1 Control of Biodeterioration in Food

1.1 OVERVIEW

All food undergoes deterioration to some degree once harvested or slaughtered. The deterioration may include loss of nutritional value, organoleptic and colour changes, and most importantly, safety may become compromised. It is the challenge of the food industry to control this deterioration and maintain the safety of the food, while making sure that the food is as convenient, nutritious and available as it can possibly be.

Biodeterioration is defined as *any undesirable change in the property of a material caused by the vital activities of organisms* [1]. It is applicable to many materials for example food, wood, paper, leather, fuels, cosmetics, building materials and building structures. Biodeterioration may be a result of the metabolic processes of one of many microorganisms, or it can be caused by insect, rodent or bird damage. As an incredibly broad and diverse field, all biodeterioration has as a common theme in that it affects materials and substances that we need and value, and that it can largely be controlled by proper understanding of the materials and the possible spoilage organisms and their mechanisms of decay.

Biodeterioration is also specifically different from biodegradation in that the changes are 'undesirable'. Biodegradation occurs when complex materials are broken down by microorganisms to form simple end-products. Within a biological ecosystem, there are microorganisms that produce a host of enzymes that can biodegrade natural as well as some synthetic products; this is very important for maintaining the stability of the ecosystem and is extremely important for water purification and sewage treatment.

Food Preservation and Biodeterioration, Second Edition. Gary S. Tucker.

It is also widely used in the food industry. The main differences between biodeterioration and biodegradation are the undesirability and uncontrollability of the former [2].

Another important feature of biodeterioration is that it is caused by organisms. According to the definition, it is not the degradation that occurs naturally in some organic materials or foods caused by intrinsic enzymes. These enzymes are present in the product and cause degradation or decay after death. Loss of food quality by intrinsic enzymes is an important topic as it can cause quality deterioration and render food unacceptable. Reactions due to these enzymes will not be considered in detail in this text, but are important to bear in mind as their activities can make nutrients from the product available and accessible to microorganisms so that biodeterioration reactions can follow [2, 3].

1.2 A SUMMARY OF THE DIFFERENT KINDS OF BIODETERIORATION

1.2.1 Chemical biodeterioration

There are two modes of chemical biodeterioration. Both have a similar result, that is the material becomes spoilt, damaged or unsafe (see Table 1.1 and Fig. 1.1), but the cause or biochemistry of the two is quite different [2, 4]:

- Biochemical assimilatory biodeterioration – the organism uses the material as food or an energy source. Growth of mould on bread is an example of this type of biodeterioration.
- Biochemical dissimilatory biodeterioration – the chemical change in the food is as a result of waste products from the organisms in question. Examples of this are pH changes in food that arise from acids generated from the metabolic action of microorganisms such as bacteria, yeast and mould.

1.2.2 Physical biodeterioration

- Mechanical biodeterioration – this occurs when the material is physically disrupted or damaged by the growth or activities of the organisms. Examples of physical damage can be seen as yeast and mould break down the surfaces of biological materials over time.
- Soiling or fouling – with this kind of biodeterioration the material or product is not necessarily unsafe, but as its appearance has been compromised, it is rendered unacceptable. An example is the building up of biofilms on the surface of a material that can affect the performance of that material.

Table 1.1 Examples of the diversity of biodeterioration.

Affected material	Example	Type of biodeterioration
Stone, marble, concrete	Deterioration of stone monuments	Chemical assimilatory: where calcium and other minerals are used as a food source Chemical dissimilatory: where acid by-products dissolve the surfaces Mechanical: where root damage can undermine and weaken structures Fouling: where biofilms can affect the aesthetics of the structure
Wood	Rotting of wooden floorboards and timber structures	Chemical assimilatory: where the cellulose and lignin in the wood are used as food by fungi and other organisms Dissimilatory: where acid and other by-products result in breakdown of the structure
Leather	Loss of strength and structure of leather objects	Chemical assimilatory: by proteolytic bacteria, which break down the proteins
Paper	Degradation of books	Chemical assimilatory: most commonly by fungi
Paint	Water-based paints	Chemical assimilatory: by bacteria and fungi, results in thinning of the paint and production of off odours
Museum artefacts	Discoloration and degradation of valuable relics	Chemical assimilatory and chemical dissimilatory: by bacteria and mould, resulting in weakening of structures and discoloration of the objects
Food	All foods: animal matter and vegetable based	The most important is chemical assimilatory: the food is used as a food source as it is nutritionally compromised and can have toxins associated with it as by-products of the microbial activity
Metal	Biodeterioration of the wreck of the RMS *Titanic*	Chemical assimilatory: attack on the steel by communities of bacteria and fungi
Fuels	Fuels in tanks	Chemical assimilatory: most commonly the C-10 to C-18 hydrocarbons are broken down to form shorter chain hydrocarbons that, together with the biofilms, can clog fuel lines
Lubricants	Lubricants in metal working lines	Chemical assimilatory: resulting in the loss of lubricating properties and therefore functionality
Teeth	Tooth decay	Chemical dissimilatory: waste products from oral acidogenic bacterial growth cause tooth decay
Glass	Leaching, staining of stained glass windows	Chemical dissimilatory: by waste products from growth of fungi and Cyanobacteria Mechanical: filamentous organisms can cause stress cracking

Living organisms can be divided on the basis of their nutritional requirements into autotrophs and heterotrophs (see Table 1.2). Autotrophic organisms see all inorganic materials as a potential source of nutrients, while heterotrophic organisms can only use organic matter. The organisms responsible for biodeterioration of food are usually chemoheterotrophs; however, it is important to realize that even the packaging that the food is stored in, and the warehouses themselves, can be a source of nutrients for some microorganisms, and it is therefore important to control the humidity, temperature and duration of storage of food, as far as possible [4].

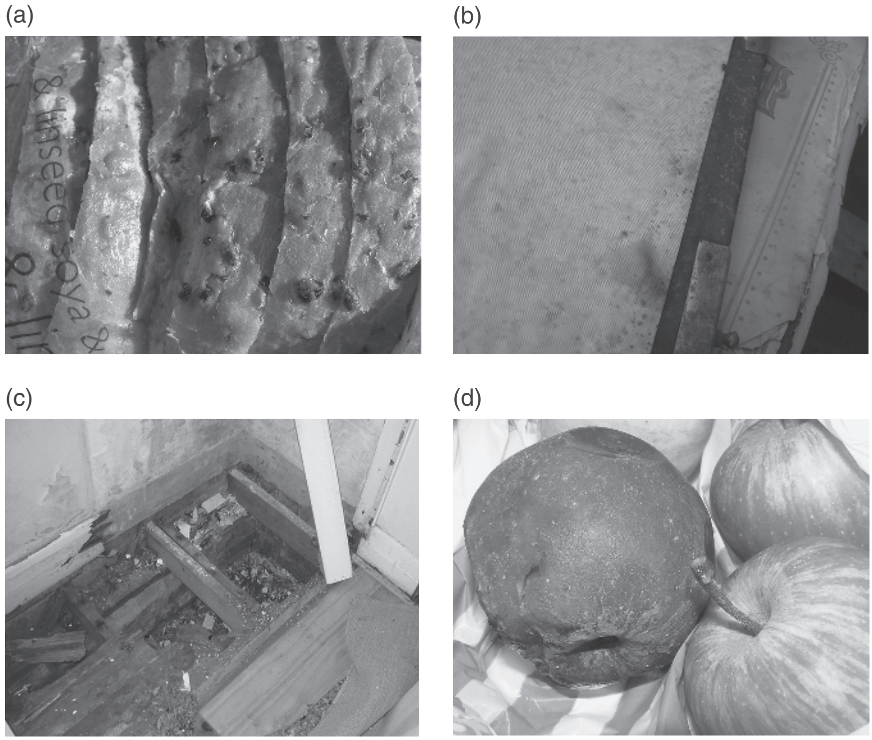

Fig. 1.1 Some common biodeterioration problems. (a) Mouldy bread; (b) mould on antique book; (c) rotten floorboards; (d) soft rot on apples. (*See insert for colour representation of the figure.*)

Table 1.2 Classification of microorganisms on the basis of their nutritional requirements.

Nutritional classification	Source of energy	Source of carbon	Examples of organisms
Autotrophs			
Photoautotroph (photolithotroph)	Sunlight (light energy)	Carbon dioxide (CO_2)	*Aerobic* Algae Cyanobacteria Purple sulphur bacteria Green sulphur bacteria
Chemoautotroph (chemolithotroph)	Redox reactions (chemical energy)	Carbon dioxide (CO_2)	*Aerobic* Hydrogen bacteria Sulphur-oxidizing bacteria Nitrifying bacteria Iron bacteria
Heterotrophs			
Photoheterotroph (photo-organotroph)	Sunlight (light energy)	Organic carbon or carbon dioxide (CO_2)	*Aerobic* Photosynthetic bacteria *Anaerobic* Purple non-sulphur bacteria
Chemoheterotroph (chemo-organotroph)	Redox reactions (chemical energy)	Organic carbon	*Aerobic* Respiratory bacteria Fungi *Anaerobic* Fermentative bacteria Sulphur reducing bacteria Denitrifying bacteria

1.3 KINDS OF LIVING ORGANISMS INVOLVED IN BIODETERIORATION

Living organisms that can cause biodeterioration are referred to as *biodeteriogens* [2]. Animals, insects and higher plants can be easily identified by visual observation and by examining their morphological and physiological characteristics. Organisms like bacteria, fungi and algae are less easy to identify and need to be isolated to be examined. Growth of these organisms under laboratory conditions is often difficult and specialized methods using fluorescent dyes and antibodies or examination using a scanning electron microscope must be used. In some instances, identification can only be made using DNA techniques.

