sp.	Salmon ( <i>Salmo salar</i> )	Nordvi, Egelandstad, Langsrud, Ofstad, & Slinde (2007)
<i>Rakorret</i>			Trout ( <i>Salmo trutta</i> )	
<i>Tidbits and Surströmming</i>			Atlantic herring ( <i>Clupea harengus</i> )	
<i>Jeotgal</i>	Korean	<i>Staphylococcus equorum</i> , <i>Halanaerobium saccharolyticum</i> , <i>Salimicrobium luteum</i> , <i>Halomonas jeotgali</i>	Shrimp ( <i>Acetes japonicus</i> )	Han et al. (2014)
<i>Terasi</i>	Indonesia	LAB ( <i>Tetragenococcus halophilus</i> group <i>Tetragenococcus mutaticus</i> )	Shrimp paste	Kobayashi et al. (2003)
<i>Balao-balao</i>	Philippines	<i>L. mesenteroides</i> , <i>P. cerevisiae</i> , <i>S. faecalis</i>	Shrimp ( <i>Penaeus indicus</i> ) and salt	Sanchez (2008)
<i>Alamang</i>	Philippines	Yeast and LAB	Small shrimp	Melchor (2008)
<i>Burong Talangka</i>	Philippines	LAB	Live shore crabs ( <i>Varuna litterata</i> )	Sanchez (2008)

N-oxide. However, the bacterial count decreases during the process of drying resulting in poor or no ammonia and TMA quantities. According to Skåra et al. (2015), several studies over the years confirm *Lactobacillus* as the dominant microbial species in the *Rakfisk*, another fermented product of Europe. The bacteria are found in lower quantities in the raw material followed by increase in their number to  $10^8$ – $10^9$  cells/mL at the end of 4-weeks. *Lactobacillus* also dominates another fermented fish product called *Surströmming*. However, further ripening in the packed-canned product is mediated by a strict anaerobic halophile called *Halanaerobium* providing a unique odour to the final product (Skåra et al., 2015).

A variety of microorganisms including range of Gram-positive and Gram-negative bacteria have been identified in various African fermented fish products. Some of the most prominent species found in *Lanhoun* include *Bacillus* sp., *Staphylococcus* sp., *Micrococcus* sp., *Streptococcus* sp. and *Corynebacterium* sp. That might have come from the salt used during the treatment of the fish which favour the growth of salt tolerant microorganisms and inhibiting the growth of lactic acid bacteria. Because, in the processing of *Lanhoun*, dressing of the fish is done prior to ripening in order to minimize the chances of any spoilage due to the presence of microorganisms of the gut. In addition to some of these species, *Momone* also contains populations like *Klebsiella* and moulds like *Aspergillus*, whereas *Guedj* contains *Proteus* sp., *Shewanella putrefaciens* and *Bacillus* sp. as the predominant microbial populations. *Momone* is prepared in method similar to *Lanhoun*.

Unlike African and European fermented fish products, major products in Asia mainly comprise of whole fish, fish sauce and fish paste. Studies indicate a significant increase in the bacterial count in fish sauces in the first 8 days of fermentation that decreases thereafter. It is also seen that the fish sauces containing spices show a comparative decrease in the microbial count as compared to those that lack spices (Kilinc et al., 2006). In case of fish pastes, like *bagoong* which is native of Philippines, studies indicate that aerobic microorganisms predominate during the beginning of the fermentation process which is replaced by microaerophiles and anaerobic microorganisms. However, in certain cases, harmful microorganisms could also persist as part of the microflora. *Pla-som* a fermented fish product of Thailand contains Lactic acid bacteria isolates of *Pediococcus pentosaceus*, *Lactobacillus alimentarius*, *Weissella confusa*, *Lactobacillus plantarum* and *Lactococcus garvieae* and yeasts like *Zygosaccharomyces rouxii* as the dominant species. A study indicates an increase in number of these microorganisms within the first week of fermentation with numbers varying depending upon the salt concentration used (Paludan-Müller et al., 2002). Yeast is mainly responsible for the flavouring of this product. However, in another Thai product *som-fak*, growth of yeast indicates spoilage.

#### 11.5.3.2 Biochemical Changes

Fermentation of the fish causes the breakdown of the fish protein by both the intrinsic enzymes of the fish and enzymes of the halophilic and halotolerant and microorganisms resulting in the formation of simple proteins, peptides, free amino acids

and ammonia. However, depending upon the amount of salt added to a product and duration of fermentation, the amount and types of these compounds present in the product varies from region to region. Some of the compounds produced by such fermentation include alcohols, acids, aldehydes, ketones, nitrogenous compounds and aromatic compounds. One of group of these compounds is responsible for distinct taste and flavour in different types of fermented fish products.

