CHAPTER 4

The Microbiology of Food Preservation

In Chapter 3 we outlined the physical and chemical factors that influence microbial growth and survival in foods. We have also seen how knowledge of these helps us to form a qualitative picture of a food's microflora and how mathematical models can be formulated which give a quantitative description of microbial growth under differing conditions. Manipulation of the factors affecting microbial behaviour is the basis of food preservation (Table 4.1). In this chapter we will survey the principal techniques of food preservation, with the notable exception of fermentation which is discussed separately in Chapter 9. Since our main concern here is the effect of preservation treatments on micro-organisms, technological features will only occasionally be touched on. For more detail on these aspects, readers are referred to more specialized texts on food technology.

4.1 HEAT PROCESSING

4.1.1 Pasteurization and Appertization

Foods are subject to thermal processes in a number of different contexts (Table 4.2). Often, their main objective is not destruction of micro-organisms in the product, although this is an inevitable and frequently useful side effect.

Credit for discovering the value of heat as a preservative agent goes to the French chef, distiller and confectioner, Nicolas Appert. In 1795 the French Directory offered a prize of 12 000 francs to anyone who could develop a new method of preserving food. Appert won this prize in 1810 after he had experimented for a number of years to develop a technique based on packing foods in glass bottles, sealing them, and then heating them in boiling water. He described his technique in detail in 1811 in a book called the ' . . . Art of Conserving all kinds of Animal and Vegetable Matter for several Years'. A similar technique was used by the

Table 4.1 *Mechanisms of principal food preservation procedures*

Procedure	*Factor influencing growth or survival*
Cooling, chill distribution and storage	Low temperature to retard growth
Freezing, frozen distribution and storage	Low temperature and reduction of water activity to prevent growth
Drying, curing and conserving	Reduction in water activity sufficient to delay or prevent growth
Vacuum and oxygen-free 'modified atmosphere' packaging	Low oxygen tension to inhibit strict aerobes and delay growth of facultative anaerobes
Carbon dioxide-enriched 'modified atmosphere'packaging	Specific inhibition of some micro-organisms by carbon dioxide
Addition of acids	Reduction of pH value and sometimes additional inhibition by the particular acid
Lactic fermentation	Reduction of pH value *in situ* by microbial action and sometimes additional inhibition by the lactic and acetic acids formed and by other microbial products, *e.g.* ethanol, bacteriocins
Emulsification	Compartmentalization and nutrient limitation within the aqueous droplets in water-in-oil emulsion foods
Addition of preservatives	Inhibition of specific groups of micro-organisms
Pasteurization and appertization	Delivery of heat sufficient to inactivate target micro-organisms to the desired extent
Radurization, radicidation and radappertization	Delivery of ionizing radiation at a dose sufficient to inactivate target micro-organisms to the desired extent
Application of high hydrostatic pressure Pascalization	Pressure-inactivation of vegetative bacteria, yeasts and moulds

Adapted from Gould (1989)

Table 4.2 *Heat processes applied to foods*

Heat process	*Temperature*	*Objective*
Cooking baking boiling frying grilling	$\leqslant 100\,^{\circ}C$	Improvement of digestibility, *e.g.* starch gelatinization, collagen breakdown during cooking of meat. Improvement of flavour. Destruction of pathogenic micro-organisms
Blanching	$< 100\,^{\circ}C$	Expulsion of oxygen from tissues. Inactivation of enzymes
Drying/ Concentration	$< 100\,^{\circ}C$	Removal of water to enhance keeping quality
Pasteurization	60–80 °C	Elimination of key pathogens and spoilage organisms
Appertization	$> 100\,^{\circ}C$	Elimination of micro-organisms to achieve 'commercial sterility'

Englishman Saddington in 1807 to preserve fruits and for which he too received a prize, this time of five guineas, from the Royal Society of Arts. British patents describing the use of iron or metal containers were issued to Durand and de Heine in 1810 and the firm of Donkin and Hall

established a factory for the production of canned foods in Bermondsey, London around 1812.

Appert held the view that the cause of food spoilage was contact with air and that the success of his technique was due to the exclusion of air from the product. This view persisted with sometimes disastrous consequences for another 50 years until Pasteur's work established the relationship between microbial activity and putrefaction. Today, the two types of heat process employed to destroy micro-organisms in food, pasteurization and appertization, bear the names of these eminent figures.

Pasteurization, the term given to heat processes typically in the range 60–80 °C and applied for up to a few minutes, is used for two purposes. First is the elimination of a specific pathogen or pathogens associated with a product. This type of pasteurization is often a legal requirement introduced as a public health measure when a product has been frequently implicated as a vehicle of illness. Notable examples are milk, bulk liquid egg (see Section 7.10.5) and ice cream mix, all of which have a much improved safety record as a result of pasteurization. The second reason for pasteurizing a product is to eliminate a large proportion of potential spoilage organisms, thus extending its shelf-life. This is normally the objective when acidic products such as beers, fruit juices, pickles, and sauces are pasteurized.

Where pasteurization is introduced to improve safety, its effect can be doubly beneficial. The process cannot discriminate between the target pathogen(s) and other organisms with similar heat sensitivity so a pasteurization which destroys say *Salmonella* will also improve shelf-life. The converse does not normally apply since products pasteurized to improve keeping quality are often considered as being intrinsically safe due to other factors such as low pH. This may be less true than was previously thought following several food poisoning outbreaks associated with unpasteurized fruit juices (see Section 7.7.5).

On its own, the contribution of pasteurization to extension of shelf-life can be quite small, particularly if the pasteurized food lacks other preservative factors such as low pH or a_w. Thermoduric organisms such as spore formers and some Gram positive vegetative species in the genera *Enterococcus*, *Microbacterium* and *Arthrobacter* can survive pasteurization temperatures. They can also grow and spoil a product quite rapidly at ambient temperatures, so refrigerated storage is often an additional requirement for an acceptable shelf-life.

Appertization refers to processes where the only organisms that survive processing are non-pathogenic and incapable of developing within the product under normal conditions of storage. As a result, appertized products have a long shelf-life even when stored at ambient

temperatures. The term was coined as an alternative to the still widely used description *commercially sterile* which was objected to on the grounds that sterility is not a relative concept; a material is either sterile or it is not. An appertized or commercially sterile food is not necessarily sterile – completely free from viable organisms. It is however free from organisms capable of growing in the product under normal storage conditions. Thus for a canned food in temperate climates, it is not a matter of concern if viable spores of a thermophile are present as the organism will not grow at the prevailing ambient temperature.

4.1.2 Quantifying the Thermal Death of Micro-organisms: D and *z* Values

In Chapter 3 we described how a micro-organism can grow only over a restricted range of temperature, defined by three cardinal temperatures. When the temperature is increased above the maximum for growth, cells are injured and killed as key cellular components are destroyed and cannot be replaced. This occurs at an increasing rate as the temperature increases.

The generally accepted view is that thermal death is a first order process, that is to say, at a given lethal temperature, the rate of death depends upon the number of viable cells present. We can express this mathematically as:

$$\mathrm{d}N/\mathrm{d}t = -cN \tag{4.1}$$

where $\mathrm{d}N/\mathrm{d}t$ is the rate of death, N is the number of viable cells present and c is a proportionality constant. The minus sign signifies that N is decreasing.

To obtain information about the number of cells surviving after different periods of heating, this equation can be integrated between time zero and time t to give:

$$\log_{\mathrm{e}}(N/N_0) = -ct \tag{4.2}$$

or

$$N = N_0 \mathrm{e}^{-ct} \tag{4.3}$$

where N and N_0 are the numbers of viable cells present at times t and 0 respectively.

It is more convenient to represent Equation (4.2) in terms of logarithms to the base 10 as:

$$\log_{10}(N/N_0) = -kt \tag{4.4}$$

where $k = c/\log_{\mathrm{e}}10 = c/2.303$.

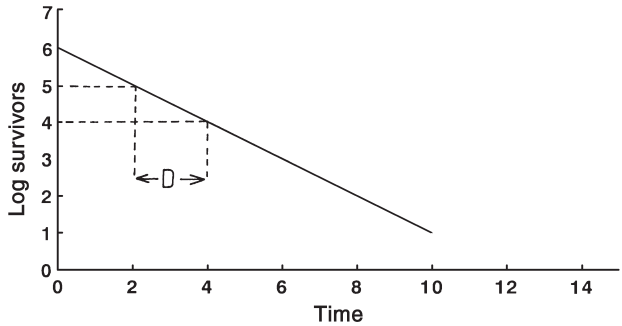

Figure 4.1 *The D value*

From Equation (4.4), it is clear that a plot of the log of the number of surviving cells at a given temperature against time should give a straight line with negative slope, k (Figure 4.1). As the temperature increases, so the slope of the survivor curve increases.

From this relationship we can derive a measure of an organism's heat resistance that is useful in calculating the lethality of heat processes. The D value or decimal reduction time is defined *as the time at a given temperature for the surviving population to be reduced by 1 log cycle, i.e.* 90%. The temperature at which a D value applies is indicated by a subscript, *e.g.* D_{65}.

A D value can be obtained from a plot of $\log_{10}$ survivors *versus* time, where it is the reciprocal of the slope, $1/k$. [You can confirm this by substituting $N_0 = 10N$ and $t = D$ in Equation (4.4).]

Alternatively, it can be calculated from:

$$D = (t_2 - t_1)/(\log N_1 - \log N_2) \tag{4.5}$$

where N_1 and N_2 are survivors at times t_1 and t_2 respectively.

One consequence of Equation (4.5) is that one can never predict with certainty how many decimal reductions a heat process must achieve (its lethality) for a product to be sterile since there is no log N_2 for $N_2 = 0$. When the initial microbial population in a batch of product is 10^n and a heat process producing n decimal reductions (nD) is applied, there will be one surviving organism in the product ($\log 1 = 0$). If you apply a more severe heat process, $(n+1)$D, $(n+2)$D or $(n+4)$D say, then the number of survivors will be 10^{-1},10^{-2} or 10^{-4} respectively. Physically, it is meaningless to talk of a fraction of an organism surviving, so these figures are interpreted as a probability of survival, corresponding to there being a 1 in 10, 1 in 100 and a 1 in 10 000 chance respectively that an organism will survive the heat process.

