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The Science Underpinning Food Fermentations

Use the word 'biotechnology' nowadays and the vast majority of people will register an image of genetic alteration of organisms in the pursuit of new applications and products, many of them pharmaceutically relevant. Even the *Merriam-Webster Dictionary* tells us that biotechnology is 'biological science when applied especially in genetic engineering and recombinant DNA technology'. Fortunately the *Oxford English Dictionary* gives the rather more accurate definition as 'the branch of technology concerned with modern forms of industrial production utilising living organisms, especially microorganisms, and their biological processes'.

Accepting the truth of the second of these, then we can realise that biotechnology is far from being a modern concept. It harks back historically vastly longer than the traditional milepost for biotechnology, namely Watson and Crick's announcement in the Eagle pub in Cambridge (and later, more formally, in *Nature*) that they had found 'the secret of life'.

Eight thousand years ago our ancient forebears may have been, in their own way, no less convinced that they had hit upon the essence of existence when they made the first beers and breads. The first micro-organism was not seen until draper Anton van Leeuwenhoek peered through his microscope in 1676 and neither were such agents firmly causally implicated in food production and spoilage until the pioneering work of Needham, Spallanzani and Pasteur and Bassi de Lodi in the eighteenth and nineteenth centuries.

Without knowing the whys and wherefores, the dwellers in the Fertile Crescent were the first to make use of living organisms in fermentation processes. They truly were the first biotechnologists. And so beer, bread, cheese, wine and most of the other foodstuffs being considered in this book come from the oldest of processes. In some cases these have not changed very much in the ensuing aeons.

Unlike the output from modern biotechnologies, for the most part we are considering high volume, low value commodities. However for products such as beer, there is now a tremendous scientific understanding of the science that underpins the product, science that is none the less tempered with the pressures of tradition, art and emotion. For all of these food fermentation products, the customer *expects*. As has been realised by those who would apply molecular biological transformations to the organisms involved in the manufacture of foodstuffs, there is vastly more resistance to this than for applications in, say, the pharmaceutical area. You don't mess with a person's meal!

Historically, of course, the micro-organisms employed in these fermentation processes were adventitious. Even then, however, it was realised that the addition of a part

Food, Fermentation, and Micro-organisms, Second Edition. Charles W. Bamforth and David J. Cook.

of the previous process stream to the new batch could serve to 'kick off' the process. In some businesses this was called 'back slopping'. We now know that what the ancients were doing was seeding the process with a hefty dose of the preferred organism(s). Only relatively recently have the relevant microbes been added in a purified and enriched form to knowingly trigger fermentation processes.

The two key components of a fermentation system are the organism and its feedstock. For some products, such as beer, there is a radical modification of the properties of the feedstock, rendering them more palatable (in the case of beer, the grain extracts pre-fermentation are most unpleasant in flavour; by contrast, grape juice is much more acceptable). For other products the organism is less central, albeit still important. One thinks for instance of bread, where not all styles involve yeast in their production.

For some products, such as cheese, the end product is quite distinct from the raw materials as a result of a series of unit operations. For products such as beer, wine and vinegar the product is actually the spent growth medium – the excreta of living organisms if one was to put it crudely. Only occasionally is the product the actual micro-organism itself – for example the surplus yeast generated in a brewery fermentation or that generated in a microbial biomass ('single cell protein') operation such as the production of mycoprotein.

The organisms employed in food fermentations are many and diverse. Key players are the lactic acid bacteria, in dairy products for instance, and yeast, in the production of alcoholic beverages and bread. The lactic acid bacteria, to illustrate, may also have a positive role to play in the production of certain types of wines and beers, but equally they represent major spoilage organisms for many such products. It truly is a case of the organism being in the right niche for the product in question.

In this chapter we will focus on the generalities of science and technology that underpin fermentations and the organisms that are involved. We will look at commonalities in terms of quality – for example the Maillard reaction that is of widespread significance as a source of colour and aroma in many of the foods that we are considering. The reader will discover (and this betrays the primary expertise of the authors) that many of the examples given are from beer making. It must be said, however, that the scientific understanding of the brewing of beer is somewhat more advanced than that for most if not all of the other foodstuffs described in this book. Many of the observations made in a brewing context very much translate to what must occur in the less well-studied foods and beverages.

1.1 Micro-Organisms

Microbes can essentially be divided into two categories: the prokaryotes and the eukaryotes.

The former, which embrace the bacteria, are substantially the simpler, in that essentially they comprise a protective cell wall, surrounding a plasma membrane, within which is a nuclear region immersed in cytoplasm (Figure 1.1). This is a somewhat simplistic description, but sufficient for our needs. The nuclear material (deoxyribonucleic acid, DNA) of course figures as the genetic blueprint of the cell. The cytoplasm contains the enzymes that catalyse the reactions necessary to the growth, survival and reproduction of the organisms (the sum total of reactions of course being referred to as *metabolism*). The membrane regulates the entry and exit of materials into and from the cell.

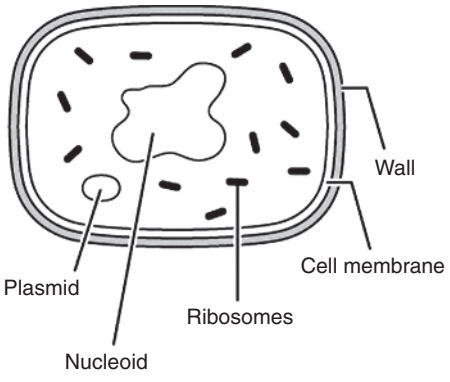


Figure 1.1 A simple representation of a prokaryotic cell. The major differences between Gram positive and Gram negative cells concerns their outer layers, with the latter having an additional membrane outwith the wall in addition to a different composition of the wall itself.

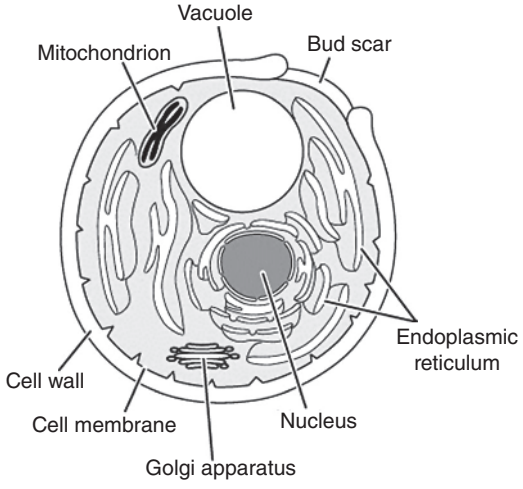


Figure 1.2 A simple representation of a eukaryotic cell.

The eukaryotic cell (of which bakers or brewers yeast, *Saccharomyces cerevisiae*, a unicellular fungus, is the model organism) is substantially more complex (Figure 1.2). It is divided into organelles, the intracellular equivalent of our bodily organs. Each has its function. Thus the DNA is located in the nucleus which, like all the organelles, is bounded by a membrane. All the membranes in eukaryotes (and prokaryotes) comprise lipid and protein. Other major organelles in eukaryotes are the mitochondria, wherein energy is generated, and the endoplasmic reticulum. The latter is an interconnected network of tubules, vesicles and sacs with various functions including protein and sterol synthesis, sequestration of calcium, production of the storage polysaccharide glycogen and insertion of proteins into membranes. Both prokaryotes and eukaryotes have polymeric storage materials located in their cytoplasm.

Table 1.1 lists some of the organisms that are mentioned in this book. Some of the relevant fungi are unicellular, for example Saccharomyces. However the major class of fungi, namely the filamentous fungi with their hyphae (moulds), are of significance for a number of the foodstuffs, notably those Asian products involving solid state fermentations, e.g. sake and miso, as well as the only successful and sustained single cell protein operation (Chapter 17).

Table 1.1 Some micro-organisms involved in food fermentation processes.

Bacteria		Fungi	
Gram negative[a]	**Gram positive[a]**	**Filamentous**	**Yeasts and non-filamentous fungi**
Acetobacter	Arthrobacter	Aspergillus	Brettanomyces
Acinetobacter	Bacillus	Aureobasidium	Candida
Alcaligenes	Bifidobacterium	Fusarium	Cryptococcus
Escherichia	Cellulomonas	Mucor	Debaromyces
Flavobacterium	Corynebacter	Neurospora	Endomycopsis
	Lactobacillus	Penicillium	Geotrichum
Gluconobacter	Lactococcus	Rhizomucor	Hanseniaspora (Kloeckera)
Klebsiella	Leuconostoc	Rhizopus	Hansenula
Methylococcus	Micrococcus	Trichoderma	Kluyveromyces
Methylomonas	Mycoderma		Monascus
Propionibacter	Staphylococcus		Pichia
Pseudomonas	Streptococcus		Rhodotorula
Thermoanaerobium	Streptomyces		Saccharomyces
Xanthomonas			Saccharomycopsis
Zymomonas			Schizosaccharomyces
			Torulopsis
			Trichosporon
			Yarrowia
			Zygosaccharomyces

[a] Danish microbiologist Hans Christian Gram (1853–1928) developed a staining technique used to classify bacteria. A basic dye (crystal violet or gentian violet) is taken up by both Gram-positive and Gram-negative bacteria. However the dye can be washed out of Gram-negative organisms by alcohol, such organisms being counterstained by safranin or fuchsin. The latter stain is taken up by both Gram-positive and Gram-negative organisms, but does not change the colour of Gram-positive organisms, which retain their violet hue.

1.2 Microbial Metabolism

In order to grow, any living organism needs a supply of nutrients that will feature as, or go on to form, the building blocks from which that organism is made. These nutrients must also provide the energy that will be needed by the organism to perform the functions of accumulating and assimilating those nutrients, to facilitate moving around, etc.

The microbial kingdom comprises a huge diversity of organisms that are quite different in their nutritional demands. Some organisms (*phototrophs*) can grow using light as a source of energy and carbon dioxide as a source of the carbon, the latter being the key element in organic systems. Others can get their energy solely from the oxidation of inorganic materials (*lithotrophs*).

All of the organisms considered in this book are *chemotrophs*, insofar as their energy is obtained by the oxidation of chemical species. Furthermore, unlike the *autotrophs*,

which can obtain all (or nearly all) their carbon from carbon dioxide, the organisms that are at the heart of fermentation processes for making foodstuffs are *organotrophs* (or *heterotrophs*) in that they oxidise organic molecules, of which the most common class is the sugars.

1.2.1 Nutritional Needs

The four elements required by organisms in the largest quantity are carbon, hydrogen, oxygen and nitrogen. This is because these are the elemental constituents of the key cellular components of carbohydrates (Figure 1.3), lipids (Figure 1.4), proteins (Figure 1.5) and nucleic acids (Figure 1.6). Phosphorus and sulphur are also important in this regard. Calcium, magnesium, potassium, sodium and iron are demanded at the milligram level, whilst microgram amounts of copper, cobalt, silicon, manganese, molybdenum, selenium and nickel are needed. Finally, organisms need a pre-formed supply of any material that is essential to their well-being, but that they cannot themselves synthesise, namely the vitamins (Table 1.2). Micro-organisms differ greatly in their ability to make these complex molecules. In all instances the vitamins form a part of coenzymes and prosthetic groups that are involved in the functioning of the enzymes catalysing the metabolism of the organism.

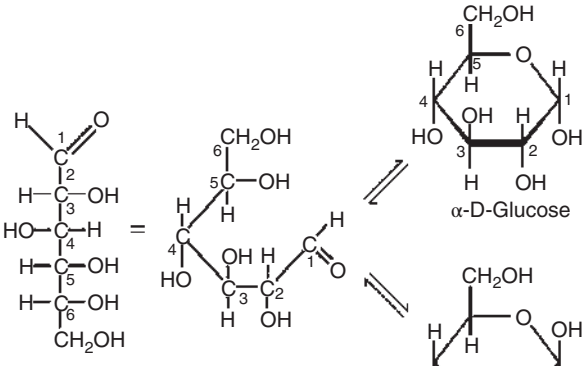
As the skeleton of all the major cellular molecules (other than water) comprises carbon atoms, there is a major demand for carbon.

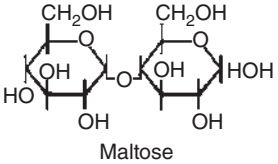
Hydrogen and oxygen originate from substrates such as sugars, but of course also come from water.

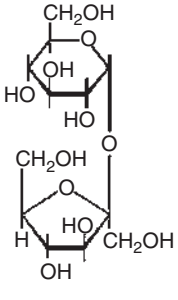
The oxygen molecule, O_2, is essential for organisms growing by aerobic respiration. Although fermentation is a term that has been most widely applied to an anaerobic process in which organisms do not use molecular oxygen in respiration, even those organisms that perform metabolism in this way generally do require a source of this element. To illustrate, a little oxygen is introduced into a brewer's fermentation so that the yeast can use it in reactions that are involved in the synthesis of the unsaturated fatty acids and sterols that are essential for it to have healthy membranes. Aerobic metabolism, too, is necessary for the production of some of the foodstuffs mentioned in this book, for example in the production of vinegar.

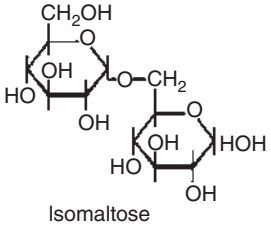
All growth media for micro-organisms must incorporate a source of nitrogen, typically at $1–2\,g\,l^{-1}$. Most cells are about 15% protein by weight, and nitrogen is a fundamental component of protein (and nucleic acids).