Alcohols are one of the main products of any fermentation process. Ethanol is produced as a result of the microbial fermentation of sugars present in the fish. 1-pentanol, 2-methyl 1-butanol, 2-methyl 1-propanol and 3-methyl 1-butanol are some of the alcohols present in the fermented fish products found to be responsible for their aroma. 3-methylbutanal, 2-methylbutanal and 2-methylpropanal are some of the aldehydes produced as a result of oxidation of lipids or deamination of amino acids present in the fish products over the period of fermentation. These aldehydes are responsible for characteristics like fishy and grassy; nutty and pungent smell in different fermented fish products. *Budu* is one such fish sauce that contains high concentration of aldehydes. Ketones like 2-ethylfuran and 3-pentylfuran, on the other hand, provide a cheesy odour to the fish sauces (Mohamed, Man, Mustafa, & Manap, 2012). A study by Fukami et al. (2002) has identified 2-methylpropanal, 2-methylbutanal, 2-pentanone, 2-ethylpyridine, dimethyltrisulphide, 3-(methylthio)-propanal and 3-methylbutonic acid as principal contributors to odours of fishy note, sweaty note, faecal note, cheesy note, rancid note, burnt note and meaty note in fish sauce (Fukami et al., 2002).

Besides, nitrogenous compounds like 2,6-dimethylpyrazine and aromatic compounds like benzaldehyde and benzeneacetaldehyde are some of the most common compounds that contribute to the aroma of fish sauces (Mohamed et al., 2012). During the fermentation process, the protein content increases and carbohydrate content decreases. Oyster sauces have been found to contain higher amounts of amino acids like aspartic acid, lysine, glutamic acid, glycine and alanine than other free amino acids. These amino acids have been found to be essential for taste in oyster sauces.

### 11.5.3.3 Other Changes

The microbiological changes contribute to a specific microbial cell population that along with the intrinsic enzymes of the fish help bring out biochemical changes resulting in the production of wide range of compounds that ultimately help achieve specific characteristics in the final product. Besides, the microbial population and the salt concentration help produce changes in the pH, moisture content, aroma, colour and texture.

Changes in pH occur during the course of fermentation due to the releases of different compounds like organic acids, ammonia, free amino acids, etc. *Hákarl* fermentation results in an increase in pH from 6 to 9 due to the conversion of urea present in the fish to ammonia over the fermentation process. In certain cases, the concentration of salt used and the temperature at which the fermentation occurs regulates the changes in pH of the product. For instance, in *rakfisk*, fermentation is mainly



by autocatalytic process at low temperatures whereas temperatures of 5–10 °C, it is mediated by microorganisms like lactic acid producing bacteria. Therefore, at temperatures of around 5–10 °C and 5–6 % salt concentrations, pH initially drops from 6.5 to 4.5 and rises at the later stage (Skåra et al., 2015). Studies on similar fermented fish products indicate changes in the moisture content and viscosity of the final products that indicate differences in the raw materials and different concentrations of salt used for curing (Harikiedua, Wijaya, & Adawiyah, 2012).

Fermentation also provides a specific colour to the final product. This is due to the release of carotenoids by the autocatalytic process. It is found that prolonged fermentation causes the release of pigment from the red–orange–coloured protein–pigment complexes (Astaxanthin) in shrimp resulting in increased redness of *Kapi* (Faithong & Benjakul, 2014). Several studies have identified sensory attributes related to taste and odour that also help differentiate the fermented fish products. Some of these attributes are sulphury meaty odour associated with garlic and meat, overripe cheese, acid vinegar, burnt odour associated to overheated product, salty taste, Umami taste associated with monosodium glutamate and sour and bitter (Harikiedua et al., 2012). These sensory attributes are supposed to be related to specific physiochemical properties, where these attributes could be positively or negatively correlated to the moisture content, salt concentration and viscosity of the fermented product.

#### **11.5.4 Value-Added Products from Fermented Marine Animals/Organisms**

Research over the decades has always emphasized on the marine animals as reservoirs of resources of human benefit. This not only remains confined to their use as food but also to wide range of value-added products that can be extracted from whole marine animals and their waste. These value-added products could be enzymes, peptides, fats and oils, vitamins, minerals, etc. Table 11.2 provides the names of some fermented products of proven value-added properties. They might also provide additional health benefits due to which they are of increased importance in pharmaceutical, nutraceutical and functional food industries. These value-added products could be derived either from the waste that is generated during the processing of marine-based products or from the fermented products itself.