To give an example: if the D_{72} of *Salmonella* Senftenberg 775 W (the most heat-resistant salmonella) in milk is 1.5 s, then HTST pasteurization (15 s at 72 °C) will produce a 10D reduction in viable numbers. If we

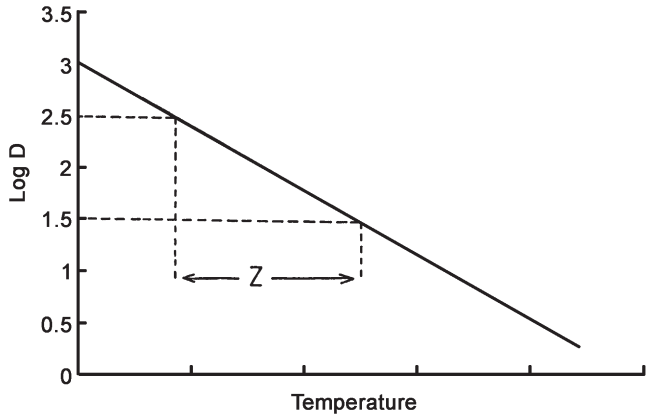

Figure 4.2 *The z-value*

assume the incidence of salmonella in the raw milk to be 1 colony-forming unit (cfu) 1^{-1}, then after pasteurization it will be reduced to 10^{-10} cfu 1^{-1} or 1 cfu $(10^{10}\ 1)^{-1}$. This means that if the milk is packed in 1 litre containers, one pack in 10 billion (10^{10}) would contain salmonella. If however the level of salmonella contamination was higher, say 10^4 cfu 1^{-1}, then the same heat treatment would result in contamination of one in a million packs. Such simple calculations underestimate the true lethal effect since they assume instantaneous heating and cooling.

As the temperature is increased so the D value decreases. This is an exponential process over the range of temperatures used in the heat processing of food so that plotting log D against temperature gives a straight line. From this we can derive another important parameter in heat processing, *z*: *the temperature change which results in a tenfold (1 log) change in D* (Figure 4.2).

$$z = (T_2 - T_1)/(\log D_1 - \log D_2) \tag{4.6}$$

Knowledge of an organism's *z* value is important if we are to take into account the lethal effect of the different temperatures experienced during a heat process.

4.1.3 Heat Sensitivity of Micro-organisms

The heat sensitivity of various micro-organisms is illustrated by Table 4.3 which shows their D values. Generally psychrotrophs are less heat resistant than mesophiles, which are less heat resistant than thermophiles; and Gram-positives are more heat resistant than Gram-negatives. Most vegetative cells are killed almost instantaneously at 100 °C and their D values are measured and expressed at temperatures appropriate to pasteurization.

Table 4.3 *Microbial heat resistance*

Vegetative organisms ($z \sim 5\,^{\circ}C$)	*D (mins)*	
Salmonella sp.	D_{65}	0.02–0.25
Salmonella Senftenberg	D_{65}	0.8–1.0
Staphylococcus aureus	D_{65}	0.2–2.0
Escherichia coli	D_{65}	0.1
Yeasts and moulds	D_{65}	0.5–3.0
Listeria monocytogenes	D_{60}	5.0–8.3
Campylobacter jejuni	D_{55}	1.1
Bacterial Endospores ($z \sim 10\,^{\circ}C$)	D_{121}	
B. stearothermophilus		4–5
C. thermosaccharolyticum		3–4
Desulfotomaculum nigrificans		2–3
B. coagulans		0.1
C. botulinum types A & B		0.1–0.2
C. sporogenes		0.1–1.5
C. botulinum type E	D_{80}	0.1–3.0
	D_{110}	<1 second

Bacterial spores are usually far more heat resistant than vegetative cells; thermophiles produce the most heat resistant spores while those of psychrotrophs and psychrophiles are most heat sensitive. Since spore inactivation is the principal concern in producing appertized foods, much higher temperatures are used in appertization processes and in the measurement of spore D values.

Yeast ascospores and the asexual spores of moulds are only slightly more heat resistant than the vegetative cells and will normally be killed by temperatures at or below 100 °C, *e.g.* in the baking of bread. Ascospores of the mould *Byssochlamys fulva*, and a few other ascomycetes do show a more marked heat resistance and can be an occasional cause of problems in canned fruits which receive a relatively mild heat process (see Section 5.5.4).

The heat resistance exhibited by the bacterial endospore is due mainly to its ability to maintain a very low water content in the central DNA-containing protoplast; spores with a higher water content have a lower heat resistance. The relative dehydration of the protoplast is maintained by the spore cortex, a surrounding layer of electronegative peptidoglycan which is also responsible for the spore's refractile nature. The exact mechanism by which it does this is not known, although it may be some combination of physical compression of the protoplast by the cortex and osmotic extraction of the water. As the cortex is dissolved during germination and the protoplast rehydrates, so the spore's heat resistance declines. Suspension of a germinated spore population in a strong solution of a non-permeant solute such as sucrose will reverse this process of rehydration and restore the spore's

heat resistance. The total picture is probably more complex than this however, since other features of the spore such as its high content of divalent cations, particularly calcium, are thought to make some contribution to heat resistance.

Thermal sensitivity as measured by the D value can vary with factors other than the intrinsic heat sensitivity of the organism concerned. This is most pronounced with vegetative cells where the growth conditions and the stage of growth of the cells can have an important influence. For example, stationary phase cells are generally more heat resistant than log phase cells. Heat sensitivity is also dependent on the composition of the heating menstruum; cells tend to show greater heat sensitivity as the pH is increased above 8 or decreased below 6. Fat enhances heat resistance as does decreasing a_w through drying or the addition of solutes such as sucrose. The practical implications of this can be seen in the more severe pasteurization conditions used for high sugar or high fat products such as ice cream mix and cream compared with that used for milk. This effect is quite dramatic in the instance of milk chocolate where the D_{70} value of *Salmonella* Senftenberg 775 W has been measured as between 6 and 8 hours compared with only a few seconds in milk. A more specialized example of medium effects on heat sensitivity occurs in brewing where the ethanol content of beer has been shown to have a profound effect on the heat sensitivity of a spoilage *Lactobacillus*; an observation that has implications for the pasteurization of low-alcohol beers (Figure 4.3).

At present all thermal process calculations are based on the assumption that the death of micro-organisms follows the log-linear kinetics described by Equation 4.4. Though this is often the case, deviations from log-linear behaviour are also often observed (Figure 4.4). Sometimes these deviations can be rationalized on the basis of some special property of the organism. For example, an apparent increase in viable numbers of organisms or a lag at the start of heating may be ascribed to heat activation of spores so that in the first moments of heating the number of spores being activated equals or exceeds the number being destroyed. Alternatively a lag phase may reflect the presence of clumps of cells, all of which require to be inactivated before that colony forming unit is destroyed. The frequently observed tailing of the curves, which has greater practical significance, may be due to sub-populations of cells that are more heat resistant. These deviations from the accepted model tend to be observed more often when studying the thermal death of vegetative organisms and in some cases may reflect inadequacy of the logarithmic death concept in this situation.

The primary assumption which gives rise to log-linear kinetics is that at a constant temperature each cell has an equal chance of inactivation at any instant. This can be explained on theoretical grounds if there is a

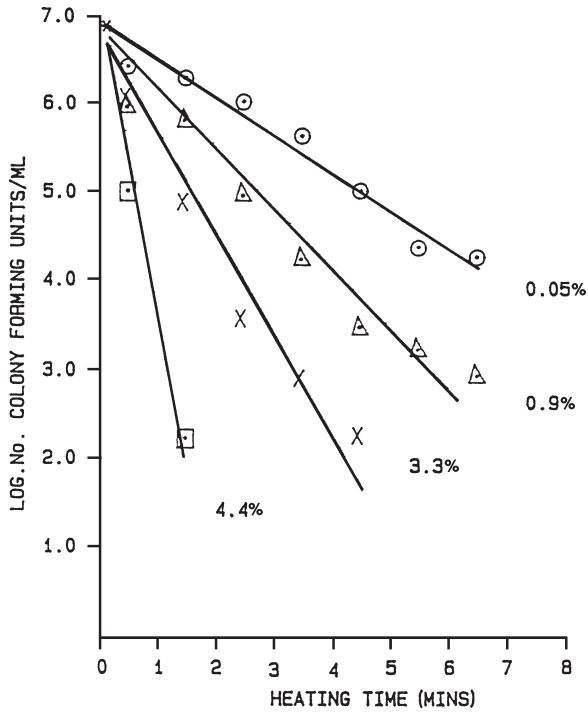

Figure 4.3 *Effect of ethanol on the survival of a lactobacillus in beer at 60 °C*

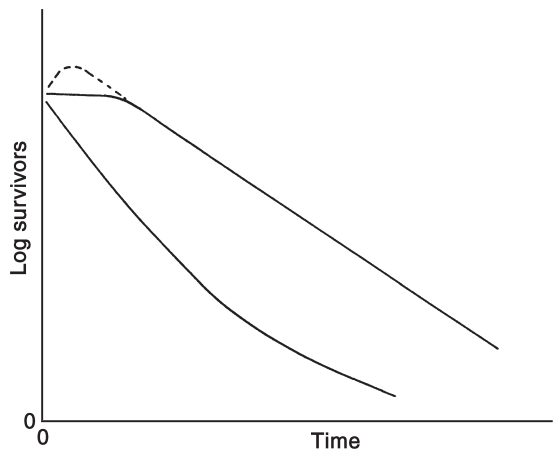

Figure 4.4 *Deviation from log-linear death kinetics*

single target molecule in each cell whose inactivation causes death. If this is not the case and there is, say, a large number of the same target molecules present or a large number of different targets, then several inactivation events would be required for cell death and a lag would be observed in the thermal death curve.

Damage to DNA has been identified as the probable key lethal event in both spores and vegetative cells. In spores, however, inactivation of germination mechanisms is also important. If this inactivation can be bypassed in some way, then apparently dead spores may be cultured. This has been demonstrated by the inclusion of lysozyme in recovery media where the enzyme hydrolyses the spore cortex, replacing the spore's own inactivated germination system.

Deviations from log-linear kinetics in the thermal death of vegetative cells probably reflect a greater multiplicity of target sites for thermal inactivation such as the cytoplasmic membrane, key enzymes, RNA and the ribosomes. This type of damage can be cumulative rather than instantly lethal. Individual inactivation events may not kill the cell but will inflict sub-lethal injury making it more vulnerable to other stresses. If however injured cells are allowed time in a non-inhibitory medium, they can repair and recover their full vigour. Examples of sub-lethal damage can be seen when cells do not grow aerobically but can be cultured anaerobically or in the presence of catalase, or when selective agents such as bile salts or antibiotics, which are normally tolerated by the organism, prove inhibitory.

