CHAPTER 5

Microbiology of Primary Food Commodities

In this chapter we will examine aspects of the microbiology of some specific commodity groups describing the microflora with particular emphasis on spoilage.

5.1 WHAT IS SPOILAGE?

According to the Oxford English Dictionary to spoil is to 'deprive of good or effective qualities'. When a food is spoiled its characteristics are changed so that it is no longer acceptable. Such changes may not always be microbiological in origin; a product may become unacceptable as a result of insect damage, drying out, discolouration, staling or rancidity for instance, but by and large most food spoilage is a result of microbial activity. Microbiological food spoilage can manifest itself in several different ways, some of which often occur in combination. Visible microbial growth may be apparent in the form of surface slime or colonies, degradation of structural components of the food can cause a loss of texture, but the most common manifestation will be chemical products of microbial metabolism, gas, pigments, polysaccharides, off-odours and flavours.

Spoilage is also a subjective quality; what is spoiled for one person may be perfectly acceptable to another. The perception of spoilage is subject to a number of influences, particularly social; foods acceptable in some cultures are unacceptable in others. Some products such as well matured cheeses and game birds that have been hung for extended periods are esteemed by some people and highly objectionable to others. Affluence is another contributory factor – many are not in the position to be able to discard food due to some slight sensory defect. In the 18th and 19th century navy, sailors often preferred to eat in dark corners so that they could not see the weevils and maggots in their food.

A general feature of microbial spoilage is its relatively sudden onset – it does not appear to develop gradually, day by day a little worse, but more often as an unexpected and unpleasant revelation. This is a reflection of the exponential nature of microbial growth (see Section 3.1) and its consequence that microbial metabolism can also proceed at an exponentially increasing rate. If a microbial product associated with spoilage, for example an off odour, has a certain detection threshold, the level will be well below this threshold for most of the product's acceptable shelf-life. Once reached, however, it will be rapidly passed so that in a comparatively short time after, levels will be well in excess of the threshold and the product will be profoundly spoiled. This is represented in Figure 5.1 where growth of a spoilage organism is plotted on a linear scale.

Prediction or early detection of spoilage is not always easy since the mechanisms underlying microbiological spoilage can be quite complex. It is generally far easier to identify the chemical responsible for a particular off-odour than to identify the organism(s) responsible. This and the relative speed of chemical analysis have led to the use of chemical indices of spoilage in some areas, but more often the ultimate judgement as to whether a food is spoiled remains subjective. Total microbial counts are generally a poor indicator of spoilage potential. Many of the organisms enumerated may not grow in the food and many of those that do will not be responsible for spoilage. The value of microbial enumeration techniques can be improved if they are specific to those organisms associated

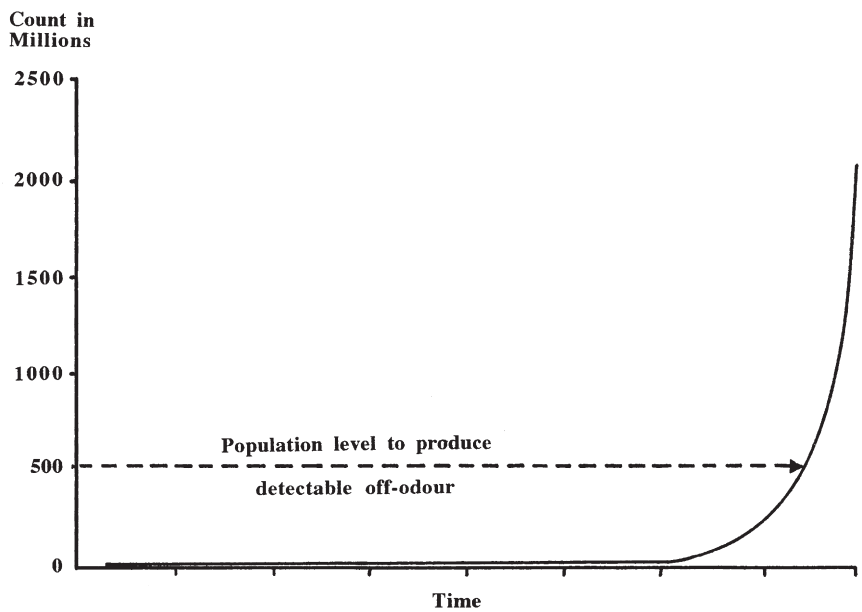


Figure 5.1 *Microbial growth and spoilage*

with spoilage, so called specific spoilage organisms (SSO). This approach has met with some success with aerobically stored fish products and the use of media to detect organisms capable of producing H_2S.

5.2 MILK

5.2.1 Composition

Milk is the fluid, excluding colostrum, secreted by mammals for the nourishment of their young. Colostrum is a much more concentrated liquid containing up to 25% total solids, mainly protein, secreted immediately after parturition. A number of animals are used to produce milk for human consumption, although the cow is by far the most important in commercial terms.

The principal components of milk are water, fat, protein and lactose. The precise composition varies between species so, for example, human milk has lower protein but higher lactose levels than cow's milk (Table 5.1). Generally the protein content of the milk reflects the growth rate of the young animal – the higher the growth rate, the more protein the milk contains.

There can be considerable compositional difference between breeds of a single species – Jersey and Guernsey milks, for instance, are noted for their higher fat content which is reflected in a richer, creamier taste. Even within a single breed variations in composition can arise depending on factors such as the stage of lactation, the stage of milking, the intervals between milking, the time of day, the number of previous lactations and the general nutritional state and health of the cow.

A more detailed analysis of cow's milk is presented in Table 5.2. The lipid content is the most variable feature. It is comprised mainly of C_{14},

Table 5.1 *Typical Milk Composition [% weight(volume)$^{-1}$]*

	Fat	*Protein*	*Lactose*	*Total solids*
Human	3.8	1.0	7.0	12.4
Cow	3.7	3.4	4.8	12.7
Jersey	5.1	3.8	5.0	14.5
Ayrshire	4.0	3.5	4.8	13.0
Short-horn	3.6	4.9	4.9	12.6
Sheep	7.4	5.5	4.8	19.3
Goat	4.5	2.9	4.1	13.2
Water buffalo	7.4	3.8	4.8	17.2
Horse	1.9	2.5	6.2	11.2

Table 5.2 *Composition of fresh cow's milk*

	Concentration g litre^{-1}	
LIPIDS	37	
	of which	% w/w
Triglycerides		95–96
Diglycerides		1.3–1.6
Free fatty acids		0.1–0.5
Total phospholipids		0.8–1.0
PROTEINS	34	
Casein	26	
α_{S1}	11.1	
α_{S2}	1.7	
β	8.2	
γ	1.2	
κ	3.7	
Whey proteins		
α-lactalbumin	0.7	
β-lactoglobulin	3.0	
serum albumin	0.3	
immunoglobulins	0.6	
NON-PROTEIN NITROGEN	1.9	
LACTOSE	48	
CITRIC ACID	1.75	
ASH	7.0	
CALCIUM	1.25	
PHOSPHORUS	0.96	

C_{16}, C_{18}, and $C_{18:1}$ fatty acids and is present in fresh milk mainly in the form of fat globules surrounded by a phospholipid rich layer known as the milk fat globule membrane. Typically these globules have a diameter of about 5 μm and the milk contains about 10^{12} fat globules per litre. If fresh milk is allowed to stand, the fat rises to the surface of the milk to produce a distinct cream line. The tendency for this to happen is reduced if the size of the globules is reduced by passing the milk through a small orifice under pressure; a process known as homogenization.

About 80–85% of the protein in milk is present as caseins. These are milk-specific proteins which are precipitated from milk by decreasing the pH to 4.6. This pH corresponds approximately to their isoelectric point which is relatively low due to the predominance of acidic amino acids and the presence of phosphorylated serine residues in the molecules. There are five main classes of caseins (see Table 5.2); these aggregate together in association with calcium phosphate in milk to form colloidal particles known as micelles. Milk contains around 10^{15} casein micelles l^{-1} with an average diameter of around 0.2 μm. The stability of the micelle is maintained by the presence of κ-casein near or on the surface of the particle. Loss of this stabilizing effect occurs when κ-casein is cleaved

by chymosin during cheese production and leads to the micelles sticking together to form a coagulum (see Section 9.6).

The balance of the protein in milk is made up of the whey proteins. These mainly comprise the compact globular proteins β-lactoglobulin and α-lactalbumin but also a number of blood-derived proteins such as serum albumin and immunoglobulins. The latter are present at higher levels in colostrum where they presumably confer some resistance to infection in the newborn calf.

5.2.2 Microflora of Raw Milk

Its high water activity, moderate pH (6.4–6.6) and ample supply of nutrients make milk an excellent medium for microbial growth. This demands high standards of hygiene in its production and processing; a fact recognized in most countries where milk was the first food to be the focus of modern food hygiene legislation.

Milk does possess a number of antimicrobial features (discussed in Section 3.2.4), present either to protect the udder from infection or to protect the newborn calf. Generally these are present at too low a concentration in cow's milk to have a very marked effect on its keeping quality or safety. In some cases the antimicrobial activity is antagonized by other milk constituents such as the effect of citrate and bicarbonate on lactoferrin activity. Stimulation of lactoperoxidase activity through the addition of exogenous hydrogen peroxide has been investigated as a means of preserving raw milk in developing countries where ambient temperatures are high and refrigeration is not often available. In one trial in Africa, use of this technique increased the proportion of samples passing the 10 minute resazurin quality test from 26% to 88%.

Three sources contribute to the micro-organisms found in milk: the udder interior, the teat exterior and its immediate surroundings, and the milking and milk-handling equipment.

Bacteria that get on to the outside of the teat may be able to invade the opening and thence the *udder interior*. Aseptically taken milk from a healthy cow normally contains low numbers of organisms, typically fewer than 10^2–10^3 cfu ml^{-1}, and milk drawn from some quarters may be sterile. The organisms most commonly isolated are micrococci, streptococci and the diptheroid *Corynebacterium bovis*. Counts are frequently higher though due to mastitis, an inflammatory disease of the mammary tissue, which is a major cause of economic loss in the dairy industry. In England and Wales, where it has been estimated to cost the industry around £90 million annually, about 1–2% of cows have a clinical infection at any one time. In the early acute stage of illness the bacterial count in mastitic milk can exceed 10^8 cfu ml^{-1} and macroscopic changes are often visible in the milk. Mastitis is also diagnosed by the presence of

high numbers of polymorphonuclear leukocytes which can rise to levels of $10^7\,ml^{-1}$ in infected milk.

In addition to acute mastitis, a substantial proportion of the national dairy herd is subclinically infected. In these cases there may be no obvious signs of infection yet the causative organism can be present in the milk at about $10^5\,cfu\,ml^{-1}$ and will contribute to an increase in the overall count of bulked milk.

Many organisms can cause mastitis, the most important being *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus agalactiae*, *Strep. dysgalactiae*, *Strep. uberis*, *Pseudomonas aeruginosa* and *Corynebacterium pyogenes*. Several of these are potential human pathogens and a number of other human pathogens such as *Salmonella*, *Listeria monocytogenes*, *Mycobacterium bovis* and *Mycobacterium tuberculosis* are also occasionally reported.

