quality of the food up to the time of consumption must be optimised. Some foods are processed in order to achieve commercial sterility of the product and its packaging, such that pathogenic bacteria are effectively eliminated. The definition of commercial sterility arose from the canning industry, where the primary aim is to eliminate pathogens and reduce the number of spoilage organisms capable of growth to an acceptably small number under intended storage conditions.

2.2 Agents of food biodeterioration

2.2.1 Enzymes

Enzymes are complex globular proteins found in living organisms and act as catalysts for speeding up the rate of biochemical reactions. Enzymes are naturally present in foods and can therefore potentially catalyse reactions which could lead to food biodeterioration. The action of enzymes can be used to beneficial effect by the food industry to produce food products and is, for example, commonly used in the manufacture of hard cheese. However, it is usually necessary to inactivate enzymes (i.e. denature the protein) present in food and on packaging surfaces using heat or chemical means in order to preserve and extend the shelf life of foods.

Fruit and vegetables being major sources of enzymes provide many examples of the nature and action of these agents of food spoilage. Enzymes associated with the deterioration of fruits and vegetables include peroxidase, lipoxygenase, chlorophyllase and catalase. Peroxidase is relatively heat-resistant and comprises a mixture of several enzymes of varying heat resistance. Some of these can be inactivated by mild heat treatments, whereas others require several minutes at sterilisation temperatures to effect a complete peroxidase inactivation. During the ripening of fruit, the activity of some enzymes (e.g. pectinesterase and polygalacturanase) increases and consequently causes a softening of the tissue. In potatoes, enzyme inhibitors play an important role in balancing the rate of biochemical reactions in relation to sugar accumulation. This has commercial importance for the storage and conditioning of potatoes prior to processing, where the presence of reducing sugars is undesirable because they can lead to enhanced browning reactions that cause discoloration. The active enzyme is invertase, which produces reducing sugars from sucrose, especially at low temperatures. At higher temperatures the inhibitor is active and reducing sugars are prevented from forming.

Another problem with fruits and vegetables is enzymic browning that results from damage or cutting of the surface and exposure to the air. This is due to the action of polyphenoloxidase, which in the presence of air oxidises phenolic constituents to indole quinone polymers. These reactions are particularly undesirable and several methods are used to prevent this type of browning.
These include the use of citric acid, malic acid or phosphoric acid to inactivate the enzyme, or alternatively preventing oxygen from coming into contact with the food by immersion in brine. Packing the fruit in an atmosphere that excludes air will reduce the extent of the browning reactions but can lead to quality problems and does not provide a viable solution.

Enzymes are also produced during microbial spoilage of foods and are often involved in the breakdown of texture. Many of the microorganisms that secrete enzymes are moulds; however, there are bacterial species (e.g. *Bacillus subtilis*, *Bacillus amyloliquefaciens* and *Bacillus licheniformis*) that produce heat-stable amylases. Amylase enzymes degrade starches, particularly naturally occurring starches, with the effect that the viscosity of the food is reduced as the macromolecular starch granules are broken down into their constituent sugars. Moulds are of particular concern for packaged foods because it is common for many species to produce spores as part of their reproductive cycle. Spores are easily carried in the air and can contaminate inside surfaces of exposed packaging. Other complex reactions can occur, for example *Rhizopus* species causes softening of canned fruits by producing heat-stable pectolytic enzymes that attack the pectins in the fruit. *Mucor piriformis* and *Rhizopus* species also cause the breakdown of texture in sulphite-treated strawberries as a result of similar production of enzymes. *Byssochlamys* species have been considered responsible for the breakdown in texture of canned foods, particularly strawberries, in which it is commonly found. This is a heat-resistant mould that requires temperatures in excess of 90°C for several minutes to adequately destroy it.

Failure to inactivate enzymes completely often shortens the storage life of packaged foods. This is rarely an issue with canned foods but is a factor to consider with frozen fruits and vegetables that receive only a blanching process prior to freezing. Blanching is intended to inactivate the majority of the enzymes, without imposing excessive thermal damage to the food, and hence it uses relatively mild temperatures (90–100°C) and short heating times (1–10min). The renewed activity that often seems to be present in the thawed food after frozen storage is attributed to enzyme regeneration.