Two other factors also contribute to deviations from log-linear behaviour in vegetative organisms. Individual cells within a population may exhibit a broader range of heat resistance than is seen with spores and, since vegetative cells are not metabolically inert, they may also respond and adapt to a heating regime modifying their sensitivity.

4.1.4 Describing a Heat Process

Heating processes are neither uniform nor instantaneous. To be able to compare the lethal effect of different processes it is necessary for us to have some common currency to describe them. For appertization processes this is known as the F value; a parameter which expresses the integrated lethal effect of a heat process in terms of minutes at a given temperature indicated by a subscript. A process may have an F_{121} value of say 4, which means that its particular combination of times and temperatures is *equivalent* to instantaneous heating to 121 °C, holding at that temperature for four minutes and then cooling instantly, it does not even necessarily imply that the product ever reaches 121 °C. The F value will depend on the z value of the organism of concern; if $z = 10$ °C then 1 minute at 111 °C has an $F_{121} = 0.1$, if $z = 5$ °C then the F_{121} value will be 0.01. It is therefore necessary to specify both the z value and the temperature when stating F. For spores z is commonly about 10 °C and the F_{121} determined using this value is designated F_0.

To determine the F_0 value required in a particular process one needs to know the D_{121} of the target organism and the number of decimal reductions considered necessary.[†]

$$F_{121} = D_{121}(\log N_0 - \log N) \tag{4.7}$$

In this exercise, the canner will have two objectives, a safe product and a stable product. From the point of view of safety in low acid canned foods (defined as those with a pH > 4.5) *Clostridium botulinum* is the principle concern. The widely accepted minimum lethality for a heat process applied to low-acid canned foods is that it should produce 12 decimal reductions in the number of surviving *C. botulinum* spores ($\log N_0 - \log N = 12$). This is known as the 12D or botulinum cook. If D_{121} of *C. botulinum* is 0.21 minutes then a botulinum cook will have an F_0 of $12 \times 0.21 = 2.52$ min. The effect of applying a process with this F_0 to a product in which every can contains one spore of *C. botulinum* ($N_0 = 1$) will be that a spore will survive in one can out of every 10^{12}.

The canner also has the objective of producing a product which will not spoil at an unacceptably high rate. Since spoilage is a more acceptable form of process failure than survival of *C. botulinum*, the process lethality requirements with respect to spoilage organisms do not need to be so severe. In deciding the heat process to be applied, a number of factors have to be weighed up.

(1) What would be the economic costs of a given rate of spoilage?
(2) What would be the cost of additional processing to reduce the rate of spoilage?
(3) Would this additional processing result in significant losses in product quality?

Most canners would regard an acceptable spoilage rate due to underprocessing as something around 1 in 10^5–10^6 cans and this is normally achievable through 5–6 decimal reductions in the number of spores with spoilage potential (the USFDA use 6D as their yardstick). PA3679, *Clostridium sporogenes* is frequently used as an indicator for process spoilage and typically has a D_{121} of about 1 min. This will translate into a process with an F_0 value of 5–6; sufficient to produce about 24–30 decimal reductions in viable *C. botulinum* spores – well in excess of the minimal requirements of the botulinum cook. Some typical F_0 values used in commercial canning are presented in Table 4.4.

[†] For pasteurization treatments, an analogous procedure is used to express processes in terms of their P value or pasteurization units. Once again the reference temperature must be specifed. This can be 60 °C, in which case: $P_{60} = D_{60}(\log N_0 - \log N)$.

Table 4.4 *Typical F_0 values for some canned foods*

Food	*F_0 (min)*
Asparagus	2–4
Beans in tomato sauce	4–6
Carrots	3–4
Peas	4–6
Milk pudding	4–10
Meats in gravy	8–10
Potatoes	4–10
Mackerel in brine	3–4
Meat loaf	6
Chocolate pudding	6

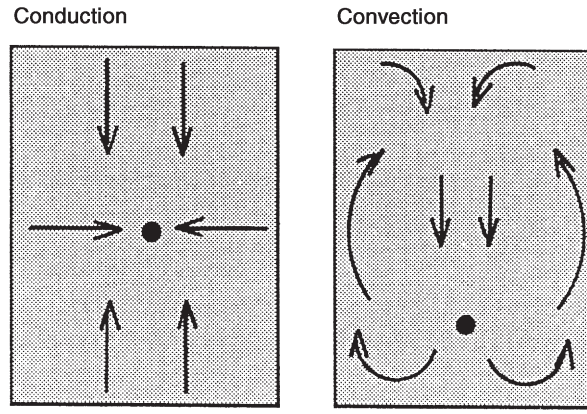

Figure 4.5 *Conduction and convection heating in cans.* ● *Denotes slowest heating point*

Having decided the *F* value required, it is necessary to ensure that the F_0 value actually delivered by a particular heating regime achieves this target value. To do this, the thermal history of the product during processing is determined using special cans fitted with thermocouples to monitor the product temperature. These must be situated at the slowest heating point in the pack where the F_0 value will be at a minimum. The precise location of the slowest heating point and the rate at which its temperature increases depend on the physical characteristics of the can contents. Heat transfer in solid foods such as meats is largely by conduction which is a slow process and the slowest heating point is the geometric centre of the can (Figure 4.5). When fluid movement is possible in the can, heating is more rapid because convection currents are set up which transfer heat more effectively. In this case the slowest heating point lies on the can's central axis but nearer the base.

The slowest heating point is not always easy to predict. It may change during processing as in products which undergo a sol–gel transition during heating, producing a broken heating curve which shows a phase

of convection heating followed by one of conduction heating. In most cases heating is by conduction but some can contents show neither pure convection nor pure conduction heating and the slowest heating point must be determined experimentally.

Movement of material within the can improves heat transfer and will reduce the process time. This is exploited in some types of canning retort which agitate the cans during processing to promote turbulence in the product.

The F value can be computed from the thermal history of a product by assigning a lethal rate to each temperature on the heating curve. The lethal rate, L_R, at a particular temperature is the ratio of the microbial death rate at that temperature to the death rate at the lethal rate reference temperature. For example, using 121 °C as the reference temperature:

$$L_R = D_{121}/D_T \tag{4.8}$$

where L_R is the lethal rate at 121 °C.

Since

$$z = (T_2 - T_1)/(\log D_1 - \log D_2) \tag{4.9}$$

and substituting $T_2 = 121$ °C; $T_1 = T$; $D_2 = D_{121}$ and $D_1 = D_T$

$$L_R = 1/10^{(121-T)/z} \tag{4.10}$$

Lethal rates calculated in this way can be obtained from published tables where the L_R can be read off for each temperature (from about 90 °C and above) and for a number of different z values (Table 4.5). Nowadays though this is unnecessary since the whole process of F value calculation tends to be computerized.

Total lethality is the sum of the individual lethal rates over the whole process; for example 2 minutes at a temperature whose L_R is 0.1 contributes 0.2 to the F_0 value, 2 minutes at a L_R of 0.2 contributes a further 0.4, and so on. Another way of expressing this is that the area

Table 4.5 *Selected lethal rate values ($F_{121.1}$ min^{-1})*

	z value					
Temp. (°C)	*7*	*8*	*9*	*10*	*11*	*12*
100.0	0.001	0.002	0.005	0.008	0.012	0.017
101.0	0.001	0.003	0.006	0.010	0.015	0.021
102.0	0.002	0.004	0.008	0.012	0.018	0.026
103.0	0.003	0.005	0.010	0.015	0.023	0.031
104.0	0.004	0.007	0.013	0.019	0.028	0.038
105.0	0.005	0.010	0.016	0.024	0.034	0.045
110.0	0.026	0.041	0.058	0.077	0.098	0.119
115.0	0.134	0.172	0.209	0.245	0.278	0.310
120.0	0.694	0.727	0.753	0.774	0.793	0.808

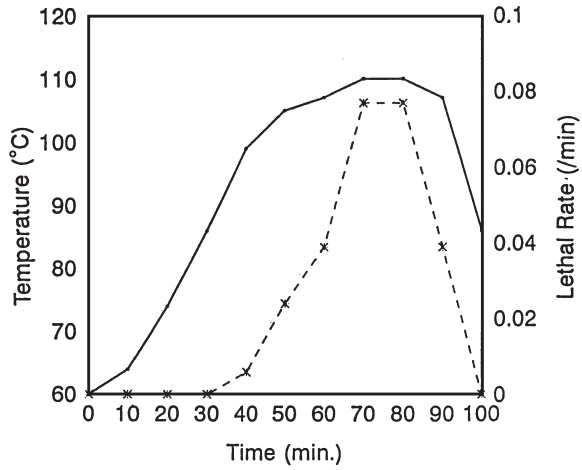

Figure 4.6 *A lethal rate plot.* ● *Product temperature;* × *lethal rate*

under a curve describing a plot of lethal rate against time gives the overall process lethality, F_0 (Figure 4.6).

$$F_0 = \int L_R \, dt \tag{4.11}$$

This procedure has safeguards built into it. If the slowest heating point receives an appropriate treatment then the lethality of the process elsewhere in the product will be in excess of this. A further safety margin is introduced by only considering the heating phase of the process; the cooling phase, although short, will also have some lethal effect.

Process confirmation can also be achieved by microbiological testing in which inoculated packs are put through the heat process and the spoilage/survival rate determined. Heat penetration studies though give much more precise and useable information since inoculated packs are subject to culture variations which can affect resistance and also recovery patterns.

A change in any aspect of the product or its preparation will require the heat process to be re-validated and failure to do this could have serious consequences. An early example of this was the scandal in the mid-nineteenth century when huge quantities of canned meat supplied to the Royal Navy putrefied leading to the accusation that the meat had been bad before canning. It transpired that the problem arose because cans with a capacity of 9–14 lb were being used instead of the original 2–6 lb cans. In these larger cans the centre of the pack took longer to heat and did not reach a temperature sufficient to kill all the bacteria. More recently, replacement of sugar with an artificial sweetener in hazelnut puree meant that spores of *C. botulinum* surviving the mild heat process

given to the product were no longer prevented from growing by the reduced a_w (see Section 7.5.5).