Fermented of different marine organisms like fish, sharks, shrimps, squids, etc. into products like paste and sauces bring about major changes in the fish proteins by both endogenous enzymes present in the fish or microbial degradation. Enzymes like pepsin, trypsin, carboxypeptidase, amylases and lipase present in the fish starts acting on the flesh as soon as the fish is dead. Also, wide range microorganism present on the flesh or from external solvents like brines of different salt concentration start acting upon the fish proteins into simpler molecules that could then not be spoiled by the degrading microorganisms. It is this process of fermentation that forms key to the preservation of any fish-based product increasing their storage life. During this process, a wide range of compounds are formed that provide the product with specific flavour and aroma. Many of these like alcohols, aldehydes and acids have



**Table 11.2** Value-added properties of some marine fermented products

Marine-organisms-based product	Value-added component	Function	References
Anchovy sauce	Angiotensin Converted Enzyme (ACE-I) inhibitor peptides	Tendency to lower blood pressure	Ichimura, Hu, Aita, & Maruyama (2003)
Anchovy sauce	Hydrophobic peptide fraction	Induction of apoptosis in human lymphoma cell line U937	Lee et al. (2003)
Marine blue mussel sauce	Mussel-derived Radical Scavenging Peptides (MRSP)	Antioxidant and radical scavenging properties	Rajapakse et al. (2005)
Fermented Oyster sauce	ACE-Inhibitor peptide	Antihypertensive effect in hypertensive rats	Je, Park, Jung, Park, & Kim (2005)
Shrimp paste (Kapi Ta Dam and Kapi Ta Deang)	Dipeptides (Ser-Val, Ile-Phe; Trp-Pro)	ACE-Inhibitory activity; radical scavenging activity	Kleekayai et al. (2015)
Seacure® (Fermented fish protein concentrate)	Small peptides	Capacity to enhance non-specific host defence mechanism	Duarte, Vinderola, Ritz, Perdígón, & Matar (2006)

already been discussed in the previous sections. Free amino acids, peptides, dipeptides and oligopeptides are formed during the fermentation of fish products that possess antihypertensive, anticoagulant, antioxidant and anticancerous properties.

Researchers have further identified certain other compounds in some of the fish-based products that possess certain properties in additions to their nutritive importance. One such product is a fermented fish-based product commercially known as Seacure® which is a dried fish protein concentrate (Duarte et al., 2006). It is produced by controlled proteolytic fermentation of pacific whiting (*Merluccius productus*) by yeast. This product is found to introduce biological gut repair and integrity in rat model (Fitzgerald et al., 2005). In another study, hydrophobic peptide fractions separated from anchovy fish sauce have been studied for their anticarcinogenic properties. These peptide fractions were studied for their role in induction of apoptosis of cancer cells in human lymphoma cell lines (U937) indicating their cancer chemopreventive effects (Lee, Kim, Lee, Kim, & Lee, 2003). A similar study on marine blue mussel sauce identified peptides with strong scavenging effects on radicals and their antioxidant properties against free radicals (Rajapakse, Mendis, Jung, Je, & Kim, 2005).

Research has also shown that the production of these value-added compounds also vary depending upon the duration of fermentation. For instance, a study on *Kapi*, a traditional fermented Shrimp sauce, found that prolonged fermentation throughout the first 8 months could result in accumulation of short chain peptides and amino acids and Millard reaction products that help in enhancing the antioxidant properties (Faithong & Benjakul, 2014). Another research studies the antioxidant activities of three Thai traditional fermented shrimp products *Kapi*, *Jaloo* and *Koong-Som* (Faithong, Benjakul, Phatcharat, & Binsan, 2010). These works testify the

value-added properties of marine-based products which are mainly due to the fermentation process over period of time that introduces changes in the native proteins, resulting in a wide range of compounds. Since microbial cells tolerant to high salt concentration, also contribute to the fermentation process. The growth of these microorganisms over the period of fermentation releases several important proteins and enzymes. *Jeotgal* is one such fermented fish product in Korea, that serves as the substrate for the production of  $\beta$ -1, 3-1, 4-glucanase, an extracellular protease by *Bacillus spp.* (Kim, Kim, Kim, Choi, & Kong, 2009).

Besides the final fermented products, the waste generated during the processing of marine-based products is another major source of value-added products. Huge tonnes of waste generated from the processing units are already a great cause of concern across all the countries of the world. In order to deal with this problem of waste disposal, several treatment measures are being worked upon. These treatment plants further costs extra amounts to the fishery sector. However, there are enough evidences suggest that this waste is still rich in high value compounds. Shrimp waste comprising of head and shell, for instance is rich in amino acids, peptides, proteins and other nutrients with bioactive properties (Dey & Dora, 2014). Chitin, chitosan and protein hydrolysates have been successfully extracted from shrimp waste (Manni, Ghorbel-Bellaaj, Jellouli, Younes, & Nasri, 2010).