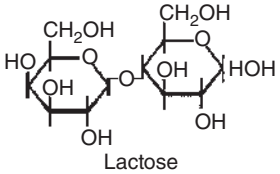
As well as being physically present in the growth medium, it is equally essential that the nutrient should be able to enter into the cell. This transport is frequently the rate-limiting step. Few nutrients enter the cell by passive diffusion and those that do tend to be lipid-soluble. Passive diffusion is not an efficient strategy for a cell to employ as it is very concentration dependent. The rate and extent of transfer depend on the relative concentrations of the substance inside and outside the cell. For this reason, facilitated transportation is the major mechanism for transporting materials (especially the water-soluble ones) into the cell, with proteins known as permeases selectively and specifically catalysing the movement. These permeases are only synthesised as and when the cell requires them. In some instances energy is expended in driving a substance into the cell if a thermodynamic hurdle has to be overcome – e.g. a higher concentration of the molecule inside than outside. This is known as 'active transport'.

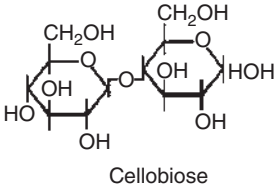












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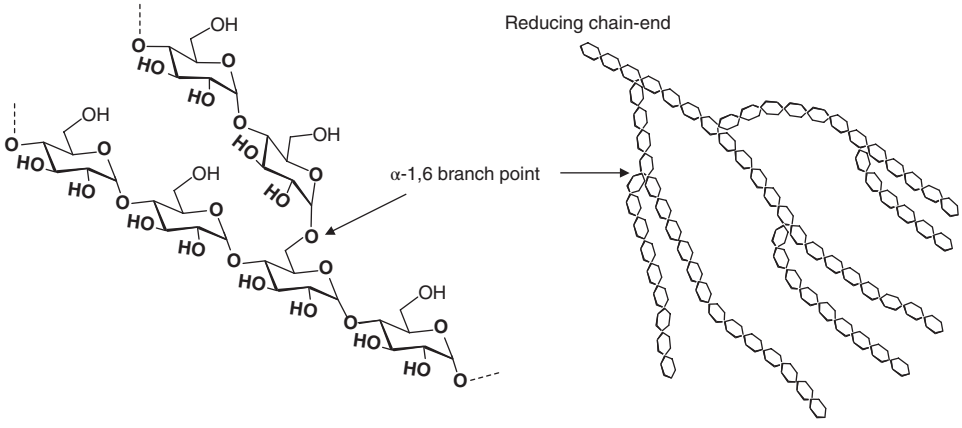


Figure 1.3 (Cont'd) carbohydrates. (a) Hexoses (sugars with six carbons) such as glucose exist in linear and cyclic forms in equilibria (top). the numbering of the carbon atoms is indicated. In the cyclic form if the OH at C_1 is lowermost the configuration is α. If the OH is uppermost then the configuration is β. At C_1 in the linear form is an aldehyde grouping, which is a reducing group. adjacent monomeric sugars (monosaccharides, in this case glucose) can link (condense) by the elimination of water to form disaccharides. Thus maltose comprises two glucose moieties linked between C_1 and C_4, with the OH contributed by the C_1 of the first glucosyl residue being in the α configuration. Thus the bond is $\alpha 1 \rightarrow 4$. For isomaltose the link is $\alpha 1 \rightarrow 6$. For cellobiose the link is $\beta 1 \rightarrow 4$. Sucrose is a disaccharide in which glucose is linked $\beta 1 \rightarrow 4$ to a different hexose sugar, fructose. similarly lactose is a disaccharide in which galactose (note the different conformation at its C_4) is linked $\beta 1 \rightarrow 4$ to glucose. (b) Successive condensation of sugar units yields oligosaccharides. This is a depiction of part of the amylopectin fraction of starch, which includes chains of $\alpha 1 \rightarrow 4$ glucosyl units linked by $\alpha 1 \rightarrow 6$ bonds. The second illustration depicts the amylopectin fraction of starch. Note that there is only one reducing chain-end, all the others being bound up in glycosidic linkages.

An additional challenge is encountered with high molecular weight nutrients. Whereas some organisms, e.g. the protozoa, can assimilate these materials by engulfing them (*phagocytosis*), micro-organisms secrete extracellular enzymes to hydrolyse the macromolecule outside the organism, with the resultant lower molecular weight products then being assimilated. These extracellular enzymes are nowadays produced commercially in fermentation processes that involve subsequent recovery of the spent growth medium containing the enzyme and various degrees of ensuing purification. A list of such enzymes and their current applications is given in Table 1.3.

1.2.2 Environmental Impacts

A range of physical, chemical and physicochemical parameters impact the growth of micro-organisms, of which we shall consider temperature, pH, water activity, oxygen, radiation, pressure and 'static' agents.

1.2.2.1 Temperature

The rate of a chemical reaction was shown by Svante Arrhenius (1859–1927) to increase twofold to threefold for every 10 °C rise in temperature. However cellular macromolecules, especially the enzymes, are prone to denaturation by heat, and this accordingly limits the temperatures that can be tolerated. Although there are organisms that can

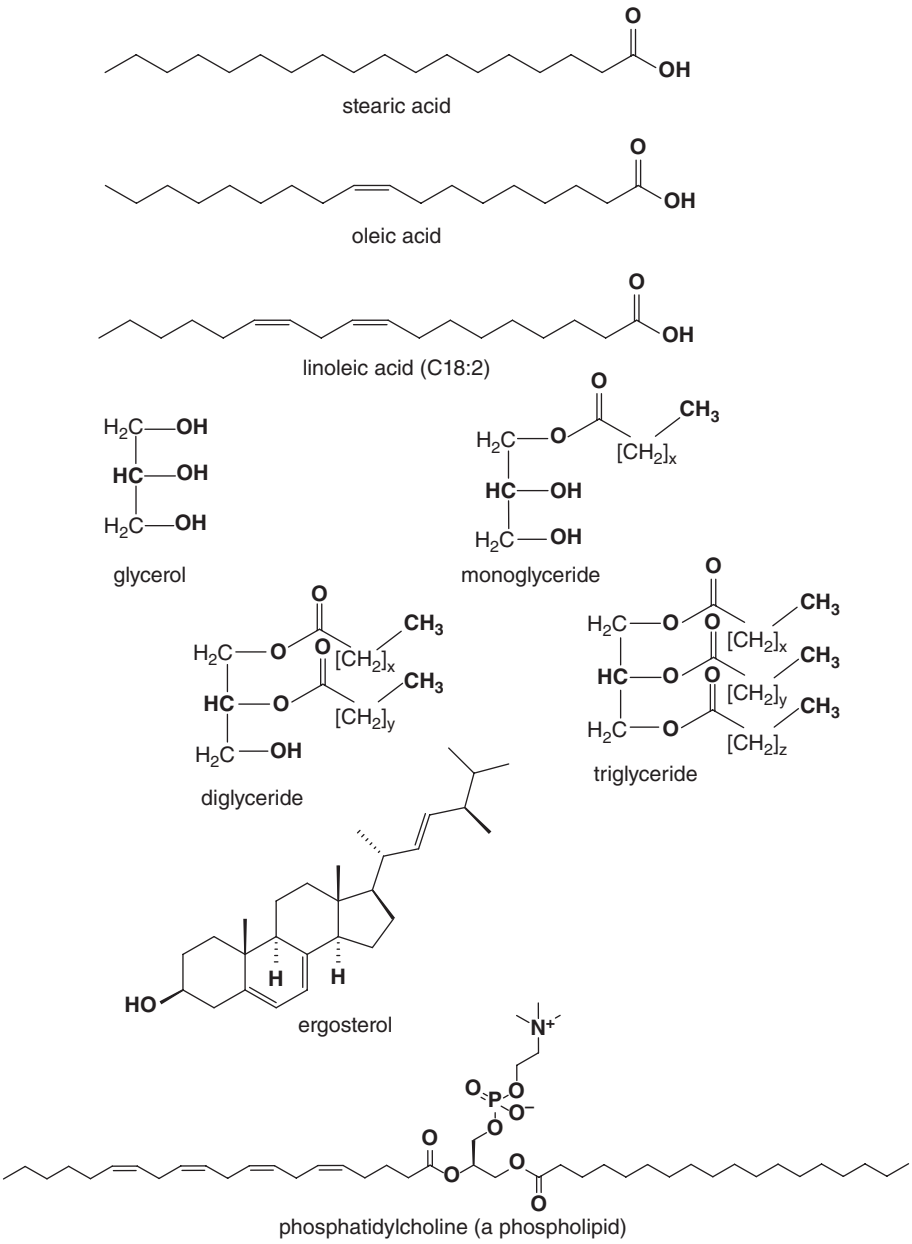
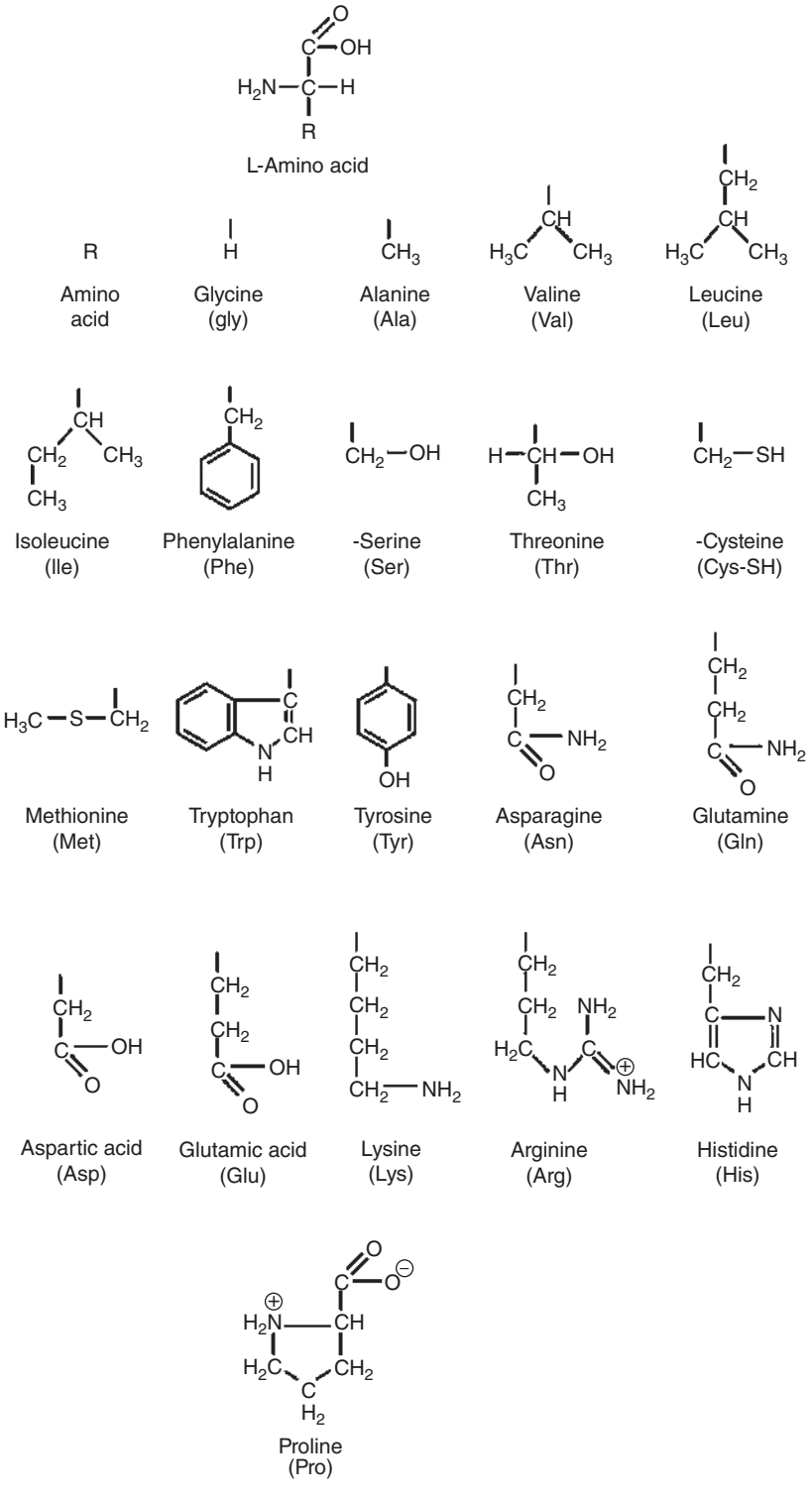


Figure 1.4 Lipids. Fatty acids comprise hydrophobic hydrocarbon chains varying in length, with a single polar carboxyl group at C_1. Three different fatty acids with 18 carbons (hence C_{18}) are shown. They are the 'saturated' fatty acid stearic acid (so-called because all of its carbon atoms are either linked to another carbon or to hydrogen with no double bonds) and the unsaturated fatty acids, oleic acid (one double bond, hence $C_{18:1}$) and linoleic acid (two double bonds, $C_{18:2}$). Fatty acids may be in the free form or attached through ester linkages to glycerol, as glycerides.