4.1.5 Spoilage of Canned Foods

If a canned food contains viable micro-organisms capable of growing in the product at ambient temperatures, then it will spoil. Organisms may be present as a result of an inadequate heat process, underprocessing, or of post process contamination through container leakage. Spoilage by a single spore former is often diagnostic of underprocessing since rarely would such a failure be so severe that vegetative organisms would survive.

A normal sound can will either be under vacuum with slightly concave ends or have flat ends in those cases where the container is brimful. Spoilage often manifests itself through microbial gas production which causes the ends to distend and a number of different terms are used to describe the extent to which this has occurred (Table 4.6). The spore-forming anaerobes *Clostridium* can be either predominantly proteolytic or saccharolytic but both activities are normally accompanied by gas production causing the can to swell. Cans may sometimes swell as a result of chemical action. Defects in the protective lacquer on the inside of the can may allow the contents to attack the metal releasing hydrogen. These hydrogen swells can often be distinguished from microbiological spoilage since the appearance of swelling occurs after long periods of storage and the rate at which the can swells is usually very slow.

In cases where microbial growth occurs without gas production, spoilage will only be apparent once the pack has been opened. *Bacillus* species, with the exceptions of *B. macerans* and *B. polymyxa*, usually break down carbohydrates to produce acid but no gas giving a type of spoilage known as a 'flat sour', which describes the characteristics of both the can and the food.

The heat process a product receives is determined largely by its acidity: the more acidic a product is, the milder the heat process applied.

Table 4.6 *Description of blown cans*

Name	*Description*
Flat	No evidence of swelling.
Hard swell	Both ends of the can are permanently and firmly bulged and do not yield readily to thumb pressure.
Soft swell	Both ends bulged but not tightly; they yield to thumb pressure.
Springer	One end flat, the other bulged. When the bulged end is pressed in then the flat one springs out.
Flipper	A can with a normal appearance which when brought down sharply on a flat surface causes a flat end to flip out. The bulged end can be forced back by very slight pressure.

Although more complex schemes have been described, the essential classification of canned foods is into low acid (pH > 4.5, or 4.6 in the United States) and acid foods (pH < 4.5 or 4.6). We have already seen how this is applied to assure safety with the requirement that products with a pH > 4.5 must undergo a botulinum cook to ensure 12 decimal reductions of *C. botulinum* spores. This is not a concern in acid foods as *C. botulinum* cannot grow and the F_0 applied to products with a pH in the range 4.0–4.5 such as canned tomatoes and some canned fruits is generally 0.5–3.0. In higher acidity products such as canned citrus fruits (pH < 3.7) the heat process is equivalent only to a pasteurization.

A product's acidity also determines the type of spoilage that may result from underprocessing since it can prevent the growth of some spoilage organisms. At normal ambient temperatures (< 38 °C) only mesophilic species will grow. Typical examples would be *C. botulinum*, *C. sporogenes* and *B. subtilis* in low acid products and *C. butyricum* and *C. pasteurianum* in products with a pH below 4.5.

Cans are cooled rapidly after processing to prevent spoilage by thermophiles. Thermophilic spores are more likely to survive the normal heat process but would not normally pose a problem. If however a large assemblage of cans is allowed to cool down naturally after retorting, the process will be slow and the cans will spend some time passing through the thermophilic growth range. Under these conditions surviving thermophilic spores may be able to germinate and grow, spoiling the product before it cools. This may also occur if cans are stored at abnormally high ambient temperatures (>40 °C) and canned foods destined for very hot climates may receive a more stringent process to reduce thermophilic spoilage.

Thermophilic organisms commonly associated with spoilage of low acid canned foods are the saccharolytic organism *C. thermosaccharolyticum, B. stearothermophilus* and *Desulfotomaculum nigrificans*. The last of these causes a type of spoilage known as 'sulfur stinker'. It produces hydrogen sulfide which does not usually distend the can but does give the product an objectionable smell and reacts with iron from the can to cause blackening.

Leakage is the most common cause of microbiological spoilage in canned foods. Cans are the most common containers used for retorted products, although glass jars, rigid plastic containers and soft pouches are also sometimes used. Cans are usually made of two or three parts: the three-part can consists of a base, body and lid while in two part cans the body and base are made from a single piece of metal. In a three-part can the body seam is electrically welded but the lid on all cans is held in place by a double seam (Figure 4.7). The correct formation and integrity of this seam are crucial to preventing leakage and monitoring seam integrity is an important aspect of quality control procedures in canning.

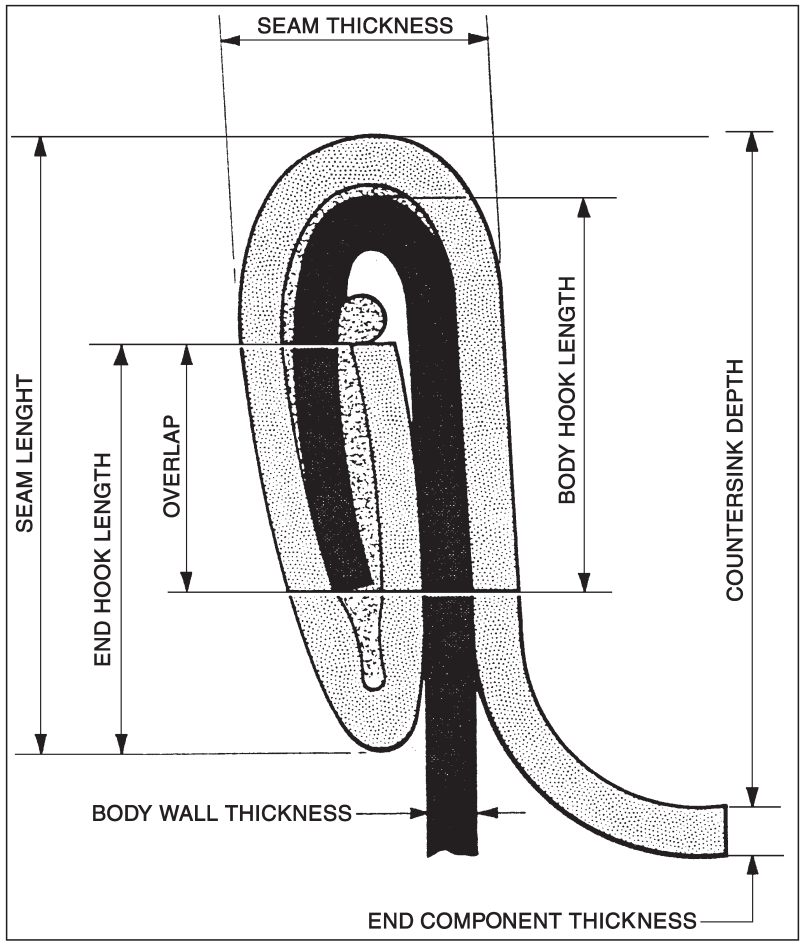

Figure 4.7 *The can double seam (Carnaud Metalbox)*

During processing cans are subjected to extreme stress, particularly when the hot can is cooled down rapidly from processing temperature. The negative pressure created in the can under these conditions could lead to micro-organisms on the container's surface or in the cooling water being sucked inside through a small defect in the seam. The defect in the hot can that allowed leakage to occur may seal up and be undetectable when the can is cool so leaker spoilage can cause cans to blow. Since the micro-organisms enter the can after processing there is no restriction on the type of organism capable of causing leaker spoilage, therefore the presence of a mixed culture or non-sporing organisms is almost certainly a result of can leakage.

To prevent leaker spoilage it is essential that the outside of cans is clean and uncontaminated and that chlorinated water is used to cool them. Failures in this respect have been the cause of a large typhoid outbreak in Aberdeen, Scotland where cans of corned beef made in the Argentine had been cooled with river water contaminated with *Salmonella* Typhi and in an outbreak of botulism associated with canned salmon where the *C. botulinum* type E spores which were associated with the raw product contaminated the outside of the cans after processing and were sucked into one can during cooling.

There have been occasional reports of pre-process spoilage in canned foods where there was an unacceptable delay between preparing the product and heat processing. During this time spoilage may occur although the organisms responsible will have been killed by the heat process.

4.1.6 Aseptic Packaging

Up until now in our consideration of appertized foods we have discussed only retorted products; those which are hermetically sealed into containers, usually cans, and then subjected to an appertizing heat process in-pack. While this has been hugely successful as a long-term method of food preservation, it does require extended heating periods in which a food's functional and chemical properties can be adversely affected.

In UHT processing the food is heat processed before it is packed and then sealed into sterilized containers in a sterile environment. This approach allows more rapid heating of the product, the use of higher temperatures than those employed in canning, typically 130–140 °C, and processing times of seconds rather than minutes. The advantage of using higher temperatures is that the z value for chemical reactions such as vitamin loss, browning reactions and enzyme inactivation is typically 25–40 °C compared with 10 °C for spore inactivation. This means that they are less temperature sensitive so that higher temperatures will increase the microbial death rate more than they increase the loss of food quality associated with thermal reactions.

F_0 values for UHT processes can be estimated from the holding temperature (T) and the residence time of the fastest moving stream of product, t.

$$F_0 = t.10^{(T-121)/10} \tag{4.12}$$

Initially UHT processing and aseptic packaging were confined to liquid products such as milk, fruit juices and some soups which would heat up very quickly due to convective heat transfer. If a food contained solid particles larger than about 5 mm diameter it was unsuited to the rapid processing times due to the slower conductive heating of the particulate

phase. Scraped surface heat exchangers have been used to process products containing particles up to 25 mm in diameter but at the cost of overprocessing the liquid phase. To avoid this, one system processes the liquid and solid phase separately. A promising alternative is the use of ohmic heating in which a food stream is passed down a tube which contains a series of electrodes. An alternating voltage is applied across the electrodes and the food's resistance causes it to heat up rapidly. Most of the energy supplied is transformed into heat and the rate at which different components heat up is determined by their conductivities rather than heat transfer.

A common packing system used in conjunction with UHT processing is a form/fill/seal operation in which the container is formed in the packaging machine from a reel of plastic or laminate material, although some systems use preformed containers. Packaging material is generally refractory to microbial growth and the level of contamination on it is usually very low. Nevertheless to obtain commercial sterility it is given a bactericidal treatment, usually with hydrogen peroxide, sometimes coupled with UV irradiation.