## 11.6 Marine Plant-Based Fermented Products

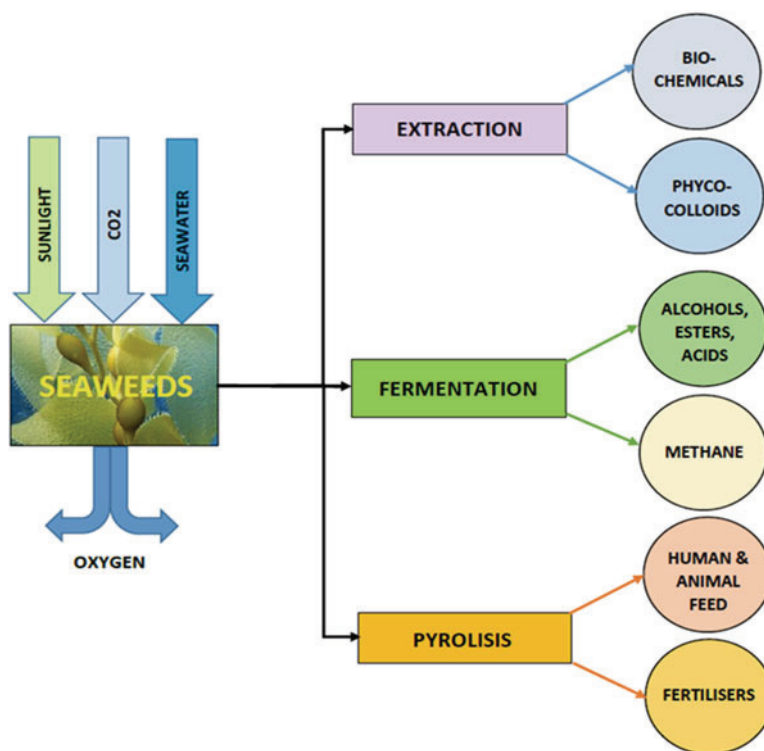
Marine agriculture is one of the fast developing sectors of the world food production and international economy with 26.1 million tonnes of aquatic algae produced in 2013 worldwide by 33 countries and territories, and 13.5 million tonnes of aquatic algae is produce yearly in China along. The dominating cultivated marine plants are *Eucheuma* seaweeds (*Kappaphycus alvarezii* and *Eucheuma* sp.) followed by Japanese kelp, or kombu (*Laminaria japonica*), *Gracilaria* sp., Wakame (*Undaria pinnatifida*), *Porphyra* sp. and other seaweeds and microalgae. Filamentous algae (*Spirulina*, *Spirogyra*, *Cladophora* and *Hydrodictyon*), seaweeds (*Ulva lactuca*, *Gracilaria* sp. and *Porphyra tenera*), floating aquatic macrophytes (*Azolla* sp., *Ipomoea aquatica*, *Pistia stratiotes*, *Salvinia* sp. and *Hydrocharis dubia*), duckweed which is including 37 species belonging to the four genera, water hyacinths (*Eichhornia crassipes*), as well as submerged and emergent aquatic macrophytes are well-known for its exceptional nutritional value and economic importance, and effectively used as natural fertilizers, animal and aqua feeds, source of rare ingredients used in medicine, skincare and cosmetics.

Algae are an excellent source of the natural compounds which have a huge variety of applications in different industries. They are excellent source of proteins and nutrients (Chlorella, Spirulina, Kelp), as well as pigments, lipids (omega-3 ( $\omega$ 3) and omega-6 ( $\omega$ 6)), carbohydrates, macroalgal polysaccharides (agar, alginates and carrageenans) and vital minerals. The algae lipids include acylglycerols, free fatty acids (FFA), phospholipids and glycolipids, and fatty acids biosynthesis is carried

out by biochemical pathways for *acetyl*-CoA production. Microalgae are also an excellent source of high-quality polyunsaturated fatty acids (PUFAs), such as  $\alpha$ -Linolenic, docosapentaenoic, docosahexaenoic (DHA) and eicosapentaenoic acids (EPA;  $\omega$ -3),  $\gamma$ -Linolenic and arachidonic acids (ARA;  $\omega$ -6). The long chain  $\omega$ -3 PUFA which are healthy development of the foetal brain (ARA and DHA), they are also effective in reduction cardiac diseases (high blood pressure, stroke and arrhythmia), depression, arthritis, asthma, Crohn's disease, ulcerative colitis, psoriasis, lupus, cystic fibrosis and cancer, especially EPA (Pulz & Gross, 2004).

Algin derived from *L. japonica* is also widely used in manufacturing of plastics, rubber products, pesticides, paints and paper. Some of the red, green and brown seaweeds and some filamentous algae are shown to be an excellent source of vitamins A, B, C, D, calcium, magnesium, potassium iodine, sulphur, selenium and zinc, proteins, algin, agar, chlorophyll, etc., super nutritious food additives for human diet. Many aquatic plants used in production of biofuel, hydrogen, paper (cellulose and hemi-cellulose), building material, animal and aquatic feeds, marine ingredients supply and medical use. One of the most popular marine plant product is seaweed, which is a great source of polyphenols, peptides and polysaccharides (Zhang et al., 2007), extracted from plant using variety of methods. The diagram of the basic products manufactured from the seaweed using different methods (extraction, fermentation and pyrolysis) is shown in Fig. 11.1.