Infected cows are treated by injection of antibiotics into the udder. Milk from these cows must be withheld from sale for several days following treatment because antibiotic residues can cause problems in sensitive consumers and inhibit starter culture activity in fermented milks. Attempts to control mastitis by good milking hygiene, use of a disinfectant teat dip after milking and an antibiotic infusion at the end of lactation have helped to reduce streptococcal and staphylococcal infections but have had little success in preventing *E. coli* mastitis.

The *udder exterior and its immediate environment* can be contaminated with organisms from the cow's general environment. This is less of a problem in summer months when cows are allowed to graze in open pasture and is worst when they are housed indoors and under wet conditions. Heavily contaminated teats have been reported to contribute up to $10^5\,cfu\,ml^{-1}$ in the milk. Contamination from bedding and manure can be a source of human pathogens such as *E. coli*, *Campylobacter*, and *Salmonella* and *Bacillus* species may be introduced from soil. Clostridia such as *C. butyricum* and *C. tyrobutyricum* can get into milk from silage fed to cows and their growth can cause the problem known as late blowing in some cheeses.

A number of measures can be taken to minimize milk contamination from the udder exterior and considerable advice on this topic is available to dairy farmers. Some of the recommendations made by the Milk Marketing Board, formerly the principal purchaser of milk in England and Wales, included:

(1) providing enough clean bedding and replacing it as necessary;
(2) removing slurry (faeces and urine) from concrete areas at least twice daily;
(3) preventing muddy areas wherever possible;
(4) shaving udders and trimming tails;

(5) washing teats with warm water containing disinfectant and drying individually with paper towels;
(6) keeping the milking parlour floor clean during milking;
(7) thoroughly cleaning teat cups if they fall off during milking and discarding foremilk.

Although such procedures certainly improve the microbiological quality of milk, economic constraints such as increasing size of individual dairy herds and decreased manning levels in milking parlours encouraged their neglect. The introduction of total bacterial count as a basis for payment in 1982 provided an incentive for their more zealous application and led to a marked decline in bacterial count of milk (see below).

Milk-handling equipment such as teat cups, pipework, milk holders and storage tanks, is the principal source of the micro-organisms found in raw milk. As the overall quality of the milk decreases so the proportion of the microflora derived from this source increases. Milk is a nutritious medium and, if equipment is poorly cleaned, milk residues on surfaces that are frequently left wet will act as a focus for microbial growth which can contaminate subsequent batches of milk. Occasional neglect of cleaning and sanitizing procedures is usually less serious since, although it may contribute large numbers of micro-organisms to the product, these tend to be fast growing bacteria that are heat sensitive and will be killed by pasteurization. They are also sensitive to sanitizing practices used and will be eliminated once effective cleaning is resumed. If cleaning is persistently neglected though, the hydrophobic, mineral-rich deposit known as milkstone can build up on surfaces, particularly heated ones. This will protect organisms from sanitizers and allow slower growing organisms to develop such as micrococci and enterococci. Many of these are thermoduric and may not be removed by pasteurization.

To encourage farmers to apply the available advice on animal husbandry practices, milking procedures, types and design of equipment and cleaning schedules which contribute to good bacteriological quality milk, the Milk Marketing Board (MMB) in England and Wales introduced in 1982 a system of paying farmers based on the total bacterial count (TBC) of their milk. Similar schemes have been introduced in a number of countries but details of the MMB's scheme are presented as Table 5.3. For four months prior to introduction of the scheme, farmers were notified of the TBC count of their milk and in anticipation of its start a dramatic fall in the count was noted (Figure 5.2). Now more than 76% of the milk produced in England and Wales falls into Band A with a mean count of 1.7×10^3 cfu ml^{-1}. The Milk Marketing Board no longer exists as the monopoly purchaser of farm milk in England and Wales, but the bodies that replaced it recognized the value of a payment scheme which includes microbiological quality and have retained similar systems.

Table 5.3 *Milk Marketing Board (England and Wales) total bacterial count payment scheme*

Grade	*Count (cfu ml^{-1})*	*Price adjustment (pence l^{-1})*
A	$<2\times10^4$	+0.23
B	$>2\times10^4$ but $<10^5$	0
C_1	$>10^5$ but no price deduction in previous 6 months	−1.5
C_2	$>10^5$ and Grade C_1 produced in previous 6 months	−6.0
C_3	$>10^5$ and Grade C_2 or C_3 deduction has been applied	−10.0

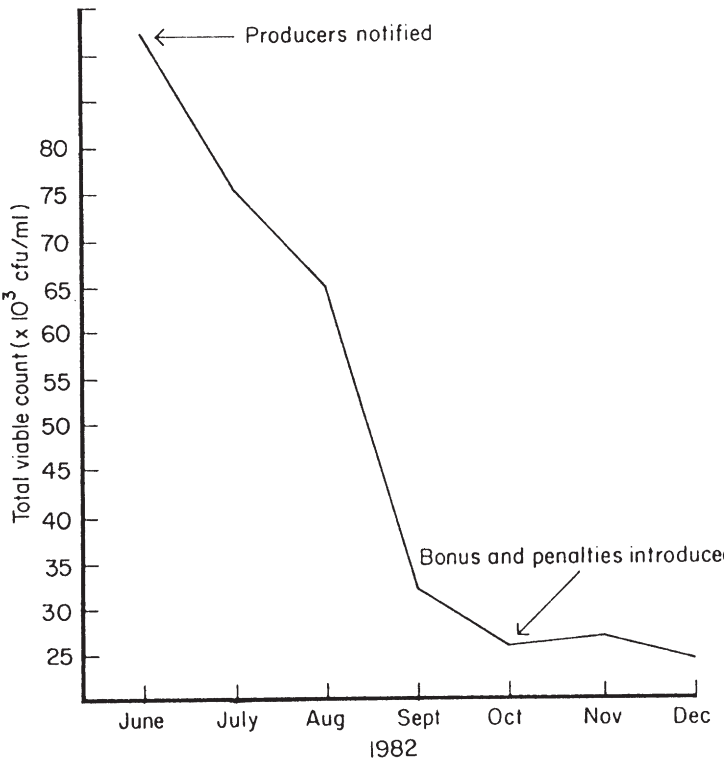


Figure 5.2 *Raw milk counts and the bonus payments scheme. Reproduced from 'Micro-organisms in Agriculture', SAB Symposium Series No. 15*

In most developed countries milk is chilled almost immediately after it issues from the cow and is held at a low temperature thereafter. It is stored in refrigerated holding tanks before being transported by a refrigerated or insulated lorry to the dairy where it is kept in chill storage tanks until use. Throughout this time, its temperature remains below

7 °C and the only organisms capable of growing will be psychrotrophs. There are many psychrotrophic species, but those most commonly found in raw milk include Gram-negative rods of the genera *Pseudomonas, Acinetobacter, Alcaligenes, Flavobacterium*, psychrotrophic coliforms, predominantly *Aerobacter* spp., and Gram-positive *Bacillus* spp.

One consequence of the current extensive use of refrigeration and the change to a microflora dominated by psychrotrophs is that traditional tests for the microbiological quality of milk based on the reduction of a redox dye such as methylene blue or resazurin have become obsolete. Psychrotrophs tend to reduce these dyes poorly and the tests are not very sensitive to low numbers of bacteria.

5.2.3 Heat Treatment of Milk

Proposals for the heat treatment of milk were made as early as 1824, forty years before Pasteur's work on the thermal destruction of micro-organisms in wine and beer. When milk pasteurization was introduced by the dairy industry around 1890, it was as much to retard souring as to prevent the spread of disease. This had become an important commercial requirement since large quantities of milk were now being transported by rail into the large cities rather than being produced locally in cramped and insanitary cowhouses.

Milk has long been recognized as an agent in the spread of human disease and within a few years it was appreciated that pasteurization was also providing protection against milk-borne disease. Nowadays it is safety rather than spoilage considerations which determine the minimum legal requirements for pasteurization.

Originally the main health concerns associated with milk were tuberculosis caused by *Mycobacterium bovis* and *Mycobacterium tuberculosis* (see Section 7.10) and brucellosis caused by *Brucella* spp. (see Section 7.3). In some parts of the world milk is still a significant source of these infections but in the UK and some other countries they have now been effectively eliminated from the national dairy herd by a programme of regular testing and culling of infected animals. Such programmes must be constantly maintained to be effective and there have been occasional problems. Initiatives such as the culling of badgers, thought to be a reservoir of *M. bovis*, have been the subject of some controversy and in 2002 there was an outbreak of brucellosis in a dairy herd in Cornwall, although this was the first recorded in England for ten years. Enteric pathogens such as *Salmonella* and *Campylobacter* are still however prevalent in raw milk and pasteurization remains the most effective measure for their control.

The four types of heat treatment applied to milk are described in Table 5.4. Specification of pasteurization temperatures to the first decimal

Table 5.4 *Heat treatment of milk*

LOW TEMPERATURE HOLDING (LTH)	62.8 °C for 30 min
HIGH TEMPERATURE SHORT TIME (HTST)	71.7 °C for 15 s
ULTRA HIGH TEMPERATURE	135 °C for 1 s
'STERILIZED'	> 100 °C typically 20–40 min

place is not some arcane feature of thermal processing but is simply a result of conversion from the Fahrenheit scale in which they were originally prescribed.

Low temperature holding (LTH) is a batch process that has been superseded in most countries by continuous high temperature/short time (HTST) pasteurization using a plate heat exchanger. Originally the temperatures prescribed for LTH pasteurization were slightly lower. They were increased in 1950 in response to the observation that the rickettsia *Coxiella burnetii*, the causative agent of Q fever, could survive this original process if present in high numbers. Spread of this organism through infected milk is a greater problem in the United States than most of Europe, where transmission appears to be mainly through aerosols in the farm environment. More recent fears that *Listeria* could survive conventional pasteurization treatments appear to be unfounded (see Section 7.9.2).

A simple test, the phosphatase test, is applied to determine whether milk has been properly pasteurized. Milk contains the enzyme alkaline phosphatase which is inactivated by the time/temperature combinations applied during pasteurization. To determine whether a milk sample has been satisfactorily pasteurized and is free from contaminating raw milk, a chromogenic substrate is added. If active phosphatase is still present then it will hydrolyse the substrate producing a colour which can be compared to standards to determine whether the milk is acceptable or not. The same principle is used in the α-amylase test applied to bulk liquid egg (see Section 7.12.5).

The microbiological quality of pasteurized milk is now also governed by EU-based regulations which require pasteurized milk to contain less than 1 coliform ml^{-1}, to have a count at 30 °C of less than 3×10^4, and also that, after 5 days storage at 6 °C, its count at 21 °C should be less than 10^5 cfu ml^{-1}.

UHT milk is a commercially sterile product of the sort described in Section 4.1. It is interesting to note that although UHT milk is a low-acid appertized food, the minimum heat process specified falls well short of the botulinum cook required for equivalent canned foods. It has been claimed that the redox potential in milk is too high to support the growth of *Clostridium botulinum* but more probable explanations for the fact that botulism has never been associated with this product are a low

incidence of *C. botulinum* spores in milk and the fact that manufacturers employ heat processes well in excess of the minimum legal requirement.