### 2.2.2 Microorganisms

The term microorganism includes all small living organisms that are not visible to the naked eye. They are found everywhere in the atmosphere, water, soil, plants and animals. Microorganisms can play a very important role in breaking down organic material. This action of degrading organic material is what food preservation techniques aim to counteract.

Temperature regulation is the most commonly used method to kill or control the number of microorganisms present within foods and on packaging material surfaces. Five categories of temperature-sensitive microorganisms are used to define the preferred temperature range for their growth.
• *Psychrotrophic* (cold tolerant), which can reproduce in chilled storage conditions, sometimes as low as 4°C. Having evolved to survive in extremes of cold, these are the easiest to destroy by heat.
• *Psychrophilic* (cold loving), which have an optimum growth temperature of 20°C.
• *Mesophilic* (medium range), which have an optimum growth temperature between 20 and 44°C. These are of greatest concern with packaged foods.
• *Thermophilic* (heat loving), which have an optimum growth temperature between 45 and 60°C. In general, these organisms are only of concern if packaged foods are produced or stored in temperate climates.
• *Thermoduric* (heat enduring), which can survive above 70°C, but cannot reproduce at these temperatures.

### 2.2.2.1 Bacteria

Bacteria are single-celled microorganisms that normally multiply by binary fission, that is they divide into two cells following a period of growth. If conditions are favourable for reproduction, one bacterium can divide into two by fission, so that after 11 hrs there can be more than 10 million cells (\(>1 \times 10^{12}\)). This is a level where organoleptic spoilage of the food is apparent due to the production of off-flavours, unpleasant odours and slime or it can result in toxin release. There are four stages in bacterial growth (Fig. 2.1):

- **lag phase**, during which the bacteria are acclimatising to their environment, which can be several hours long;
- **log phase**, during which reproduction occurs logarithmically for the first few hours. Conditions for growth are ideal during this period;
- **stationary phase**, during which the bacteria’s reproduction rate is cancelled by the death rate;
- **mortality or decline phase**, during which exhausted nutrient levels or the levels of toxic metabolites in the environment prevent reproduction, with the result that the bacteria gradually die off.

The simplest method of identifying bacteria is according to their appearance, which approximates to spherical (cocci), rod (bacilli) and spiral shapes. Cocci occur in different formulations, for example diplococci in pairs, staphylococci in clusters and streptococci in chains. Bacilli, mostly form chains. Cocci can vary in size between 0.4 and 1.5 μm, whereas bacilli can vary between 2 and 10 μm in length. Some cocci and many bacilli can move within their liquid environment by using flagella, which are similar in appearance to hairs. The most widely used method of identifying bacteria was introduced by the Danish bacteriologist Gram and is called Gram dyeing. Bacteria are divided into two main groups according to their Gram stain characteristics: red, Gram negative and blue, Gram positive.
Bacteria require water, proteins, carbohydrates and lipids for growth. In addition, small quantities of vitamins and trace elements are needed to support and catalyse metabolism. Nutrients must be available in soluble form to aid transport through the cytoplasmic cell membrane. Large and complex organic molecules cannot pass through the membrane without first being broken down into smaller molecules. This is achieved by the bacteria releasing enzymes into the surrounding food that catalyse the break down of complex molecules into a form which can be used by the organism. An example of this is the release of amylase by various *Bacillus* species such as *subtilis*, *amyloliquefaciens* and *licheniformis*, which breaks down complex carbohydrates into simple sugars that can be absorbed through the cell membrane.