4.2 IRRADIATION

Electromagnetic (e.m.) radiation is a way in which energy can be propagated through space. It is characterized in terms of its wavelength λ, or its frequency ν, and the product of these two properties gives the speed, c, at which it travels (3×10^8 m sec^{-1} in a vacuum).

$$\lambda\nu = \text{c} \tag{4.13}$$

The range of frequencies (or wavelengths) that e.m. radiation can have is known as the electromagnetic spectrum and is grouped into a number of regions, visible light being only one small region (Figure 4.8).

The energy carried by e.m. radiation is not continuous but is transmitted in discrete packets or quanta; the energy, E, contained in each quantum being given by the expression:

$$E = h\nu \tag{4.14}$$

where h is a constant (6.6×10^{-27} ergs sec^{-1}) known as Planck's constant. Thus, the higher the frequency of the radiation the higher its quantum energy.

As far as food microbiology is concerned, only three areas of the e.m. spectrum concern us; microwaves, the UV region and gamma rays. We will now consider each of these in turn.

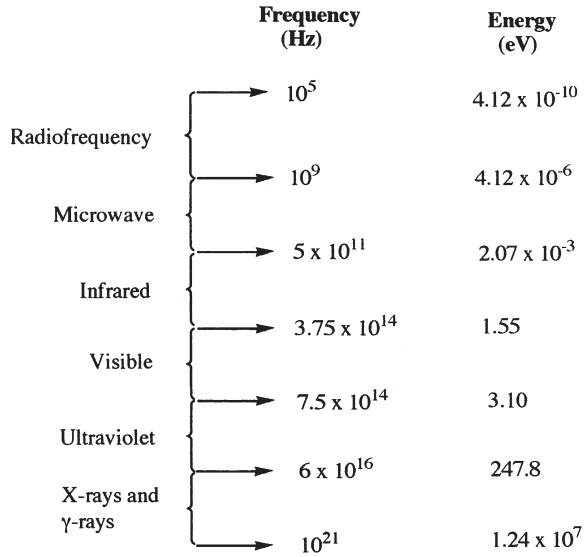

Figure 4.8 *The electromagnetic spectrum*

4.2.1 Microwave Radiation

The microwave region of the e.m. spectrum occupies frequencies between 10^9 Hz up to 10^{12} Hz and so has a relatively low quantum energy. For the two frequencies used in food processing, 2450 MHz and 915 MHz, this is around 10^{-18} ergs or 10^{-6} eV. Domestic microwave ovens use 2450 MHz which is less penetrating than the lower frequency.

Unlike the other forms of radiation we will discuss, microwaves act indirectly on micro-organisms through the generation of heat. When a food containing water is placed in a microwave field, the dipolar water molecules align themselves with the field. As the field reverses its polarity about 2.5×10^9 times each second the water molecules are continually oscillating. This kinetic energy is transmitted to neighbouring molecules leading to a rapid rise in temperature throughout the product. In foods with a high salt content, surface heating due to ions acquiring kinetic energy from the microwave field can also contribute, but this is generally of minor importance.

Microwaves are generated using a magnetron, a device first developed in the UK during research into radar during the Second World War. Magnetrons are used both commercially and domestically, but their biggest impact has been in the domestic microwave oven and in catering where their speed and convenience have enormous advantages. The principal problem associated with the domestic use of microwaves is non-uniform heating of foods, due to the presence of cold spots in the oven, and the non-uniform dielectric properties of the food. These can

lead to cold spots in some microwaved foods and concern over the risks associated with consumption of inadequately heated meals has led to more explicit instructions on microwaveable foods. These often specify a tempering period after heating to allow the temperature to equilibrate.

Microwaves have been slow to find industrial applications in food processing, although they are used in a number of areas. Microwaves have been used to defrost frozen blocks of meat prior to their processing into products such as burgers and pies thus reducing wear and tear on machinery. There has also been a limited application of microwaves in the blanching of fruits and vegetables and in the pasteurization of soft bakery goods and moist (30% H_2O) pasta to destroy yeasts and moulds. In Japan, microwaves have been used to pasteurize high-acid foods, such as fruits in syrup, intended for distribution at ambient temperature. These are packed before processing and have an indefinite microbiological shelf-life because of the heat process and their low pH. However, the modest oxygen barrier properties of the pack has meant that their biochemical shelf-life is limited to a few months.

4.2.2 UV Radiation

UV radiation has wavelengths below 450 nm ($\nu \simeq 10^{15}$ Hz) and a quantum energy of 3–5 eV (10^{-12} ergs). The quanta contain energy sufficient to excite electrons in molecules from their ground state into higher energy orbitals making the molecules more reactive. Chemical reactions thus induced in micro-organisms can cause the failure of critical metabolic processes leading to injury or death.

Only quanta providing energy sufficient to induce these photochemical reactions will inhibit micro-organisms, so those wavelengths that are most effective give us an indication of the sensitive chemical targets within the cell. The greatest lethality is shown by wavelengths around 260 nm which correspond to a strong absorption by nucleic acid bases. The pyrimidine bases appear particularly sensitive, and UV light at this wavelength will, among other things, induce the formation of covalently linked dimers between adjacent thymine bases in DNA (Figure 4.9). If left intact these will prevent transcription and DNA replication in affected cells.

The resistance of micro-organisms to UV is largely determined by their ability to repair such damage, although some organisms such as micrococci also synthesize protective pigments. Generally, the resistance to UV irradiation follows the pattern:

$$\textit{Gram-negatives} < \textit{Gram-positives} \approx \textit{yeasts} < \textit{bacterial spores} < \textit{mould spores} < < \textit{viruses}.$$

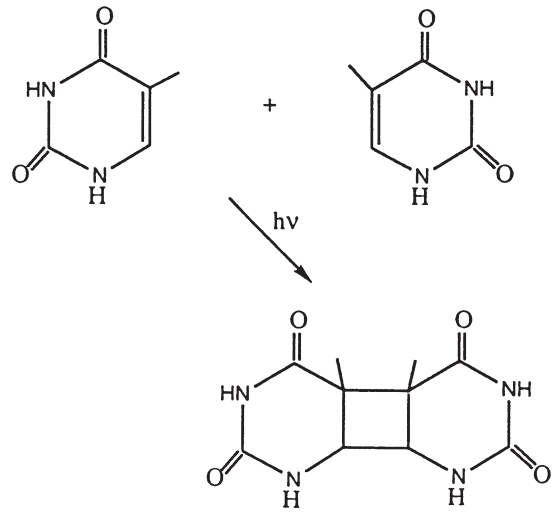

Figure 4.9 *The photochemical dimerization of thymine*

Table 4.7 *UV resistance of some selected food-borne micro-organisms*

Species	*D (ergs × 10^2)*
E. coli	3–4
Proteus vulgaris	3–4
Serratia marcescens	3–4
Shigella flexneri	3–4
Pseudomonas fluorescens	3–4
Bacillus subtilis (vegetative cells)	6–8
Bacillus subtilis (spores)	8–10
Micrococcus luteus	10–20
Staph. aureus	3–4
Aspergillus flavus	50–100
Mucor racemosus	20–50
Penicillium roquefortii	20–50
Rhizopus nigrificans	>200
Saccharomyces cerevisiae	3–10

Data from 'Microbial Ecology of Foods'. Vol. 1.ICMSF.

Death of a population of UV-irradiated cells demonstrates log-linear kinetics similar to thermal death and, in an analogous way, D values can be determined. These give the dose required to produce a tenfold reduction in surviving numbers where the dose, expressed in ergs or μWs, is the product of the intensity of the radiation and the time for which it is applied. Some published D values are presented in Table 4.7.

Determination of UV D values is not usually a straightforward affair since the incident radiation can be absorbed by other medium components and has very low penetration. Passage through 5 cm of clear water

will reduce the intensity of UV radiation by two-thirds. This effect increases with the concentration of solutes and suspended material so that in milk 90% of the incident energy will be absorbed by a layer only 0.1 mm thick. This low penetrability limits application of UV radiation in the food industry to disinfection of air and surfaces.

Low-pressure mercury vapour discharge lamps are used: 80% of their UV emission is at a wavelength of 254 nm which has 85% of the biological activity of 260 nm. Wavelengths below 200 nm are screened out by surrounding the lamp with an absorbent glass since these wavelengths are absorbed by oxygen in the air producing ozone which is harmful. The output of these lamps falls off over time and they need to be monitored regularly.

Air disinfection is only useful when the organisms suspended in air can make a significant contribution to the product's microflora and are likely to harm the product; for example, in the control of mould spores in bakeries. UV lamps have also been mounted in the head space of tanks storing concentrates, the stability of which depends on their low a_w. Fluctuations in temperature can cause condensation to form inside the tank. If this contacts the product, then areas of locally high a_w can form where previously dormant organisms can grow, spoiling the product. Process water can be disinfected by UV; this avoids the risk of tainting sometimes associated with chlorination, although the treated water will not have the residual antimicrobial properties of chlorinated water. UV radiation is commonly used in the depuration of shellfish to disinfect the water recirculated through the depuration tanks. Chlorination would not be suitable in this situation since residual chlorine would cause the shellfish to stop feeding thus stopping the depuration process.

Surfaces can be disinfected by UV, although protection of micro-organisms by organic material such as fat can reduce its efficacy. Food containers are sometimes treated in this way and some meat chill store rooms have UV lamps to retard surface growth. UV can however induce spoilage of products containing unsaturated fatty acids where it accelerates the development of rancidity. Process workers must also be protected from UV since the wavelengths used can cause burning of the skin and eye disorders.

4.2.3 Ionizing Radiation

Ionizing radiation has frequencies greater than 10^{18} Hz and carries sufficient energy to eject electrons from molecules it encounters. In practice three different types are used.

(1) *High-energy electrons*. in the form of β particles produced by radioactive decay or machine generated electrons. Strictly

speaking they are particles rather than electromagnetic radiation, although in some of their behaviour they do exhibit the properties of waves. Because of their mass and charge, electrons tend to be less penetrating than ionizing e.m. radiation; for example, 5 MeV β particles will normally penetrate food materials to a depth of about 2.5 cm.

(2) *X-rays* generated by impinging high energy electrons on a suitable target.

(3) *Gamma γ rays* produced by the decay of radioactive isotopes. The most commonly used isotope cobalt 60, ^{60}Co, is produced by bombarding non-radioactive cobalt, ^{59}Co, with neutrons in a nuclear reactor. It emits high-energy γ-rays (1.1 MeV) which can penetrate food up to a depth of 20 cm (*cf.* β particles). An isotope of caesium, ^{137}Cs, which is extracted from spent nuclear fuel rods, has also been used but is less favoured for a number of reasons.