Sterilized milk described in Table 5.4 is a rather specialized product of diminishing importance. In the UK it is defined as a product which is heat processed in-bottle at temperatures above 100 °C for sufficient time for it to pass the turbidity test. This test is based on the principal that the heat process is sufficient to denature whey proteins. In it, casein is precipitated with ammonium sulfate and filtered off. The filtrate is then heated; if it remains clear the milk is acceptable because the whey proteins have already been denatured and removed with the precipitated casein. Turbidity indicates an inadequate heat process which has left some whey protein undenatured.

Gram-negative psychrotrophs will not survive pasteurization, although some pseudomonads produce extracellular lipases and proteases which are heat resistant. If enough of these bacteria are present ($>10^5\,ml^{-1}$), sufficient enzyme can be produced pre-pasteurization to cause rancidity and casein degradation in the processed milk.

Raw milk may also contain a number of organisms known as thermodurics that can survive mild pasteurization treatments. These are generally Gram-positives such as the sporeforming bacteria and members of the genera *Microbacterium, Micrococcus, Enterococcus* and *Lactobacillus*, but 1–10% of strains of the Gram-negative *Alcaligenes tolerans* may also survive.

In the main, spoilage of pasteurized milk is due to the growth of psychrotrophic Gram-negative rods such as *Pseudomonas, Alcaligenes*, and *Acinetobacter*, introduced as post-pasteurization contaminants. Product shelf-life will depend on the number of contaminants introduced and the efficiency of the cold chain; a 2 °C decrease in storage temperature will approximately double the shelf life, but pasteurized milk produced under conditions of good manufacturing practice should keep for more than 10 days under refrigeration. Spoilage usually manifests itself as off odours and flavours described as fruity or putrid but visual defects such as clotting due to proteolytic activity can also arise. The souring traditionally associated with milk spoilage and due to the growth of lactic acid bacteria is now rare.

In milk which is subject to very low levels of post-pasteurization contamination, spoilage can result from the growth of thermoduric *Bacillus* spp. This may be associated with flavour defects but the most studied example is the bitty cream phenomenon produced by the lecithinase activity of *Bacillus cereus*. This enzyme hydrolyses the phospholipids associated with the milk fat globule membrane to produce small proteinaceous fat particles which float on the surface of hot drinks and adhere to surfaces of crockery and glasses. Bitty cream is associated mostly with milk that has been subject to temperature abuse, although

psychrotrophic *Bacillus* species are becoming increasingly associated with the spoilage of refrigerated milk.

To extend the shelf life of milk beyond 5–15 days requires measures to reduce the number of spores present. UHT processing achieves this but introduces a cooked flavour which is not always appreciated. To avoid this problem, alternative techniques to produce so-called extended shelf life (ESL) milks which retain the taste of pasteurised milk have been explored. These include direct heating by steam injection to temperatures of 85–127 °C for 2 seconds, the removal of spores by centrifugation combined with a pasteurisation treatment, or microfiltration of skim milk and its subsequent recombination with UHT treated cream. Such techniques combined with hygienic handling and an effective cold chain can achieve shelf lives of 21 days.

5.2.4 Milk Products

Fresh milk is the starting point for a number of other food products, some of which are shown in Figure 5.3. A number of these are described in detail elsewhere in this book. Yoghurt, cheese and other fermented products are dealt with in Chapter 9, dried milk in Section 4.7, and the principles behind the production of evaporated milk and UHT milk are described in Section 4.1.

Fat can be concentrated from milk by centrifugal separation to produce a number of different types of cream. These are distinguished

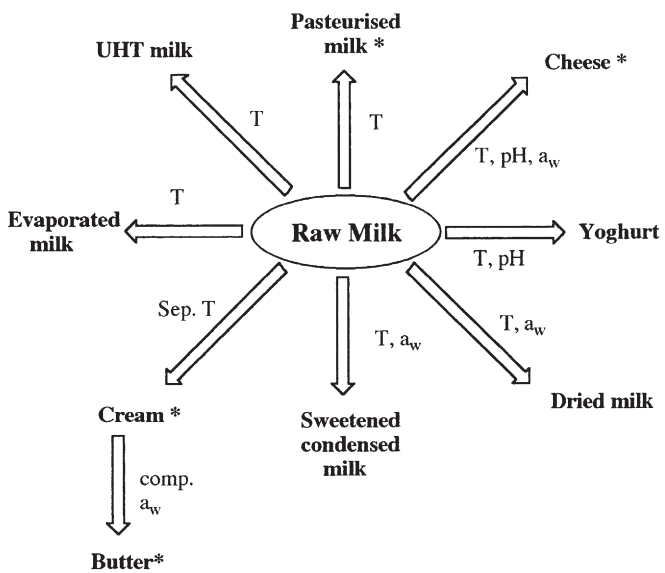


Figure 5.3 *Milk and milk products.* T *indicates elevated temperature; pH, reduced pH;* a_w*, reduced* a_w*; sep., separation, comp., compartmentalization; and * stored at chill temperatures*

primarily by their fat content, which can vary from around 12% for half cream up to 55% in clotted cream, but also by differences in other aspects of their processing such as the heat treatment they receive and whether they are homogenized, whipped or fermented.

After separation the microbial count of the cream fraction is usually higher than that of the skim milk and, despite the fact that some bacteria are removed as slime from the separator, the combined count of skim milk and cream often exceeds that of the original milk. These observations are a result mainly of physical processes for, although mechanical separators operate at temperatures at which growth can occur (25–30 °C), there is limited time for microbial growth during efficient processing. During separation it is thought that the fat globules rising through the milk act as a moving sieve to which bacteria adhere and become concentrated in the fat layer. The increase in combined count of the two fractions is attributed to the breaking up of bacterial clumps which increases the number of colony forming units, a phenomenon also noticed during the churning of cream to produce butter.

Pasteurization treatments applied to cream are generally in excess of those used with milk because of the protective effect of fat and also because a longer product shelf-life is often necessary. As with milk, the spoilage of cream is due to growth of post-pasteurization contaminants such as pseudomonads and surviving thermodurics such as *B. cereus*. Generally lipolytic activity leading to rancidity is a more important feature of spoilage than proteolysis. Butter, made from cream, is described in Section 4.9 as the principal example of food preservation by compartmentalization.

5.3 MEAT

Originally meat was a term used to describe any solid food, but has now come to be applied almost solely to animal flesh. As such, it has played a significant role in the human diet since the days of hunting and gathering, and animals (sheep) were first domesticated at the beginning of the Neolithic revolution around 8500 BC. Though abjured by some on moral or religious grounds, meat eating remains widely popular today. In the main, this is due to its desirable texture and flavour characteristics, although meat protein does also have a high biological value.

Meat consumption is often something of a status symbol and is generally far greater in wealthy societies. This is because large-scale meat production is a relatively inefficient means of obtaining protein. It requires agriculture to produce a surplus of plant proteins which can be fed to animals: with modern production techniques, it takes two kilos of grain to obtain 1 kilo of chicken, four for 1 kilo of pork and eight for 1 kilo of beef.

Though numerous species are used as a source of meat around the world, ranging from flying foxes to frogs and from kangaroos to crocodiles, the meat animals of principal importance in economic terms are cattle, pigs, sheep, goats and poultry.

5.3.1 Structure and Composition

Edible animal flesh comprises principally the muscular tissues but also includes organs such as the heart, liver, and kidneys. Most microbiological studies on meat have been conducted with muscular tissues and it is on these that the information presented here is based. Though in many respects the microbiology will be broadly similar for other tissues, it should be remembered that differences may arise from particular aspects of their composition and microflora.

Structurally muscle is made up of muscle fibres; long, thin, multinucleate cells bound together in bundles by connective tissue. Each muscle fibre is surrounded by a cell membrane, the sarcolemma, within which are contained the myofibrils, complexes of the two major muscle proteins, myosin and actin, surrounded by the sarcoplasm. The approximate chemical composition of typical adult mammalian muscle after *rigor mortis* is presented in Table 5.5. Its high water activity and abundant nutrients make meat an excellent medium to support microbial growth. Though many of the micro-organisms that grow on meat are proteolytic, they grow initially at the expense of the most readily utilized substrates—the water soluble pool of carbohydrates and non-protein nitrogen. Extensive proteolysis only occurs in the later stages of decomposition when the meat is usually already well spoiled from a sensory point of view.

The carbohydrate content of muscle has a particularly important bearing on its microbiology. Glycogen is a polymer of glucose held in the liver and muscles as an energy store for the body. During life, oxygen is supplied to muscle cells in the animal by the circulatory system and glycogen can be broken down to provide energy by the glycolytic and respiratory pathways to yield carbon dioxide and water. After death the supply of oxygen to the muscles is cut off, the redox potential falls and respiration ceases, but the glycolytic breakdown of glycogen continues leading to an accumulation of lactic acid and a decrease in muscle pH. Provided sufficient glycogen is present, this process will continue until the glycolytic enzymes are inactivated by the low pH developed. In a typical mammalian muscle the pH will drop from an initial value of around 7 to 5.4–5.5 with the accumulation of about 1% lactic acid. Where there is a limited supply of glycogen in the muscle, acidification will continue only until the glycogen runs out and the muscle will have a higher ultimate pH. This can happen if the muscle has been exercised before slaughter

Table 5.5 *Chemical composition of typical adult mammalian muscle after rigor mortis*

		% weight
Water		75.0
Protein		19.0
Myofibrillar	11.5	
Sarcoplasmic	5.5	
Connective	2.0	
Lipid		2.5
Carbohydrate		1.2
Lactic acid	0.9	
Glycogen	0.1	
Glucose and glycolytic intermediates	0.2	
Soluble non-protein nitrogen		1.65
Creatine	0.55	
Inosine monophosphate	0.30	
NAD/NADP	0.30	
Nucleotides	0.10	
Amino acids	0.35	
Carnosine, anserine	0.35	
Inorganic		0.65
Total soluble phosphorus	0.20	
Potassium	0.35	
Sodium	0.05	
Magnesium	0.02	
Other metals	0.23	
Vitamins		

After R.A. Lawrie, 'Meat Science', 3rd edn., Pergamon Press, Oxford, 1979

but can also result from stress or exposure to cold. When the ultimate pH is above 6.2, it gives rise to dark cutting meat, a condition also known as dry, firm, dark (DFD) condition. Because the pH is relatively high, the meat proteins are above their isoelectric point and will retain much of the moisture present. The fibres will be tightly packed together giving the meat a dry, firm texture and impeding oxygen transfer. This, coupled with the higher residual activity of cytochrome enzymes, will mean that the meat has the dark colour of myoglobin rather than the bright red oxymyoglobin colour. The higher pH will also mean that microbial growth is faster so spoilage will occur sooner.

Another meat defect associated with post mortem changes in muscle carbohydrates is known as pale, soft, exudative (PSE) condition. This occurs mainly in pigs and has no microbiological implications but does give rise to lower processing yields, increased cooking losses and reduced juiciness. The PSE condition results when normal non-exercised muscle

is stimulated just before slaughter leading to a rapid post mortem fall in pH while the muscle is still relatively warm. This denatures sarcoplasmic proteins, moisture is expelled from the tissues which assume a pale colour due to the open muscle texture and the oxidation of myoglobin to metmyoglobin.