1.3.1 Bacteria

Bacteria are a large diverse group of microscopic, prokaryotic, unicellular organisms. They can be of various shapes (spherical, rod-like or spiral) and may be motile or non-motile. They include both autotrophic and heterotrophic species, and can be aerobic or anaerobic, and many species can thrive under either condition. They have relatively simple nutritional needs and are easily adaptable and can readily change to suit their environment.

1.3.2 Fungi

Fungi are a large group of small chemoheterotrophic organisms. They do not contain chlorophyll and, therefore, cannot make their own food using sunlight. They are, however, extremely adaptable and can utilize almost any organic material. Their growth is characterized by unicellular or multicellular filamentous hyphae, which can often be the cause of physical biodeterioration.

1.3.3 Algae, mosses and liverworts

Algae, mosses and liverworts are eukaryotic unicellular or multicellular organisms. They are photoautotrophic and need moisture, light and inorganic nutrients to grow.

1.3.4 Higher plants

Higher plants are photoautotrophic organisms with specialized tissues and organs that show functional specialization.

1.3.5 Insects

Insects include a large group of aerobic heterotrophic organisms. They need to feed on organic matter, but as a group are diverse in what they can consume. They can feed off all processed and unprocessed foods, as well as non-food items like binding materials and adhesives. Since some insects are attracted to the tight, dark places that abound in storage areas, insects often do significant damage before they are discovered. Some examples of insect pests are silverfish, psocids, cockroaches, borer beetles, weevils and moths. Insects can be infected by disease-causing organisms such as bacteria, viruses and fungi. Besides causing significant biodeterioration themselves, insects can contaminate food or other organic matter.

1.3.6 Birds, mammals and reptiles

Birds, mammals and reptiles are aerobic heterotrophic organisms that have fairly sophisticated food requirements. They can be very resourceful in their acquiring of food and can cause extensive physical damage. Their waste products can also serve as a source of nutrients for other biodeteriogens and can also be corrosive.

1.4 FOOD BIODETERIORATION

From man's earliest history, control of biodeterioration of food has been a concern. The basic principles for control that were applied thousands of years ago are still applicable today:

- Eat food as soon after harvesting as possible.
- Physically protect food from pests by storing in sealed containers.
- Preserve by drying, salting or adding spices.

In our modern, urbanized world we find it impractical to eat food immediately after harvesting and there are times that it must travel thousands of kilometres to get to our plate. Therefore other appropriate methods of food preservation have been developed.

Food is a target for microorganisms and pests. Some microorganisms are better adapted to food spoilage than others and hence knowing and understanding food and the organisms that cause biodeterioration will help in ensuring that they do not get an opportunity to thrive and cause spoilage of the food [5]. All of the issues mentioned above will be considered in this text.

In addition to the microbiological aspects of food biodeterioration, it is important to ensure that food is not degraded, spoiled or rendered susceptible to further or unnecessary spoilage owing to poor procedures and hygiene in farming, harvesting, storage and distribution. The impact of insects and mammals on the damage to cereals and other dry staples and on fruit and vegetables is enormous. These infestations are also initiation points in that their action renders the food susceptible to microbial attack. This is particularly relevant to developing economies in less well-resourced parts of the world where dependence on primary staples is critical.

Some general examples of this sort of biodeterioration include borers, worms, pecking, gnawing, physical bruising and so on Some examples include:

- flies that carry pathogenic bacteria, but which can also cause damage because they lay eggs, the larvae of which then invade the meat or foodstuff causing further deterioration,
- snails on salad leaves,
- aphids on various crops.

1.4.1 The composition of food

Food can be of animal or plant origin, made up mainly from varying proportions of carbohydrates, fats and proteins that provide energy and are the building blocks for growth and essential for maintaining a healthy body. There are also small amounts of vitamins and minerals that are essential for the body to function properly. Water is an important component of food and is vital for cellular functions (Table 1.3).

1.4.1.1 Water

Water is essential for life and is abundant in all food products (unless there have been steps taken to remove it or formulate it without water). As microorganisms cannot grow without water, the presence or absence of water is very important to the status of food and its potential for biodeterioration. Many food processing techniques use the availability of water as the basis for preservation, by making it unavailable to the microorganisms so they cannot grow. Examples include drying, salting, freezing, emulsification and the creation of gels [5, 6].

The chemical formula for water is H_2O. Each molecule of water is made up from two hydrogen atoms and one oxygen atom. A strong covalent bond holds the hydrogen atoms to the oxygen atom, but as the oxygen atom attracts the electrons more strongly than the hydrogen, the bond is slightly ionic, with the hydrogen being slightly positively charged and the oxygen being slightly negatively charged. As a result of this, the water molecule is polar, and there are weak bonds (hydrogen bonds) between the negative and positive charges between molecules. The hydrogen bond, although weak, is very important because this is what causes water to be liquid at room temperature. It influences much of its chemistry and allows it to bond with other molecules that contain charged groups such as sugars, pectins, starches and proteins.

Table 1.3 The composition of some common foods.

Food	% Water	% Protein	% Fat	% Carbohydrate	% Sugar	% Starch
Fruit						
Apple (fresh)	87.7	0.3	0.1	8.9	8.9	Trace
Banana, flesh only (ripe, fresh)	75.1	1.2	0.3	23.2	20.9	2.3
Grapes (fresh)	81.8	0.4	0.1	15.4	15.4	0.0
Cherries (fresh)	82.8	0.9	0.1	11.5	11.5	0.0
Cherries (glace)	23.6	0.4	Trace	66.4	66.4	0.0
Peaches (fresh)	88.9	1.0	0.1	7.6	7.6	0.0
Raisins	13.2	2.1	0.4	69.3	69.3	0.0
Vegetables						
Baked beans (canned in tomato sauce)	71.5	5.2	0.6	15.3	5.9	9.4
Carrots (raw, young)	88.8	0.7	0.5	6.0	5.6	0.2
Potato (raw)	81.7	1.7	0.3	16.1	1.3	14.8
Pumpkin (raw)	95.0	0.7	0.2	2.2	1.7	0.3
Sweet potato (raw)	73.7	1.2	0.3	21.3	5.7	15.6
Meat/fish						
Bacon, streaky	41.8	14.6	39.5	0.0	0.0	0.0
Beef, rump steak (raw)	66.7	18.9	13.5	0.0	0.0	0.0
Beef, salted and dried	29.4	55.4	1.5	0.0	0.0	0.0
Cod fillets (raw)	82.1	17.4	0.7	0.0	0.0	0.0
Pilchards, in tomato sauce (canned)	70.0	18.8	5.4	0.7	0.6	0.1
Miscellaneous						
Bread, white	37.3	8.4	1.9	49.3	2.6	46.7
Butter	15.6	0.5	81.7	Trace	Trace	0.0
Cheese, cheddar	36.0	25.5	34.4	0.1	0.1	Trace
Eggs (chicken)	75.1	12.5	10.8	Trace	Trace	0.0
Flour, white, plain	14.0	9.4	1.3	77.7	1.5	76.2
Milk, cows, whole	87.8	3.2	3.9	4.8	4.8	0.0

Data from Holland *et al.* [6].

Another important characteristic of water, as far as food science is concerned, is that frozen water is less dense than liquid water. In liquid water the molecules are free to pack together closely and 'slide' past each other, whereas in ice the molecules form more-or-less rigid bonds with adjacent molecules. This creates a solid structure but also holds the molecules further apart. This means that ice floats on liquid water, but more importantly, when food is frozen, the volume increases by about 9%.

1.4.1.2 Carbohydrates

Carbohydrates are organic compounds that contain carbon, oxygen and hydrogen. They can be simple sugars or complex molecules based on sugars as building blocks. They have the general formula $C_nH_{2n}O_n$. Food carbohydrates include monosaccharides (e.g. glucose), disaccharides (e.g. lactose, sucrose) and polysaccharides (e.g. dextrins, starches, celluloses and pectins).

Monosaccharides and disaccharides are also referred to as sugars. They are readily digested and metabolized by the human body to supply energy, but can also be easily metabolized (fermented) by microorganisms. Glucose is the main sugar used by the body for energy. It is a small molecule that can pass through the semi-permeable membranes in cells. It is either delivered as a glucose molecule as part of the sugar within a food or it is broken down by amylase enzymes from starch during digestion.

1.4.1.3 Fats

Fats are the second most important source of energy in the diet, after carbohydrates. The yield of energy from fats is greater than that of carbohydrates, with fats yielding more than double the amount of energy from an equivalent weight of carbohydrate. They are an essential part of the diet and are utilized in membrane, cell, tissue and organ structures. Fats or oils (triglycerides) are a group of naturally occurring organic compounds comprised of three molecules of fatty acid covalently bonded to one molecule of glycerol. The properties of a fat are determined by the type and length of fatty acids bonded to the glycerol molecule.

Fats are designated as saturated or unsaturated, depending on whether the fatty acid moieties contain all the hydrogen atoms they are capable of holding (saturated) or whether they have capacity for additional hydrogen atoms (unsaturated). To put it another way, all the carbon–carbon bonds are single bonds in saturated fats, but unsaturated fats/oils have at least one carbon–carbon double bond. Saturated fats are generally solid at room temperature, whereas unsaturated and polyunsaturated fats are liquids and referred to as oils. Unsaturated fats may be converted to saturated fats by the chemical addition of hydrogen atoms (hydrogenation).