Ionizing radiation can affect micro-organisms directly by interacting with key molecules within the microbial cell, or indirectly through the inhibitory effects of free radicals produced by the radiolysis of water (Figure 4.10). These indirect effects play the more important role since in the absence of water, doses 2–3 times higher are required to obtain the same lethality. Removal of oxygen also increases microbial resistance 2–4 fold and it is thought that this may be due to the ability of oxygen to participate in free radical reactions and prevent the repair of radiation induced lesions. As with UV irradiation, the main site of damage in cells is the chromosome. Hydroxyl radicals cause single- and double-strand breaks in the DNA molecule as a result of hydrogen abstraction from deoxyribose followed by β-elimination of phosphate which cleaves the molecule. They can also hydroxylate purine and pyrimidine bases.

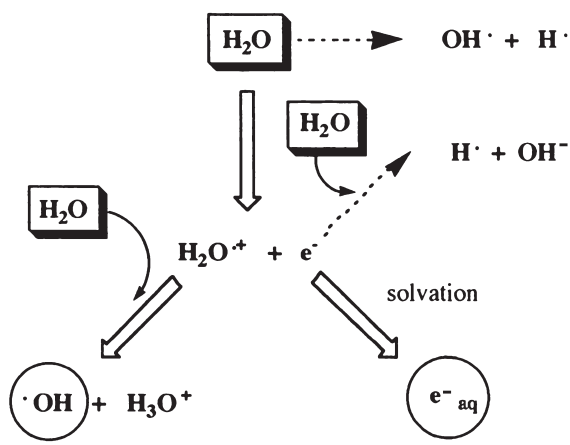

Figure 4.10 *The radiolysis of water.* ○ *Denotes main reactive radicals*

Resistance to ionizing radiation depends on the ability of the organism to repair the damage caused. Inactivation kinetics are generally logarithmic, although survival curves often appear sigmoidal exhibiting a shoulder and a tail to the phase of log-linear death. The shoulder is usually very slight but is more pronounced with bacteria which have more efficient repair mechanisms where substantially more damage can be accumulated before death ensues.

D values can be derived from the linear portion of these curves and Table 4.8 presents 6D values (the dose to produce a millionfold reduction) reported for a number of foodborne organisms. These are expressed in terms of the absorbed dose of ionizing radiation which is measured in Grays ($1\ \text{Gy} = 1\ \text{joule}\,\text{kg}^{-1}$). Resistance generally follows the sequence:

Gram-negative < *Gram-positive* ≈ *moulds* < *spores* ≈ *yeasts* < *viruses*.

Food-associated organisms do not generally display exceptional resistance, although spores of some strains of *Clostridium botulinum* type A have the most radiation resistant spores. Since studies on food irradiation started, a number of bacteria which are highly resistant to radiation have been isolated and described. Although one of these, *Deinococcus radiodurans*, was first isolated from meat, their role in foods is not significant in the normal course of events.

Although patents describing the use of ionizing radiation in the treatment of food appeared soon after the discovery of radioactivity at the turn of the 20th century, it was not until after the Second World War that food irradiation assumed commercial potential. This was largely due to technological advances during the development of nuclear weapons,

Table 4.8 *Radiation resistance of some foodborne micro-organisms*

Species	*6D dose (KGy)*
E. coli	1.5–3.0
Salmonella Enteritidis	3–5
S. Typhimurium	3–5
Vibrio parahaemolyticus	<0.5–1
Pseudomonas fluorescens	0.5–1
Bacillus cereus	20–30
B. stearothermophilus	10–20
C. botulinum type A	20–30
Lactobacillus spp.	2–7.5
Micrococcus spp.	3–5
Deinococcus radiodurans	>30
Aspergillus flavus	2–3
Penicillium notatum	1.5–2
S. cerevisiae	7.5–10
Viruses	>30

Data from 'Microbial Ecology of Foods'. Vol. 1. ICMSF.

but also to a strong desire to demonstrate that nuclear technology could offer the human race something other than mass destruction. In particular, food irradiation has the advantage of being a much more precisely controlled process than heating, since penetration is deep, instantaneous and uniform. It also retains the fresh character of the product as low level irradiation produces no detectable sensory change in most products.

This failure of low doses of radiation to produce appreciable chemical change in the product has been an obstacle to the development of simple tests to determine whether a food has been irradiated. Although availability of such a test is not essential for the control of irradiation, it is generally accepted that it would facilitate international trade in irradiated food, enhance consumer confidence and help enforce labelling regulations. A number of methods have been developed that are applicable to specific types of food. Free radicals created by irradiation can be detected using electron spin resonance when they are trapped in solid matrices such as bone, seeds and shells. The energy stored in grains of silicate minerals as a result of irradiation can be measured in foods such as herbs and spices using thermoluminescence and long chain volatile hydrocarbons and 2-alkylcyclobutanones produced by irradiation of fatty foods can be detected using gas chromatography. One microbiological test for irradiated food is based on the ratio between an assessment of total microbial numbers using the DEFT technique (see Chapter 10) and a plate count to determine the number of viable bacteria present.

Food irradiation is not without its disadvantages, but a lot of the concerns originally voiced have proved to be unfounded. In 1981 an expert international committee of the FAO/WHO and the International Atomic Energy Authority recommended general acceptance of food irradiation up to a level of 10 kGy. They held the view that it 'constitutes no toxicological risk. Further toxicological examinations of such treated foods are therefore not required'.

It had been thought that irradiation could lead to pathogens becoming more virulent but, apart from one or two exceptions, it has been found that where virulence is affected it is diminished. In the exceptions noted, the effect was slight and not sufficient to compensate for the overall reduction in viable numbers. No example has been found where a non-pathogenic organism has been converted to a pathogen as a result of irradiation. Although it has been reported that spores of some mycotoxigenic moulds which survive irradiation may yield cultures with increased mycotoxin production.

Morphological, biochemical and other changes which may impede isolation and identification and increased radiation resistance have been noted as a result of repeated cyclic irradiation. However, these experiments were performed under the most favourable conditions and for this to occur in practice would require extensive microbial regrowth after

each irradiation; a condition that is readily preventable by good hygienic practices and is most unlikely to occur.

The levels of radiation proposed for foods are not sufficient to induce radioactivity in the product and there is no evidence that consumption of irradiated foods is harmful. Food irradiation facilities do require stringent safety standards to protect workers but that is already in place for the irradiation of other materials such as the sterilization of medical supplies and disposables.

By far the greatest obstacle to the more widespread use of food irradiation is not technical but sociological in the form of extensive consumer resistance and distrust. Much of this is based on inadequate information and false propaganda and parallels very closely earlier arguments over the merits of milk pasteurization. Among the same objections raised then were that pasteurization would be used to mask poor quality milk and would promote poor practices in food preparation. While it has to be agreed that those who take the most cynical view of human nature are often proved correct, this did not prove to be the case with milk pasteurization where the production standards and microbiological quality of raw milk are now higher than they have ever been.

Depending on the lethality required, food irradiation can be applied at two different levels. At high levels it can be used to produce a safe shelf stable product in a treatment known as *radappertization*. Though this has been investigated in the context of military rations, it is unlikely to be a commercial reality in the forseeable future. *C. botulinum* spores are the most radiation resistant known, so very high doses are required to achieve the minimum standard of a 12D reduction ($\approx$45 kGy) for low-acid foods. In the event of a process failure, the growth of more resistant, non-pathogenic clostridia would not act as a warning as it can in thermal processing. High radiation doses are also more likely to produce unacceptable sensory changes and the product has to be irradiated in the frozen state to minimize migration of the radiolytic species that cause such changes. These considerations would not apply when the food was inhibitory to the growth of *C. botulinum* as a result of low pH or the presence of agents such as curing salts.

Two terms are used to distinguish different types of radiation pasteurization. *Radicidation* is used to describe processes where the objective is the elimination of a pathogen, as, for example, in the removal of *Salmonella* from meat and poultry. *Radurization* applies to processes aiming to prolong shelf-life. This distinction may be thought a little over elaborate since, as with thermal pasteurization, irradiation treatments are relatively non-discriminating and will invariably improve both safety and shelf-life.

Several potential applications have been identified (Table 4.9) and food irradiation for specific applications is now permitted in more than

Table 4.9 *Applications of food irradiation*

Application	*Commodity*	*Dose (kGy)*
Inhibition of sprouting	Potatoes Onions Garlic Mushrooms	0.1–3
Decontamination of food ingredients	Spices Onion powder	3–10
Insect disinfestation	Grains	0.2–7
Destruction of parasites	Meats	0.3–0.5
Inactivation of *Salmonella*	Poultry Eggs Shrimps and frog's legs	3–10
Delay in fruit maturation	Strawberries Mangoes Papayas	2–5
Mould and yeast reduction		1–3

Table 4.10 *Foods which may be treated with ionizing radiation in the UK*

	Maximum permitted dose (kGy)
Fruit and mushrooms	2
Vegetables	1
Cereals	1
Bulbs and tubers	0.2
Spices and condiments	10
Fish and shellfish	3
Poultry	7

50 countries, the USA, South Africa, the Netherlands, Thailand and France being among the leading exponents. In South Africa 1,754 tonnes of herbs and spices were irradiated in 2004 and in the USA in 2003 22,000 tonnes of hamburgers were irradiated. In the UK, the applications listed in Table 4.10 have been permitted since 1991, although consumer resistance and the requirement that irradiated foods are labelled as such have meant that, to-date, only one licence has been granted covering the treatment of some herbs and spices.

4.3 HIGH-PRESSURE PROCESSING–PASCALIZATION

Hite, working at the University of West Virginia Agricultural Experimental Station at the turn of the 20th century, showed that high hydrostatic pressures, around 650 MPa (6500 atm), reduced the microbial load in foods such as milk, meats and fruits. He found that 680 MPa applied for 10 min at room temperature reduced the viable count of milk from 10^7 cfu ml^{-1} to 10^1–10^2 cfu ml^{-1} and that peaches and pears

subjected to 410 MPa for 30 min remained in good condition after 5 years storage. He also noted that the microbicidal activity of high pressure is enhanced by low pH or temperatures above and below ambient.

Since then, microbiologists have continued to study the effect of pressure on micro-organisms, although this work has centred on organisms such as those growing in the sea at great depths and pressures. Interest in the application of high pressures in food processing, sometimes called *pascalization*, lapsed until the 1980s when progress in industrial ceramic processing led to the development of pressure equipment capable of processing food on a commercial scale and a resurgence of interest, particularly in Japan.