5.3.2 The Microbiology of Primary Processing

The tissues of a healthy animal are protected against infection by a combination of physical barriers and the activity of the immune system. Consequently, internal organs and muscles from a freshly slaughtered carcass should be relatively free from micro-organisms. Microbial numbers detected in aseptically sampled tissues are usually less than $10\,\text{cfu}\,\text{kg}^{-1}$, although there is evidence that numbers can increase under conditions of stress and they will of course be higher if the animal is suffering from an infection. Since some animal diseases can be transmitted to humans, meat for human consumption should be produced only from healthy animals. Visual inspection before and after slaughter to identify and exclude unfit meat is the general rule, although it will only detect conditions which give some macroscopic pathological sign. In the UK this and other duties are performed by the Meat Hygiene Service, a body established in 1995 to protect public and animal health and unify all aspects of meat inspection and enforcement.

The most heavily colonized areas of the animal that may contaminate meat are the skin (fleece) and gastrointestinal tract. Numbers and types of organisms carried at these sites will reflect both the animal's indigenous microflora and its environment. The animal hide, for example, will carry a mixed microbial population of micrococci, staphylococci, pseudomonads, yeasts and moulds as well as organisms derived from sources such as soil or faeces. Organisms of faecal origin are more likely to be encountered on hides from intensively reared cattle or from those transported or held in crowded conditions.

The various processing steps in slaughter and butchering are described in Chapter 11 for beef and will be summarized only briefly here. With reasonable standards of hygienic operations, contamination of meat carcasses from processing equipment, knives and process workers is less important than contamination from the animals themselves. The greatest opportunity for this occurs during dressing, the stages during which the head, feet, hides, excess fat, viscera and offal are separated from the bones and muscular tissues.

Skinning can spread contamination from the hide to the freshly exposed surface of the carcass through direct contact and *via* the skinning knife or handling. Washing the animal prior to slaughter can reduce microbial numbers on the hide but control is most effectively exercised by

skillful and hygienic removal of the hide. The viscera contain large numbers of micro-organisms, including potential pathogens, and great care must be taken to ensure the carcass is not contaminated with visceral contents either as a result of puncture or leakage from the anus or oesophagus during removal.

After dressing, carcasses are washed to remove visible contamination. This will have only a minor effect on the surface microflora, although bactericidal washing treatments such as hot water (80 °C), chlorinated water ($50\,mg\,l^{-1}$) or dilute lactic acid (1–2%) have been shown to reduce the surface microflora by amounts varying between about 1 and 3.5 log cycles.

After dressing the carcass is cooled to chill temperatures during which cold shock may cause some reduction in numbers. At chill temperatures, microbial growth among the survivors is restricted to those psychrotrophs present and these can be further inhibited by the partial surface drying that takes place. Surface numbers of bacteria at the end of dressing will typically be of the order of 10^2–$10^4\,cfu\,cm^{-2}$. Counts are generally higher in sheep carcasses than beef and higher still in pigs which are processed differently, the skin not being removed from the carcass but scalded and dehaired.

Psychrotrophic organisms form only a small percentage of the initial microflora but come to predominate subsequently as the meat is held constantly at chill temperatures. An increase in microbial numbers is seen during cutting and boning, but this is due less to microbial growth, since the operation is usually completed within a few hours at temperatures below 10 °C, than to the spreading of contamination to freshly exposed meat surfaces by equipment such as knives, saws and cutting tables.

The primary processing of poultry differs from red meat in a number of respects that have microbiological implications. First among these is the sheer scale of modern poultry operations where processing plants can have production rates up to 12 000 birds per hour. This leaves little opportunity for effectively sanitizing equipment and exacerbates problems associated with some of the procedures and equipment used which favour the spread of micro-organisms between carcasses.

During transport to the plant contamination can be spread between birds by faeces and feathers and from inadequately cleaned transport cages. Once at the plant, birds are hung by their feet on lines, electrically stunned and killed by cutting the carotid artery. The close proximity of the birds and the flapping wings further contribute to the spread of contamination. This is followed by scalding where the birds are immersed in hot water at about 50 °C to facilitate subsequent removal of the feathers. Each bird contributes large numbers of micro-organisms to the scald water and these will be spread between birds. This can be reduced to some extent by using a counter-current flow of birds and water so that the birds leaving the scalder are in contact with the cleanest

water. Higher scald water temperatures will eliminate most vegetative bacteria but cause an unacceptable loosening of the skin cuticle.

After scalding, birds are mechanically defeathered by a system of rotating rubber fingers. A number of studies have demonstrated how these can pass organisms, for example *Salmonella*, from one carcass to others following it and when the fingers become worn or damaged they are liable to microbial colonization. As the poultry carcass is not skinned, skin-associated organisms will not be removed.

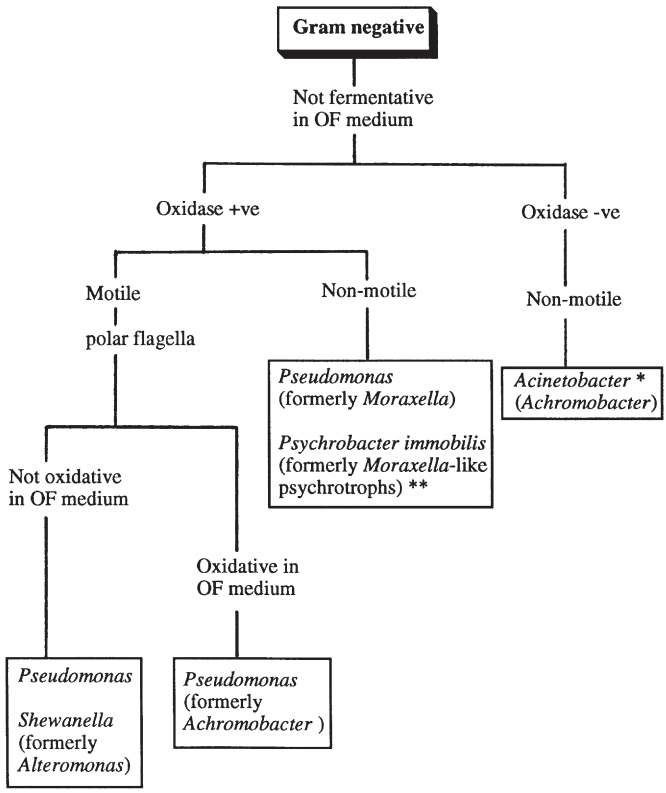
The intestinal tract of poultry will contain high numbers of organisms including pathogens such as *Salmonella* and *Campylobacter*. Poultry evisceration therefore poses similar microbiological hazards to those with other animals but the size and structure of the carcass make it a much more difficult operation to execute hygienically. To allow high processing rates, poultry evisceration is usually automated but this too leads to a high incidence of carcass contamination with gut contents. Since the carcasses are not split like those of sheep and cattle, effective washing of the gut cavity after evisceration is more difficult.

Poultry to be frozen is usually chilled in water and this offers a further opportunity for cross contamination. This is controlled by chlorination of the cooling water, use of a counter-current flow as in scalding, and a sufficient flow rate of water to avoid the build up of contamination.

5.3.3 Spoilage of Fresh Meat

Aerobic storage of chilled red meats, either unwrapped or covered with an oxygen permeable film, produces a high redox potential at the meat surface suitable for the growth of psychrotrophic aerobes. Non-fermentative Gram-negative rods grow most rapidly under these conditions and come to dominate the spoilage microflora that develops. Taxonomic description of these organisms has been somewhat unsettled over the years with some being described as *Moraxella* and *Moraxella*-like. Such terms have now been largely abandoned in favour of a concensus that has emerged from numerical taxonomy studies. In this, the principal genera are described as *Pseudomonas, Acinetobacter* and *Psychrobacter* with *Pseudomonas* species such as *P. fragi, P. lundensis* and *P. fluorescens* generally predominating. A dichotomous key describing the differential characteristics of these organisms and some of the names used previously to describe them is presented as Figure 5.4. Other organisms are usually only a minor component of the spoilage microflora, but include psychrotrophic Enterobacteriaceae such as *Serratia liquefaciens* and *Enterobacter agglomerans*, lactic acid bacteria and the Gram-positive *Brochothrix thermosphacta*.

The first indication of spoilage in fresh meat is the production of off odours which become apparent when microbial numbers reach around



* see Bouvet, P.J.M. & Grimont, P.A.D. (1986). Taxonomy of the genus *Acinetobacter*. *International Journal of Systematic Bacteriology* **36**, 228-240.
see Juni, E. & Heym, G.A. (1986). *Psychrobacter immobilis* gen. nov., sp. nov. Genospecies composed of Gram-negative, aerobic, oxidase-positive coccobacilli. *International Journal of Systematic Bacteriology* **36, 388-391.

Figure 5.4 *Characteristics of some Gram-negatives associated with meat*

10^7 cfu cm^{-2}. At this point it is believed that the micro-organisms switch from the diminishing levels of glucose in the meat to amino acids as a substrate for growth. In meat with lower levels of residual glucose this stage is reached earlier (10^6 cfu cm^{-2}) and this accounts for the earlier onset of spoilage in high pH meat.

Bacterial metabolism produces a complex mixture of volatile esters, alcohols, ketones and sulfur-containing compounds which collectively comprise the off odours detected. Such mixtures can be analysed by a combination of gas chromatography and mass spectrometry and the origin of different compounds can be established by pure culture studies. These have confirmed the predominant role of pseudomonads in spoilage of aerobically stored chilled meat. Usually the different spoilage taints appear in a sequence reflecting the order in which components of the meat are metabolized. The first indication of spoilage is generally the

buttery or cheesy odour associated with production of diacetyl (2,3-butanedione), acetoin (3-hydroxy-2-butanone), 3-methyl-butanol and 2-methylpropanol. These compounds are produced from glucose by members of the Enterobacteriaceae, lactic acid bacteria and *Brochothrix thermosphacta*. Pseudomonads then begin to increase in importance and the meat develops a sweet or fruity odour. This is due to production of a range of esters by *Pseudomonas* and *Moraxella* species degrading glucose and amino acids and by esterification of acids and alcohols produced during the first phase of spoilage. Ester production is particularly associated with *Pseudomonas fragi* which can produce ethyl esters of acetic, butanoic and hexanoic acids from glucose, but other pseudomonads and *Moraxella* species are also capable of producing esters when grown on minced beef. As the glucose becomes exhausted, the meat develops a putrid odour when *Pseudomonas* species and some *Acinetobacter* and *Moraxella* species turn their entire attention to the amino acid pool, producing volatile sulfur compounds such as methane thiol, dimethyl sulfide and dimethyl disulfide.

In the later stages of spoilage an increase in the meat pH is seen as ammonia and a number of amines are elaborated. Some of these have names highly evocative of decay and corruption such as putrescine and cadaverine but in fact do not contribute to off odour. When microbial numbers reach levels of around 10^8 cfu cm^{-2}, a further indication of spoilage becomes apparent in the form of a visible surface slime on the meat.