Water is essential for bacterial growth because it facilitates the transport of small molecules through the outer cytoplasmic membrane of the bacterial cell via osmotic pressure gradients. Bacteria require higher levels of available water than yeasts or moulds. At 20% available water, their growth is good, but it is limited when reduced to 10%, and at 5% there is no bacterial growth. The available water or water activity ($a_w$) is the amount of free water in a food and excludes moisture that is bound and unavailable for the microorganisms to use. Table 2.1 shows typical $a_w$ values for a range of foods. Most bacteria cannot grow below an $a_w$ of 0.91–0.94. The equilibrium relative humidity (ERH), defined as the ratio of the vapour pressure of food over that of water. ERH is calculated by $a_w \times 100$ and is expressed as a percentage. Therefore a food with an $a_w$ of 0.90 has an ERH of 90%. Section 2.3.3 discusses methods of drying and control of water activity.

All bacteria need a supply of oxygen to oxidise their food in order to produce energy and for growth. Some bacteria obtain their oxygen directly from the air (aerobic bacteria), whereas others obtain oxygen from their food (anaerobic

![Figure 2.1 Typical growth curve for a bacterium (Phases: a – lag, b – log, c – stationary, d – mortality).](image-url)
bacteria). The latter are usually killed when exposed to oxygen from the air. Some bacteria can be facultatively anaerobic, which means that they can consume oxygen from the air if present, but can also grow in its absence. Adjusting the atmosphere above a packaged food is used as a means to prevent bacterial growth, typically in combination with chilled storage as a further hurdle to growth. This allows foods to be manufactured with minimal or no heat processing, yet delivers an extended shelf life.

Light is not an essential requirement for bacterial growth because the cells do not synthesise food using light energy. Instead, light has a destructive effect on bacteria because of the ultraviolet (UV) component that causes chemical changes in the cell proteins. Bacteria prefer to grow in conditions where light is excluded. This effect is utilised by using UV light to sterilise bottled water where the limitation of transparency is not a restriction.

If the environmental conditions become adverse to the growth of certain bacterial species they have the ability to form a protective spore. Examples of conditions that can initiate spore formation are extremes of temperature, presence of adverse chemical environments (e.g. disinfectants) and low quantities of available moisture and low concentrations of nutrients. During spore formation, the vegetative part of the cell dies and will only form again if the environmental conditions become favourable. Spores do not metabolise and hence can lie dormant for years, in conditions that could not support the growth of the bacteria. This presents a challenge to the food processor, in that it is often necessary to kill both bacterial cells and spores that collect on the surface of exposed packaging. Destruction of spores requires more severe conditions of heat or disinfectant than that required to destroy vegetative bacterial cells. In some situations, the bacteria can develop resistance against the adverse conditions as a result of their rapid evolution time.

_Clostridium botulinum_ is a spore former that is of concern to the heat processed foods sector because of the resistant nature of the resulting spore and the deadly toxin produced by the bacteria. _C. botulinum_ spores will germinate if the pH is above 4.5 and there is an absence of oxygen (i.e. anaerobic condition). Since it is the most heat-resistant and lethal pathogen all sterilisation processes used to kill it will also destroy the other less heat-resistant pathogens.

Several bacteria need consideration when designing a packaging and processing line. Of primary concern from a public health perspective are those that produce toxins such as _C. botulinum, Listeria monocytogenes, Salmonella, Escherichia coli, Staphylococcus aureus, Bacillus cereus_ and _Campylobacter_. These can be controlled by the use of sterilising solutions and/or heat, with the aim to achieve the condition of commercial sterility for the packaged food. The most lethal of these is _C. botulinum_, which produces a toxin that attacks the nervous system. The industry is fully aware of the risks associated with this organism and has taken measures to prevent it from being present in food.
C. botulinum spores only germinate in anaerobic conditions where there is available moisture, nutrient and where the pH is over 4.5. Products with pH over 4.5 are often referred to as low acid foods, whereas products with pH of 4.5 and below are referred to as high acid foods. This critical pH limit is an important determinant as to whether heat-preserved foods receive a pasteurisation or sterilisation treatment. Sterilisation processes (typically in the range 115.5–135°C) using heat have greater cooking effects on product quality than the relatively mild heat treatment of pasteurisation processes (typically 75–105°C). It is important to ensure that spoilage organisms in a high-acid food do not cause a shift in pH to the low acid level, thereby allowing the potential development of C. botulinum. Pasteurisation often requires foods to be acidified prior to thermal treatment (e.g. pickled vegetables). Refer to the work of Adam and Dickinson (1959) for pH values of various products.