1.4.1.4 Proteins

Proteins are the most abundant molecules in cells, making up about 50% of the dry mass. Protein molecules range from soluble globules that can pass through cell membranes and set off metabolic reactions (e.g. enzymes and hormones) to the long insoluble fibres that make up connective tissue and hair. Proteins are made up from amino acids, of which 20 are used by living organisms. Each amino acid has specific properties, depending on its structure, and when they combine together to form a protein, a unique complex molecule is formed. All proteins have unique shapes that allow them to carry out a particular function in the cell. All amino acids are organic compounds that contain both an amino (NH_2) and a carboxyl (COOH) group.

Proteins are very important foods, both nutritionally and as functional ingredients. They serve primarily to build and maintain cells, but their chemical breakdown also provides energy, yielding almost the same amount of energy as carbohydrates on a weight-for-weight basis.

1.4.1.5 Minerals and trace elements

Living organisms need countless numbers of minerals and trace elements for them to be able to function adequately. Among these are calcium, iodine, iron, magnesium, manganese, phosphorus, selenium and zinc.

1.5 DESCRIPTION OF THE MECHANISMS OF FOOD BIODETERIORATION

1.5.1 Fermentation

Many different types of fermented foods are consumed worldwide (See Fig. 1.2). Many countries have their own unique types of fermented food, representing the staple diet and the (raw) ingredients available in that particular place. Some of the more obvious fermented fruit and vegetable products are the alcoholic beverages that include beer, cider and wine. However, several fermented fruit and vegetable products arise from lactic acid fermentation and are extremely important in meeting the nutritional requirements of a large proportion of the global population [7].

Food fermentation can be brought about by bacteria, yeast or mould. When microorganisms metabolize and grow, they release by-products

Fig. 1.2 Examples of foods derived from fermentation. (*See insert for colour representation of the figure.*)

of the metabolism. In food fermentation some of the by-products have a preserving effect in the food by lowering the pH and/or producing preservation materials such as alcohols (e.g. ethanol in beer, wine, cider) and carboxylic acids (e.g. propionic acid in bread dough). Most food poisoning bacteria and some spoilage bacteria cannot survive in either alcoholic or acidic environments. The production of these by-products can protect the food safety and prevent it from spoilage so extending shelf life. Fermentation by-products also change the texture and flavour of the food substrate, for example, in cheese manufacture, the lactic acid causes the precipitation of milk proteins to solid curd [8–11].

The most important bacteria in desirable food fermentation are the *Lactobacillaceae*, which produce lactic acid from carbohydrates, and the acetic acid producing *Acetobacter* species. Sour dough cultures rely on these organisms to ferment wheat flour and generate a range of beneficial chemicals that offer texture, flavour and preservation benefits to the bread. Bread preservation is enhanced by these relatively small molecules that can penetrate the cells walls of microorganisms.

Yeasts also play a beneficial role in the fermentation processes such as the leavening of bread and the production of alcohol and invert sugar. The most beneficial yeasts in terms of desirable food fermentation are from the *Saccharomyces* family, especially *S. cerevisiae*.

Moulds, on the other hand, do not play a significant role in the desirable fermentation of fruit and vegetable products, with most mould action being undesirable. However, some do impart characteristic flavours to foods and others produce beneficial enzymes that are used elsewhere (e.g. fungal amylases for bread production). An example of this is mould from the genus *Penicillium* that is associated with the ripening and flavour of cheeses. Most moulds are aerobic and therefore require oxygen for growth. They produce a large variety of enzymes and can colonize and grow on most types of food.

As stated previously, many changes that occur during fermentation of foods are the result of enzymes produced by the microorganisms. Enzymes are complex proteins produced by living cells in order to carry out specific biochemical reactions. They initiate and control reactions, rather than being used as part of a reaction. They are sensitive to temperature, pH, moisture content, nutrient concentration and the concentration of any inhibitors. Enzymes have specific requirements for optimum performance. Extremes of temperature and pH will denature the proteins and destroy enzyme activity.

Most food fermentation is the result of more than one microorganism, either working together or in a sequence. There are very few pure culture fermentations. Different species of bacteria, yeast and mould each have their own optimum growing conditions. An organism that initiates fermentation will grow until the by-products it produces inhibit further growth and activity. During this initial growth period, other organisms develop that are ready to take over when the conditions become favourable for them. Generally, growth is initiated by bacteria, followed by yeasts and then moulds.

Fermentation usually results in the breakdown of complex organic substances into smaller molecules. Food fermentation includes many important chemical reactions, for example the enzyme lactase, produced by bacteria, causes the lactose in milk to be converted into lactic acid, in alcoholic fermentation. Similarly zymase, secreted by yeast, converts simple sugars (e.g. glucose and fructose) into ethanol and carbon dioxide. Some fermentation reactions are desirable, but others are not, such as the formation of butanoic acid when butter becomes rancid and that of acetic acid when wine turns sour.

The use of fermentation during food production contributes about 20–35% of the daily calorific intake. It is generally desirable in food for various reasons:

- Many desirable flavours and odours are generated as part of fermentation reactions.
- It makes the nutrients more available.
- Microorganisms are anabolic as well as catabolic, that is they also synthesise nutrients like riboflavin and other vitamins.

Some examples of specific fermentations and examples when they change from being useful to being biodegradation reactions are as follows [12]:

1.5.1.1 Pickles

The preservation of food by lactic acid bacteria fermentation is one of the most important methods of food conservation for thousands of years. Pickled products are made from many different fruits and vegetables (e.g. cucumbers, olives, cabbages, peppers, green tomatoes, okra, carrots and mangoes). If the food contains sufficient moisture, a pickling brine may be produced simply by adding dry salt to the vegetables to draw out excess water, then allowing natural fermentation to create an acidic brine solution containing lactic acid (e.g. sauerkraut). Other pickles are made by placing the vegetable in a brine solution (e.g. cucumbers) and allowing enough time for the subsequent fermentation reactions to take place.

The salinity of the brine solution, the temperature of fermentation, the exclusion of oxygen and the acidity of the brine all determine which microorganisms dominate, as well as the flavour of the end product. For example, when the salt concentration and temperature are low, *Leuconostoc mesenteroides* dominates, producing a mix of acids, alcohol and aroma compounds. When the temperature is higher, *Lactobacillus plantarum* dominates, which produces primarily lactic acid. Many commercial pickles have starter cultures added and start with *Leuconostoc*, and change to *Lactobacillus* with higher acidity.

The manufacture of cucumber pickles The cucumber is one of the oldest vegetables cultivated by man and is thought to have its origin in Asia more than 3,000 years ago. Pickled cucumbers are sold commercially worldwide. While many different recipes are popular, the basic method of manufacture from traditional fermentation is as follows. Immature cucumbers are picked and care is taken not to bruise or damage them. The washed cucumbers are placed in large tanks and 4.0–5.3% salt brine (15–20° by salometer) is added. The cucumbers are submerged in the brine, ensuring that none float on the surface. The strong brine draws water out of the cucumbers, which reduces the salinity of the solution. This is monitored daily, and more salt is added when necessary. The concentration of salt must be maintained above 3.1%, or 12° by salometer, or spoilage will occur through putrefaction and softening. Shortly after the cucumbers have been placed in brine the fermentation process, which generates heat and acids, begins. During fermentation, visible changes take place that are important in judging the progress of the process. The colour of the surface of the cucumbers changes from bright green to a dark olive green as acids interact with the chlorophyll. The interior of the cucumber changes from white to a waxy translucent shade as air is forced out of the cells. The specific gravity of the cucumbers also increases, and they begin to sink in the brine rather than floating on the surface.

In the primary stage of fermentation there are many microorganisms, but after a few days Gram-positive cocci, for example *Leuconostoc mesenteroides*, predominate. This species is more resistant to temperature changes and tolerates higher salt concentration than the competitor and subsequent species. As fermentation proceeds and the acidity increases, *Lactobacilli* and *Pediococcus* take over from the *Leuconostoc*. After about 10–14 days all extraneous and undesirable microorganisms should have disappeared. A complete fermentation lasts for between 10 and 30 days, depending upon the temperature of the fermentation. The optimum temperature for *Lactobacilli cucumeris* is 29–32°C. During the fermentative period, the acidity increases to about 1% (as lactic acid) and the pH can go as low as 3.3. If sugar or acetic acid is added to the fermenting mixture during this time it increases the production of acid.

Some problems encountered in the production of pickled cucumbers Soft texture can result from many conditions such as excessive aeration, poor salting procedure and varying temperatures. When the normal sequence of bacterial growth is altered or disturbed, it can result in a soft product. There are three main pectolytic enzymes responsible for softening: polygalacturonase, pectin methylesterase and polygalacturonic acid *trans*-eliminase. These can be produced by a host of bacteria and sometimes fungi, including various species of *Bacillus* and *Achromobacter*, *Aerobacter* and *Escherichia*. Factors that have been found to contribute to the softening of cucumbers include unusually high numbers of pectinolytic bacteria in the initial microbiological population, pH of brine above 5.5, delay in lactic acid fermentation and brine concentrations of 5–8%.

Floating or bloated cucumbers are caused by gas producing micro-organisms (yeasts and bacteria) that can grow inside the cucumbers causing internal cavities, loss of structure and texture.

1.5.1.2 Yogurt

Yogurt is made when bacteria ferment milk sugar (lactose) to lactic acid, and in doing so, lower the pH and cause the characteristic curd to form. Importantly, acid restricts the growth of food-poisoning bacteria. During the fermentation of yogurt flavours are produced that give it its characteristic taste. To be named yoghurt, the product should at least contain the bacteria *Streptococcus salivarius* ssp. *thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. Often these are co-cultured with other lactic acid bacteria (*L. acidophilus*, *Lactobacillus casei* and *Bifidobacterium* species) for either taste or health effects (probiotics).