Vacuum and modified-atmosphere packing of meat (see also Section 4.6) changes the meat microflora and consequently the time-course and character of spoilage. In vacuum packs the accumulation of CO_2 and the absence of oxygen restrict the growth of pseudomonads giving rise to a microflora dominated by Gram-positives, particularly lactic acid bacteria of the genera *Lactobacillus, Carnobacterium* and *Leuconostoc*.

Spoilage of vacuum packed meat is characterized by the development of sour acid odours which are far less objectionable than the odour associated with aerobically stored meat. The micro-organisms reach their maximum population of around 10^7 cfu cm^{-2} after about a week's storage but the souring develops only slowly thereafter. Organic acids may contribute to this odour, although the levels produced are generally well below the levels of endogenous lactate already present. Some work has suggested that methane thiol and dimethyl sulfide may contribute to the sour odour.

The extension of shelf-life produced by vacuum packing is not seen with high pH (>6.0) meat. In this situation *Shewanella putrefaciens*, which cannot grow in normal pH meat, and psychrotrophic Enterobacteriaceae can grow and these produce high levels of hydrogen sulfide giving the meat an objectionable odour.

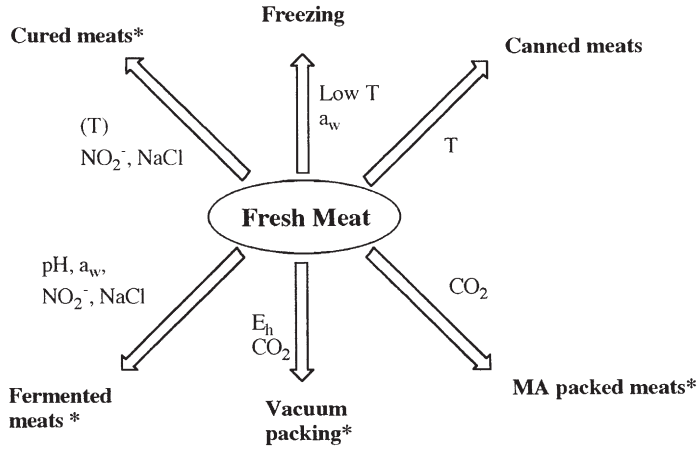


Figure 5.5 *Meat and meat products.* T *indicates elevated temperature;* E_h*, low redox potential; pH, reduced pH;* a_w*, reduced* a_w*; and * stored at chill temperatures*

In modified-atmospheres containing elevated levels of both CO_2 and O_2 growth of pseudomonads is restricted by the CO_2 while the high levels of O_2 maintain the bright red colour of oxygenated myoglobin in the meat. Here the microflora depends on the type of meat, its storage temperature, and whether it was vacuum packed or aerobically stored previously. In general terms though, the microflora and spoilage tend to follow a similar pattern to that of vacuum packed meat. Heterofermentative lactic acid bacteria can be more numerous due to the stimulatory effect of oxygen on their growth and, under some circumstances, *Brochothrix thermosphacta*, Enterobacteriaceae and pseudomonads can be more important.

Meat can be processed in a number of different ways which affect its characteristics, shelf-life and microbiology. The variety of these is illustrated by Figure 5.5; they will not be discussed further here but are treated in greater detail under the generic technologies in Chapters 4 and 9.

5.4 FISH

Here we are mainly concerned with what most people think of as fish; principally the free swimming teleosts and elasmobranchs. The same term can also encompass all seafoods including crustaceans with a chitinous exoskeleton such as lobsters, crabs and shrimp, and molluscs such as mussels, cockles, clams and oysters. Microbiologically these share many common features with free swimming fish but some specific aspects are discussed in Section 5.4.3.

Historically the extreme perishability of fish has restricted its consumption in a reasonably fresh state to the immediate vicinity of where

the catch was landed. This has detracted only slightly from it playing a significant role in human nutrition as, throughout the world, traditional curing techniques based on combinations of salting, drying and smoking were developed which allowed more widespread fish consumption. The importance of dried salted cod in economic and social history has already been alluded to in Section 4.7.

Poor keeping quality is a special feature of fish which sets it apart even from meat and milk. The biochemical and microbiological reasons for this are discussed in Section 5.4.4.

5.4.1 Structure and Composition

Although broadly similar in composition and structure to meat, fish has a number of distinctive features. Unlike meat, there are no visually obvious deposits of fat. Although the lipid content of fish can be up to 25%, it is largely interspersed between the muscle fibres. A further feature which contributes to the good eating quality of fish is the very low content of connective tissue, approximately 3% of total weight compared with around 15% in meat. This, and the lower proportion of body mass contributed by the skeleton, reflect the greater buoyancy in water compared with that in air.

Muscle structure also differs. In land animals it is composed of very long fibres while in fish they form relatively short segments known as myotomes separated by sheets of connective tissue known as myocommata. This gives fish flesh its characteristically flaky texture.

Fish flesh generally contains about 15–20% protein and less than 1% carbohydrate. In non-fatty fish such as the teleosts cod, haddock and whiting, fat levels are only about 0.5%, while in fatty fish such as mackerel and herring, levels can vary between 3 and 25% depending on factors such as the season and maturity.

5.4.2 The Microbiology of Primary Processing

As with meat, the muscle and internal organs of healthy, freshly caught fish are usually sterile but the skin, gills and alimentary tract all carry substantial numbers of bacteria. Reported numbers on the skin have ranged from 10^2–10^7 cfu cm^{-2}, and from 10^3–10^9 cfu g^{-1} in the gills and the gut. These are mainly Gram-negatives of the genera *Pseudomonas, Shewanella, Psychrobacter, Vibrio, Flavobacterium* and *Cytophaga* and some Gram-positives such as coryneforms and micrococci. Since fish are cold blooded, the temperature characteristics of the associated flora will reflect the water temperatures in which the fish live. The microflora of fish from northern temperate waters where the temperatures usually range between -2 and $+12\,^{\circ}C$ is predominantly psychrotrophic or

psychrophilic. Most are psychrotrophs with an optimum growth temperature around 18 °C. Far fewer psychrotrophs are associated with fish from warmer tropical waters and this is why most tropical fish keep far longer in ice than temperate fish.

Bacteria associated with marine fish should be tolerant of the salt levels found in sea water. Though many do grow best at salt levels of 2–3%, the most important organisms are those that are not strictly halophilic but euryhaline, *i.e.* they can grow over a range of salt concentrations. It is these that will survive and continue to grow as the salt levels associated with the fish decline, for example when the surface is washed by melting ice.

After capture at sea, fish are commonly stored in ice or refrigerated sea water until landfall is made. It is important that fresh, clean cooling agent is used as re-use will lead to a rapid build up of psychrotrophic contaminants and accelerated spoilage of the stored fish. Gutting the fish prior to chilling at sea is not a universal practice, particularly with small fish and where the time between harvest and landing is short. It does however remove a major reservoir of microbial contamination at the price of exposing freshly cut surfaces which will be liable to rapid spoilage. Similarly any damage to the fish from nets, hooks, *etc.* that breaches the fish's protective skin will provide a focus for spoilage. Subsequent processing operations such as filleting and mincing which increase the surface area to volume of the product also increase the rate of spoilage.

Fish can be further contaminated by handling on board, at the dock and at markets after landing, particularly where they are exposed for sale and are subject to contamination with human pathogens by birds and flies. Generally though, fish have a far better safety record than mammalian meat. A number of types of foodborne illness are associated with fish (Table 5.6), and these are discussed in detail in Chapters 7 and 8.

5.4.3 Crustaceans and Molluscs

The propensity of crustaceans to spoil rapidly (see Section 5.4.4) can be controlled in the case of crabs and lobsters by keeping them alive until

Table 5.6 *Foodborne illness and pathogens associated with fish*

Vibrio cholerae
Vibrio parahaemolyticus
Vibrio vulnificus
Clostridium botulinum Type E
Enteric viruses
Scombroid fish poisoning
Paralytic shellfish poisoning

immediately before cooking or freezing. This is not possible with shrimp or prawns, which are of far greater overall economic importance but die soon after capture. In addition to their endogenous microflora, shrimp are often contaminated with bacteria from the mud trawled up with them and are therefore subject to rapid microbiological deterioration following capture. Consequently they must be processed either by cooking or by freezing immediately on landing.

Some aspects of the production and processing of frozen cooked peeled prawns can pose public health risks. Increasingly prawns are grown commercially in farms where contamination of the ponds, and thence the product, with pathogenic bacteria can occur *via* bird droppings and fish feed. After cooking, which should be sufficient to eliminate vegetative bacterial contaminants derived from the ponds, the edible tail meat is separated from the chitinous exoskeleton. Peeling machines are used in some operations but large quantities are still peeled by hand, particularly in countries where labour is cheap. The handling involved gives an opportunity for the product to be contaminated with human pathogens after the bactericidal cooking step and prior to freezing.

The flesh of molluscs such as cockles, mussels, oysters and clams differs from that of crustaceans and free swimming fish by containing appreciable ($\approx 3\%$) carbohydrate in the form of glycogen. Though many of the same organisms are involved, spoilage is therefore glycolytic rather than proteolytic, leading to a pH decrease from around 6.5 to below 5.8.

Molluscs are usually transported live to the point of sale or processing where the flesh can often be removed by hand. Although contamination may occur at this stage, the significant public health problems associated with shellfish arise more from their ability to concentrate viruses and bacteria from surrounding waters, the frequent pollution of these waters with sewage and the practice of consuming many shellfish raw or after relatively mild cooking. This is discussed in more detail in Chapter 9.

5.4.4 Spoilage of Fresh Fish

A number of factors contribute to the unique perishability of fish flesh. In the case of fatty fish, spoilage can be non-microbiological; fish lipids contain a high proportion of polyunsaturated fatty acids which are more reactive chemically than the largely saturated fats that occur in mammalian meat. This makes fish far more susceptible to the development of oxidative rancidity.

In most cases though, spoilage is microbiological in origin. Fish flesh naturally contains very low levels of carbohydrate and these are further depleted during the death struggle of the fish. This has two important consequences for spoilage. Firstly it limits the degree of post mortem acidification of the tissues so that the ultimate pH of the muscle is 6.2–6.5

Table 5.7 *Nitrogen-containing extractives in fish*

	Cod	*Herring*	*Dogfish*	*Lobster*
Total nitrogenous extractives gkg^{-1}	12	12	30	55
Free amino acids ($mM\,l^{-1}$)	7	30	10	300
TMAO ($mM\ l^{-1}$)	5	3	10	2
Urea ($mM\ l^{-1}$)	0	0	33	0
Creatine ($mM\ l^{-1}$)	3	3	2	0
Betaine ($mM\ l^{-1}$)	0	0	2	1
Anserine	1	0	0	0

Adapted from D.M. Gibson, 'Microbial spoilage of foods in Micro-organisms in Action: Concepts and Applications in Microbial Ecology'. J. M.Lynch and J. E.Hobbie, (eds.) Blackwell, Oxford, 1988

compared with around 5.5 in mammalian muscle. Fish which have a lower pH such as halibut (approx. 5.6) tend to have better keeping qualities. Secondly, the absence of carbohydrate means that bacteria present on the fish will immediately resort to using the soluble pool of readily assimilated nitrogenous materials, producing off-odours and flavours far sooner. This can be less pronounced in fish produced by intensive aquaculture since they are normally fed to satiation which increases glycogen levels in the liver and muscle.