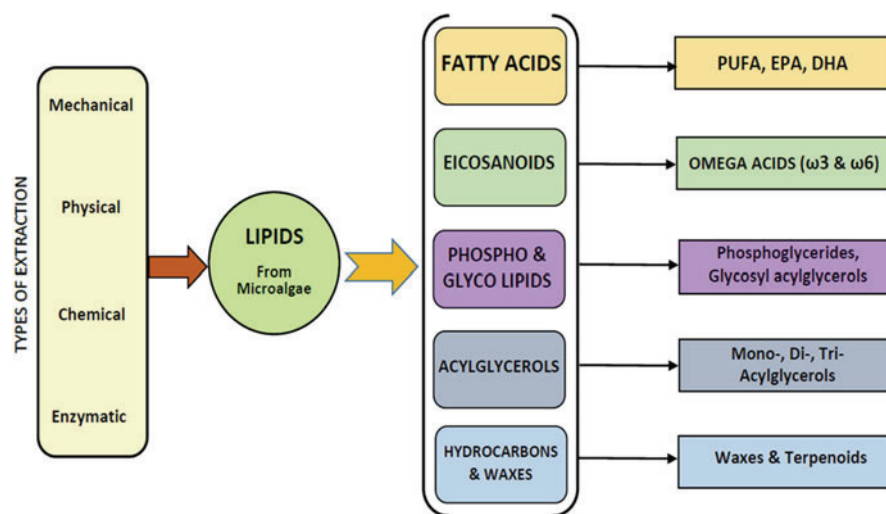
Marine algae by-products obtained using fermentation process using different types of microbes, such as *Bacillus subtilis*, *Pediococcus acidilacti*, *P. pentosaceus*, etc. The seaweed fermentation showed an increase in immunoglobulin concentration and stimulation of the immune system in poultry and mammals (Allen & Pond, 2002). Many active compounds derived from seaweed exhibit anticoagulant, anti-inflammatory, antiviral and anticancer properties. Usually, the basic marine plants fermentation process is triggered in distilled water supplemented with yeast extract (0.1 %) and glucose (0.5 %), followed by hydrolysis using cellulose enzyme (4 %), and incubation under static condition. Cosmeceuticals, a novel class of products, which is a combination of cosmetics and pharmaceuticals, include extracts made from algae, seaweeds and sea minerals often possess UV some antioxidant protection properties. A variety of bioactive compounds isolated from marine macroalgae have many great health benefits and demonstrate antioxidant, antibacterial, antiviral, anti-inflammatory, apoptotic and anticoagulant activity, as well as present rich source of water-soluble dietary fibre (50–85 % dry weight). The polysaccharides, such as fucoidan, alginate, laminarin and others derived from marine macroalgae, used as a prebiotic in animal feed and human health products. *Digenea* is an effective vermifuge agent, red algae from family *Dumontiaceae* inhibit the *Herpes simplex virus* and carrageenans have been patented as antiviral agents. *Kelp*, *Saccharina* and *Sargassum* polysaccharides could be effective against the breast and other types of cancer, as well as protect from heavy metal toxicity (Andrade et al., 2010). Fucoidans from *Ascophyllum nodosum*, *Saccharina japonica*, *U. pinnatifida*, *Alaria* sp. and *Fucus evanescens* demonstrate powerful antitumorigenic potential (Vishchuk, Ermakova, & Zvyagintseva, 2013). To obtain the bioactives from the marine plants, researchers used acid–base hydrolysis, hot water or solvent extraction and enzymatic digestion (Ekanayake et al., 2008).



**Fig. 11.1** The basic diagram of the types of production from the seaweed plants (Copyright @ by Dr. M. Emerald, 2016)

*Pachymeniopsis elliptica*, *Sargassum horneri* and *Ulva pertusa* natural fermentation by adding sugar, water and keeping at room temperature (25 °C) for 3 months has been proposed as a method of active compounds release for potential anticoagulant activity. It has been found that the fermentation process improving the anticoagulant properties of the polysaccharide compound (Ekanayake et al., 2008).

Due to a limited availability of fish oil, and the focus on the use of microalgae as one of the main sources for Omega-3 production, the Omega-3 fatty acid market is expected to grow from current 32B to 36B by the end of 2016. Microalgae are also an abundant source of the omega-3 fatty acids which are crucial for a good health, and a variety of microalgal strains from genera *Phaeodactylum*, *Nannochloropsis*, *Thraustochytrium* and *Schizochytrium* has highest content of total lipids, over 50 % of dry weight, both EPA and/or DHA. Omega-3 fatty acids ( $\omega$ -3) are used in the treatment of rheumatoid, Crohn's disease, ulcerative colitis, neurological conditions, psoriasis, asthma, lupus and cystic fibrosis. The fermentation and conversion of the carbohydrate fraction into glucose prior to lipid extraction caused 15 % increase in additional lipids, as well as improved the solvent extractability of lipids from the algae (Trzcinski, Hernandez, & Webb, 2012) (Fig. 11.2).