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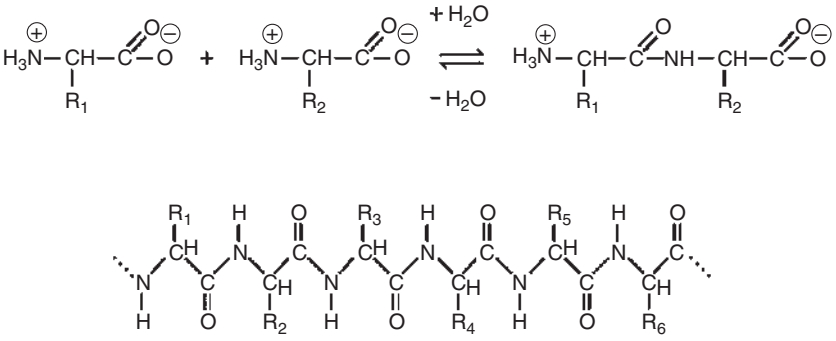


Figure 1.5 (Cont'd) Proteins. (a) the monomeric component of proteins are the amino acids, of which there are 19 major ones and the imino acid proline. The amino acids have a common basic structure and differ in their R-group. The amino groups in the molecules can exist in free ($-NH_2$) and protonated forms ($-NH_3^+$) depending on the pH. Similarly the carboxyl groups can be in the protonated (–COOH) and non-protonated ($-COO^-$) states. (b) adjacent amino acids can link through the 'peptide' bond. Proteins contain many amino acids thus linked. Such long high molecular weight molecules adopt complex 3-dimensional forms through interactions between the amino acid R groups, such structures being important for the properties that different proteins display.

thrive at relatively high temperatures (*thermophiles*), most of the organisms discussed in this book do not fall into that class. Neither do they tend to be *psychrophiles*, which are organisms capable of growth at very low temperatures. They have a minimum temperature at which growth can occur, below which the lipids in the membranes are insufficiently fluid. It should be noted that many organisms can *survive* (if not grow) at lower temperatures and advantage is taken of this in the storage of pure cultures of defined organisms (see later). Organisms that prefer the less-extreme temperature brackets, say 10–40 °C, are referred to as *mesophiles*.

1.2.2.2 pH

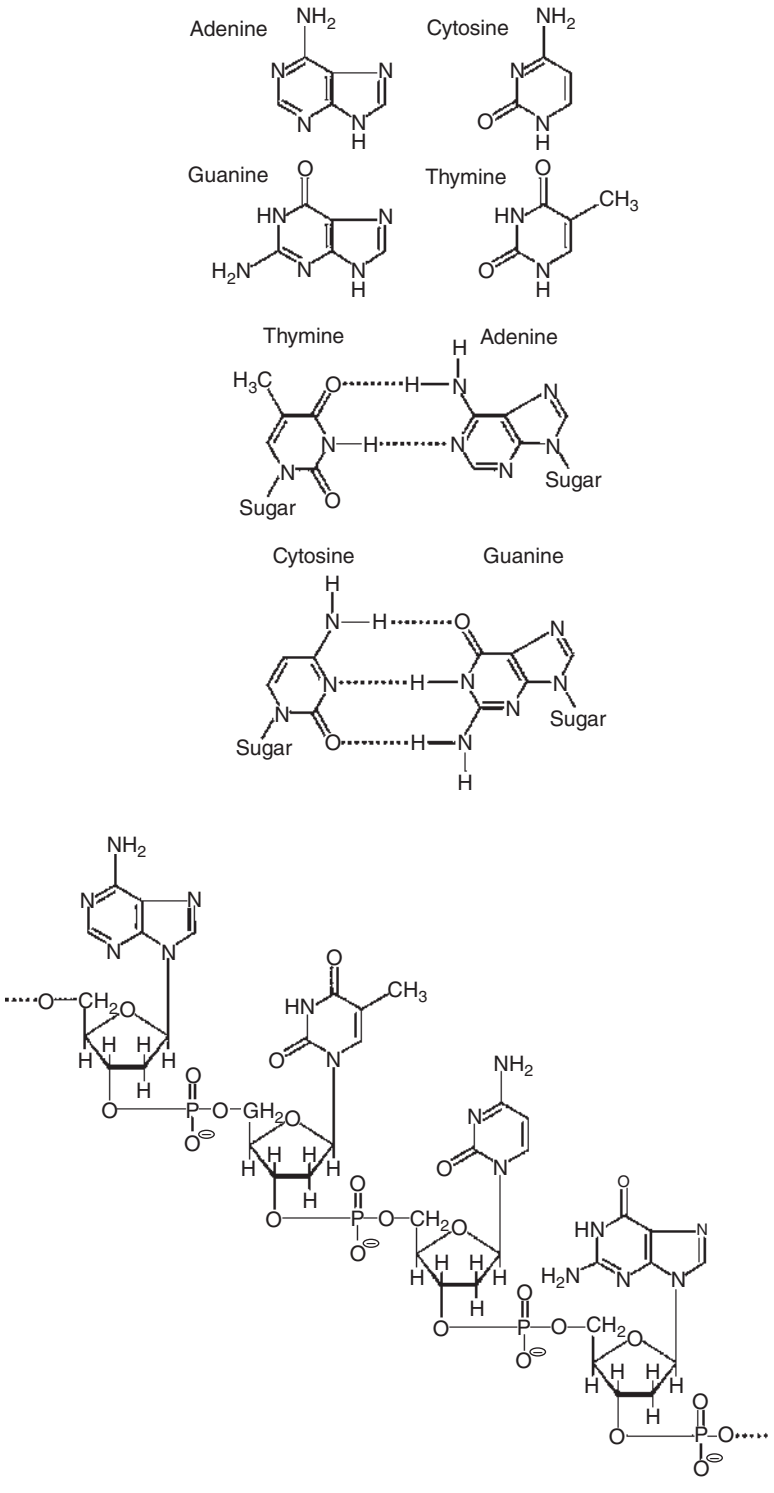
Most organisms have a relatively narrow range of pH within which they grow best. This tends to be lower for the fungi than it is for bacteria. The optimum pH of the medium reflects the best compromise position in respect of:

- the impact on the surface charge of the cells (and the influence that this has on behaviours such as flocculation and adhesion).
- the ability of the cells to maintain a desirable intracellular pH and, in concert with this, the charge status of macromolecules (notably the enzymes) and the impact that this has on their ability to perform.

1.2.2.3 Water Activity

The majority of microbes comprise between 70 and 80% water. Maintaining this level is a challenge when an organism is exposed variously to environments that contain too little water (dehydrating or *hypertonic* locales) or excess water (*hypotonic*).

The water that is available to an organism is quantifiable by the concept of water activity (a_w). The latter is defined as the ratio of the vapour pressure of water in the solution surrounding the micro-organism to the vapour pressure of pure water. Thus pure water itself has an a_w of 1 whilst an absolutely dry, water-free entity would have an



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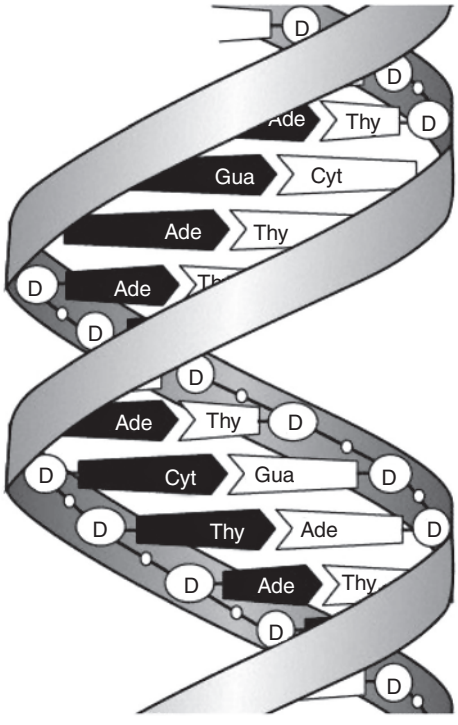

Figure 1.6 (Cont'd) Nucleic acids. (a) Nucleic acids comprise three building blocks: bases, pentose sugars (with five carbon atoms) and phosphate. There are four bases in deoxyribonucleic acid (DNA): The purines adenine (A) and guanine (G) and the pyrimidines thymine (T) and cytosine (C). A and T or G and C can interact through hydrogen bonds (dotted lines) and this binding affords the linking between adjacent chains in DNA. The bases are linked to the sugar-phosphate backbone. (b) In the famous double-helix form of DNA adjacent strands of deoxyribose (D) – phosphate (○) are linked through the bases. The sequence of bases represents the genetic code that determines the properties of any living organism. In ribonucleic acid (RNA) there is only one strand, thymine is replaced by another pyrimidine (uracil) and the sugar is ribose, whose C_2 has an –OH group rather than two H atoms.

Table 1.2 Role of vitamins in micro-organisms.

Vitamin	Coenzyme it forms part of
Thiamine (vitamin B_1)	Thiamine pyrophosphate
Riboflavin (B_2)	Flavin adenine dinucleotide, Flavin mononucleotide
Niacin (B_3)	Nicotinamide adenine dinucleotide
Pantothenate (B_5)	Coenzyme A
Pyridoxine (B_6)	Pyridoxal phosphate
Biotin (B_7)	Prosthetic group in carboxylases
Folate (B_9)	Tetrahydrofolate
Cobalamin (B_{12})	Cobamides

a_w of 0. Micro-organisms differ greatly in the extent to which they will tolerate changes in a_w. Most bacteria will not grow below a_w of 0.9, so drying is a valuable means for protecting against spoilage by these organisms. By contrast many of the fungi that can spoil grain (a_w 0.7) can grow at relatively low moisture levels and are said to be *xerotolerant*. Truly *osmotolerant* organisms will grow at an a_w of 0.6.

Table 1.3 Exogenous enzymes.

Enzyme	Major sources	Application in foods
α-Amylase	Aspergillus, Bacillus	Syrup production, baking, brewing
β-Amylase	Bacillus, Streptomyces, Rhizopus	Production of high maltose syrups, brewing
Glucoamylase	Aspergillus, Rhizopus	Production of glucose syrups, baking, brewing, wine-making
Glucose isomerase	Arthrobacter, Streptomyces	Production of high fructose syrups
Pullulanase	Klebsiella, Bacillus	Starch (amylopectin) degradation
Invertase	Kluyveromyces, Saccharomyces	Production of invert sugar, production of soft-centred chocolates
Glucose oxidase (coupled with catalase)	Aspergillus, Penicillium	Removal of oxygen in various foodstuffs
Pectinase	Aspergillus, Penicillium	Fruit juice and wine production, coffee bean fermentation
β-Glucanases	Bacillus, Penicillium, Trichoderma	Brewing, fruit juices, olive processing
Pentosanases	Cryptococcus, Trichosporon	Baking, brewing
Proteinases	Aspergillus, Bacillus, Rhizomucor, Lactococcus, recombinant Kluyveromyces, Papaya	Baking, brewing, meat tenderization, cheese
Catalase	Micrococcus, Corynebacterium, Aspergillus	Cheese (see also glucose oxidase above)
Lipases	Aspergillus, Bacillus, Rhizopus, Rhodotorula	Dairy and meat products
Urease	Lactobacillus	Wine
Tannase	Aspergillus	Brewing
β-Galactosidase	Aspergillus, Bacillus, Escherichia, Kluyveromyces	Removal of lactose
Acetolactate decarboxylase	Thermoanaerobium	Accelerated maturation of beer
Prolyl endoproteinase	Aspergillus, Myxococcus	Removal of haze-sensitive polypeptides from beer; potential value in production of foodstuffs based on barley, wheat and oats that would be tolerable by patients suffering from celiac disease

1.2.2.4 Oxygen

Microbes differ substantially in their requirements for oxygen. *Obligate aerobes* must have oxygen as the terminal electron acceptor for aerobic growth (Figure 1.7). *Facultative anaerobes* can use oxygen as terminal electron acceptor, but they can function in its absence. *Microaerophiles* need a relatively small proportions of oxygen in order to

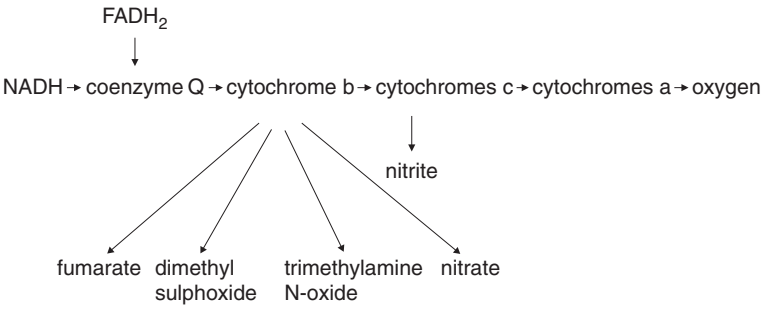

Figure 1.7 Electron transport chains. Reducing power captured as NADH or $FADH_2$ is transferred successively through a range of carriers until ultimately reducing a terminal electron acceptor. In aerobic organisms this acceptor is oxygen, but other acceptors found in many microbial systems are illustrated. This can impact parameters such as food flavour – for example reduction of trimethylamine N-oxide affords trimethylamine (fishy flavour) while reduction of dimethyl sulphoxide yields dimethyl sulphide, which is important in the flavour of many foodstuffs.

perform certain cellular activities, but the oxygen exposure should not exceed 2–10% v/v (cf. the atmospheric level of 21% v/v). *Aerotolerant anaerobes* do not use molecular oxygen in their metabolism but are tolerant of it. *Obligate anaerobes* are killed by oxygen.

Clearly these differences impact on the susceptibility of foodstuffs to spoilage. Most foods when sealed are (or rapidly become) relatively anaerobic, thus obviating the risk from the first three categories of organism.

Irrespective of which class an organism falls into, oxygen is still a potentially damaging molecule when it becomes partially reduced and converted into radical forms, the so-called 'Reactive Oxygen Species' (Figure 1.8). Organisms that can tolerate oxygen have developed a range of enzymes that scavenge radicals, amongst them superoxide dismutase, catalase and glutathione peroxidase.