It is thought that yogurt fermentation was discovered, probably by accident, by Balkan tribes several thousands of years ago. Yogurt remained mainly a food of Eastern Europe until the 1900s when the biologist Mechnikov postulated the theory that *Lactobacilli* bacteria in yogurt are responsible for the longevity of Bulgarian people.

Many types of yogurt are manufactured commercially, and each type and manufacturing company has their own recipes. A mixture of pasteurized fat-free, low-fat or full-cream milk, usually with some milk solids added and, depending on the type of yogurt, with sweeteners and stabilizers, is inoculated with a starter culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. The mixture is held at 30–43°C for 3–16 hours to allow the fermentation to take place. It is then ready to be filled and packaged.

Typical problems that can be found with yogurt are flavour related (i.e. the lack of flavour development, or too intense or unnatural flavours), slimy texture or lack of body. These occur when the added bacteria are out-competed by spoilage bacteria that are introduced from the processing environment. Careful control of hygiene during yogurt production is critical to avoid spoilage.

1.5.1.3 Bread

Bread is one of the oldest foods. Part of the manufacture of bread is called leavening, which is the process of adding gas to the dough before baking, to produce bread that is lighter in texture and has a subtle flavour. Leavening can be done chemically or with yeast. The development of leavened bread possibly began in prehistoric times; however, the earliest archaeological evidence is from ancient Egypt, where scientists using scanning electron microscopy have detected yeast cells in some ancient Egyptian loaves. The yeast used for leavening bread is *Saccharomyces cerevisiae*, which ferments carbohydrates in the flour, producing carbon dioxide and ethanol.

Flour (made from grain that has been ground into a powdery consistency) is the main ingredient, with wheat flour being the most popular because of its ability to form gluten. Some other common grains are rye, barley and maize. There is a large variety of breads made with a variety of techniques and from many different ingredients. A basic method of bread manufacture is as follows. Flour, salt and fat are mixed with yeast and water to form dough. This is kneaded to develop gluten from the flour proteins. In the fermentation stage the dough is left to 'prove' in a warm environment so yeast cells can generate carbon dioxide gas to inflate the bubbles, giving the dough structure and shape. The dough is kneaded again, and then left to prove one last time, usually to double in size. Finally, the bread is baked at a high temperature (about 220°C) to set the proteins and starches, and to generate colour and flavour.

The most common cause of spoilage in bread is by surface mould growth from airborne mould spores, introduced to the bread surfaces after baking. This is more prevalent in packaged bread because of the higher humidity environment created within the packaging. Crusty bread tends to remain free from surface mould because the surface dries to below the threshold water activity for mould growth. On rare occasions 'ropiness' in bread is found, which is caused by outgrowth of *Bacillus* spores (e.g. *B. subtilis*, *B. megaterium*) during slow cooling and in warm storage conditions. It is characterized by strands of syrupy material generated by amylase enzymes from the bacilli. The addition of a preservative based on propionic acid is a common way to control rope.

1.5.1.4 Wine

Wine is an alcoholic beverage produced by the fermentation of sugar in fruit, typically grapes, though a number of other fruits are also popular. A brief description of how wine is made in a modern winery is as follows:

- Grapes are crushed in a perforated, rotating drum that allows the juice and skins of the grapes to pass through, but keep the stems inside the drum. The crushed grapes and juice are called 'must'.
- Red-grape must is sent directly to the fermentation tanks. White-grape must is sent first to a wine press, where the juice is separated from the skins.
- Fermentation tanks are large, airtight, stainless steel vessels, cooled to about 4°C. The winemaker adds sugar and yeast to start the process of fermentation. The type of yeast and the amount of sugar added depend on the type of grape.
- When the yeast gets mixed into the must, the concentration of glucose ($C_6H_{12}O_6$) is very high. The yeast breaks down the glucose via a metabolic process called glycolysis. The products of glycolysis are two three-carbon sugars, called pyruvates, and some ATP (adenosine triphosphate). ATP supplies energy to the yeast and allows it to multiply. The pyruvates are then converted by the yeast into CO_2 and ethanol (CH_3CH_2OH), which is the alcohol in wine.
- The fermentation process takes about 2–4 weeks. During this time, the winemaker samples the fermenting must and measures the pH or acid levels to determine whether the fermentation process is proceeding as it should.
- Once the fermentation process is completed, red wines are sent to the press to separate the skins from the wine and are then filtered to remove the yeast. White wines are allowed to settle and are filtered to remove the yeast.
- With the yeast removed, the wines are stored in either stainless steel storage tanks or oak barrels (oak gives many wines a characteristic flavour), depending on the type of wine. In some red wines, a second type of fermentation, called malolactic fermentation, is undertaken while in storage. In malolactic fermentation, the winemaker adds a bacterium to the wine that breaks down malic acid, a by-product of aerobic metabolism, into lactic acid, a by-product of anaerobic metabolism. Lactic acid is a milder acid than malic acid. The ageing process can be anywhere from 3 months to 3 years.
- After the wine has aged sufficiently, as determined by the winemaker, it is time to bottle and package it for sale.

A wine fault or defect is an unpleasant characteristic of a wine often resulting from poor winemaking practices or storage conditions. Some examples are listed below:

- *Acetaldehyde* production is associated with the presence of surface film-forming yeasts and bacteria, such as acetic acid bacteria, which form the compound by the decarboxylation of pyruvate. Acetaldehyde is an intermediate product of yeast fermentation; however, it is more commonly associated with ethanol oxidation catalysed by the enzyme ethanol dehydrogenase. Wine with levels above 100–125 ppm can be described as ‘green apple’, sour and metallic.

- *Acetic acid* in wine, often referred to as volatile acidity or vinegar taint, can be contributed by many wine spoilage yeasts and bacteria. This can be from either a by-product of fermentation or due to the spoilage of finished wine. Acetic acid bacteria, such as those from the genera *Acetobacter* and *Gluconobacter*, produce high levels of acetic acid.
- *Ethyl acetate* is formed in wine by the esterification of ethanol with acetic acid. Therefore, wines with high acetic acid levels are more likely to see ethyl acetate formation, but the compound does not contribute to the volatile acidity. It is a common microbial fault produced by wine spoilage yeasts, particularly *Pichia anomala*, *Kloeckera apiculata* and *Hanseniaspora uvarum*. High levels of ethyl acetate are also produced by lactic acid bacteria and acetic acid bacteria. Low levels of ethyl acetate can give an added richness and sweetness to the wine, but above about 150–200 ppm an aroma characteristic of nail polish remover, glue or varnish can be detected.
- *Hydrogen sulphide* (H_2S) is generally thought to be a metabolic by-product of yeast fermentation in nitrogen-limited environments. It is formed when yeast ferments via the sulphate reduction pathway. Hydrogen sulphide can further react with wine compounds to form mercaptans and disulphides.
- *Geosmin* is a compound with a very distinct earthy, musty, beetroot, even turnip flavour and aroma and has an extremely low sensory threshold of down to 10 ppm. Its presence in wine is usually derived as a metabolite from the growth of filamentous actinomycetes such as *Streptomyces*, and moulds such as *Botritis cinerea* and *Penicillium expansum* on grapes. Geosmin is also thought to be a contributing factor to cork taint.
- *Lactic acid bacteria* have a useful role to play in winemaking by converting malic acid to lactic acid in malolactic fermentation. However, after this function has been completed, the bacteria may still be present within the wine, where they can metabolize other compounds and produce wine faults. Wines that have not undergone malolactic fermentation may be contaminated with lactic acid bacteria, leading to refermentation of the wine and it becoming turbid, ‘swampy’ and slightly effervescent. Lactic acid bacteria can also be responsible for wine taints.
- *Ropiness* is manifested as an increase in viscosity and a slimy or fatty mouth-feel of a wine. It is caused by the production of dextrins and polysaccharides by certain lactic acid bacteria, particularly of the genera *Leuconostoc* and *Pediococcus*.

- *Mousiness* is a wine fault most often attributed to *Brettanomyces*, but it can also originate from the lactic acid bacteria *Lactobacillus brevis*, *Lactobacillus fermentum* and *Lactobacillus hilgardii*. The compounds responsible are lysine derivatives, and the taints are not volatile at the pH of wine, and therefore not obvious as an aroma. However, when mixed with the neutral pH of saliva, they can become very apparent on the palate especially at the back of the mouth, as 'mouse cage' or 'mouse urine' flavour.
- *Refermentation/secondary fermentation* is caused by yeasts refermenting the residual sugar present within bottled wine. It occurs when sweet wines are bottled in non-sterile conditions, allowing the presence of microorganisms. The most common yeast to referment wine is the standard wine fermentation yeast *Saccharomyces cerevisiae*, but has also been attributed to *Schizosaccharomyces pombe* and *Zygosaccharomyces bailii*. The main issues associated with the fault include turbidity, excess ethanol production, carbonation and some coarse odours.

1.5.1.5 Beer

Beer is one of the oldest beverages humans have produced, dating back to at least the 5th millennium BC, and recorded in the written history of Ancient Egypt and Mesopotamia. It is most likely that beer-like beverages were independently developed among various cultures throughout the world. Beer is produced by the fermentation of many starch-based materials, though commonly barley, cassava, millet, sorghum, potato and agave are used. Because the ingredients and processes used to make beer differ dramatically, characteristics such as taste and colour also vary.