The composition of the non-protein nitrogen fraction differs significantly from that in meat (Table 5.7). Trimethylamine oxide (TMAO) occurs in appreciable quantities in marine fish as part of the osmoregulatory system. TMAO is used as a terminal electron acceptor by non-fermentative bacteria such as *Shewanella putrefaciens* and this allows them to grow under microaerophilic and anaerobic conditions. The product of this reduction is trimethylamine which is an important component in the characteristic odour of fish (Figure 5.6.) TMAO also contributes to a relatively high redox potential in the flesh since the E_h of the TMAO/TMA couple is +19 mV.

Elasmobranchs such as dogfish and shark contain high levels of urea. Bacterial urease activity in the flesh can produce ammonia very rapidly giving the product a pungent odour. Not only does this render the flesh itself uneatable but it can also taint the flesh of other fish stored nearby. It is for this reason that in many areas fishermen will discard all but the fins of shark when they catch them.

Shellfish such as lobster have a particularly large pool of nitrogenous extractives and are even more prone to rapid spoilage; a factor which accounts for the common practice of keeping them alive until immediately prior to consumption.

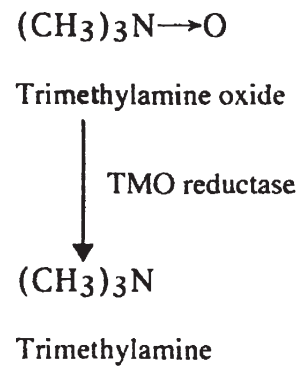


Figure 5.6 *Reduction of trimethylamine oxide*

Fish proteins are less stable than mammalian protein. As with meat, extensive proteolysis does not become apparent until the product is already well spoiled, but limited protein degradation may improve bacterial access to the nutrient pool of extractives.

The speed with which a product spoils is also related to the initial microbial load on the product: the higher the count the sooner spoilage occurs. Since fish from cold waters will have a larger proportion of psychrotrophs among their natural microflora, this can shorten the chill shelf-life appreciably.

Spoilage of chilled fish is due principally to the activity of psychrotrophic Gram-negative rods also encountered in meat spoilage, particularly *Shewanella putrefaciens* and *Pseudomonas* spp. The uniquely objectionable smell of decomposing fish is the result of a cocktail of chemicals, many of which also occur in spoiling meat. Sulfurous notes are provided by hydrogen sulfide, methyl mercaptan and dimethyl sulfide and esters contribute the 'fruity' component of the odour. A number of other amines in addition to TMA are produced by bacterial catabolism of amino acids. Skatole, a particularly unpleasant example produced by the degradation of tryptophan, also contributes to the smell of human faeces. The level of volatile bases in fish flesh has provided an index of spoilage, although this and other chemical indices used are often poor substitutes for the trained nose and eyes.

Figure 5.7 illustrates some of the different products made from fish, most of which are discussed elsewhere in terms of the general processing technologies used. One interesting aspect that relates to some of the discussion above will be discussed here. The combination of a near neutral pH and availability of TMAO as an alternative electron acceptor means that vacuum and modified-atmosphere packing of fish does not produce the same dramatic extension of keeping quality seen with meat. Typically the shelf-life extension of vacuum and modified-atmosphere-packed cod will vary from less than 3 days to about 2 weeks. *Shewanella*

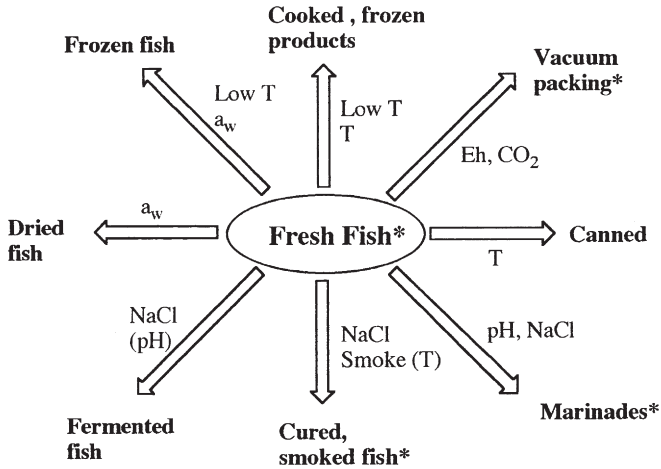


Figure 5.7 *Fish and fish products.* T *indicates elevated temperature;* E_h *low redox potential: pH, reduced pH;* a_w*, reduced* a_w*, and * stored an chill temperatures*

putrefaciens can grow under these conditions producing TMA and hydrogen sulfide to spoil the product. Work in Denmark has also demonstrated that CO_2 tolerant marine vibrios like *Photobacterium phosphoreum* may be responsible for a non-sulfurous spoilage of these products in some instances. This is a large (5 μm diameter), almost yeast-like, bacterium that has been isolated from the intestines of several different fish. Because of its size it produces 10–100-fold more TMA per cell than smaller organisms such as *Shewanella* and therefore can cause spoilage at lower populations, typically around 10^7 cfu g^{-1} compared with 10^8 cfu g^{-1} for more conventional bacteria. Whether *Shewanella* or *Photobacterium* is ultimately responsible for spoilage in MAP fish products probably depends on relative numbers present initially and whether any other selective factors operate.

5.5 PLANT PRODUCTS

The plant kingdom provides a considerable part of human food requirements and, depending on the particular plant species, use is made of every part of the plant structure from root, tuber, stem, leaves, fruit and seeds. Plants have evolved many strategies to survive the predation of herbivores and omnivores, including humans, and these strategies include, not only protective mechanisms to protect vegetative parts such as leaves, stems and roots, but also the development of rich, succulent fruits to encourage animals to help in the dispersal of seeds.

As the human race settled down from the nomadic hunter–gatherer state to form increasingly large stable communities so a wider range of plants have been brought into cultivation. Plant products are grown on

an ever larger scale and many are stored for significant periods after harvest and may be transported from one part of the world to another.

Microbiological problems may occur at all stages in the production of plant products. During growth in the field there is a wide range of plant pathogens to contend with and, although these are dominated by the fungi, there are a significant number of bacteria and viruses. A further range of micro-organisms may cause post-harvest spoilage although there cannot be an absolutely clear boundary between plant pathogens and spoilage organisms because many plant products are made up of living plant tissue even after harvest.

Plants have evolved many mechanisms to prevent microbial invasion of their tissues. The outer surface is usually protected by a tough, resistant cuticle although the need for gas exchange requires specialized openings in parts of the leaf surface, the stomata and lenticels, which may provide access by some micro-organisms to plant tissue. Plant tissues may contain antimicrobial agents which are frequently phenolic metabolites, indeed the complex polyphenolic polymer known as lignin is especially resistant to microbial degradation. Many plants produce a special group of antimicrobial agents, the phytoalexins, in response to the initiation of microbial invasion. The low pH of the tissues of many fruits provides considerable protection against bacteria and the spoilage of these commodities is almost entirely by fungi. In contrast, many vegetables have somewhat higher pH and may be susceptible to bacterial spoilage (Table 5.8).

Another physical factor influencing the pattern of spoilage is the availability of water. Cereals, pulses, nuts and oilseeds are usually dried post harvest and the low water activity should restrict the spoilage flora to xerophilic and xerotolerant fungi. These three groups of plant products, *i.e.* fruits, vegetables and cereals *etc.*, are sufficiently distinct that they will now be considered separately.

Table 5.8 *pH values of some fruits and vegetables*

Fruits	*pH*	*Vegetables*	*pH*
Apples	2.9–3.3	Asparagus	5.4–5.8
Apricots	3.3–4.4	Broccoli	5.2–6.5
Bananas	4.5–5.2	Cabbage	5.2–6.3
Cherries	3.2–4.7	Carrots	4.9–6.3
Grapefruit	3.0	Cauliflower	6.0–6.7
Grapes	3.4–4.5	Celery	5.6–6.0
Limes	2.0–2.4	Lettuce	6.0–6.4
Melons	6.2–6.7	Parsnip	5.3
Oranges	3.3–4.3	Rhubarb	3.1–3.4
Pears	3.4–4.7	Runner beans	4.6
Plums	2.8–4.6	Spinach	5.1–6.8
Raspberries	2.9–3.5	Sweet potato	5.3–5.6
Tomatoes	3.4–4.9	Turnips	5.2–5.6

5.5.1 Cereals

The cereals, which all belong to the Gramineae or the grass family, are one of the most important sources of carbohydrates in the human diet. Some of the more important cereal crops are listed in Table 5.9. Wheat, rice and maize are by far the most important cereal crops on the basis of world-wide tonnage and well over three hundred million tons of each are produced annually. However, each cereal species is adapted to grow in a particular range of climatic conditions although plant breeding programmes have extended the ranges of several of them. The common wheat is the major cereal of temperate parts of the world, and is grown extensively in both northern and southern hemispheres, whereas the durum wheat is grown extensively in the Mediterranean region and in the warmer, drier parts of Asia, North and South America. Rye can be grown in colder parts of the world and is an important crop in central Europe and Russia. Maize, which originated from the New World, is now grown extensively throughout the tropics and subtropical regions of both northern and southern hemispheres. Similarly, although rice was originally of Asian origin, it is also grown in many parts of the world. Although sorghum and some of the millets are not very important in terms of world tonnage, they are especially well adapted to growing in warm, dry climates and may be locally the most important cereals in such regions as those bordering the southern edge of the Sahara desert.

The microbiology of cereals, during growth, harvest and storage is dominated by the moulds and it is convenient to consider two groups of fungi. The field fungi are well adapted to the sometimes rapidly changing conditions on the surfaces of senescing plant material in the field. Although they require relatively high water activities for optimum growth, genera such as *Cladosporium, Alternaria* and *Epicoccum* are able to survive the rapid changes that can occur from the desiccation of a hot sunny day to the cool damp conditions of the night. The genus *Fusarium* includes species which have both pathogenic and saprophytic activities.

Table 5.9 *Some of the more important cereal crops*

Botanical name	*Common name*
Triticum aestivum	Common wheat (bread wheat)
Triticum durum	Durum wheat (pasta wheat)
Hordeum spp.	Barley
Avena sativa	Oats
Secale cereale	Rye
Zea mays	Maize (American corn)
Oryza sativa	Rice
Sorghum vulgare	Sorghum
Panicum miliaceum	Millet
Pennisetum typhoideum	Bulrush millet

Thus *F. culmorum* and *F. graminearum* can cause both stem rot and head blight of wheat and barley in the field and these field infections may lead to more extensive post harvest spoilage of these commodities if they are stored at too high a water activity. By contrast the so-called storage fungi seem to be well adapted to the more constant conditions of cereals in storage, and generally grow at lower water activities (Table 5.10). The most important genera of the storage fungi are *Penicillium* and *Aspergillus*, although species of *Fusarium* may also be involved in spoilage when grain is stored under moist conditions.