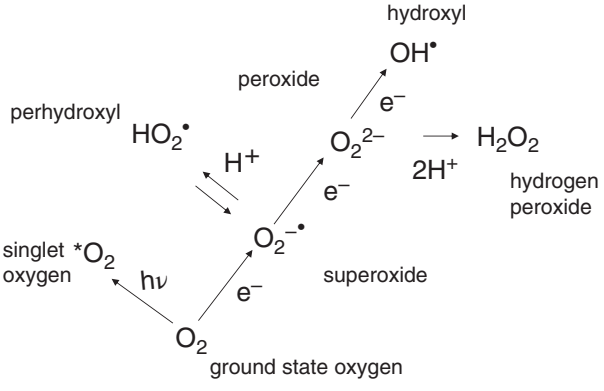

Figure 1.8 Activation of oxygen. Ground-state oxygen is relatively unreactive. By acquiring electrons it become successively more reactive – superoxide, peroxide, hydroxyl. Superoxide exists in charged and protonated forms, the latter (perhydroxyl) being the more reactive. Exposure to light converts oxygen to another reactive form, singlet oxygen.

1.2.2.5 Radiation

One of the activated forms of oxygen, singlet oxygen, is produced by exposure to visible light. An even more damaging segment of the radiation spectrum is the ultra violet, exposure to which can lead to damage of DNA. Ionising radiation such as gamma rays causes the production of an especially reactive oxygen-derived radical, hydroxyl (OH•), and one of the numerous impacts of this is the breakage of DNA. Thus radiation is a very powerful technique for removing unwanted microbes – e.g. in food treatment operations.

1.2.2.6 Hydrostatic Pressure

In nature many microbes do not encounter forces exceeding atmospheric pressure (1 atm = 101.3 kPa = 1.013 bar). Increasing the pressure tends to at least inhibit if not destroy an organism. Pressure is of increasing relevance in food fermentation systems, because modern fermenters hold such large volumes that pressure may exceed 1.5 atm in some instances. Whilst not necessarily killing organisms, high pressures do impact how organisms behave, including their tendency to aggregate and certain elements of their metabolism. The latter is at least in part due to the accumulation of carbon dioxide occurring when the pressure is increased.

1.2.3 Controlling or Inhibiting Growth of Micro-organisms

It is important to regulate which organisms are present during the making of fermentation products and also those that are able to grow and survive in the finished product. On the one hand we have nowadays the deliberate seeding of the desired organism(s), which therefore gain a selective advantage in outgrowing other organisms. Conversely there are physical or chemical '-cidal' treatments or sterilisation procedures that are employed to achieve the depletion or total kill of organisms.

Relevant factors are

- how many organisms are present
- the types of organism that are present
- the concentration of antimicrobial agents that are present or the intensity of the physical treatment
- the prevailing conditions of temperature, pH, viscosity, water activity
- the period of exposure, and
- the concentration of organic matter.

Fermentation of itself comprises a procedure that originally emerged as a means for preserving the nutritive value of foodstuffs. Through fermentation there was either the lowering of levels of substances that contaminating organisms would need to support their growth, or the development of materials or conditions that would prevent organisms from developing – e.g. a lowering of pH. In the case of a product like beer, there is the deliberate introduction of antiseptic agents, in this case the bitter acids from hops.

1.2.3.1 Heating

Moist heat is used for sterilising a greater diversity of materials than dry heat. Moist heat employs steam under pressure and is very effective for the sterilisation of

production vessels and pipe work. Dry heat is less efficient, and requires a higher temperature (e.g. 160 °C as opposed to 120 °C); it is used in systems like glassware and for moisture-sensitive materials.

The microbial content of finished food products is frequently lowered by heat treatment. Ultra-high temperature (UHT) treatments are used where especially high kills are necessary. Pasteurisation is a milder process, one in which the temperature and the time of exposure are regulated to achieve a sufficient kill of spoilage organisms without deleteriously impacting the other properties of the foodstuff. In batch pasteurisation, filled containers (e.g. bottles of beer) are held at, say, 62 °C for 10 minutes in chambers through which the product slowly passes on a conveyor (tunnel pasteurisation). In flash pasteurisation the liquid is heated as it flows through heat exchangers en route to the packaging operation. Residence times are much shorter so temperatures are higher (e.g. 72 °C for 15 seconds). In the specific example of beer, this might be the way in which beer destined for kegs is processed. One pasteurisation unit (PU) is defined as exposure to 60 °C for one minute. As the temperature is increased, so does a shorter exposure time equate to 1 PU. The more organisms, the more extensive is the heat treatment, so the onus is on the operator to minimise the microbial populations by good hygienic practice.

1.2.3.2 Cooling

The ability of organisms to grow is curtailed as the temperature is lowered (refrigeration, freezing).

1.2.3.3 Drying

As organisms usually require significant amounts of water (see earlier), drying affords preservation. Thus, for example, starting materials for fermentation (such as grains and fruits) may be subjected to some degree of drying if they are to be stored successfully prior to use. The other way in which water activity can be lowered is by adding solutes such as salt or sugar. In this book we will encounter several instances where there is deliberate salting during processing to achieve food preservation – e.g. in fermented fish production.

1.2.3.4 Irradiation

The use of irradiation to eliminate spoilage organisms is charged with emotion. Critics hit on the tendency of the technique to reduce the food value, e.g., by damaging vitamins. However the procedure really should be considered on a case-by-case basis, and only if there is some definite negative impact on the quality of a product should it necessarily be avoided. Thus, to take beer as our example again, there is evidence for the increased production of hydrogen sulphide when beer is irradiated.

1.2.3.5 Filtration

Undesirable organisms can be removed by physically filtering them from the product. Depth filters operate by trapping and adsorbing the cells in a fibrous or granular matrix. Membrane filters possess defined pore sizes through which organisms of greater dimensions cannot pass. Typically these pore sizes may be 0.45 μm or, for especially rigorous 'clean-up', 0.2 μm. Practical systems may employ successive filters – for example a depth filter followed by membranes of different sizes. The approach may be most valuable for heat-sensitive products.

1.2.3.6 Chemical Agents

Modern food production facilities are designed so that they are readily cleanable between production runs by chemical treatment regimes, often called 'cleaning in place' or CIP. This demands fabrication with resilient materials, e.g. stainless steel, as well as design that ensures that the agent reaches all nooks and crannies. CIP protocols generally involve an initial water rinse to remove loose soil, followed by the 'detergent' wash. This is not so much a detergent proper as sodium hydroxide or nitric acid and it is targeted at the tougher adhering materials. Next is another water rinse to eliminate the detergent, followed by a sterilant. Various chemical sterilants are available, the most commonly used are chlorine, chlorine dioxide and peracetic acid.

Some foodstuffs are formulated so that they contain preservatives (Table 1.4). In other foodstuffs there are natural antimicrobial compounds present – e.g. polyphenols and the hop iso-α-acids in beer. Furthermore, the end products of some fermentations are historically the basis of protection for fermented foodstuffs, e.g. the low pH, organic acids, alcohol, carbon dioxide. Of especial interest here is the nisin (Figure 1.9) that is a natural product from lactic acid bacteria and able to counter the invasion of other bacteria.

An essential aspect of the long-term success of lactic acid bacteria as protective agents within the fermentation industries is the multiplicity of ways in which they counter the growth of competing organisms. Apart from nisin and other bacteriocins, we might draw attention to the production of:

- organic acids, such as lactic, acetic and propionic acids, with acetic being especially valuable in countering bacteria, yeasts and moulds.
- hydrogen peroxide, which as we have seen is an activated (and therefore potentially damaging) derivative of oxygen.
- diacetyl and acetaldehyde, although some argue that the levels developed are not of practical significance as antimicrobial agents.

Table 1.4 Food grade antimicrobial agents.

Preservative
Acetic acid and its sodium, potassium and calcium salts
Benzoic acid and its sodium, potassium and calcium salts
Biphenyl
Formic acid and its sodium and calcium salts
Hydrogen peroxide
p-Hydroxybenzoate, ethyl-, methyl- and propyl variants and their sodium salts
Lactic acid
Nisin
Nitrate and nitrite, and their sodium and potassium salts
o-Phenylphenol
Propionic acid and its sodium, potassium and calcium salts
Sorbic acid and its sodium, potassium and calcium salts
Sulphur dioxide, sodium and potassium sulphites, sodium and potassium bisulphites, sodium and potassium metabisulphites (disulphites)
Thiabendazole

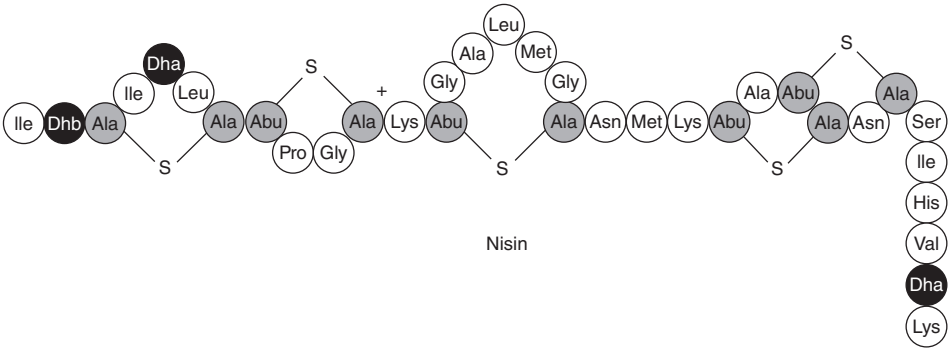

Figure 1.9 Nisin. This antimicrobial destroys Gram positive organisms by making pores in their membranes. It includes some unusual amino acids, including dehydrated serine (Dha), dehydrated threonine (Dhb), lanthionine (Ala-S-Ala) and β-methyllanthionine (Abu-S-Ala). The latter two originate from the coupling of cysteine with dehydrated serine or threonine respectively. Go to http://131.211.152.52/research_page/nisin.html

1.2.4 Metabolic Events

1.2.4.1 Catabolism

Catabolism refers to the metabolic events whereby a foodstuff is broken down so as to extract energy in the form of adenosine triphosphate (ATP), as well as reducing power (customarily generated primarily in the form of NADH but utilised as NADPH) that may subsequently be used to fuel the reactions (anabolism) wherein cellular constituents are fabricated (Figure 1.10).

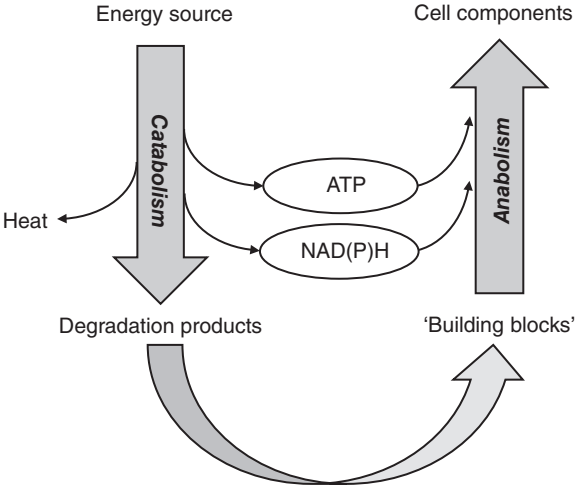

Figure 1.10 Energy sources (e.g. sugars) are successively broken down in catabolic reactions, resulting in the capture of energy in the form of adenosine triphosphate (ATP) and reducing power (as reduced nicotinamide adenine dinucleotide, NADH). Building blocks are transformed into the polymers from which cells are comprised in anabolic reactions that draw on energy (ATP) and reducing power (many of the anabolic processes use the phosphorylated form of NADH, i.e. NADPH).

In focusing on the organotrophs, and in turn even more narrowly (for the most part) on those that use sugars as the main source of carbon and energy, we first must consider the Embden-Meyerhof-Parnas pathway (Figure 1.11). This is the most common route by which sugars are converted into a key component of cellular metabolism, pyruvic acid. This pathway, for example, is central to the route by which alcoholic fermentations are performed by yeast. In this pathway the sugar is 'activated' to a more reactive phosphorylated state by the addition of two phosphates from ATP. There follows a splitting of the biphosphate to two three-carbon units that are in equilibrium. It is the glyceraldehyde 3-phosphate that is metabolised further, but as it is used up so the equilibrium is strained and dihydroxyacetone phosphate is converted to it. Hence we are in reality dealing with two identical units proceeding from the fructose biphosphate. The first

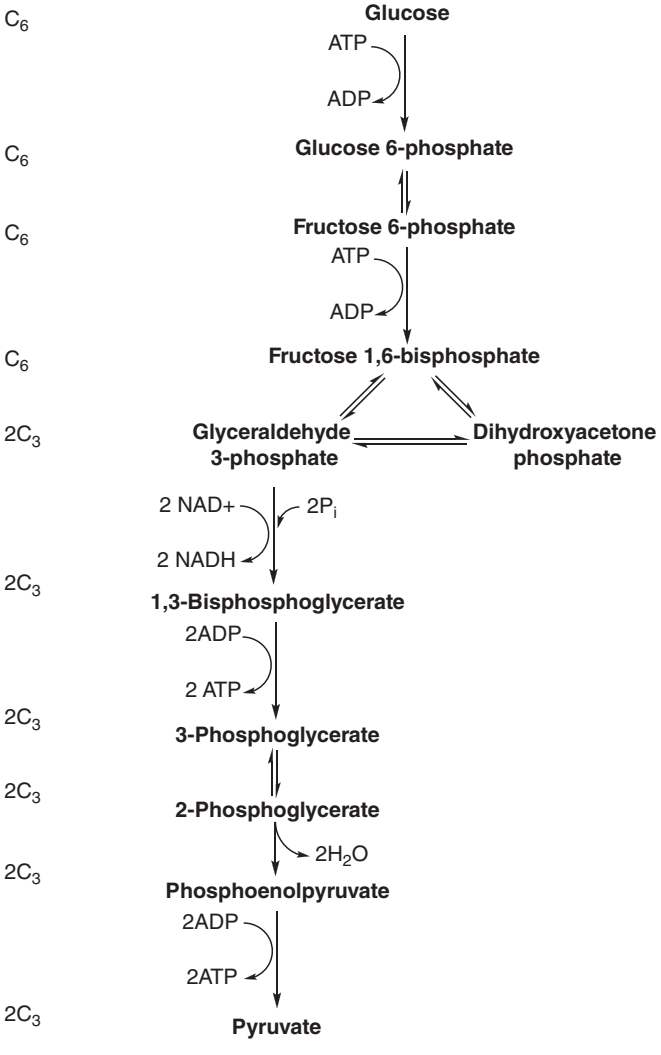

Figure 1.11 The Embden-Meyerhof-Parnas pathway.