Beer manufacturing methods vary depending on the type of raw material used. A method for commercial beers with the basic ingredients water, malted barley, yeast (*Saccharomyces cerevisiae* or *Saccharomyces uvarum*) and hops is as follows:

- *Malting*: Malt is formed from barley by soaking it in water, allowing it to start to germinate, and then drying the germinated grain in a kiln. Malting the barley produces enzymes that will eventually convert the starches into fermentable sugars.
- *Mashing*: This is the first phase of brewing, in which the malted grains are crushed and soaked in warm water in order to create a malt extract. The mash is held at a constant temperature for long enough for enzymes to convert starches into fermentable sugars.
- *Lautering*: This involves the separation of extracts formed during mashing from the spent grain. It is achieved in either a Lauter tun, a wide vessel with a false bottom, or a mash filter, a plate-and-frame filter

designed for this kind of separation. It has two stages: first wort run-off, during which the extract is separated in an undiluted state from the spent grains, and sparging, in which extract which remains with the grains is rinsed off with hot water.

- *Boiling*: The wort is boiled along with any remaining ingredients (excluding yeast), to remove excess water and kill any microorganisms. The hops are added at some stage during the boil.
- *Fermentation*: The yeast is added and the beer is left to ferment. After primary fermentation, the beer may be allowed a second fermentation, which allows further settling of yeast and other particulate matter that may have been introduced earlier in the process. Some brewers may skip the secondary fermentation and simply filter off the yeast.
- *Packaging*: At this point, the beer contains alcohol, but not much carbon dioxide. The brewer has a few options to increase CO_2 levels. The most common approach by large-scale brewers is force carbonation, via the direct addition of CO_2 gas to the keg or bottle. Smaller-scale or more classically minded brewers will add extra sugar or a small amount of newly fermenting wort to the final vessel, resulting in a short refermentation known as 'cask-' or 'bottle conditioning'.
- After brewing, the beer is usually a finished product. At this point the beer is kegged, casked, bottled or canned.

Lager is the English name for bottom-fermented beers. Lager yeast is a bottom-fermenting yeast, and typically undergoes primary fermentation at 7–12°C (the 'fermentation phase'), and then is given a long secondary fermentation at 0–4°C (the 'lagering phase'). During the secondary stage, the lager clears and mellows. The cooler conditions also inhibit the natural production of esters and other by-products, resulting in a 'crisper' tasting beer.

Ales are brewed with top-fermenting yeasts. Ale is typically fermented at temperatures between 15 and 24°C, at which temperatures yeast produces significant amounts of esters and other secondary flavour and aroma products, and the result is often a beer with slightly 'fruity' compounds resembling, but not limited to, apple, pear, pineapple, banana, plum or prune. Typical ales have a sweeter, fuller body than lagers. The important distinction for ales is that they are fermented at higher temperatures and thus ferment more quickly than lagers.

Problems encountered in beer making A stuck fermentation is a fermentation of wine or beer that has stopped before completion, that is before the anticipated percentage of sugars has been converted by yeast into alcohol. It may be caused by (i) insufficient or incomplete nutrients required to allow the yeast to complete fermentation; (ii) low temperatures, or temperature changes which have caused the yeast to stop working early or (iii) an alcohol content too high for the particular yeast chosen for the fermentation. Conditions like ropiness (beer becomes viscous and pours as an oily stream), sourness (caused by elevated levels of acetic acid) and turbidity are just some of the conditions caused by undesirable microbiological activity.

1.5.1.6 Cheese

Cheese is an ancient food, the origins of which are debated; estimates range from around 8000 BC (when sheep were domesticated) to around 3000 BC. It was probably discovered in Central Asia or the Middle East and taken from there to Europe. The earliest cheeses would probably have been quite sour and salty, similar in texture to feta. When basic cheese-making found its way into Europe, the cooler climates meant less aggressive salting was needed for preservation. With moderate salt and acidity, cheese became a suitable environment for a variety of beneficial microbes and moulds, which are what give aged cheeses their pronounced and interesting flavours.

Cheese is a solid food made from the milk of cows, goats, sheep, buffalo or other mammals. Their milk is curdled using some combination of bacterial acidification and the enzyme rennet (or a rennet substitute). Bacteria that turn milk sugars into lactic acid acidify the milk and play a role in defining the texture and flavour of most cheeses. Some cheeses also feature moulds, either on the outer rind or throughout (See Fig. 1.3).

Cheese making

- *Curdling*: This is the only strictly required step in making any sort of cheese and results in separating the milk into solid curds (protein) and liquid whey. This is done by acidifying the milk (usually by starter

Fig. 1.3 Sample of typical cheeses. (*See insert for colour representation of the figure.*)

bacteria from the *Lactococci*, *Lactobacilli* or *Streptococci* families) and adding the enzyme rennet. The starter bacterium converts milk sugars into lactic acid, and generates enzymes that are responsible for the eventual flavour of aged cheeses. Some fresh cheeses are curdled only by acidity, but rennet is also used in most cheeses. Rennet sets the cheese into a strong and rubbery gel (compared to the fragile curds produced by acidic coagulation alone).
- *Curd processing*: At this point the cheese has set into a very moist gel. Some soft cheeses are now essentially complete and can be drained, salted and packaged. For most other cheeses, the curd is cut into small cubes that allow water to drain from the individual pieces of curd. Salt has a number of roles in cheese besides adding a salty flavour. It preserves cheese from spoiling, draws moisture from the curd and firms up the cheese texture by interacting with its proteins. Some cheeses are salted from the outside with dry salt or brine washes but most cheeses have the salt mixed directly into the curds.
- *Ageing/ripening*: A new cheese usually has a salty bland flavour, and the harder varieties are rubbery in texture. Cheeses are usually left to rest under carefully controlled conditions. This ageing period can last from a few days to several years. As cheese ages, microorganisms and enzymes transform its texture and intensify its flavour. This transformation is largely a result of the breakdown of casein proteins and milk fat into a complex mix of amino acids, amines and fatty acids.

There are many potential problems associated with cheese making, as for each cheese type there are many very specific parameters that have to be complied with, for example pH control in the initial fermentation is significant in the control of curdling. At many stages there can be the development of off or uncharacteristic flavours as well as uncharacteristic textures.

Flavour defects include:

- *Sourness/acidity* caused by excessive fermentation and/or inadequate washing of the curds.
- *Bitterness* caused by abnormal protein degradation.
- *Fruity and fermented flavour* caused by anaerobic spore-formers. This occurs when the pH is high and the salt content is low.

Texture defects include:

- *Corky*, caused by inadequate acid development of and/or excessive washing of the curds.
- *Weak/pasty*, caused by too much moisture or too little salt.

- *Gassiness*, caused by the growth of various bacteria and yeasts.
- *Openness*, caused when whey is trapped between curds (and the opening remains after draining).

1.5.2 Fermentation biochemistry

Prokaryotic and eukaryotic cells share a major metabolic pathway in which glucose is catalysed to pyruvate through several enzymatic pathways. The pyruvate is further broken down to compounds like ethanol and lactic acid. This requires no oxygen and produces energy in the form of adenosine triphosphate (ATP). Although this is not a very efficient way of producing energy, it is fast [13, 14].

Metabolically fermentation is a process that is important for an organism in anaerobic conditions when there is no oxidative phosphorylation to maintain the production of ATP by glycolysis. During fermentation, pyruvate is metabolized to various different compounds. Homolactic fermentation is the production of lactic acid from pyruvate; alcoholic fermentation is the conversion of pyruvate into ethanol and carbon dioxide; and heterolactic fermentation is the production of lactic acid as well as other acids and alcohols (see Fig. 1.4) [13].

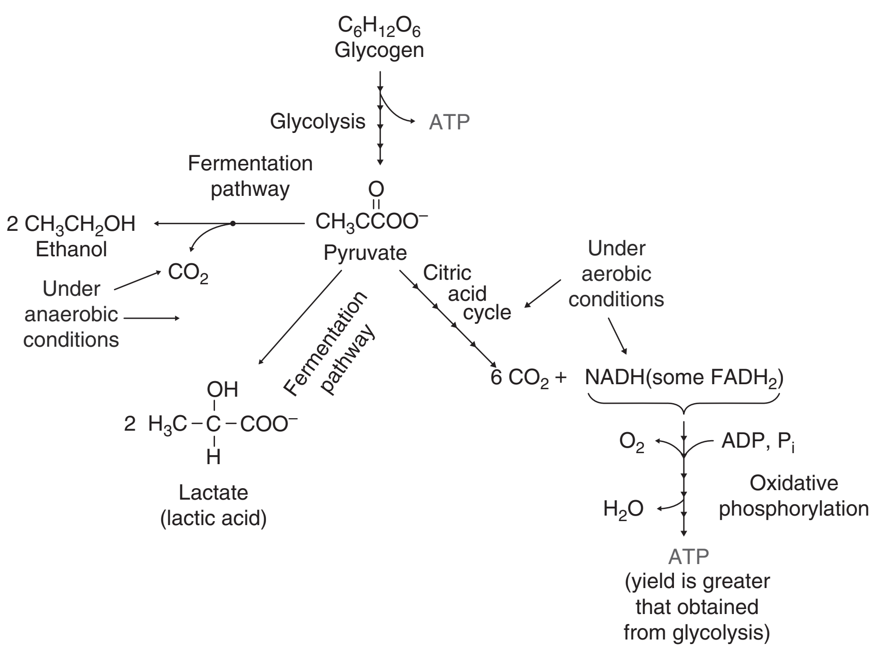

Fig. 1.4 Metabolization of glucose (glycogen) via pyruvate.

(a) $C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 +$ energy released
Glucose → Ethanol + Carbon dioxide

(b) $C_6H_{12}O_6 \rightarrow 2\ CH_3CHOHCOOH +$ energy released
Glucose → Lactic acid

(c) $C_6H_{12}O_6 \rightarrow CH_3CHOHCOOH + C_2H_5OH + 2\ CO_2 +$ Energy
Glucose → Lactic acid + Ethanol + Carbon dioxide

Fig. 1.5 Examples of anaerobic fermentation.