**Fig. 11.2** The basic diagram of the types of extraction and final products obtained from the micro-algae lipids (Copyright @by Dr. M. Emerald, 2016)

### 11.6.1 Algal-Based Fermented Food and Value-Added Products

Most fermented foods are made from marine animals like fish and shrimp; however, no food products have yet been developed from marine plant materials like algae. Seaweed *Gracilaria fisheri* has been utilized for the production of a fermented beverage (Prachyakij, Charernjiratrakul, & Kantachote, 2008). Gupta, Abu-Ghannam, and Scannell (2011) used different brown Irish seaweed as sole source of nutrition for LAB and suggested the potential of fermentation of seaweeds with a possibility towards the development of functional food products. The same authors also explored *Saccharina latissima* and *Laminaria digitata* seaweed as a sole source of nutrition for *Lactobacillus Rhamnosus* probiotic bacterium for the development of possible fermented products with health-promoting properties (Gupta, Abu-Ghannam, & Rajauria, 2012). Wijesinghe et al. (2012) fermented the processing by-product of *Ecklonia cava* brown seaweed by the yeast *Candida utilis*. The study demonstrated that fermentation enhanced the polyphenolic content and resultant antioxidant activities, thus suggested fermented *E. cava* processing waste could be a potential alternate for the development of functional food and cosmetic products. Similarly, the red seaweed waste, obtained after extraction of agar-agar, has been fermented with LAB and yeast and the product has been utilized as a fertilizer (Ennouali, Ouhssine, Ouhssine, & Elyachioui, 2006). In another study, Felix and Pradeepa (2012) produced a fermented seaweed-based food for shrimp larvae wherein the *Ulva* sp. of seaweed was fermented with *L. plantarum* (LAB) and food grade *S. cerevisiae* yeast from grape. The substrate was also fortified with potato and soya powder as a sugar and nitrogen substrate, respectively. The findings concluded that the fermented seaweed product was an ideal material for feeding shrimp larvae. The seaweed biomass

is also considered a possible carbon source for lactic acid production which is one of the vital component of food, pharmaceutical, leather and textile sectors (Wee, Kim, & Ryu, 2006). Research showed that sugar from seaweed biomass are more promising or even more beneficial substrate than lignocellulosic biomass for L-lactic acid fermentation and production with different LAB species (Hwang, Lee, Kim, & Lee, 2011). Table 11.3 lists some of the most common algal species and microorganisms used for fermentation for value-added products preparation.

Though, both macroalgae (seaweeds) and microalgae are possible materials for lactic acid fermentation, but their potential have not been explored for any commercial fermented product development. Recent research demonstrated that fermented sauce can be one of the possible products to be developed from *U. pinnatifida* and *Ulva* sp. of seaweed. However, the developed product has limited commercial value because of the shortage of amino acid compounds, which can be explained by the low protein content of seaweed. Additionally, seaweed contains exclusive polysaccharides such as alginate, laminarin, fucoidan and galactan which require novel enzymes to initiate the saccharification process, a very important step of many kinds of fermentations. Therefore, the advancement in enzyme products is essential for saccharification as well as future algal food product development (Uchida, 2011; Uchida & Miyoshi, 2013).

### 11.6.2 Other Non-food Algal-Based Fermented Products

Apart from food products, algal species have shown a great potential to produce bioenergy (such as biodiesel and bioethanol) through fermentation. The renewable energy production with high energy yields is a vital new direction on the way of prevention and overcome of negative and potentially irreversible effects of fossil fuels and environmental pollution on the global climate with emissions of greenhouse gases. Anaerobic fermentation from various marine macroalgae such as *U. lactuca*, *P. tenera*, *U. pinnatifida* and especially *L. japonica* (the great source of carbon) are widely used for production of hydrogen (Mohan, 2010). The standard routes for H<sub>2</sub> production from the algae are based on using fermentation, anaerobic fermentation, enzymatic and microbial electrolysis. The further development of the process including combination of fermentation, microbial electrolysis, bioaugmentation and multiple process integration directed towards improvement of the process efficiency. The most important factors for the water-splitting photosynthesis, photofermentation, dark fermentation and microbial electrolysis directed towards the optimal and effective H<sub>2</sub> production are (a) specific type of algae (e.g. green algae); (b) appropriate substrate and supplementation with nutrients, carbon and nitrates; (c) the optimal concentrations of phosphate; (d) supplementation with suitable metal ions; (e) presence of a specific bacteria for anoxygenic photofermentation (*Allochromatium vinosum*, *Thiocapsa roseopersicina*, *Rhodobacter sphaeroides*, *Chlorobium vibrioforme*, *Desulfuromonas acetoxidans* and *Chloroflexus aurantiacus*) (Ghosh, Sobro, & Hallenbeck, 2012) and dark fermentation (*Enterobacter aerogenes*, *Enterobacter cloacae*, *Escherichia coli*, *Citrobacter intermedius*,