Water activity and temperature are the most important environmental factors influencing the mould spoilage of cereals, and the possible production of mycotoxins, and Table 5.11 shows the relationship between water content and water activity for barley, oats and sorghum while Figure 3.10 shows the same information as an isotherm for wheat. Although xerophilic moulds such as *Eurotium* spp. and *Aspergillus*

Table 5.10 *Minimum water activity requirements of some common field and storage fungi*

Species	*Minimum* a_w
Field Fungi	
Fusarium culmorum	0.89
Fusarium graminearum	0.89
Alternaria alternata	0.88
Cladosporium herbarum	0.85
Storage fungi	
Penicillium aurantiogriseum	0.82
Penicillium brevicompactum	0.80
Aspergillus flavus	0.78
Aspergillus candidus	0.75
Eurotium amstelodami	0.71
Wallemia sebi	0.69

Table 5.11 *Equilibrium relative humidity, water activity and moisture content (as % wet weight) of cereals at 25 °C*

Equilibrium relative humidity (%)	*Water activity* a_w	*Water potential (MPa)*	*Water content (% wet weight)*		
			Barley	*Oats*	*Sorghum*
15	0.15	−261	6.0	5.7	6.4
30	0.30	−166	8.4	8.0	8.6
45	0.45	−110	10.0	9.6	10.5
60	0.60	−70	12.1	11.8	12.0
75	0.75	−39	14.4	13.8	15.2
90	0.90	−14.5	19.5	18.5	18.8
100	1.00	0.0	26.8	24.1	21.9

restrictus may grow very slowly at the lower limit of their water activity range (0.71 corresponding to about 14% water content in wheat at 25 °C) once they start growing and metabolizing they will produce water of respiration and the local water activity will steadily rise allowing more rapid growth. Indeed it could increase sufficiently to allow mesophilic mould spores to germinate and grow; the process being, in a sense, autocatalytic. There is a sequence of observable consequences of the process of mould growth on cereals starting with a decrease in germinability of the grain. This is followed by discolouration, the production of mould metabolites including mycotoxins, demonstrable increase in temperature (self-heating), the production of musty odours, caking and a rapid increase in water activity leading finally to the complete decay with the growth of a wide range of microorganisms.

5.5.2 Preservation of High-moisture Cereals

Although not directly relevant to human foods, the availability of high-moisture cereals, such as barley, provides a highly nutritious winter feed for cattle. Long-term storage of such material can be achieved by a lactic acid fermentation comparable to the making of silage, or by the careful addition of fatty acids such as propionic acid. If this process is not carried out carefully then it may be possible to have sufficient propionic acid to inhibit the normal spoilage moulds associated with cereals in a temperate climate, but not enough to inhibit *Aspergillus flavus*. It has been shown that, even though partially inhibited in its growth, this mould can produce aflatoxin B_1 at enhanced levels under these conditions. If such material is fed to dairy cattle there is the possibility of aflatoxin M_1 being secreted in the milk and it then becomes a problem in human foods and not just a problem of animal feeds (see Section 8.4.2).

5.5.3 Pulses, Nuts and Oilseeds

The pulses are members of the huge legume family of plants, the Fabaceae also known as the Papilionaceae and Leguminosae, which form a major source of vegetable proteins and include such important crops as peas, beans, soya, groundnuts and lentils. Although many species of peas and beans are familiar to us as fresh vegetables, millions of tons of the mature seeds of soya beans and groundnuts are harvested for longer term storage every year and may be susceptible to mould spoilage if not stored under appropriate conditions. Several of the leguminous seeds, such as groundnuts and soya beans, are also valuable sources of vegetable oils but there are plants from many other diverse families which are now used to provide food quality vegetable oils, rapeseed from the crucifers, sunflower seed from the daisy family,

oilpalm and olives to mention just a few. Edible nuts may also come from a botanically wide range of tree species and many of them are rich in oil and give similar microbiological problems as oilseeds.

Seeds rich in oil, such as groundnuts, have a much lower water content at a particular water activity than cereals, thus groundnuts with a 7.2% water content have a water activity of about 0.65–0.7 at 25 °C. Apart from the problem of mycotoxin formation in moulded oilseeds, several mould species have strong lipolytic activity leading to the contamination of the extracted oils with free fatty acids which may in turn undergo oxidation to form products contributing to rancidity. The most important lipolytic moulds are species of *Aspergillus*, such as *A. niger* and *A. tamarii, Penicillium* and *Paecilomyces*, while at higher water activities species of *Rhizopus* may also be important. Figure 5.8 shows the influence of moisture content, and damage on the formation of free fatty

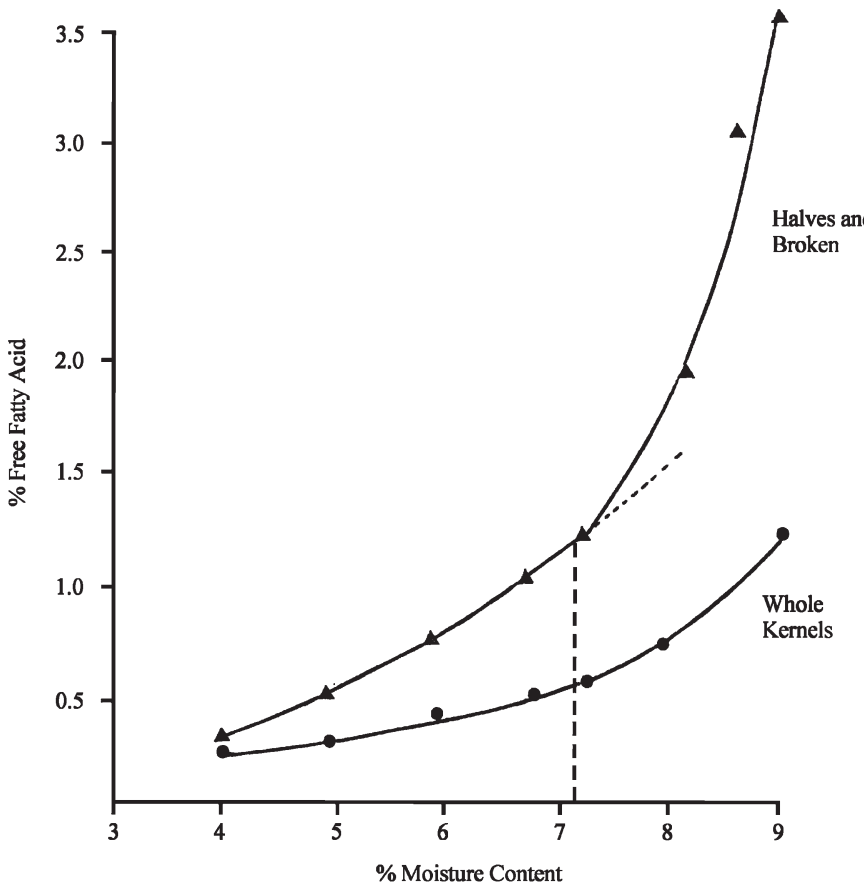


Figure 5.8 *The influence of moisture content on free fatty acid formation in groundnuts stored for four months. 7.2% moisture content corresponds to* a_w *0.65–0.70 at 25 °C so fungal growth may occur*

acids (FFA) in groundnuts stored for 4 months. It can be seen that in whole nuts there is a steady increase in FFA formation with an increase in moisture content and this is due to the plants own lipolytic enzymes. However, damaged groundnuts show a more rapid rise at low moisture contents, presumably due to increased contact of enzymes and substrate as a result of damage, but they also show an especially rapid rise in FFA at moisture contents greater than 7.2% corresponding to the active growth of lipolytic moulds.

If cereals, pulses, oilseeds and tree nuts are harvested with as little damage as possible and dried to an appropriate water content it should be possible to store them for considerable periods of time so long as they are not exposed to excessive temperature abuse during storage. The problems which may arise when large storage facilities, such as silos, are not carefully designed to avoid temperature differentials arising within the stored commodity were discussed in Section 3.3.1. The migration of water in these circumstances can result in the germination of fungal spores and the growth of mycelium creating a localized region of fungal activity releasing further water of respiration into the region. In this way, despite the commodity initially going into store at what was judged to be a safe water content, it may nevertheless go mouldy over a period of time.

It should be noted that, although a commodity may be dry enough to avoid direct microbiological spoilage, it may not be secure against the ravages of pests such as insects and rodents and their activity may lead to secondary invasion and mould spoilage.

5.5.4 Fruits and Fruit Products

Despite the high water activity of most fruits, the low pH leads to their spoilage being dominated by fungi, both yeasts and moulds but especially the latter. The degree of specificity shown by many species of moulds, active in the spoilage of harvested fruits in the market place or the domestic fruit bowl, reflects their possible role as pathogens or endophytes of the plant before harvest. Thus *Penicillium italicum* and *P. digitatum* show considerable specificity for citrus fruits, being the blue mould and green mould respectively of oranges, lemons and other citrus fruits. *Penicillium expansum* causes a soft rot of apples and, although the rot itself is typically soft and pale brown, the emergence of a ring of tightly packed conidiophores bearing enormous numbers of blue conidiospores, has led to this species being referred to as the blue mould of apples. This particular species has a special significance because of its ability to produce the mycotoxin patulin which has been detected as a contaminant in unfermented apple juices but not in cider (see Section 8.4.3).

Other common diseases of apples and pears include the black spot or scab, caused by the ascomycete *Venturia inaequalis* (anamorph *Spilocaea pomi* = *Fusicladium dendriticum*), and a brown rot caused by another ascomycete, *Monilinia fructigena* (= *Sclerotinia fructigena*, anamorph *Monilia fructigena*). Apple scab spoils the appearance of fruit, and would certainly reduce its commercial value, but does not cause extensive rotting of the tissue. The brown rot, however, can lead to extensive damage of fruit both on the tree and in storage. The typical brown rot is usually associated with rings of brown powdery pustules of the imperfect, or anamorph, stage, however fruit which is infected, but apparently healthy when it goes into store, can be reduced to a shiny black mummified structure in which much of the fruit tissue has been replaced by fungal material and the whole apple has become a functional sclerotium, or overwintering resting body, of the fungus. Although rarely seen in the United Kingdom, it is this structure which may germinate in the spring to produce the stalked apothecia of the perfect, or teleomorph, stage.

An especially widespread mould on both fruits and vegetables is the grey mould *Botrytis cinerea*, which is the imperfect stage of another ascomycete, *Botryotinia fuckeliana* (= *Sclerotinia fuckeliana*). Its role in the spoilage of strawberries was described in Section 2.5. Infection of grapes on the vine by this same mould can lead to drying out of the grape and an increase in sugar concentration and wines made from such contaminated fruit are considered to be very special. Under these circumstances the fungus has been referred to as *La Pourriture Noble* – the noble rot!

To avoid excessive mould spoilage of harvested fruit during storage and transport it is necessary to harvest at the right stage of maturity and avoid damage and bruising. Mouldy fruit should be removed and destroyed and good hygiene of containers and packaging equipment is essential to prevent a build-up of mould propagules. The development of international trade in many fruit species has led to the use of some biocides (Figure 5.9) to prevent mould spoilage. Benomyl has proved useful where it can be applied to the surface of fruits, such as citrus and bananas, in which the skins would normally be discarded (this, of course, is not the case for citrus used for marmalade and other preserves). In some parts of the world moulds like *Penicillium digitatum* have developed increased resistance to benomyl. Biphenyl is quite an effective protectant when incorporated into the wrapping tissues of fruit such as oranges when they are individually wrapped. Captan has been used as a spray for strawberries in the field to control *Botrytis* but its use must be stopped well before harvest.