$C_2H_5OH + O_2 \rightarrow CH_3COOH + H_2O +$ energy released
Ethanol + Oxygen → Acetic acid + Water

Fig. 1.6 An example of aerobic fermentation.

Lysine $\xrightarrow{\textit{decarboxylase}}$ $H_2N(CH_2)_5NH_2$ (Cadaverine)

Fig. 1.7 Production of cadaverine.

Biochemically, fermentation is the anaerobic metabolic breakdown of carbohydrate nutrients, like glucose, without net oxidation (see Fig. 1.5) [13]. Fermentation does not release all the available energy in a molecule; it merely allows glycolysis to continue by replenishing reduced coenzymes. Depending on where it is taking place, fermentation may yield lactic acid, acetic acid, ethanol, butyric acid, acetone and other reduced metabolites. Food fermentation uses the term more broadly, and fermentation refers to the anaerobic or aerobic growth (see Fig. 1.6) of microorganisms on a substrate. It refers to the chemical changes in organic substances produced by the action of specific enzymes, produced by microorganisms such as moulds, bacteria and yeasts.

1.5.3 Putrefaction

Putrefaction is the breakdown of proteins by microbial enzymes, usually produced by anaerobic spoilage microorganisms. It results in 'off' odours referred to as putrid odours. The odours are caused by the diamines cadaverine (pentamethylenediamie) (Fig. 1.7) and putrescine (butanediamine) (Fig. 1.8) and are the end products of spoilage. Putrefaction occurs in protein rich products like meat, fish and certain vegetables.

1.5.4 Lypolysis

Lypolysis is the breakdown of fat into glycerol and free fatty acids. Lipolysed fat has a rancid taste and smell. The lypolysis reaction is controlled by enzymes called lipases, which are produced by microorganisms. As with many enzymatic reactions, high storage temperatures encourage

$$\text{Ornithine or arginine} \xrightarrow{\textit{decarboxylase}} H_2N(CH_2)_4NH_2 \text{ (Putrescine)}$$

Fig. 1.8 Production of putrescine.

lipolysis. In foods like fatty fish, the fish oils are largely composed of glycerol combined with fatty acids to form glycerides. Splitting of the glycerides and formation of free fatty acids (FFA) results in reduced quality of the oil. In dairy products, where the fat component is a significant part of the whole product, much effort has to be taken in processing, storage and distribution to ensure that lypolysis is minimized.

1.6 MICROORGANISMS INVOLVED IN BIODETERIORATION REACTIONS

1.6.1 Factors that affect microbial growth

There are many complex reactions and conditions that either inhibit or encourage microbial growth. The availability of oxygen, temperature (hot or cold), light and other radiation, moisture and dryness, the activity of natural enzymes and the amount of spoilage microorganisms that are present will all affect the growth of spoilage organisms. A few of the basic physical properties of the food or the storage environment are discussed briefly below [11, 12]. Further details on these mechanisms of preservation are discussed in later chapters.

1.6.1.1 pH

pH refers to the hydrogen ion concentration of a solution, a measure of the solution's acidity. It is defined as the negative logarithm of the concentration of H^+ ions.

$$\text{pH} = -\log_{10}\left[H^+\right] \tag{1.1}$$

where $[H^+]$ is the concentration of H^+ ions in moles per litre.

Most microorganisms grow best at neutral pH and only a few are able to grow at a pH lower than 4.0. Bacteria are more fastidious about their pH requirements than are yeast and mould. The fact that pH can limit microbial growth is a basic principle of food preservation and has been used for thousands of years. Fermentation and pickling extend the shelf life of food products by lowering the pH. The fact that no known spore-forming pathogenic bacteria can grow at pH $<$ 4.6 is the basis for the food sterilization principle for low acid and acid foods. See Fig. 1.9 for examples of pH growth ranges of various microorganisms.

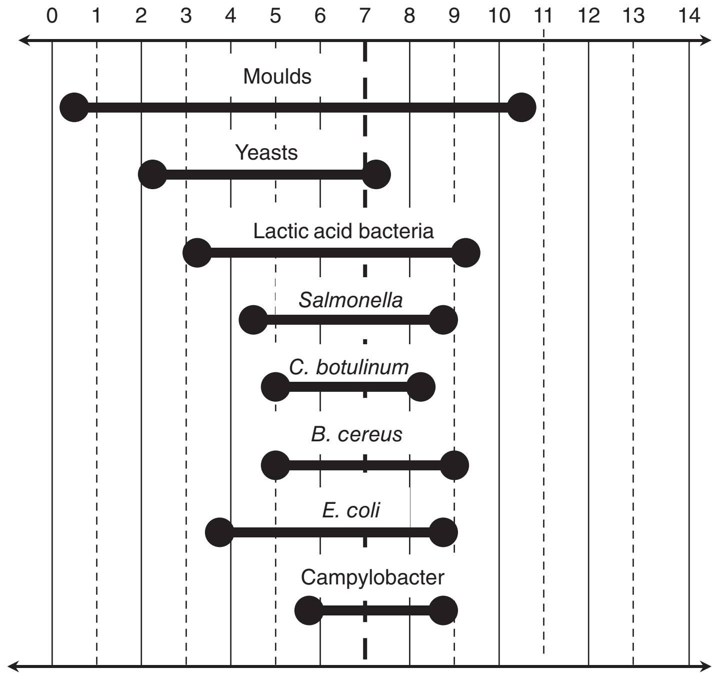

Fig. 1.9 pH growth ranges for selected microorganisms.

Table 1.4 The pH of selected foods.

Food	pH	Food	pH	Food	pH
Fruit		*Vegetables*		*Miscellaneous*	
Apple	3.3–4.0	Asparagus	6.0–6.7	Bread, white	5.0–6.2
Banana	5.0–5.3	Avocado	6.3–6.6	Bread, whole	5.5–5.9
Cherries	3.3–3.6	Beans (soy)	6.0–6.6	Wheat	
Grapes	3.5–3.8	Beans in tomato sauce	5.3	Cheddar cheese	5.9
Grapefruit	3.0–3.8			Cottage cheese	4.7–5.0
Lemon	2.0–2.6	Carrots	5.9–6.4	Eggs	6.6
Peaches	3.3–4.1	Mushrooms	6.0–6.7	Egg, white	7.9
Pineapple	3.2–4.0	Olives	6.0–7.5	Egg, yolk	6.1
Fish and meat		Olives,	3.6–4.6	Honey	3.7–4.2
Fish	5.8–6.8	Fermented		Ketchup	3.9
Beef	5.1–6.2	Peas	6.2–6.8	Milk, cow's	6.4–6.8
Pork	5.3–6.9	Sweet potatoes	5.3–5.6	Marmalade	3.0–3.3
Lamb	5.4–6.7			Peanut butter	6.3
Chicken	6.2–6.4	Tomatoes	4.0–4.9	Tea	7.2

The pH of some food is inherently low, and therefore, the kinds of organisms that can cause biodegradation of that food is limited. Some foods are acidified to preserve them or to provide one of the food preservation hurdles. See Table 1.4 for some examples of the pH of foods.

1.6.1.2 Moisture content of the food

Microorganisms cannot grow in a water-free environment, as enzyme activity is absent, and most chemical reactions are greatly slowed down. Fresh vegetables, fruit, meat, fish and some other foods naturally have a high moisture content, which averages about 80%. Drying is one of the oldest methods of food preservation. Drying reduces the availability of moisture, thereby limiting the number and types of microorganisms that can grow as well as reducing their growth rate. A measure of this parameter is called *water activity* and is defined by the ratio of the water vapour pressure in the food substrate to the vapour pressure of pure water at the same temperature, and is denoted by a_w.

$$a_w = \frac{p}{p_o} \tag{1.2}$$

where p = vapour pressure of solution

p_o = vapour pressure of solvent (usually water)

Water activity is a measure of the water that is available to microorganisms. Pure water has a water activity of 1.0, while most fresh foods have a water activity of about 0.99. In general, bacteria require a higher a_w for growth than yeasts and moulds. Most spoilage bacteria cannot grow at $a_w < 0.91$, with *Clostridium botulinum* having a minimum growth level of 0.94. *Staphylococcus aureus*, has, however, been found to grow at a_w as low as 0.84. The lowest reported a_w value for a bacterial growth is 0.75. Most spoilage moulds cannot grow at $a_w < 0.80$. The lowest reported a_w for any mould growth is 0.65, and for yeasts it is 0.61.

1.6.1.3 Humidity of the environment

The humidity of the environment is important as it can affect the food a_w as well as the moisture on its surface. Food can pick up moisture from the atmosphere if the food a_w is less than that of the environment. Under conditions of high humidity storage (e.g. in a refrigerator), surface spoilage can take place unless food is adequately protected by packaging.

1.6.1.4 Temperature

Storage temperature can be considered the most important factor that affects biodegradation of food. Microorganisms have been reported to grow over a wide temperature range; the lowest reported is below −30°C and the highest close to 100°C. All microorganisms do, however, have an optimum temperature as well as a range in which they will grow. This preference for temperature forms the basis of dividing microorganisms into groups.

- Psychrotrophs have an optimum from 20 to 30°C, but can grow at or below 7°C.
- Mesophiles have an optimum of 30–40°C, but can grow between 20 and 45°C.
- Thermophiles grow optimally between 55 and 65°C, but can grow at a temperature as low as 45°C.

Just as moulds are able to grow over a wide range of pH values and moisture conditions, they can also tolerate a wider temperature range than bacteria. Many moulds can grow in the refrigerator, albeit slowly. Yeasts are not usually found growing in the thermophilic temperature range, but prefer psychrotrophic and mesophilic temperatures.

While lower storage temperature generally slows down microbial growth, it is not suitable to store all foods in the freezer (−18°C) or even the refrigerator (<8°C), as they lose texture and other desirable features.