**Table 11.3** Fermentation of marine plants and microorganisms involved in the process

Raw material	Microorganisms	Value-added component	References
Microalgae ( <i>Pavlova lutheri</i> )	Yeast ( <i>Hansenula polymorpha</i> )	Peptide for its protective effect against oxidative stress as well as inhibitory effect on melanogenesis	Oh et al. (2015)
Brown seaweed ( <i>Sargassum</i> sp.)	Marine LAB isolates ( <i>Pediococcus acidilactici</i> , <i>Weissella paramesenteroides</i> <i>Pediococcus pentosaceus</i> , and <i>Enterococcus faecium</i> )	Polyphenol and polysaccharides for antioxidant and anticoagulant activity	Shobharani, Nanishankar, Halami, & Sachindra (2014)
Brown, green and red seaweed ( <i>Undaria pinnatifida</i> , <i>Ulva</i> sp. and <i>Porphyra</i> sp.)	LAB ( <i>Lactobacillus brevis</i> , <i>Lactobacillus casei</i> and <i>Lactobacillus plantarum</i> )	Seaweed sauce as a source of protein	Uchida & Miyoshi (2013)
Red and brown seaweed ( <i>Porphyra</i> sp. and <i>U. pinnatifida</i> )	–	Diet for Stockbreeding animals as a source of minerals	Uchida & Miyoshi (2013)
Brown seaweed ( <i>Saccharina latissima</i> and <i>Laminaria digitata</i> )	<i>Lactobacillus rhamnosus</i>	Seaweed with higher antioxidant activity for probiotic product development	Gupta et al. (2012)
Brown seaweed ( <i>Ecklonia cava</i> )	Yeast ( <i>Candida utilis</i> )	Fermented by-product as a potential antioxidant	Wijesinghe et al. (2012)
Blue-green alga <i>Spirulina</i> ( <i>Arthrospira platensis</i> )	LAB ( <i>Lactobacillus acidophilus</i> , <i>Bifidobacterium bifidum</i> , <i>L. casei</i> , <i>B. infantis</i> , <i>B. longum</i> , <i>Lactococcus lactis</i> )	Production of phycocyanin for antioxidant, anti-inflammatory, and UV protective activities	Liu, Hou, Lee, Chuang, & Lin (2011)
Brown seaweed ( <i>Himanthalia elongata</i> , <i>L. digitata</i> and <i>Laminaria saccharina</i> )	<i>Lactobacillus plantarum</i>	Fermented seaweed as a source of a range of functional foods	Gupta et al. (2011)
Seaweed sugars	LAB ( <i>L. rhamnosus</i> (KCTC 3237), <i>L. casei</i> , <i>L. brevis</i> , <i>L. diolivorans</i> , <i>L. collinoides</i> , <i>L. salivarius</i> , and <i>L. plantarum</i> )	Biomass feedstock	Hwang et al. (2011)
Green seaweed ( <i>Ulva reticulata</i> )	<i>Lactobacillus plantarum</i> and <i>Saccharomyces cerevisiae</i>	Silage preparation for prawn larval development	Felix and Pradeepa (2012)
Brown seaweed ( <i>Sargassum fulvellum</i> )	–	Anticoagulant activity of sulphated polysaccharide	De Zoysa, Nikapitiya, Jeon, Jee, & Lee (2008)
Brown seaweed ( <i>U. pinnatifida</i> )	Mixture of LAB ( <i>Lactobacillus brevis</i> ) and yeasts ( <i>Debaryomyces hansenii</i> and <i>Candida</i> sp.)	Marine silage preparation for young pearl oysters	Uchida, Numaguchi, & Murata (2004)

*Clostridium beijerinckii*, *Clostridium paraputrificum*, *Ruminococcus albus* and others). The appropriate substrate and temperature (the maximal yield of H<sub>2</sub> in mixed microbial population is obtained at 35–45 °C) are different and important for different types of bacteria to have a maximum efficiency of the fermentation process. The maximum optimal pH for the efficient H<sub>2</sub> production is maintained at 6.0 (Van Ginkel, Sung, & Lay, 2001). The enzymes (e.g. hydrogenase), co-enzymes and Fe<sup>+</sup> concentrations presented in the substrate are essential for the microbial growth, development and molecular transport, as well as for the effective fermentation process (Lee, Miyahara, & Noike, 2001). The high concentration of Mg<sup>+</sup>, Na<sup>+</sup> and Zn<sup>+</sup> were found to be essential to achieve the maximum yields of H<sub>2</sub>. The use of the organic substances from the fermentation effluents is a novel proposal. There is also a variety of secondary processes involved, such as methanogenesis, acidogenic fermentation for H<sub>2</sub> and photobiological processes for H<sub>2</sub> production.