High hydrostatic pressure acts primarily on non-covalent linkages, such as ionic bonds, hydrogen bonds and hydrophobic interactions, and it promotes reactions in which there is an overall decrease in volume. It can have profound effects on proteins, where such interactions are critical to structure and function, although the effect is variable and depends on individual protein structure. Some proteins such as those of egg, meat and soya form gels and this has been employed to good effect in Japan where high pressure has been used to induce the gelation of fish proteins in the product surimi. Other proteins are relatively unaffected and this can cause problems when they have enzymic activity which limits product shelf-life. Pectin esterase in orange juice, for instance, must be inactivated to stabilize the desired product cloudiness. Nonprotein macromolecules can also be affected by high pressures so that pascalized starch products often taste sweeter due to conformational changes in the starch which allow salivary amylase greater access.

Adverse effects on protein structure and activity obviously contribute to the antimicrobial effect of high pressures, although the cell membrane also appears to be an important target. Membrane lipid bilayers have been shown to compress under pressure and this alters their permeability. As a general rule vegetative bacteria and fungi can be reduced by at least one log cycle by 400 MPa applied for 5 min.

Bacterial endospores are more resistant to hydrostatic pressure, tolerating pressures as high as 1200 MPa. Their susceptibility can be increased considerably by modest increases in temperature, when quite low pressures (100 MPa) can produce spore germination, a process in which the spores lose their resistance to heat and to elevated pressure.

High pressure processing is typically a batch process employing a pressure vessel, the pressure transmission fluid (usually water) and pumps to generate the pressure. Although the capital cost of equipment is quite high, hydrostatic processing has a number of appealing features for the food technologist. It acts instantly and uniformly throughout a food so that the processing time is not related to container size and there are none of the penetration problems associated with heat processing.

With the exceptions noted above, adverse effects on the product are slight; nutritional quality, flavour, appearance and texture resemble the fresh material very closely. To the consumer it is a 'natural' process with none of the negative associations of processes such as irradiation or chemical preservatives.

Initially, commercial application of high-pressure technology was limited mainly to acidic products. The yeasts and moulds normally responsible for spoilage in these products are pressure sensitive and the bacterial spores that survive processing are unable to grow at the low pH. In 1990, the Meidi-Ya company in Japan launched a range of jams treated at 400–500 MPa in pack. These have a chill shelf-life of 60 days and have sensory characteristics quite different from conventional heat-processed jams since more fresh fruit flavour and texture are retained. Refrigeration is necessary to limit residual enzyme activities which give rise to browning and flavour changes. Other products introduced include salad dressings, fruit sauces, and fruit flavoured yoghurts. More recently a number of pressure-treated foods have been introduced in Europe, the United States and elsewhere. These include fruit purees and juices and some more novel products such as guacamole, cooked ham and oysters. Pressure-treated guacamole has been a success in the USA where pressures of around 500 MPa for 2 minutes extend its chill shelf life from 7 to 30 days. Similar treatments are applied to packs of sliced cooked ham and other delicatessen meat products in Italy, Spain, Germany, the USA and Japan to reduce the risk posed by any post-cooking contamination with *Listeria monocytogenes*. In the United States and South Korea, pressure-treated oysters are also available. The process used releases the adductor muscle which holds the oyster shell closed, so it has the dual safety benefits of eliminating any *Vibrio* species present as well as reducing the number of stab injuries incurred during abortive attempts to open the shell.

In the future, the range of products may be increased by coupling moderate pressure with a heat treatment equivalent to pasteurization. In one trial, shelf stable, low acid foods were produced by combining a pressure of just 0.14 MPa with heating at temperatures of 82–103 °C. Other developments such as equipment capable of semi- or fully-continuous operation will also considerably improve commercial feasibility, so that we may see and hear a lot more about pascalization.

4.4 LOW-TEMPERATURE STORAGE – CHILLING AND FREEZING

The rates of most chemical reactions are temperature dependent; as the temperature is lowered so the rate decreases. Since food spoilage is usually a result of chemical reactions mediated by microbial and

endogenous enzymes, the useful life of many foods can be increased by storage at low temperatures. Though this has been known since antiquity, one of the earliest recorded experiments was conducted by the English natural philosopher Francis Bacon who in 1626 stopped his coach in Highgate in order to fill a chicken carcass with snow to confirm that it delayed putrefaction. This experiment is less notable for its results, which had no immediate practical consequences, than for its regrettable outcome. As a result of his exertions in the snow, it is claimed Bacon caught a cold which led to his death shortly after.

Using low temperatures to preserve food was only practicable where ice was naturally available. As early as the 11th century BC the Chinese had developed ice houses as a means of storing ice through the summer months, and these became a common feature of large houses in Europe and North America in the 17th and 18th centuries. By the 19th century, the cutting and transporting of natural ice had become a substantial industry in areas blessed with a freezing climate.

Mechanical methods of refrigeration and ice making were first patented in the 1830s. These were based on the cooling produced by the vaporization of refrigerant liquids, originally ether but later liquid ammonia. Much early development work was done in Australia where there was considerable impetus to find a way of transporting the abundant cheap meat available locally to European population centres. At the 1872 Melbourne Exhibition, Joseph Harrison exhibited an 'ice house' which kept beef and mutton carcasses in good condition long enough for some of it to be eaten at a public luncheon the following year. This banquet was to send off a steamship to London carrying 20 tons of frozen mutton and beef packed in tanks cooled by ice and salt. Unfortunately it was an inauspicious start, during passage through the tropics the ice melted and most of the meat had been thrown overboard before the ship reached London. Chilled rather than frozen meat had however already been successfully shipped the shorter distance from North America to Europe and by the end of the century techniques had been refined to the extent that shipping chilled and frozen meat from North and South America and Australia to Europe was a large and profitable enterprise.

Since then, use of chilling and freezing has extended to a much wider range of perishable foods and to such an extent that refrigeration is now arguably the technology of paramount importance to the food industry.

4.4.1 Chill Storage

Chilled foods are those foods stored at temperatures near, but above their freezing point, typically 0–5 °C. This commodity area has shown a massive increase in recent years as traditional chilled products such as fresh meat and fish and dairy products have been joined by a huge

variety of new products including complete meals, prepared and delicatessen salads, dairy desserts and many others. Three main factors have contributed to this development:

(1) the food manufacturers' objective of increasing added value to their products;
(2) consumer demand for fresh foods and ease of preparation while at the same time requiring the convenience of only occasional shopping excursions; and
(3) the availability of an efficient cold chain – the organization and infrastructure which allows low temperatures to be maintained throughout the food chain from manufacture/harvest to consumption.

Chill storage can change both the nature of spoilage and the rate at which it occurs. There may be qualitative changes in spoilage characteristics, as low temperatures exert a selective effect preventing the growth of mesophiles and leading to a microflora dominated by psychrotrophs. This can be seen in the case of raw milk which in the days of milk churns and roadside collection had a spoilage microflora comprised largely of mesophilic lactococci which would sour the milk. Nowadays in the UK, milk is chilled almost immediately it leaves the cow so that psychrotrophic Gram-negative rods predominate and produce an entirely different type of spoilage. Low temperatures can also cause physiological changes in micro-organisms that modify or exacerbate spoilage characteristics. Two such examples are the increased production of phenazine and carotenoid pigments in some organisms at low temperatures and the stimulation of extracellular polysaccharide production in *Leuconostoc* spp. and some other lactic acid bacteria. In most cases, such changes probably represent a disturbance of metabolism due to the differing thermal coefficients and activation energies of the numerous chemical reactions that comprise microbial metabolism.

Though psychrotrophs can grow in chilled foods they do so only relatively slowly so that the onset of spoilage is delayed. In this respect temperature changes within the chill temperature range can have pronounced effects. For example, the generation time for one pseudomonad isolated from fish was 6.7 hours at 5 °C compared with 26.6 hours at 0 °C. Where this organism is an important contributor to spoilage, small changes of temperature will have major implications for shelf-life. The keeping time of haddock and cod fillets has been found to double if the storage temperature is decreased from 2.8 °C to −0.3 °C. Mathematical modelling techniques of the sort described in Section 3.4 can be useful in predicting the effect of temperature fluctuations on shelf-life, but, as a

general rule, storage temperature should be as low, and as tightly controlled, as possible.

The ability of organisms to grow at low temperatures appears to be particularly associated with the composition and architecture of the plasma membrane (see Section 3.3.2). As the temperature is lowered, the plasma membrane undergoes a phase transition from a liquid crystalline state to a rigid gel in which solute transport is severely limited. The temperature of this transition is lower in psychrotrophs and psychrophiles largely as a result of higher levels of unsaturated and short chain fatty acids in their membrane lipids. If some organisms are allowed to adapt to growth at lower temperatures they increase the proportion of these components in their membranes.

There seems to be no taxonomic restriction on psychrotrophic organisms which can be found in the yeasts, moulds, Gram-negative and Gram-positive bacteria. One feature they share is that in addition to their ability to grow at low temperatures, they are inactivated at moderate temperatures. A number of reasons for this marked heat sensitivity have been put forward including the possibility of excessive membrane fluidity at higher temperatures. Low thermal stability of key enzymes and other functional proteins appears to be an important factor, although thermostable extracellular lipases and proteases produced by psychrotrophic pseudomonads can be a problem in the dairy industry.

Though mesophiles cannot grow at chill temperatures, they are not necessarily killed. Chilling will produce a phenomenon known as cold shock which causes death and injury in a proportion of the population but its effects are not predictable in the same way as heat processing. The extent of cold shock depends on a number of factors such as the organism (Gram-negatives appear more susceptible than Gram-posi-tives), its phase of growth (exponential-phase cells are more susceptible than stationary phase cells), the temperature differential and the rate of cooling (in both cases the larger it is, the greater the damage), and the growth medium (cells grown in complex media are more resistant).

The principal mechanism of cold shock appears to be damage to membranes caused by phase changes in the membrane lipids which create hydrophilic pores through which cytoplasmic contents can leak out. An increase in single-strand breaks in DNA has also been noted as well as the synthesis of specific cold-shock proteins to protect the cell.