Growth of most strains of C. botulinum is inhibited at refrigeration temperatures; however, there are psychrotrophic strains that can grow at low temperatures and are increasingly giving rise for concern in foods. This is of concern with sous-vide and vacuum packed foods that only receive a mild heat treatment and rely heavily on precise control of chill temperatures to prevent out-growth. Listeria is another bacterium that can survive and grow at low temperatures, but fortunately it is killed by mild temperatures. The process used to achieve a 6-log reduction in Listeria is 70°C for 2 min, a process also applicable to Salmonella and E. coli (the group of bacteria referred to as aerobic pathogens).

Not all bacteria are pathogenic or the cause of food spoilage. Bacteria have been used to beneficial effect in food fermentation and preservation processes to extend the shelf life of certain foods. One example that has been exploited for many years is the deliberate introduction of lactic acid bacteria for the fermentation of milk to produce yoghurts. Lactic acid bacteria can be either bacilli or cocci and are facultatively anaerobic. Their energy source during growth is the milk sugar, lactose, and during fermentation this is converted to lactic acid. The result is that the level of acidity increases until a predetermined pH value is reached whereby the yoghurt is ready to be packaged. Lactic acid bacteria do not form spores, and the cells can be killed by heating to 70°C once the fermentation is complete. However, many types of yoghurt are marketed as having additional health attributes because they contain live lactobacilli that may have beneficial actions within the human digestive system. Section 2.3.5 provides further detail on fermentation methods of preservation.

2.2.2.2 Fungi

Fungi are a group of microorganisms that are found in nature on plants, animals and human beings. Different species of fungi vary a great deal in their structure and method of reproduction. Fungi may be single-celled, round or oval organisms or threadlike multi-celled structures. The threads may form a network, visible
to the naked eye, in the form of mould as seen, for example, on foods such as bread and cheese. Fungi are sub-divided into yeasts and moulds.

Yeast are single-cell organisms of spherical, elliptical or cylindrical shape. The size of yeast cells varies considerably, for example, Brewer’s yeast, *Saccharomyces cerevisiae*, has a diameter of the order of 2–8 μm and a length of 3–15 μm. Some yeast cells of other species may be as large as 100 μm. The yeast cells normally reproduce by budding, which is an asexual process, although other methods of reproduction can also occur. During budding, a small bud develops on the cell wall of the parent cell, with the cytoplasm shared by parent and offspring before the bud is sealed off from the parent cell by a double wall. The new cell does not always separate from its parent but may remain attached, while the latter continues to form new buds. The new cell can also form fresh buds of its own. This can result in large clusters of cells being attached to each other. Some yeasts can form spores, although these are part of the reproductive cycle and are quite different to bacterial spores where the spore is formed as means to survive adverse conditions. Hence yeast spores are relatively easy to kill within foods by mild heat or on packaging surfaces by mild heat or sterilising solutions.

Moulds belong to a large category of multi-celled threadlike fungi. Moulds attach themselves to their food, or substrate, using long threads called hyphae. These are the vegetative part of the mould and grow straight up from the substrate. Like yeasts, moulds can also multiply by sexual or asexual reproduction. Reproduction is normally by asexual methods and results in the production of a large number of spores. Sexual reproduction tends to be in response to changing environmental conditions, although this is not necessarily the case. Spores produced by sexual reproduction are more resistant to adverse conditions and, like bacterial spores, can lie dormant for some time. One of the key sources of contamination of exposed packaging materials is from mould spores. This is because the spores are very small and light, are produced in huge numbers and are designed to be carried by air to new environments. Moulds introduced to the package as mould spores are the usual source of post-process contamination of foods. For example, a gooseberry jam hot-filled into glass jars and capped without heat pre-treatment of closures resulted in fungal growth on the headspace surface of the jam. Steam treatment of closures, jar inversion immediately after sealing or a pasteurisation process would have prevented spoilage.