The biofuels which can be produced from the algal biomass are biodiesel, bioethanol, biobutanol, biomethane, jet fuel, biohydrogen and thermochemical conversion products such as bio-oil, biocrude and syngas (Chinnasamy, Rao, Bhaskar, Rengasamy, & Singh, 2012). A lot of research is being carried out for developing microalgal biodiesel technology by performing bioprospecting of high-lipid-containing strains as well as by inducing higher lipid production by various physiological and genetic strain improvement methods. Therefore, lipid extraction is an extremely important process for the production of microalgal biodiesel. The cost of microalgae biodiesel is 15.70 US\$/L, which is significantly lower than microalgae biodiesel produced from the photobioreactor (73.5 US\$/L) (Lam & Lee, 2014). The main adjustments which must be done in order to increase the efficiency, suggesting that an improvement in the fermentation process will reduce the production costs and increase the process efficiency. The main points to pay attention to are the substrate (glucose) feeding and process control in the fermenter must be optimized to reduce the fermentation time frame; the glucose might be changed to a cheaper feedstock, such as cassava, Jerusalem artichoke or waste molasses and high-value by-products or co-products, such as carotene and lutein, might be explored to supplement algal biodiesel production.

Microalgal biodiesel products are usually obtained by bioprospecting of high-lipid-containing strains and inducing higher lipid production by various strains. There are quite a few methods of the lipids and oil extraction from algae, which are established on the present market: Folch, Bligh, Dyer and Matyas methods, superior solvent extraction, lipid hydrolysis, supercritical in situ transesterification, ultrasonic, bead beating, expeller press, solvent extraction, osmotic pressure, isotonic solution, enzymatic and microwave extraction. The solvent extraction, osmotic pressure and isotonic solution are the simplest, economical and sustainable methods with many advantages, such as use of dry and wet algae biomass, free of toxic organic solvents. However, the combinative method (e.g. enzymatic and mechanical, and others) is the most promising and effective lipid extraction which reduces energy consumption and also increases total process efficiency.

There is a huge demand in additional research and development directed towards optimization, better efficiency and process improvement. One of the challenges is high energy consumption in the biofuel production process from the aquatic plants, especially involved into separation of the algal products from the aqueous medium, as well as contribution to greenhouse gas emissions, due to the current energy inputs



required for algal biofuel production. Research dedicated to a low-energy separation processes, use of clear waste water and setting up the nutrients recovery process need to be done in order to increase the effectiveness and to lower the costs of the biofuel production. A major biotechnology advances, such as development of super productive aquatic plants and algae strains and improvement of technical aspects, are needed to achieve sustainable, large-scale algal biofuel production.

### 11.7 Challenges and Future Trends

Though traditional fermented marine products provide preferred organoleptic quality, but their safety and inadequate shelf-life is still a matter of concern. Since live microorganisms are the integral part of the fermentation, the risk of contamination and consequent toxicity are the major challenges for fermented food products. In addition, excess and regular consumption of fermented food may also expose certain risk and health hazards. According to US federal agency report, Alaskan Eskimos possess high risk of botulism disease because of the consumption of traditional marine-based fermented food. The Eskimos ferment whole fish, fish heads, walrus, seal and whale flippers in airtight plastic container for an prolonged period of time before being consumed. The utilization of airtight plastic containers or wrappers creates anaerobic conditions thus provide favourable environment for *C. botulinum* bacteria to thrive in the microaerophilic conditions which causes botulism (Ganguly, 2012; US Federal Agency Report). Therefore, the fermented food industry is looking for some alternative or synergistic approaches that can enhance the quality and shelf-life of final products without compromising its traditional likeness. To fulfil the industrial demand, the research has been carried out to explore the application of novel non-thermal approaches like pulse electric field and high pressure processing in last two decades. These emerging techniques offer numerous alternates in developing microbiologically safe with improved shelf-life, healthy and nutritious fermented products with very slight implication on its nutrition and organoleptic characteristics (Ojha et al., 2015; Nordvi et al., 2007).

However, despite the advancement in industrial starter culture and novel process control, the fermented products have not attracted the consumers and none of the production approach has been commercialized yet (Burgess, 2014; Skåra et al., 2015). Although, few producers in Europe is practicing a standardized procedure for fermented fish product preparation but limited scientific knowledge regarding the novel processing techniques is still a biggest challenge for them. Moreover, the complexity of resident microflora and the interaction of different enzymes in gut possess another challenge for its successful implementation (Skåra et al., 2015). Nevertheless, recent advancement in non-destructive spectroscopic analytic tools and improvement in taste and other organoleptic properties could be helpful to expand this knowledge. Furthermore, a deeper knowledge of process monitoring and starter culture will help to control the fermentation and may produce new product types (Skåra et al., 2015; Svensson, Nielsen, & Bro, 2004). Despite the greater advancement in the technology, new industry-based algal fermentation still has a great potential to explore and will remain open for future research.

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