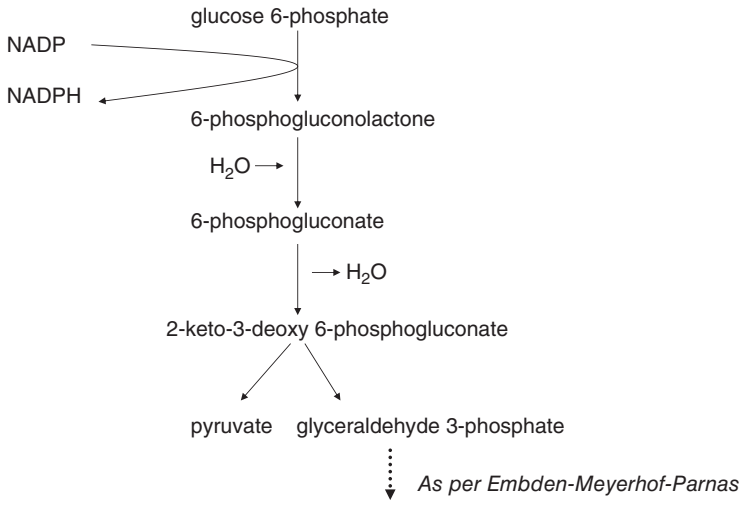

Figure 1.12 The Entner-Doudoroff pathway.

step is an oxidation, the reducing equivalents (electrons, hydrogen) being captured by NAD. En route to pyruvate there are two stages at which ATP is produced by the splitting off of phosphate – this is called *substrate-level phosphorylation*. As there are two three-carbon (C_3) fragments moving down the pathway, this therefore means that 4 ATPs are being produced per sugar molecule. As 2 ATPs were consumed in activating the sugar, there is a net ATP gain of two.

In certain fermentations, the Entner-Doudoroff pathway (Figure 1.12) is employed by the organism, a pathway differing in the earliest part of the pathway insofar as only one ATP is used. Meanwhile in certain lactic acid bacteria there is the quite different phosphoketolase pathway (Figure 1.13).

A major outlet for pyruvate is into the Krebs Cycle (Citric acid cycle; Tricarboxylic acid cycle; Figure 1.14). In particular this pathway is important in aerobically growing cells. There are four oxidative stages with hydrogen collected either by NAD or FAD. When growing aerobically, this reducing power can be recovered by successively passing the electrons across a sequence of cytochromes located in the mitochondrial membranes of eukaryotes or the plasma membrane of prokaryotes (Figure 1.7), with the resultant flux of protons being converted into energy collection as ATP through the process of oxidative phosphorylation (Figure 1.15). In aerobic systems the terminal electron acceptor is oxygen, but other agents such as sulphate or nitrate can serve the function in certain types of organism. An example of the latter would be the nitrate reducers that have relevance in certain meat fermentation processes (see Chapter 13).

In classic fermentations, where oxygen is not employed as terminal electron acceptor and indeed the respiratory chain as a whole is not used, there needs to be an alternative way for the cell to recycle the NADH produced in the EMP pathway, so that NAD is available to continue the process. Herein lays the basis of much of the diversity in fermentation end products, with pyruvate being converted in various ways (Figure 1.16). In brewers yeast the end product is ethanol. In lactic acid bacteria there are two modes of metabolism. In *homofermentative* bacteria the pyruvate is reduced solely to lactic acid. In *heterofermentative* lactic acid bacteria there are alternative end products, most

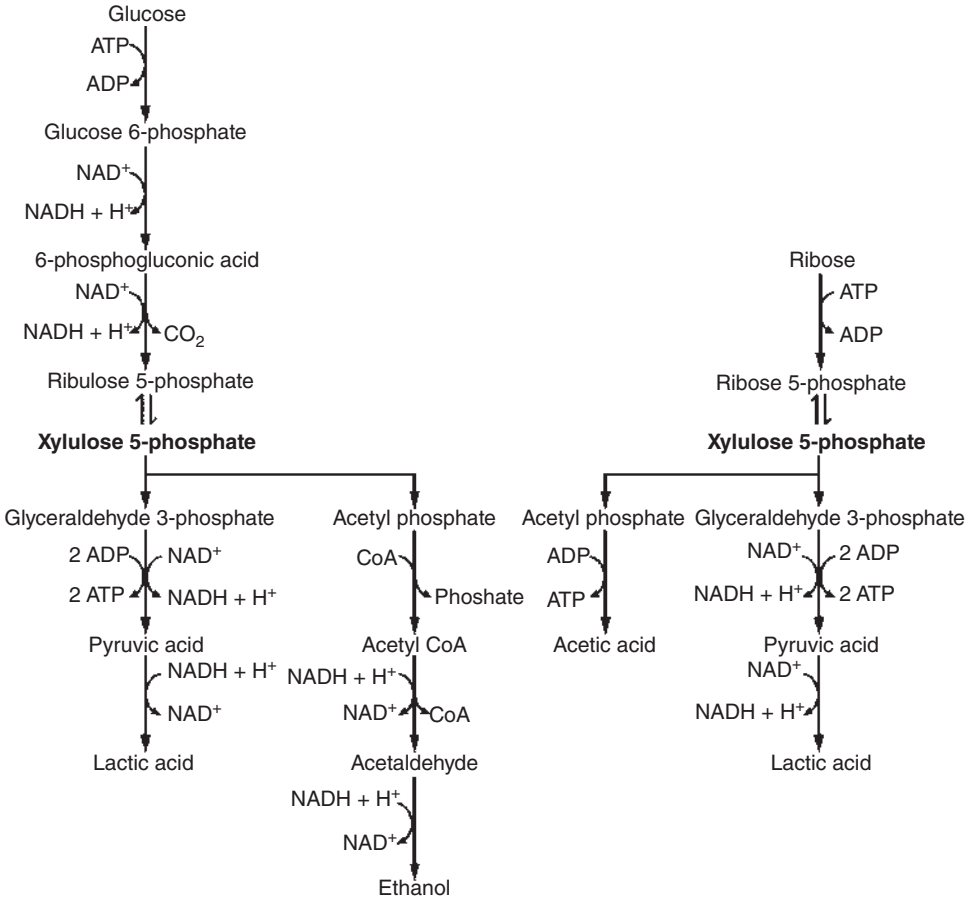

Figure 1.13 The phosphoketolase pathway.

notably lactate, ethanol and carbon dioxide, produced through the intermediacy of the phosphoketolase pathway.

As noted earlier, higher molecular weight molecules that are too large to enter into the cell *as is* are hydrolysed by enzymes secreted from the organism. The resultant lower molecular weight materials are then transported into the cell in the same manner as exiting smaller sized materials. The transport is by selective permeases, which are elaborated in response to the needs of the cell. For example, if brewing yeast is exposed to a mixture of sugars then it will elaborate the transport permeases (proteins) in a defined sequence (see Chapter 2).

1.2.4.2 Anabolism

The above named pathways are examples of how cells deal with sugars, thereby obtaining carbon, hydrogen and oxygen. As observed earlier, cells must also secure a supply of other elements from the medium. Nitrogen may be provided as amino acids (e.g. in the case of brewing yeast), urea or inorganic nitrogen forms, primarily as ammonium salts (often used in wine fermentations).

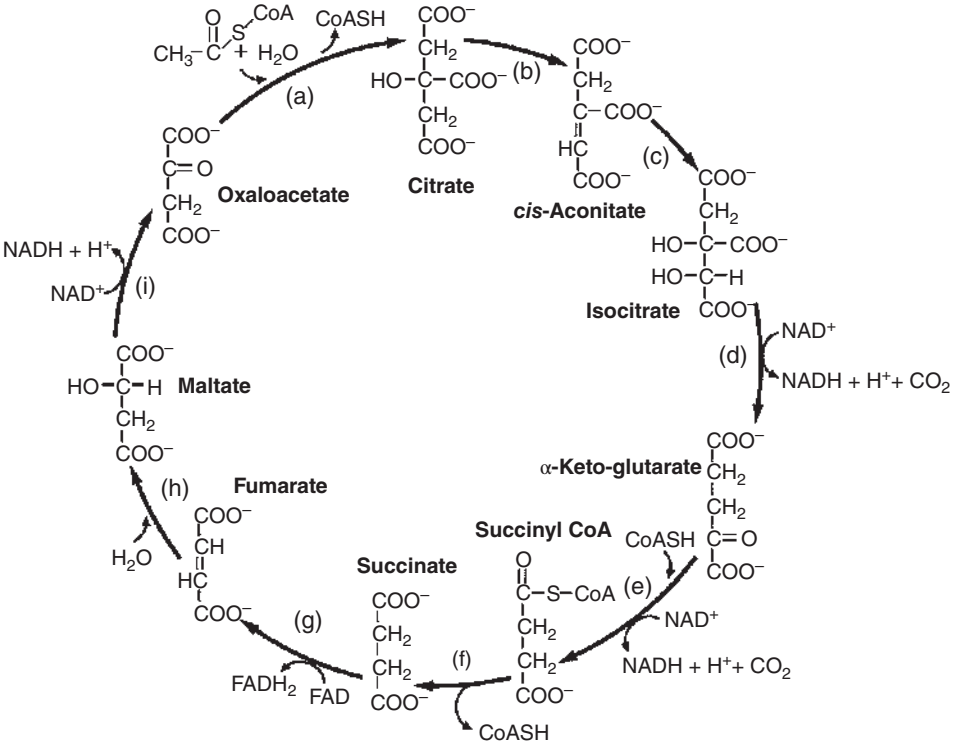

Figure 1.14 The tricarboxylic acid cycle.

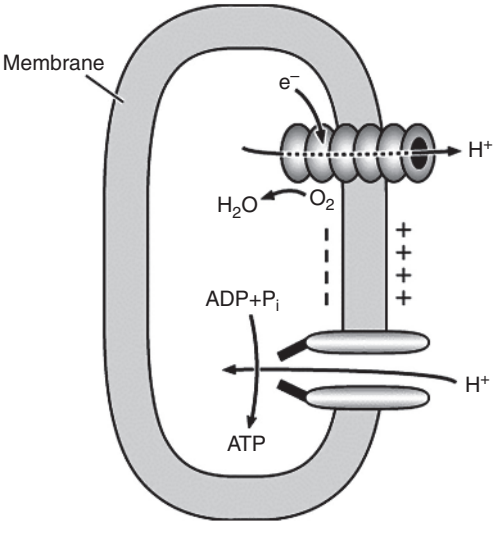

Figure 1.15 Oxidative phosphorylation. The passage of electrons through the electron transport chain is accompanied by an exclusion of protons (H^+) from the cell (or mitochondrion for a eukaryote). The energetically favourable return passage of protons 'down' a concentration gradient is linked to the phosphorylation of ADP to produce ATP.

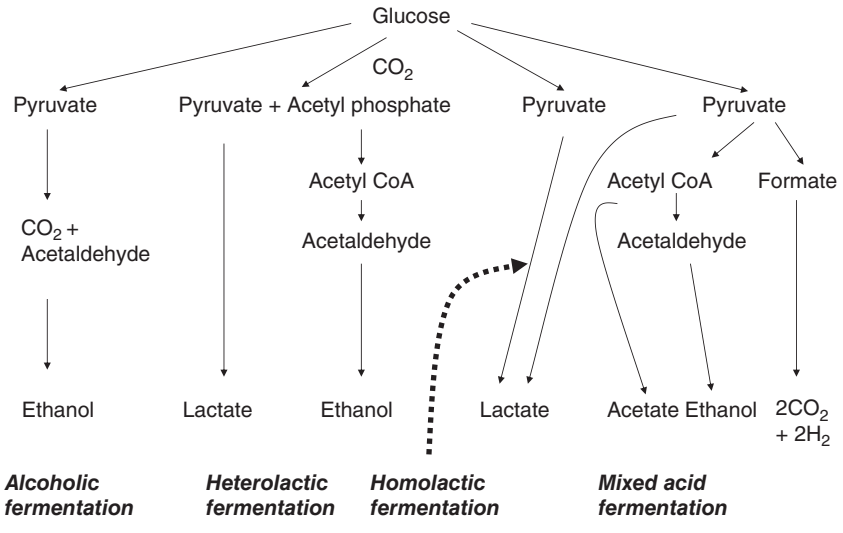

Figure 1.16 Alternative end-products in fermentation.

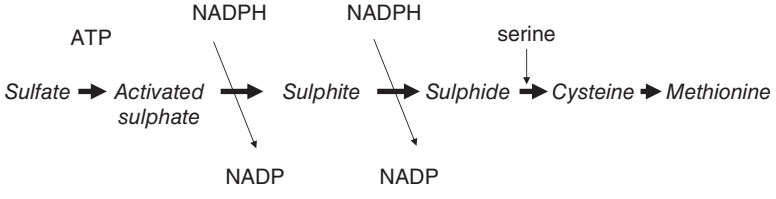

Figure 1.17 The assimilation of sulphur.