One of the most important medicines for treating bacterial infections, penicillin, is derived from the *Penicillium* mould. The appearance of the spore-forming hyphae is that of a miniature green bush and is responsible for much of the green mould found in nature. The *Penicillium* family of moulds produces powerful lipases and proteases (fat- and protein-degrading enzymes) that make them key agents in the ripening of blue cheeses (*Penicillium roqueforti*) and Camembert (*Penicillium camemberti*). Another fungus, *Oospora lactis*, which displays characteristics of both yeasts and moulds, occurs on the surfaces of
cultured milk as a white velvety coating. This organism is used for ripening soft cheeses (Tetra Laval, 1977).

Conditions for the growth of yeasts and moulds are similar to those for bacteria. They can survive at lower available water levels, which is why bread is at risk of mould spoilage but not of spoilage by bacteria which are unable to grow. Fungi also have a greater resistance to osmotic pressure than bacteria and can grow in many commercial jams and marmalades. Fungi present on packaging surfaces and in food will be killed by the heat process applied to the packaged food, typically of the order 85°C for 5 min (CCFRA, 1992), but once the jar is opened, airborne contamination from mould spores can occur. The moulds that grow in high sugar conditions do not form toxins, and so the furry growth colonies are unsightly but do not represent a public health risk.

Both yeasts and moulds are more tolerant to high acidity levels, with yeasts being able to grow between pH 3.0 and 7.5, and moulds between pH 2.0 and 8.5. Optimum pH for the growth of fungi tends to be in the pH range 4.5–5.0. Few bacteria can survive these low pH conditions, although spores of Alicyclobacillus strains have been reported to exhibit extreme acid and heat tolerance. These bacteria are referred to as acido-thermophiles and have caused spoilage problems in fruit juices where the pH can be as low as 3.0.

Generally, fungi are less tolerant to high temperatures than bacteria, an exception being ascospores from moulds such as Byssochlamys fulva. To produce a commercially sterile food in which these ascospores are the target organism would require extended heating at temperature above 90°C. Strawberries are a common source of B. fulva. Typically, yeast or mould cells are killed after only 5–10 min heating at 60°C. Temperature for optimum growth of fungi are normally between 20 and 30°C, which is the main cause for increases in spoilage outbreaks in food production during summertime.

Yeast cells are facultatively anaerobic and moulds almost exclusively aerobic. In the absence of oxygen, yeast cells break down sugar to alcohol and water, while in the presence of oxygen, sugar is broken down to carbon dioxide and water. The former reaction is used in the fermentation of alcoholic drinks; however, within the fermenting liquid, conditions lie between anaerobic and aerobic and hence alcohol and carbon dioxide are produced.

2.2.3 Non-enzymic biodeterioration

One further category of biodeterioration worthy of mentioning is that of non-enzymic browning. An important reaction in foods takes place between the sugar constituents and amine-type compounds, which results in progressive browning and the development of off-flavours. An example of foods in which this type of quality deterioration takes place is dehydrated foods, especially...
dried potato and vegetables, fruit juices, both dried and concentrated, and wine. These complex chemical reactions are known as Maillard reactions, named after the French chemist who first investigated the interaction of sugars and amines in 1912. Essentially, the aldehyde groupings of the reducing sugars react with the free amino groups of amino acids to form furfuraldehyde, pyruvaldehyde, acetal, diacetyl, hydroxydiacetyl and other sugar-degradation compounds that in turn react with amines to produce melanoide-type macromolecules (brown pigments). Despite intensive research into this subject, the only successful way of inhibiting these reactions is by using sulphurous acid and sulphites. The levels of sulphur dioxide allowed in food products are strictly controlled by legislation and also by the amount that can be tolerated before the taste becomes unacceptable. In the case of dried products, these are sulphited immediately after or during blanching. The use of sulphites for this purpose does not involve the antimicrobial protection for which these compounds are used in other applications. It is important to note that sulphite treatment of any fruit and vegetables intended for canning needs to be very tightly monitored in order to avoid the risk of severe accelerated internal de-tinning.