1.6.1.5 Availability of oxygen

Controlling the availability of free oxygen is one means of controlling microbial activity within a food. Although oxygen is essential for carrying out metabolic activities that support all forms of life, some microorganisms use free atmospheric oxygen, while others metabolize the oxygen (reduced form) which is bound to other compounds such as carbohydrates.

Microorganisms can be broadly classified into two groups – aerobic and anaerobic. Aerobes grow in the presence of atmospheric oxygen, while anaerobes grow in the absence of atmospheric oxygen. In between these two extremes are the facultative anaerobes, which can adapt and grow in either the absence or presence of atmospheric oxygen, and micro-aerophilic organisms, which grow in the presence of reduced amounts of atmospheric oxygen. In aerobic fermentation the amount of oxygen present is one of the limiting factors. It determines the type and amount of biological product obtained, the amount of substrate consumed and the energy released from the reaction.

1.6.2 Bacteria

There is a vast number of different bacteria involved with biodeterioration reactions. Below a few examples are discussed. In some instances biodeterioration has dire consequences, as it can cause food poisoning and in some instances death [9, 11, 15].

1.6.2.1 Clostridium botulinum

Food-poisoning incidents that result in fatalities are relatively rare. However, one bacterium that can cause death is *Clostridium botulinum*. The botulinum toxin is one of the most potent neurotoxins known. It has been considered a real threat for use in biological warfare. Only one gram of crystalline toxin, could, if evenly dispersed over a city and then inhaled by its inhabitants, kill more than a million people. Food contaminated with only a few nanograms (10^{-9} g) can be toxic. For this reason, while fairly uncommon, the risk is taken very seriously by all producers of products packed in an anaerobic environment and stored for an extended period of time, for example canned and vacuum-packed foods. All food processes are designed with organisms like *C. botulinum* in mind. The canning process is specifically designed to ensure that the food is safe with regard to *C. botulinum* being eliminated. Heat processing is one way of ensuring food safety, but the integrity of the can has to be maintained right up until the contents are consumed, as pathogenic bacteria can 'leak' into the can if given the opportunity.

Clostridium botulinum is a Gram-positive, obligate anaerobe, spore-forming bacterium. It is commonly found in soils throughout the world and so could contaminate vegetables cultivated in or on the soil and commonly colonises the gastrointestinal tract of fish, birds and mammals. *C. botulinum* is classified as a single species of bacterium but includes at least three genetically distinguishable groups of organisms that have been found to be toxic to humans (*C. botulinum*, *C. baratii* and *C. butyricum*). These organisms share the ability to produce the neurotoxins. The toxin types are classified as A, B, C, D, E, F and G. Human botulism has been described with the strains of *C. botulinum* that produce toxin types A, B and E.

Clostridium botulinum bacteria produce spores that are fairly heat resistant and cannot be killed simply by boiling. Canned food sterilization processes are specifically designed so as to eliminate the possibility of any spores surviving. The toxin is, however, fairly heat sensitive and heating at 80°C for 30 minutes or 100°C for 10 minutes will destroy the active toxin. If canned food like meat, fish or vegetables is under-processed or becomes contaminated after processing with *C. botulinum* the sealed can provides an ideal, anaerobic environment for the bacterium to grow. The factors which limit the growth of *C. botulinum* include: (i) low pH: it will not produce toxin in acid or acidified foods (i.e. below pH 4.5); (ii) low water activity: a minimum of 0.94 is needed to support bacterial growth and toxin production. This water activity corresponds to a 10% salt (NaCl) solution, which is why salting is sometimes used as a method of preservation; (iii) temperature: most strains grow optimally at

35–40°C, but some can grow at temperatures as low as 3°C; (iv) food preservatives: many preservatives (nitrite, sorbic acid, phenolic antioxidants, polyphosphates, etc.) inhibit *C. botulinum* growth; (v) competing microorganisms.

Botulism is a paralytic illness caused by a neuro- (nerve) toxin that is produced by the bacterium *Clostridium botulinum*. There are five types of botulism:

1) Food-borne botulism: an intoxication most commonly found in home-preserved foods, usually because of under-processing.
2) Wound botulism: caused when the bacterium manages to grow inside a wound (usually a puncture wound or gun-shot wound or with drug abusers).
3) Infant botulism: caused when the intestine of the infant (less than 1 year of age) is colonized by the bacterium, which then produces toxin. Honey is known to be a source of *C. botulinum* spores and is, therefore, not recommended for babies less than 1 year old.
4) Adult infectious botulism: this is similar to infant botulism, but is usually found in patients with intestinal diseases or after bowel surgery.
5) Inadvertent botulism: following treatment with botulinum toxin injection, used to treat various muscular problems.

1.6.2.2 Salmonella

Salmonella is a Gram-negative, rod shaped, motile bacterium, that does not form spores. It is widespread in occurrence, being found in, for example, fresh and salt water, soil and animal faeces. Various foods have been found to be associated with Salmonellosis, the illness caused by *Salmonella* spp., including raw meat, poultry and seafood, raw eggs and foods made from raw eggs, dried gelatine, cocoa, chocolate, peanut butter, yeast and coconut. Salmonellosis is caused by infection with the organism and extremely low doses (as low as 15 cells) can cause disease.

1.6.2.3 Listeria monocytogenes

Listeria are Gram-positive, motile bacteria. They do not form spores, but are quite resistant to desiccation and heat. They are found in the soil and associated with birds and animals. Infection with these bacteria causes a disease called Listeriosis, which can result in septicaemia, meningitis encephalitis and spontaneous abortions in pregnant females. *Listeria monocytogenes* has been associated with raw milk, soft cheeses, raw vegetables, raw meat, fish and poultry. It can grow at temperature as low as 3°C, which allows growth during refrigeration.

1.6.2.4 *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive, small round bacterium (coccus) that can produce a heat-stable toxin. Less than 1 μg of toxin can cause illness. *S. aureus* is found all over, but the most common source of contamination of food is via humans, that is food handlers. Staphylococci are found in the nasal passages, throat, on the skin and in the hair of more than half of healthy people. Intoxication is caused by contaminated food being kept either not hot enough (i.e. at <60°C) or not cold enough (i.e. at >8°C), which allows the organism to grow and produce its toxin. The symptoms (nausea, vomiting, cramping) of staphylococcal food poisoning come on very rapidly (within a few hours) and are usually acute.

1.6.2.5 *Clostridium perfringens*

Clostridium perfringens is a Gram-positive spore-forming anaerobic rod-shaped bacterium. It is widely distributed in nature and is also found associated with the intestines of animals and humans. *C. perfringens* produces a toxin that causes intense abdominal cramps and diarrhoea. Food poisoning from this organism is usually associated with cooked foods that have been inadequately cooled and held for several hours before consumption. It was notorious as a cause for food poisoning in wedding banquets in which large (reconstituted) meat joints were cooked then left to cool slowly in warm ambient conditions. This provided time and temperature conditions for *C. perfringens* spores to germinate and grow to number sufficient that toxin production occurred.

1.6.2.6 *Bacillus cereus*

Bacillus cereus is a Gram-positive, facultatively aerobic, spore-forming, rod-shaped bacterium. The presence of this organism in large numbers can result in two types of food poisoning illness. A protein toxin causes diarrhoeal symptoms, similar to those caused by *Clostridium perfringens*, and a heat-stable peptide causes vomiting symptoms, similar to those caused by *Staphylococcus aureus*. The diarrhoeal type of illness has been diagnosed after consumption of a variety of foods, for example milk, meat, fish and vegetables, while the vomiting type is associated with the consumption of rice products and other starchy foods like potatoes and pasta. Puddings, soups, casseroles, pastries and salads have all been implicated in food poisoning by this bacterium. Notoriety of *B. cereus* food poisoning arose from early takeaway facilities in which rice was cooked during the day and left too cool slowly in large quantities ready for reheating in the evening rush. As with *C. perfringens* poisoning, this practice allowed time for *B. cereus* spores to germinate and grow to number sufficient that toxin production occurred.

1.6.2.7 *Escherichia coli*

Escherichia coli are Gram-negative bacteria that are found in the intestines of all mammals, including humans. *E. coli* produce toxins that can cause four different classes of illnesses that are of concern to the food industry. The first class is enterovirulent (EEC) that causes gastroenteritis. This class includes *E. coli* 0157:H7 (EHEC), which is enterohaemorrhagic. The second type is referred to as enterotoxigenic (ETEC), the third is enteropathogenic (EPEC) and the fourth is enteroinvasive (EIEC). Outbreaks of food poisoning due to *E. coli* are usually associated with faecal contamination of water by food handlers, or are due to unprocessed or under-processed foods.

1.6.2.8 *Campylobacter jejuni*

Campylobacter jejuni are Gram-negative, microaerophilic, motile, rod-shaped bacteria. They are found on raw chickens, in raw milk, and are often carried in the intestines of healthy cattle. They can contaminate food via flies on farms and are also found in non-chlorinated water. Consumption of contaminated food results in diarrhoea caused by a heat-labile toxin and by the invasive organism. *Campylobacter* is currently a source of great concern for the poultry industry because of the ease in which it is spread by liquid and droplet cross-contamination.

1.6.2.9 *Lactic acid bacteria*

Lactic acid bacteria form a large, diverse group comprising bacteria from *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, and so on. These bacteria are commonly involved with fermentation reactions that are desirable, but can also cause spoilage by acid and gas formation in some products (see Fig. 1.10). They are not implicated in food poisoning incidents.