Sulphur can variously be supplied in organic or inorganic forms. Brewing yeast, for example, can assimilate sulphate, but will also take up sulphur-containing amino acids (Figure 1.17).

The major structural and functional molecules in cells are polymeric. These include:

Polysaccharides – notably the storage molecules such as glycogen in yeast, which has a structure closely related to the amylopectin fraction of starch (see later), and the structural components of cell walls, for example the mannans and glucans in yeast and the complex polysaccharides in bacterial cell walls.
Proteins – notably the enzymes and the permeases.
Lipids – notably the components at the heart of membrane structure.
Nucleic acids – DNA and RNA.

A greatly simplified summary of cellular metabolism, incorporating the essential features of anabolic reactions is given in Figure 1.18. It is sufficient in the present discussion to state that pyruvate is at the heart of metabolism. There are clearly various draws on it, both catabolic and anabolic. Of particular note is the draw off from the tricarboxylic acid cycle to satisfy biosynthetic needs, meaning that there is a failure to regenerate the oxaloacetate needed to collect a new acetyl CoA residue emerging from

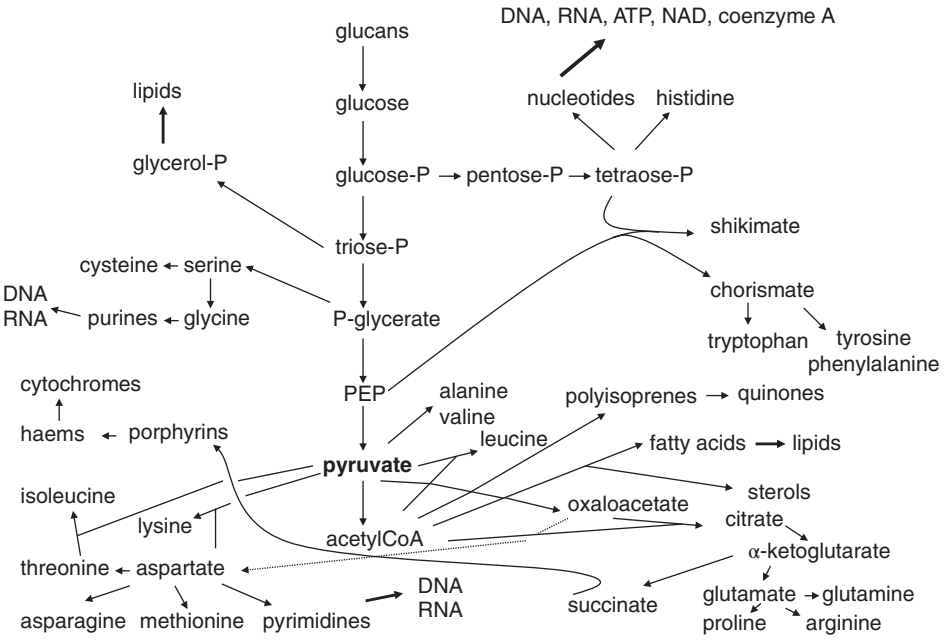

Figure 1.18 A simplified overview of intermediary metabolism.

pyruvate. Thus cells have so-called *anaplerotic* pathways by which they can replenish necessary intermediates such as oxaloacetate. The best known such pathway is the glyoxylate cycle (Figure 1.19).

It is essential that the multiplicity of reactions that as a whole constitute cellular metabolism are controlled so that they are in balance to achieve the appropriate needs of the cell under the prevailing conditions within which it finds itself. It is outside the scope of this treatise to dwell on these regulatory mechanisms, but they include coarse controls on the synthesis of the necessary permeases and enzymes (the general rule being that a protein is only synthesised as and when it is needed) and fine controls on the rate at which the enzymes are able to act. Examples of the impact of these control strategies will be encountered in this book, for example the control of whether brewing yeast degrades sugars by respiration or fermentation.

1.3 The Origins of the Organisms Employed in Food Fermentations

For the longest time the foodstuffs described in this book were prepared using endogenous microflora. Increasingly however, and starting first with the isolation of pure strains of brewing yeast by Emil Christian Hansen in 1883, many of the products employ starter cultures in their production. The organisms conform to the criterion of being Generally Recognised as Safe (GRAS). They are selected for their advantageous properties in terms of process performance and impact on final product quality.

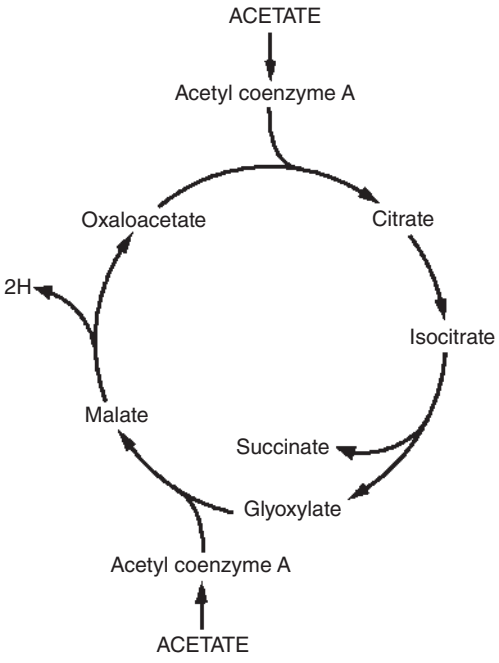

Figure 1.19 The glyoxylate cycle.

Many companies and academic laboratories are seeking newer, improved cultures. This can be achieved in what may be called 'serendipity mode' by screening a broad swathe of samples taken from multitudinous habitats, the screening employing growth media and cultivation conditions that are best suited to an organism with the desired characteristics. Alternatively, some narrowing of odds can be achieved by looking specifically in locales where certain types of organism are known to thrive, for example yeasts are plentiful on the surface of fruit. One extreme example of the latter might most reasonably be described as 'theft', with the pure culture of one company finding its way by whatever mechanism into the clutches of another corporation!

A more honest approach to the latter is by purchasing samples of pure organisms with the desired character from culture collections (Table 1.5). Nowadays the cultures are likely to be in the form of vials frozen in liquid nitrogen (–196 °C) or they may be lyophilised. For some industries, notably bread making and wine making, companies do not produce their own yeast but rather bring it into the production facility on a regular basis from a supplier company. This might be supplied frozen or merely refrigerated with cryoprotectants such as sucrose, glycerol or trehalose. The latest technology here is active dried yeast, with the organism cultured optimally to ensure its ability to survive drying in a state that will allow it to perform vigorously and representatively when rehydrated. In other industries, notably beer brewing, companies tend to maintain their own strains of yeast and propagate them themselves. This is probably on account of the fact that beer making is essentially the only industry described in this book where the surplus organism that grows in the process is re-used.

Table 1.5 Culture collections and suppliers of organisms.

Collection	Organisms	Web page
Belgian Coordinated Collections of Micro-organisms (BCCM)	Fungi, yeasts	http://bccm.belspo.be/index.php
American Type Culture Collection (ATCC)	All types	http://www.atcc.org
Centraalbureau voor Schimmelcultures	Filamentous fungi and yeasts	http://www.cbs.knaw.nl
Collection Nationale de Cultures de Microorganismes	All types	https://research.pasteur.fr/en/team/national-collection-of-cultures-of-microorganisms
Die Deutsche Sammlung von Mikroorganismen und Zellkulturen	All types	http://www.dsmz.de
Herman J. Phaff Culture Collection	Yeasts and fungi	http://phaffcollection.ucdavis.edu
National Collection of Industrial and Marine Bacteria	Bacteria	www.ncimb.com
National Collection of Yeast Cultures	Yeasts	www.ncyc.co.uk
Siebel Pure Yeast Library	Yeast	http://www.siebelinstitute.com/services/yeast.html
VTT	Fungi, bacteria	http://culturecollection.vtt.fi/m/nomenclature.html
White Labs	Yeast	http://www.whitelabs.com
Wyeast	Yeast	http://www.wyeastlab.com

An overview of starter cultures is given in Table 1.6. A starting inoculum might typically be of the order of 1% of the total fermentation batch on a volume basis. An example of how the volume can be scaled up from the pure 'slope' of the master culture to an amount to 'pitch' the most enormous of fermenters is given in Chapter 2.

There are various opportunities for enhancing the properties of the organisms that are already employed by food companies. Mutagenesis to eliminate undesirable traits has been employed. However this is a challenge for eukaryotes as such cells tend to have multiple copies of each gene (polyploidy or aneuploidy) and it is a formidable challenge to eliminate all the alleles of the undesirable gene. Classic recombination techniques (conjugation, transduction and transformation) have been pursued, but there is always the risk that an undesirable trait will be introduced as an accompaniment to the attribute of interest. Much more selectivity is afforded by modern genetic modification strategies. However, as noted earlier, this arouses far more emotion for organisms used in food production than it does in the production of, say, fuels or pharmaceuticals.

1.4 Some of the Major Micro-Organisms in This Book

Reference to the chapters that follow will highlight to the reader that a diversity of micro-organisms is involved in food fermentations. However the organisms that one encounters most widely in these processes are undoubtedly the yeasts, notably

Table 1.6 Starter cultures.

Organism	Type of organism	Foodstuff
Acetobacter aceti	Bacterium	Vinegar
Aspergillus oryzae	Mould	Miso, Soy sauce, sake
Brevibacterium linens	Bacterium	Cheese pigment and surface growth
Lactobacillus casei	Bacterium	Cheese and other fermented dairy products
Lactobacillus curvatus	Bacterium	Sausage
Lactobacillus delbrueckii ssp. Bulgaricus	Bacterium	Cheese, yoghurt
Lactobacillus helveticus	Bacterium	Cheese and other fermented dairy products
Lactobacillus lactis	Bacterium	Cheese and other fermented dairy products
Lactobacillus plantarum	Bacterium	Fermented vegetables, sausage
Lactobacillus sakei	Bacterium	Sausage
Lactobacillus sanfranciscensis	Bacterium	Sourdough bread
Leuconostoc lactis	Bacterium	Cheese and other fermented dairy products
Leuconostoc mesenteroides	Bacterium	Fermented vegetables, Cheese and other fermented dairy products
Oenococcus oeni	Bacterium	Wine
Pediococcus acidilactici	Bacterium	Fermented vegetables, sausage
Pedicococcus halophilus	Bacterium	Soy sauce
Pediococcus pentosaceus	Bacterium	Sausage
Penicillium camemberti	Mould	Surface ripening of cheese
Penicillium chrysogenum	Mould	Sausage
Penicillium roqueforti	Mould	Blue-veined cheeses
Propionibacterium freudenreichii	Bacterium	Eyes in Swiss cheese
Rhizopus microsporus	Mould	Tempeh
Saccharomyces cerevisiae	Fungus	Bread, ale, wine, cider
Saccharomyces pastorianus	Fungus	Lager
Saccharomyces sake	Fungus	Sake
Staphylococcus carnosus	Fungus	Meat
Streptococcus thermophilus	Bacterium	Cheese, yoghurt
Tetragenococcus halophila	Bacterium	Soy sauce
Zygosaccharomyces rouxii	Bacterium	Soy sauce

Saccharomyces, and the lactic acid bacteria. It is important to note in passing that if these organisms 'stray' from where they are supposed to be then they are spoilage organisms with a ruinous nature. For example, the lactic acid bacteria have a multiplicity of values in the production of many foodstuffs, including cheese, sourdough bread, some wines and a very few beers. However their development in the majority of beers, for example, is very much an undesirable source of spoilage.

1.4.1 Yeast

In most instances use of the word yeast in a food context is synonymous with *S. cerevisiae*, viz. brewer's yeast or baker's yeast. However, as we shall discover, there are other yeasts involved in fermentation processes (Table 1.7).

Yeasts are heterotrophic organisms whose natural habitats are the surfaces of plant tissues, including flowers and fruit. They mostly are obligate aerobes, although some (such as brewing yeast) are facultative anaerobes. They are fairly simple in their nutritional demands, requiring a reduced carbon source, various minerals and a supply of nitrogen and vitamins. Ammonium salts are readily used, but equally a range of organic nitrogen compounds, notably the amino acids and urea, can be used. The key vitamin requirements are biotin, pantothenic acid and thiamine.

Legras et al. (2007) argue that diversity in Saccharomyces is founded upon human history. They draw attention to genetic relatedness between strains, with bread yeasts displaying a genetic make-up intermediate between beer and wine strains with the strains used for the production of rice wine and sake being closely related to beer and bread strains. However they emphasise that local domestication makes a sizeable contribution to the genetic diversity. In the case of wine yeasts, for instance, they propose that the organism followed the migration of humans and their vines.

Table 1.7 Sources of Saccharomyces (Walker 1998).

Saccharomyces	Source
S. barnettii	Sauerkraut, soft drink
S. bayanus	Fruit juice, beer, perry, grape must
S. cariocanus	*Drosophila* sp.,
S. castellii	Soil, baboon caecum, buttermilk
S. cerevisiae	Wine, beer, fruit, soil, soft drinks, man
S. dairenensis	Fermenting grapes, dry fruit
S. exiguous	Grape must, sewage, soil
S. kluyveri	Soil, *Drosophila* spp., tree exudate
S. kudriavzevii	Decayed leaf
S. kunashirensis	Soil near hot spring
S. martiniae	Fermenting mushroom
S. mikatae	Decayed leaf, soil
S. paradoxus	Oak tree exudates, soil
S. pastorianus	Beer
S. rosinii	Soil
S. servazzii	Soil, man with HIV
S. spencerorum	Soil, larval gut
S. transvaalensis	Soil
S. unisporus	Kefyr, cheese

Focusing on brewing yeast, and following the most recent taxonomic findings, the term *S. cerevisiae* is properly applied only to ale yeasts. Lager yeasts are accurately termed *Saccharomyces pastorianus,* representing as they do organisms with a 50% larger genome and tracing their pedigree to a coupling of *S. cerevisiae* with *Saccharomyces eubayanus* (see Figure 1.20).