Reduced temperature and increased carbon dioxide concentration may also be useful in controlling mould spoilage during storage and transport but many fruits are themselves sensitive to low temperatures

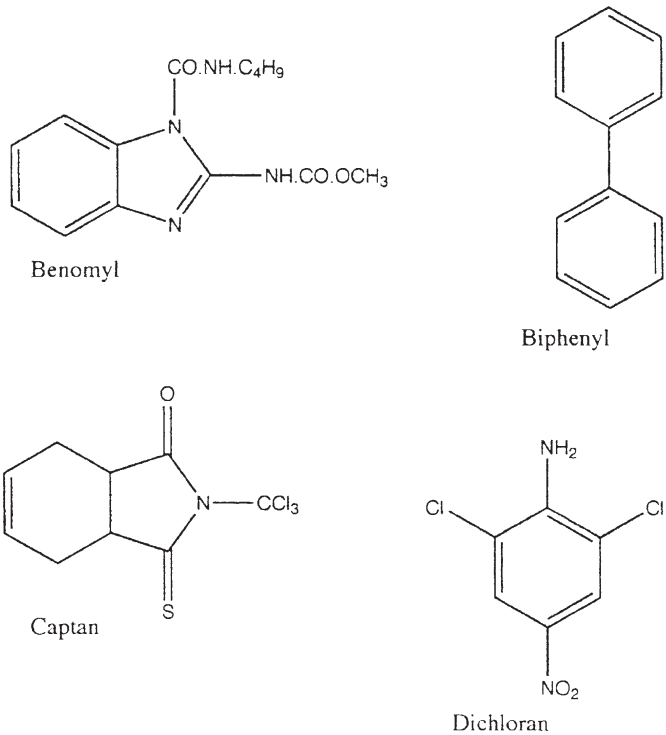


Figure 5.9 *Examples of antifungal biocides which may be used to protect fruits from mould spoilage*

and enhanced CO_2 levels and appropriate conditions need to be established for each commodity.

Canned fruits are normally given a relatively low heat treatment because of their low pH and although most mould propagules would be killed the ascospores of some members of the Eurotiales are sufficiently heat resistant to survive. Species of *Byssochlamys* are the best known but the increasing use of more exotic fruits is providing cases where spoilage of canned fruits have been due to such organisms as *Neosartorya fischeri* (anamorph *Aspergillus fischerianus*) and *Talaromyces flavus* var. *macrosporus* (anamorph *Penicillium* sp.).

5.5.5 Vegetables and Vegetable Products

The higher pH values of the tissues of many vegetables makes them more susceptible to bacterial invasion than fruits although there are also a number of important spoilage fungi of stored vegetables. The bacteria involved are usually pectinolytic species of the Gram-negative genera *Pectobacterium, Pseudomonas* and *Xanthomonas*, although pectinolytic strains of *Clostridium* can also be important in the spoilage of potatoes

Table 5.12 *Some micro-organisms involved in the spoilage of fresh vegetables*

Micro-organism	*Vegetable*	*Symptom*
BACTERIA		
Corynebacterium sepedonicum	Potato	ring rot of tubers
Ralstonia solanacearum	Potato	soft rot
Pectobacterium carotovorum var. *atrosepticum*	Potato	soft rot
Streptomyces scabies	Potato	scab
Xanthomonas campestris	Brassicas	black rot
FUNGI		
Botrytis cinerea	Many	grey mould
Botrytis allii	Onions	neck rot
Mycocentrospora acerina	Carrots	liquorice rot
Trichothecium roseum	Tomato Cucurbits	pink rot
Fusarium coeruleum	Potato	dry rot
Aspergillus alliaceus	Onion Garlic	black rot

under some circumstances, and the non-sporing Gram-positive organism *Corynebacterium sepedonicum* causes a ring rot of potatoes. Table 5.12 lists a range of micro-organisms which may cause spoilage of fresh vegetables.

The role of plant pathogens in subsequent spoilage post-harvest may be complex, thus *Phytophthora infestans* causes a severe field disease of the potato plant, frequently causing death of the plant, but it may also remain dormant within the tubers and either cause a rot of the tubers during storage, or a new cycle of disease in the next season's crop. However, the most frequent agents of spoilage are not the plant pathogens themselves but opportunistic micro-organisms which gain access to plant tissue through wounds, cracks, insect damage or even the lesions caused by the plant pathogens. All freshly harvested vegetables have a natural surface flora, including low numbers of pectinolytic bacteria, and it is becoming increasingly evident that healthy tissue of the intact plant may also contain very low numbers of viable micro-organisms (endophytic). The onset and rate of spoilage will depend on the interactions between the physiological changes occurring in the tissues after harvest and changes in microbial activity. Harvesting itself will produce physiological stress, principally as a result of water loss and wilting, and cut

surfaces may release nutrients for microbial growth. This stress may also allow growth of the otherwise quiescent endophytic flora.

The most frequently observed form of spoilage is a softening of the tissue due to the pectinolytic activity of micro-organisms. Pectin, the methyl ester of α-1,4-poly-D-galacturonic acid, and other pectic substances are major components of the middle lamella between the cells making up plant tissue and once it is broken down the tissue loses its integrity and individual plant cells are more easily invaded and killed. Pectic substances may be quite complex and include unesterified pectic acid as well as having side chains of L-rhamnose, L-arabinose, D-galactose, D-glucose and D-xylose. Several distinct enzymes are involved in the degradation of pectin and their role is illustrated in Figure 5.10.

As described in the case of fruits, the prevention of spoilage during storage and transport of vegetables must involve a range of measures. The control of the relative humidity and the composition of the atmosphere in which vegetables are stored is important but there is a limit to the reduction of relative humidity because at values below 90–95%, loss of water from vegetable tissues will lead to wilting. It is essential to avoid the presence of free water on the surfaces of vegetables and temperature

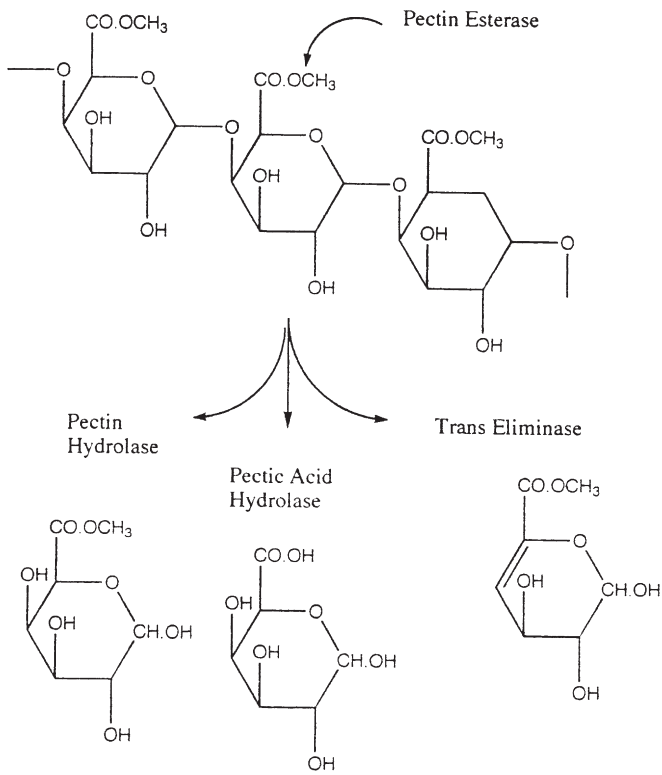


Figure 5.10 *Enzymic activities leading to the degradation of pectin*

control may be just as important to prevent condensation. The presence of a film of water on the surface will allow access of motile bacteria such as *Erwinia Pectobacterium* and pseudomonads to cracks, wounds and natural openings such as stomata. A combination of constant low temperature, controlled relative humidity, and a gas phase with reduced oxygen (*ca*. 2–3%) and enhanced CO_2 (*ca*. 2–5%) has made it possible to store the large hard cabbages used in coleslaw production for many months making the continuous production of this commodity virtually independent of the seasons.

Vegetables should not normally be a cause of public health concern but the transmission of enteric pathogens such as *Salmonella*, VTEC and *Shigella* is possible by direct contamination from farmworkers and the faeces of birds and animals, the use of manure or sewage sludge as fertilizer, or the use of contaminated irrigation water. Celery, watercress, lettuce, endive, cabbage and beansprouts have all been associated with *Salmonella* infections, including typhoid and paratyphoid fevers, and an outbreak of shigellosis has been traced to commercial shredded lettuce. Since salad vegetables are not usually cooked before consumption, it is important to follow good agricultural practices to avoid their contamination during production. Contamination can be reduced by washing produce in clean water but even chlorinated water will normally give only a 2–3 log reduction in microbial numbers as some surface bacteria are lodged in hydrophobic folds or pores and thus evade treatment.

Not all pathogens are necessarily transmitted to vegetables by direct or indirect faecal contamination. Organisms such as *Clostridium botulinum* have a natural reservoir in the soil and any products contaminated with soil can be assumed to be contaminated with spores of this organism, possibly in very low numbers. This would not normally present a problem unless processing or storage conditions were sufficiently selective to allow subsequent spore germination, growth and production of toxin. In the past, this has been seen mainly as a problem associated with underprocessed canned vegetables, but now it must be taken into consideration in the context of sealed, vacuum or modified-atmosphere packs of prepared salads. Those salads containing partly cooked ingredients, where spores may have been activated and potential competitors reduced in numbers, could pose particular problems. In 1987 a case of botulism caused by *Clostridium botulinum* type A was associated with a pre-packed rice and vegetable salad eaten as part of an airline meal. Similar risks may occur in foil-wrapped or vacuum packed cooked potatoes or film-wrapped mushrooms and in all these cases adequate refrigeration appears to be the most effective safety factor.

Another group of pathogens naturally associated with the environment includes the psychrotrophic species *Listeria monocytogenes* which is commonly associated with plant material, soil, animals, sewage and a

wide range of other environmental sources. Raw celery, tomatoes and lettuce were implicated on epidemiological grounds as a possible cause of listeriosis which occurred in several hospitals in Boston, USA in 1979, although direct microbiological evidence was missing. An outbreak of listeriosis in Canada in 1981 was associated with coleslaw (see Section 7.9.5). Strains of *L. monocytogenes* can certainly grow on shredded cabbage and salad vegetables such as lettuce at temperatures as low as 5 °C and modified-atmospheres seem to have no effect on this organism. In the UK, routine surveillance of foods by the Public Health Laboratories revealed that, out of 567 samples of processed vegetables and salads examined, 87 (15%) were found to contain *Listeria* spp. while 72 (13%) contained *L. monocytogenes* specifically.

Two other psychrotrophic organisms which are readily isolated from the environment are *Yersinia enterocolitica* and *Aeromonas hydrophila*. Both may be expected to be associated with vegetables and could grow to levels capable of causing illness if care is not taken during the growth, harvesting, storage and treatment of these commodities.