1.6.2.10 *Pseudomonas*

Pseudomonas spp. are a large group of Gram-negative rod-shaped bacteria that are responsible for spoilage of many refrigerated proteinaceous products (e.g. meat, fish, eggs). Some strains produce blue-green pigments. They are implicated in many food spoilage conditions, for example green rot, black rot, pink rot and red rot. They have very varied nutritional requirements and can even cause spoilage of bottled water.

Fig. 1.10 Spoilage of yogurt owing to presence of lactic acid bacteria. (*See insert for colour representation of the figure.*)

1.6.2.11 Toxins formed by bacterial action – Scombrotoxin

Scombroid poisoning is caused by consuming foods that contain high levels of histamine. Histamine and other amines are formed by the growth of a variety of bacteria and the subsequent action of their decarboxylase enzymes on the amino acid histadine. Any food product that contains high levels of this amino acid can be susceptible to this sort of spoilage, but it occurs most commonly with tuna, mackerel, pilchards and some other fish. Once present, the levels of histamine cannot be reduced by cooking or freezing. The symptoms of scombroid poisoning can be immediate to about 30 minutes and may include tingling or a burning sensation in the mouth, development of a rash, itching, headache, drop in blood pressure, nausea and vomiting.

1.6.3 Moulds

Moulds are filamentous fungi that rapidly grow in a mass that may cover several centimetres in a day (see Fig. 1.11). Moulds multiply by means of ascospores, zygospores or conidia. The ascospores of some moulds are particularly significant in food spoilage as they are heat resistant. Some moulds found associated with food spoilage are [12, 16]:

- *Botrytis* moulds, which cause grey mould rot: this condition affects many fruit and vegetable crops and products (e.g. grapes, strawberries, tomatoes, cauliflowers, pumpkins, cucumbers, sweet potatoes and many more). The fungus grows, causing the decay of the fruit or vegetable, and appears as a prominent grey mould.

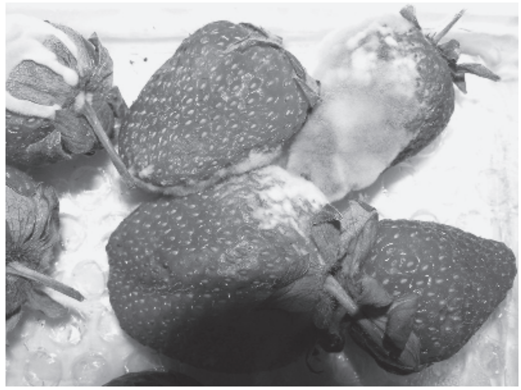

Fig. 1.11 Spoilage of strawberries owing to presence of mould. (*See insert for colour representation of the figure.*)

- *Rhizopus stolonifer* moulds, which produce pectinases that causes soft rot, which makes vegetables soft and mushy.
- *Aspergillus* moulds, which have been implicated in the spoilage of a large number of foods including bacon, bread, peanuts, fish, and so on. They can produce mycotoxins.
- *Byssochlamys* moulds, which can cause spoilage in canned fruit as a result of their heat-resistant ascospores and the pectinases that they produce. They can produce mycotoxins.
- *Fusarium* moulds, which have extensive mycelium that have tinges of brown, red, purple and pink. They cause brown rot in citrus fruit and on pineapples and can grow on cereal crops. They can produce mycotoxins.
- *Penicillium* moulds, which typically spoil fruits causing blue and blue-green coloration. They can produce many different mycotoxins.

1.6.3.1 Mycotoxins

Mycotoxins are secondary metabolites produced by moulds. The name mycotoxin comes from the Greek words mykes (mould) and toxicum (poison). There are many known types of mycotoxins, and they can contaminate a wide variety of food and animal feeds. Examples of common mycotoxins include Aflatoxin, Ocratoxin A, Patulin, Fusarin, Fumonisin, deoxynivalenol (DON) and Zearalenone. Mycotoxins are not essential to maintaining the life of the mould in a primary way (i.e. obtaining energy or synthesizing structural components). They are chemical compounds that give the mould a competitive advantage over other mould or bacteria species in their environment. They are almost all cytotoxic, disrupting various cellular structures like membranes and interfering with vital cellular processes like RNA and DNA synthesis.

The consumption of food contaminated with mycotoxin is related to several acute and chronic diseases in humans and animals, examples of which are listed below.

- In 1960, 100,000 turkey poults died after eating peanut meal that was contaminated with Aflatoxins. Aflatoxins are a group of toxins produced by *Apergillus* spp. that have a similar structure and form highly oxygenated heterocyclic compounds. There are four major Aflatoxins: B1, B2, G1 and G2. Aflatoxins have a potent carcinogenic effect. Aflatoxins occur in crops (most commonly corn, peanuts and cotton-seed) post-harvesting if the moisture level in the foodstuff is allowed to exceed the critical values for mould growth to occur. They can also be found in milk, cheese and eggs if the animals are fed contaminated feed.
- Patulin is produced by certain species of *Penicillium*, *Aspergillus* and *Byssochylamys* moulds. The moulds that produce Patulin grow on a variety of foods, including fruit (e.g. apples, grapes and pears), vegetables, grains (e.g. flour and malt) and cheese. However, owing to the nature of the food (e.g. pH, protein amino acid composition, etc.), the manufacturing processes, or consumption practices for many foods, Patulin does not appear to pose a public safety concern, except in the case of apple juice. Patulin is relatively stable in acid solutions, but is susceptible to alkaline hydrolysis. It is destroyed by fermentation, which means that it is not found in either alcoholic fruit beverages or vinegar produced by fruit juices, but it will survive pasteurization.
- Ocratoxin A is a mycotoxin produced by *Apergillus ochraceus* and a few other moulds for example *Penicillium viridicatum* and *Penicillium verrucosum*. It has both antibiotic and toxic (carcinogenic, teratogenic and nephrotoxic) properties. The moulds that produce Ocratoxin A can be found on wheat, sorghum, raisins and coffee amongst other foodstuffs.

1.6.4 Yeasts

Yeasts may be generally viewed as unicellular fungi and are much larger than bacteria. They are widely distributed in nature, and are present in orchards and vineyards, in the air and in the soil, as well as in the intestinal tracts of animals. There are many very beneficial fermentation reactions initiated by yeasts, but some can cause spoilage. Some examples of yeasts that can cause food spoilage include [9, 11, 12]:

- *Rhodotorula* is a diverse organism that can grow in high sugar concentrations and is implicated in the spoilage of jams, jellies and candies. It can grow well at refrigerator temperatures as well as on the surface of butter.
- *Zygosaccharomyces* can grow in high sugar concentrations and can cause spoilage of jams and jellies. *Z. rouxii* can grow at a pH as low as 1.8 and water activity as low as 0.62.

- *Brettanomyces* produces acetic acid from glucose under aerobic conditions and can cause spoilage in beer, wine and other fermented products.
- *Debaryomyces* can grow in 25% NaCl and at water activities as low as 0.65. It has been found to cause a slimy growth on salted meats, cheeses and in brines. It is also one of the causes of spoilage of yogurt.
- *Candida* can cause spoilage of refrigerated meat.
- *Saccharomyces bailii* is a spoilage yeast causing spoilage in mayonnaise, tomato sauce, fruit drinks and wine. It is resistant to benzoate and sorbate preservatives.

REFERENCES

1. Allsopp, D. Seal, K.J. and Gaylargde, C.C. *Introduction to Biodeterioration*, 2nd edn. Cambridge University Press, Cambridge, NY. 2003.
2. Kumar, R. and Kumar, A.V. *Biodeterioration of Stone in Tropical Environments – An Overview Research in Conservation Series*. The Getty Conservation Institute. Los Angeles 1999.
3. Cullimore, R. and Johnston, L. Biodeterioration of the RMS Titanic. www.encyclopedia-titanica.org (accessed 31 July 2015). 2001.
4. Morton, G. Things that go rot in the night – a review of biodeterioration. *Microbiology Today*. 2003, 30 (August), 103–106.
5. Frazier, W.C. *Food Microbiology*. McGraw-Hill Book Company, Inc., New York. 1958.
6. Holland, B. *et al*. *McCance and Widdowson's: The Composition of Foods*, 5th edn. Royal Society of Chemistry MAFF. Cambridge, UK 1991.
7. Battcock, M. and Azam-Ali, S. *Fermented Fruits and Vegetables. A Global Perspective*. FAO Agricultural Services Bulletin No. 134. FAO, Rome. 1998.
8. Hutton, T. *Food Manufacture: An Overview. Key Topics in Food Science and Technology, No. 3*. Campden BRI.Chipping Campden 2001.
9. Kay, J.M. *Modern Food Microbiology*. 4th edn. Chapman and Hall. New York 1992.
10. Potter, N.N. and Hotchkiss, J.H. *Food Science*, 5th edn. Chapman and Hall, New York. 1995.
11. Prescott, L.M. Harley, J.P. and Klein, D.A. *Microbiology* 3rd edn. Wm. C. Brown Publishers, Dubuque. 1996.
12. Reed, G. (ed.). *Prescott & Dunn's Industrial Microbiology* 4th edn. AVI Publishing Company, Inc., Westport, CT. 1982.
13. Bohinski, R.C. *Modern Concepts in Biochemistry*, 4th edn. Allyn and Bacon, Inc., Cambridge, MA. 1983.
14. Sykes, P. *A Guidebook to Mechanism in Organic Chemistry*, 5th edn. Longman Inc., New York. 1981.
15. FDA Bad Bug Book. Center for Food Safety and Applied Nutrition, of the Food and Drug Administration (FDA), U.S. Department of Health and Human Services. www.fda.gov (accessed 31 July 2015).
16. Albert, J.L. *Food, Nutrition and Agriculture No. 1 – Food for the Future*. FAO, Rome. 1991.