Saccharomyces is spherical or ellipsoidal. Whereas laboratory strains are haploid (one copy of each of the 16 linear chromosomes), industrial strains are polyploid (i.e. they have multiple copies of each chromosome) or aneuploid (varying numbers of each chromosome). Some 6000 genes have been identified in yeast and indeed the entire genome has now been sequenced (go to http://www.yeastgenome.org).

Brewing yeast does have a sex life – but reproduces in production conditions primarily by budding (Figure 1.21). A single cell may bud up to 20 times, each time leaving a scar, the counting of which can be used to indicate how senile the culture has become.

The surface of the wall surrounding the yeast cell is negatively charged, due to the presence of phosphate groups attached to the mannan polysaccharides that are located in the wall. It is unlikely that the fact that calcium promotes the binding together of adjacent cells is simply through ionic bonding and it is understood that there is a complex chemistry involved, including the binding of lectins on the surface. There are varying degrees of association between different strains, resulting in differing extents of flocculation, advantage of which is taken in the separation of cells from the liquid at the end of fermentation.

The underlying plasma membrane (as well as the other membranes in the cellular organelles) is comprised primarily of sterols (notably ergosterol), unsaturated fatty acids and proteins, notably the permeases (see earlier) (Figure 1.22). As oxygen is needed for the desaturation reactions involved in the synthesis of the lipids, relatively small quantities of oxygen must be supplied to the yeast, even when it is growing anaerobically by fermentation.

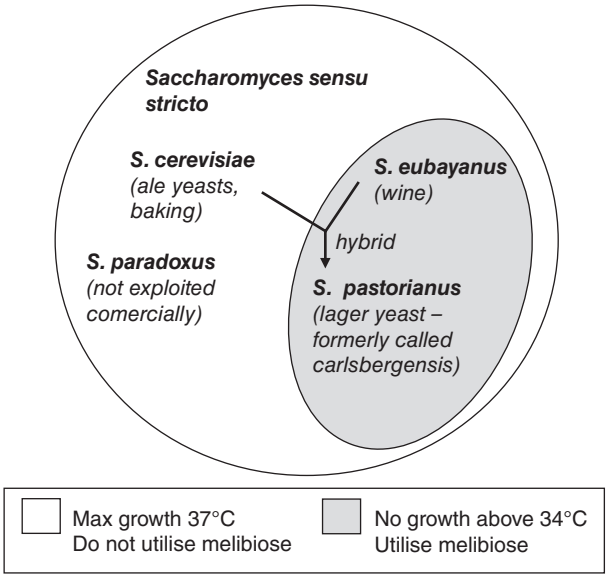

Figure 1.20 Species of yeast, genus Saccharomyces.

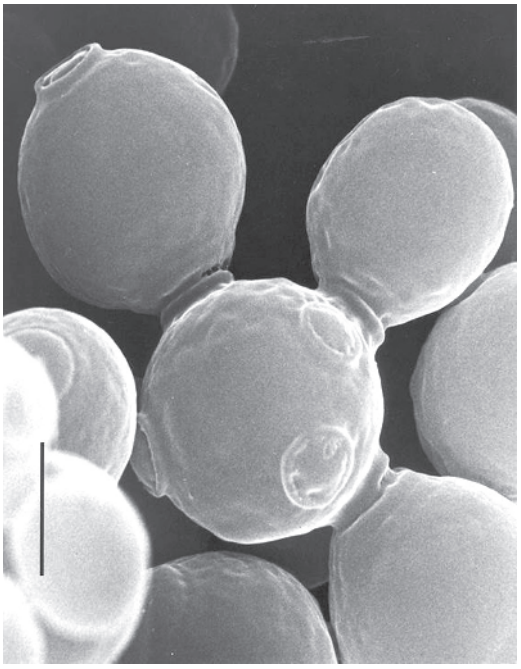

Figure 1.21 Yeast cells budding. Bud scars, where previous cell division has occurred, are visible. *Source:* Photograph courtesy of Dr. Alastair Pringle.

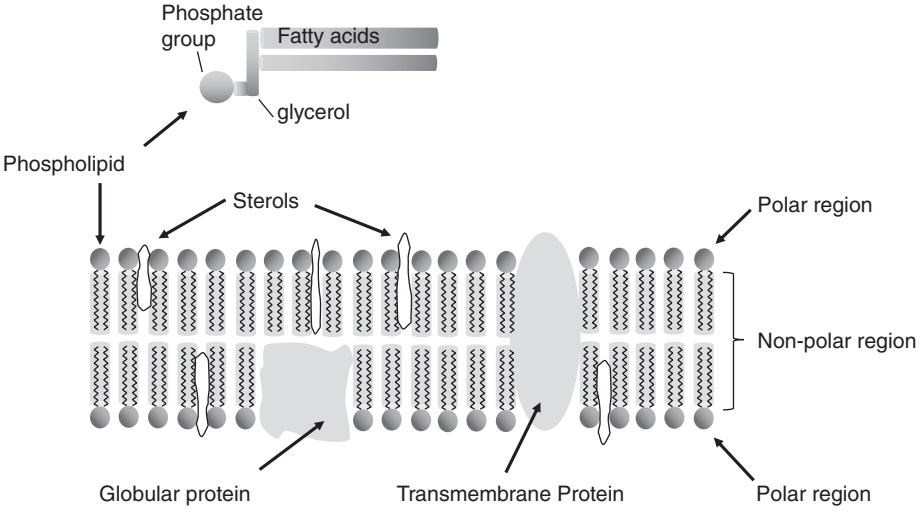

Figure 1.22 Structural features of the yeast cell membrane.

The control mechanisms that drive the mode of metabolism in the yeast cell (i.e. by aerobic respiration or by fermentation) are based on the concentration of sugar that the yeast is exposed to. At high concentrations of sugar, the cell is switched into fermentative mode, and the pyruvate is metabolised via acetaldehyde to ethanol. At low sugar concentrations, the pyruvate shunts into acetyl-CoA and the respiratory chain.

This is the so-called Crabtree Effect. The rationale is that when sugar concentrations are high the cell does not need to generate as many ATP molecules per sugar molecule, whereas if the sugar supply is limited, the yeast must maximise the efficiency with which it utilises that sugar (ATP yield via fermentation and respiration are 2 molecules and 32 molecules respectively). The significance of this in commercial fermentation processes is clear. In brewing, where the primary requirement is a high yield of alcohol, the sugar content in the feedstock (wort) is high, whereas in the production of baker's yeast, where the requirement is a high cell yield, the sugar concentration is always kept low, but the sugar is continuously passed into the fermenter ('fed batch').

1.4.2 Lactic Acid Bacteria

Throughout the centuries it has been the practice in various fermentation-based processes to add back a proportion of the previously produced food to the new batch, so called 'back slopping'. What of course this did was to seed the fermentation with the preferred micro-organism, and for many foodstuffs that organism is a lactic acid bacterium. Such bacteria are only weakly proteolytic and lipolytic, which means that they are quite 'mild' in respect of their tendency to produce pungent flavours. They are also naturally present in the intestine and the reproductive tract, so it is no surprise that nowadays we talk of probiotics and prebiotics in the context of enriching the level of lactic acid bacteria in the gut. Probiotics are organisms, notably lactobacilli and bifidobacteria, that are added to the diet to boost the flora in the large intestine. For example they are added to yoghurt. Prebiotics are nutrients that boost the growth of this type of organism that is naturally present in the digestive system.

Like the brewing and baking yeasts, lactic acid bacteria tend to be GRAS, although some strains are pathogenic. Joseph Lister isolated the first lactic acid bacterium in 1873. We now refer to this organism as *Lactococcus lactis*, a species of great significance in the fermentation of milk products.

There are 16 genera of lactic acid bacteria, some 12 of which are active in a food context. They are Gram positive organisms, are either rod-shaped, cocci (spherical) or coccobacilli. For the most part they are mesophilic, but some can grow at refrigerator temperatures (4 °C) and others as high as 45 °C. Generally they prefer a pH in the range 4.0–4.5, but certain strains can tolerate and grow at pHs above 9.0 or as low as 3.2. They need pre-formed purines, pyrimidines, amino acids and B vitamins. Lactic acid bacteria do not possess a functional Tricarboxylic Acid cycle or haem-linked electron transport systems, so they use substrate level phosphorylation to gain their energy.

As we saw previously, their metabolism can be classified as either homofermentative, where lactic acid represents 95% of the total end products, or heterofermentative, in which acetic acid, ethanol and carbon dioxide are produced alongside lactic acid.

Lactic acid bacteria produce antimicrobial substances known as bacteriocins. For the most part these are cationic amphipathic peptides that insert into the membranes of closely related bacteria, causing pore formation, leakage and an inability to sustain metabolism, ergo death. The best known of these agents is nisin (see earlier), and it has been used substantially as a 'natural' antimicrobial agent. Lactic acid bacteria also produce acids and hydrogen peroxide as antimicrobials.

1.4.2.1 Lactococcus

The most notable species within this genus is *L. lactis*, which is most important in the production of foodstuffs such as yoghurts and cheese. It is often co-cultured with Leuconostoc.

There are two sub-species of *L. lactis*: Cremoris, which is highly prized for the flavour it affords to certain cheeses, and Lactis, in particular *L. lactis* ssp. *lactis* biovar. *Diacetyllactis*, which can convert citrate to diacetyl, a compound with a strong buttery flavour highly prized in some dairy products but definitely taboo in most beers. The CO_2 produced by this organism is important for eye formation in Gouda cheese.

1.4.2.2 Leuconostoc

These are heterofermentative cocci.

Leuconostoc mesenteroides, with its three subspecies: mesenteroides, cremoris and dextranicum, and *Leuconostoc lactis* are the most important species, especially in the fermentation of vegetables. They produce extracellular polysaccharides that have value as food thickeners and stabilisers. These organisms also contribute to the CO_2 production in Gouda cheese.

Oenococcus oeni (formerly *Leuconostoc oenos*) plays an important role in malolactic fermentations in wine.

1.4.2.3 Streptococcus

These are mostly pathogens, however *Streptococcus thermophilus* is a food organism, featuring alongside *Lactobacillus delbrueckii* ssp *bulgaricus* in the production of yoghurt. Furthermore it is used in the starter cultures for certain cheeses, notably Parmesan.

1.4.2.4 Lactobacillus

There are some 60 species of such rod-shaped bacteria that inhabit the mucous membranes of the human, *ergo* the oral cavity, the intestines and the vagina. However they are equally plentiful in foodstuffs, such as plants, meats and milk products.

Lb. delbrueckii ssp *bulgaricus* is a key starter organism for yoghurts and some cheeses. However lactobacilli have involvement in other fermentations, such as sourdough and fermented sausages, e.g. salami. Conversely they can spoil beer and either fresh or cooked meats, etc.

1.4.2.5 Pediococci

Pedicococcus halophilus (now *Tetragenococcus*) is extremely tolerant of salt (> 18%) and as such is important in the production of soy sauce. Pediococci also function in the fermentation of vegetables, meat and fish. On the other hand *Pediococcus damnosus* results in ropiness in beer and the production of diacetyl as an off-flavour.

1.4.2.6 Enterococcus

These faecal organisms have been isolated from various indigenous fermented foods, however no positive contribution has been unequivocally demonstrated and their presence is debatably indicative of poor hygiene.

1.5 Providing the Growth Medium for the Organisms

The microflora is of course one of the two key inputs to a food fermentation. The other is the substrate that the organism(s) converts. With the possible exception of mycoprotein (Chapter 17), the substrates that we encounter in this book are very traditional and well-defined insofar as the end product is what it is as much because of that substrate as through the action of the microbe that deals with it. Thus for beers the final product, whether it is an ale, lager or stout, a wheat beer or a lambic has clear characteristics that are afforded by the raw materials (malt, adjunct and hops) used to make the wort that the yeast ferments. The same applies for the cereal used to make bread, the milk going to cheese and yoghurt, the meat destined for salami, the cabbage en route to sauerkraut.

In all instances there are defined preparatory steps that must be undertaken to render the substrate in the state that is ready for the microbial fermentative activity. For some foodstuffs, e.g. yoghurt, there is relatively little processing of the milk. However for a product like beer, there is prolonged initial processing, notably the malting of grain and its subsequent extraction in the brewery.

The growth substrate must always include sources of carbon, nitrogen, water and, usually, oxygen, as well as the trace elements. These nutritional considerations have already been discussed.

1.6 Fermenters

Most food fermentations are generally classified as being 'non-aseptic' to distinguish them from microbial processes where rigorous hygiene must be ensured, e.g. production of antibiotics and vaccines. This is not to say that those practising food fermentations are less than hygienic. The majority of the processes that we describe in this book are carried out in vessels that are subjects to rigorous CIP (see earlier).

A diversity of fermenter types is employed – ranging from the relatively sophisticated cylindroconical vessels in modern brewery operations (see Figure 2.27 in Chapter 2) through to the relatively crude set-ups used in some of the indigenous fermentation operations, not least the fermentation of cocoa. Key issues in all instances are the ability to maintain the required degree of cleanliness, the ability to mix, the ability to regulate temperature and change temperature smoothly and efficiently, the access of oxygen (aeration or oxygenation) and the ability to monitor and control.

1.7 Downstream Processing

For many of the foodstuffs that we will address, some form of post-fermentation clarification is necessary to remove surplus microbial cells and various other types of insoluble particles. Cells may be harvested by sedimentation (perhaps encouraged by agents such as isinglass or egg white), centrifugation or filtration. Additionally there may be other downstream treatments, such as the adsorption of materials that might (if not removed) fall out of solution and ruin the appearance of a product, e.g. polyphenols and proteins in beer. Many products have their microbial populations depleted either by

pasteurisation or filtration through depth and/or membrane filters. Finally of course they receive varying degrees of primary and secondary packaging.

Several of the products described in the present volume involve distillation stage(s) in their production. This will be described in general terms in Chapter 6.

1.8 Some General Issues for a Number of Foodstuffs

Some topics are of general significance for many of the foodstuffs considered in this book and, accordingly, reference is made to them here.

1.8.1 Non-enzymatic Browning

These are chemical reactions that lead to colour development when food is heated. The relevant chemistry is known as the Maillard reaction, which actually comprises a sequence of reactions that occur when reducing sugars are heated with compounds that contain a free amino group, e.g. amino acids, proteins and amines (Figure 1.23 and Table 1.8). In reflection of the complexity of the chemistry, there are many reaction intermediates and products. As well as colour, Maillard reaction products impact on flavour and may act as antioxidants. The antioxidants are mostly produced at higher pHs and when the ratio of amino acid to sugar is high. It must also be stressed that some of the Maillard reaction products can *promote* oxidative reactions. Other Maillard type reactions occur between amino compounds and substances other than sugars that have a free carbonyl group. These include ascorbic acid and molecules produced during the oxidation of lipids.

The Maillard reaction should not be confused with caramelisation, which is the discoloration of sugars as a result of heating in the absence of amino compounds.

In the primary Maillard reaction, the amino compound reacts with the reducing sugar to form an N-substituted glycosylamine that rearranges to 1-amino-1-deoxy-2-ketose (the so-called Amadori rearrangement product). This goes forward in a cascade of reactions in various ways depending on the pH. At the pH of most foods (4–6) the primary route involves melanoidin formation by further reaction with amino acids. Other products are Strecker aldehydes, pyrazines, pyrroles and furfurals. The substances produced in these reactions have flavours that are typical of roasted coffee and nuts, bread and cereals. The pyrrole derivatives afford bitter tastes. The Maillard reaction may also lead to aged or cooked characters in products such as processed orange juice and dried milk products.

The early products in the Maillard reaction are colourless, but when they get progressively larger they become coloured and responsible for the hue of a wide range of foods. Some of these coloured compounds have low molecular weights, but others are much larger and may include complexes produced by the heat-induced reactions of the smaller compounds and proteins.

The exact events in any Maillard-based process depend on the proportion of the various precursors, the temperature, pH, water activity and time available. Metals, oxygen and inhibitors such as sulphite also impact. The flavour developed differs depending on the time and intensity of heating for instance – high temperature for a short time gives a

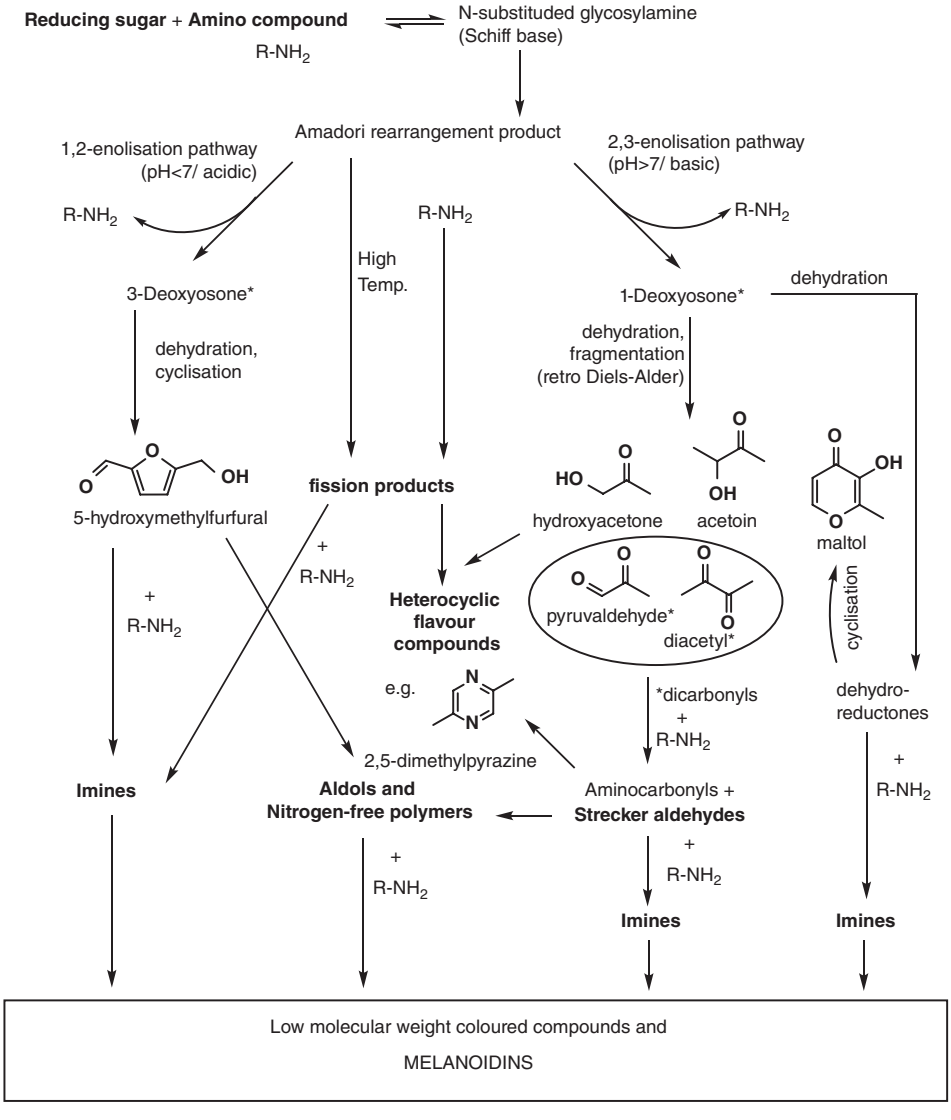

Figure 1.23 The Maillard reaction (outline scheme).

different result to low temperature for a long time. Pentose sugars react faster than do hexoses, which in turn react more rapidly than disaccharides such as maltose and lactose. With regard to the amino compounds, lysine and glycine are much more reactive than is cysteine, for instance. In addition, the flavour also depends on the amino acid. Cysteine affords meaty characters; methionine gives potato, while proline gives bready flavours.

As water is produced in the Maillard reaction, it occurs less readily in food where the water activity is high. The Maillard reaction is especially favoured at a_w 0.5–0.8.

Finally sulphite, by combining with reducing sugars and other carbonyl compounds, inhibits the reaction.

Table 1.8 Some products of the Maillard reaction.

Type of compound	Example	Flavour descriptors
(a) Products derived from interactions of sugars and amino acids		
Pyrrole	2-acetyl-1-pyrroline	Newly baked crust of wheat bread
Pyridine	2-acetyl-1,4,5,6-tetrahydropyridine	Cream crackers
Pyrazine	Methylpyrazine	Nut
Oxazole	Trimethyloxazole	Green, nutty, sweet
Thiophene	2-acetylthiphene	Onion, mustard
(b) Products derived from the sugar		
Furan	Furaneol	Caramel, strawberry
Carbonyl	Diacetyl	Butterscotch
(c) Products derived from the amino acid		
Cyclic polysulphur	5-methyl-5-pentyl-1,2,4-trithiolane	Fried chicken
Sulphur-container	Methional	Mashed potato
Thiazole	2-actylthiazole	Popcorn

1.8.2 Enzymatic Browning

This arises by the oxidation of polyphenols to o-quinones by enzymes such as polyphenol oxidase and peroxidase (Figure 1.24). A day to day example would be the browning of sliced apple. In other foods the reaction is wanted, for example in the readying of prunes, dates and tea for the marketplace.

Whereas heating boosts non-enzymatic browning, the converse applies to enzymatic browning, as the heat inactivates enzymes. The alternative strategies to avoid the reaction are to lower the levels of polyphenols (the agent polyvinylpolypyrrolidone achieves this), adjust the pH, or exclude oxygen.

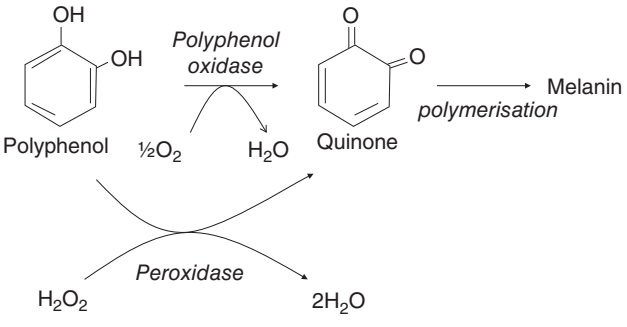

Figure 1.24 Polyphenol oxidation.

1.8.3 Caramelisation of Sugars

Caramel is still produced to this day by burning sugar, but in very controlled ways. The principal products are produced by the polymerisation of glucose by dehydration. The process is catalysed by acids or bases and requires temperatures in excess of 120 °C. In some markets the word caramel is retained for materials that are produced in the absence of nitrogen-containing compounds and these products are used for flavouring value. Where N is present then 'sugar colours' are produced and these are used for colouring purposes.

Caramel is polymeric in nature, but also contains several volatile and non-volatile lower molecular weight components that afford the characteristic flavour, compounds such maltol and isomaltol (Figure 1.25).

1.8.4 Antioxidants

There is much interest in antioxidants from the perspective of protecting foodstuffs from flavour decay, but increasingly for their potential value in countering afflictions such as cancer, rheumatoid arthritis and inflammatory bowel diseases. Figure 1.26 presents a range of these antioxidants. Many are phenolics, and act either by scavenging or neutralising (by reduction) the radicals that effect deterioration or by chelating the metal ions that cause the production of these radicals.

The tocopherols are fat-soluble and are found in vegetable oils and the fatty regions of cereals, e.g. the germ. The carotenoids (e.g. lycopene) are water-soluble and are found in fruits and vegetables. The flavonoids are water-soluble polyphenols found in fruits, vegetables, leaves and flowers. Such molecules have particular significance for some of the products discussed in this book, notably wine, beer, cider and tea. The phenolic acids, e.g. caffeic and ferulic acids and their esters, are abundant in cereal grains such as wheat and barley.

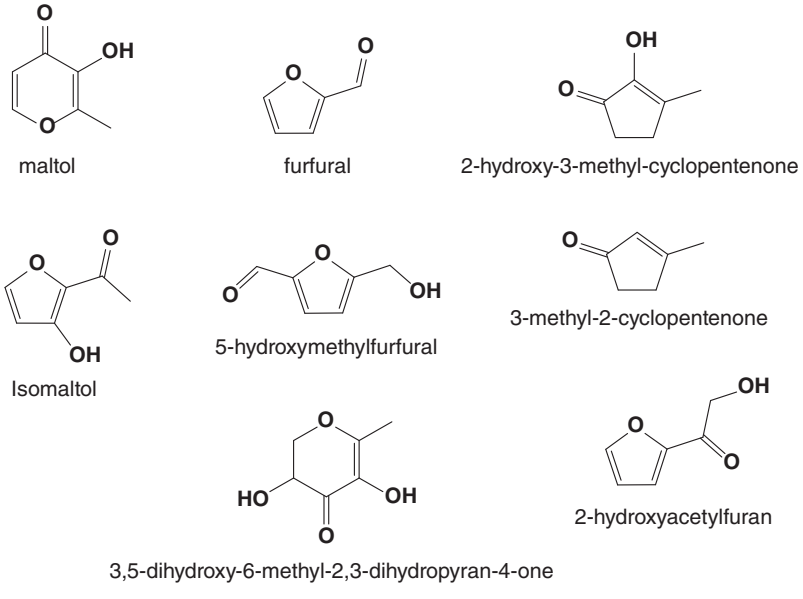

Figure 1.25 Some flavour compounds produced in caramelisation reactions.

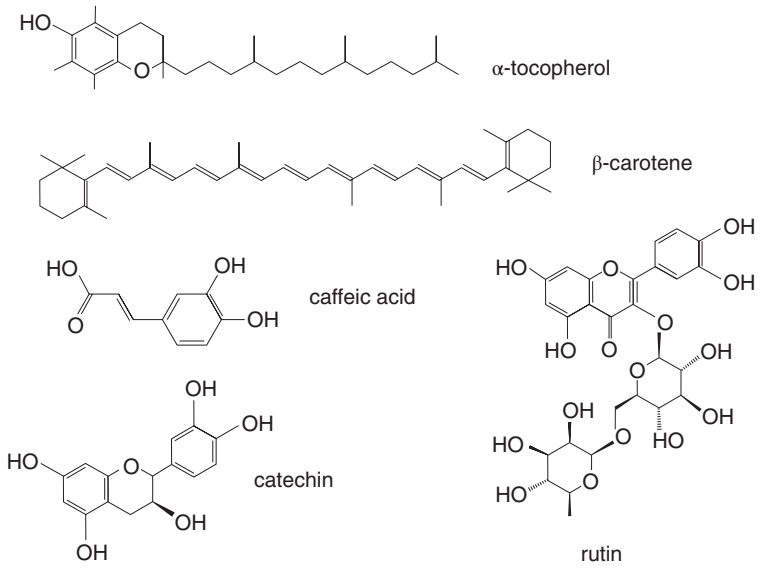

Figure 1.26 Some antioxidants.

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