Since chilling is not a bacteriocidal process, the use of good microbiological quality raw materials and hygienic handling are key requirements for the production of safe chill foods. Mesophiles that survive cooling, albeit in an injured state, can persist in the food for extended periods and may recover and resume growth should conditions later become favourable. Thus chilling will prevent an increase in the risk from mesophilic pathogens, but will not assure its elimination. There are

however pathogens that will continue to grow at some chill temperatures and the key role of chilling in the modern food industry has focused particular attention on these. Risks posed by these organisms, which are given more detailed attention in Chapter 7, may increase with duration of storage but this process is likely to be slow and dependent on the precise storage temperature and composition of the food.

Some foods are not suitable for chill storage as they suffer from cold injury where the low temperature results in tissue breakdown which leads to visual defects and accelerated microbiological deterioration. Tropical fruits are particularly susceptible to this form of damage.

4.4.2 Freezing

Freezing is the most successful technique for long-term preservation of food since nutrient content is largely retained and the product resembles the fresh material more closely than in appertized foods.

Foods begin to freeze somewhere in the range −0.5 to −3 °C, the freezing point being lower than that of pure water due to the solutes present. As water is converted to ice during freezing, the concentration of solutes in the unfrozen water increases, decreasing its freezing point still further so that even at very low temperatures, *e.g.* −60 °C, some water will remain unfrozen. The temperatures used in frozen storage are generally less than −18 °C. At these temperatures no microbial growth is possible, although residual microbial or endogenous enzyme activity such as lipases can persist and eventually spoil a product. This is reduced in the case of fruits and vegetables by blanching before freezing to inactivate endogenous polyphenol oxidases which would otherwise cause the product to discolour during storage. Freezer burn is another non-microbiological quality defect that may arise in frozen foods, where surface discolouration occurs due to sublimation of water from the product and its transfer to colder surfaces in the freezer. This can be prevented by wrapping products in a water-impermeable material or by glazing with a layer of ice.

Low temperature is not the only inhibitory factor operating in frozen foods; they also have a low water activity produced by removal of water in the form of ice. Table 4.11 describes the effect of temperature on water activity. As far as microbiological quality is concerned, this effect is only significant when frozen foods are stored at temperatures where microbial growth is possible (above −10 °C). In this situation, the organisms that grow on a product are not those normally associated with its spoilage at chill temperatures but yeasts and moulds that are both psychrotrophic and tolerant of reduced water activity. Thus meat and poultry stored at −5 to −10 °C may slowly develop surface defects such as black spots due to the growth of the mould *Cladosporium herbarum*, white spots caused by *Sporotrichum carnis* or the feathery growth of *Thamnidium elegans*.

Table 4.11 *Effect of freezing on the water activity of pure water-ice*

Temperature (°C)	a_w
0	1
−5	0.953
−10	0.907
−15	0.864
−20	0.823
−40	0.68

Micro-organisms are affected by each phase of the freezing process. In cooling down to the temperature at which freezing begins, a proportion of the population will be subject to cold shock discussed in Section 4.4.1 above. At the freezing temperature, further death and injury occur as the cooling curve levels out as latent heat is removed and the product begins to freeze. Initially ice forms mainly extracellularly, intracellular ice formation being favoured by more rapid cooling. This may mechanically damage cells and the high extracellular osmotic pressures generated will dehydrate them. Changes in the ionic strength and pH of the water phase as a result of freezing will also disrupt the structure and function of numerous cell components and macromolecules which depend on these factors for their stability. Cooling down to the storage temperature will prevent any further microbial growth once the temperature has dropped below −10 °C. Finally, during storage there will be an initial decrease in viable numbers followed by slow decline over time. The lower the storage temperature, the slower the death rate.

As with chilling, freezing will not render an unsafe product safe – its microbial lethality is limited and preformed toxins will persist. Frozen chickens are, after all, an important source of *Salmonella*.

Survival rates after freezing will depend on the precise conditions of freezing, the nature of the food material and the composition of its microflora, but have been variously recorded as between 5 and 70%. Bacterial spores are virtually unaffected by freezing, most vegetative Gram-positive bacteria are relatively resistant and Gram-negatives show the greatest sensitivity. While frozen storage does reliably inactivate higher organisms such as pathogenic protozoa and parasitic worms, food materials often act as cryoprotectants for bacteria so that bacterial pathogens may survive for long periods in the frozen state. In one extreme example *Salmonella* has been successfully isolated from ice cream stored at −23 °C for 7 years.

The extent of microbial death is also determined by the rate of cooling. Maximum lethality is seen with slow freezing where, although there is little or no cold shock experienced by the organisms, exposure to high solute concentrations is prolonged. Survival is greater with rapid freezing where

exposure to these conditions is minimized. Food freezing processes are not designed however to maximize microbial lethality but to minimize loss of product quality. Formation of large ice crystals and prolonged exposure to high osmotic pressure solutions during slow cooling also damage cells of the food material itself causing greater drip loss and textural deterioration on thawing, so fast freezing in which the product is at storage temperature within half an hour is the method of choice commercially. The rate of freezing in domestic freezers is much slower so, although microbial lethality may be greater, so too is product quality loss.

Thawing of frozen foods is a slower process than freezing. Even with moderate size material the outside of the product will be at the thawing temperature some time before the interior. So with high thawing temperature, mesophiles may be growing on the surface of a product while the interior is still frozen. Slow thawing at lower temperature is generally preferred. It does have some lethal effect as microbial cells experience adverse conditions in the 0 to $-10\,^{\circ}C$ range for longer, but it will also allow psychrotrophs to grow. Provided the product is not subject to contamination after thawing, the microflora that develops will differ from that on the fresh material due to the selective lethal effect of freezing. Lactic acid bacteria are often responsible for the spoilage of defrosted vegetables whereas they generally comprise only about 1% of the microflora on fresh chilled produce which is predominantly Gram-negative.

Freezing and defrosting may make some foods more susceptible to microbiological attack due to destruction of antimicrobial barriers in the product and condensation, but defrosted foods do not spoil more rapidly than those that have not been frozen. Injunctions against refreezing defrosted products are motivated by the loss of textural and other qualities rather than any microbiological risk that is posed.

4.5 CHEMICAL PRESERVATIVES

The addition of chemicals to food is not a recent innovation but has been practised throughout recorded history. Doubtless too, there has also always been a certain level of misuse but this must have gone largely undetected until modern analytical techniques became available. When chemical analysis and microscopy were first applied to foods in the early 19th century, they revealed the appalling extent of food adulteration then current. Pioneering work had been done by the 18th century chemist Jackson, but publication of the book 'A Treatise on Adulterations of Food, and Culinary Poisons' by Frederick Accum in 1820 marks a watershed. Accum exposed a horrifying range of abuses such as the sale of sulfuric acid as vinegar, the use of copper salts to colour pickles, the use of alum to whiten bread, addition of acorns to coffee, blackthorn leaves to tea, cyanide to give wines a nutty flavour and red lead to colour

Gloucester cheese. These and subsequent investigations, notably those sponsored by the journal *Lancet*, led directly to the introduction of the first British Food and Drugs Act in 1860. Despite the protection of a much stricter regulatory framework, occasional triumphs of human cupidity are still recorded today. Further examples include the use of ethylene glycol in some Austrian wines, the intrepid entrepreneur who sold grated umbrella handles as Parmesan cheese and the grim case of the Spanish toxic cooking oil scandal which killed or maimed hundreds.

Although some would regard all chemical additions to food as synonymous with adulteration, many are recognized as useful and are allowed. Additives may be used to aid processing, to modify a food's texture, flavour, nutritional quality or colour but, here, we are concerned with those which primarily effect keeping quality: preservatives.

Preservatives are defined as 'substances capable of inhibiting, retarding or arresting the growth of micro-organisms or of any deterioration resulting from their presence or of masking the evidence of any such deterioration'. They do not therefore include substances which act by inhibiting a chemical reaction which can limit shelf-life, such as the control of rancidity or oxidative discolouration by antioxidants. Neither does it include a number of food additives which are used primarily for other purposes but have been shown to contribute some antimicrobial activity. These include the antioxidants, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), and the phosphates used as acidity regulators and emulsifiers in some products.

Preservatives may be microbicidal and kill the target organisms or they may be microbistatic in which case they simply prevent them growing. This is very often a dose-dependent feature; higher levels of an antimicrobial proving lethal while the lower concentrations that are generally permitted in foods tend to be microbistatic. For this reason chemical preservatives are useful only in controlling low levels of contamination and are not a substitute for good hygiene practices.

Recently consumers have shown an inclination to regard preservatives as in some way 'unnatural', even though the use of salts, acid, or smoke to preserve foods goes back to the beginning of civilization. Usage of chemical preservatives is now more restricted and controlled than ever and in many areas it is declining. It is perhaps well to remember though that only the fairly recent advent of technologies such as canning and refrigeration has allowed us any alternative to chemical preservation or drying as a means of extending the food supply.

4.5.1 Organic Acids and Esters

The most important organic acids and esters that are used as food preservatives are listed in Table 4.12 along with their E-numbers (EU

Table 4.12 *Organic acid food preservatives*

Preservative	[a]ADI (mg kg^{-1} body wt).	*Typical usage and levels*	*(mg kg^{-1})*
E200 Sorbic acid E201 Sodium salt E202 Potassium salt E203 Calcium salt	25	Salad dressing bakery products fruit desserts	<2000
E210 Benzoic acid E211 Sodium salt E212 Potassium salt E213 Calcium salt	5	Cider, soft drinks, fruit products, bottled sauces	<3000
E260 Acetic acid	No limit	Pickles, sauces chutneys	up to % levels (1% = 10 000 mg kg^{-1})
E270 Lactic acid	No limit	Fermented meats dairy and vegetable products. Sauces and dressings. Drinks.	up to % levels (1% = 10 000 mg kg^{-1})
E280 Propionic acid E281 Sodium salt E282 Calcium salt E283 Potassium salt	10	Bakery goods Cheese spread	1000–5000
Parabens E214 *p*-Hydroxybenzoic acid ethyl ester E215 Sodium salt	10	Bakery goods, pickles, fruit products, sauces	<2000
E216 *p*-Hydroxybenzoic acid *n*-propyl ester E217 Sodium salt		Bakery goods, pickles, fruit products, sauces	<2000
E218 *p*-Hydroxybenzoic acid methyl ester E219 Sodium salt		Bakery goods, pickles, fruit products, sauces	<2000

[a] ADI Acceptable daily intake

codes for food additives used throughout the European Union). Their structures are presented in Figure 4.11.

The antimicrobial effect of organic acids such as *acetic* and *lactic acids* has been discussed in Chapter 3. Both are produced microbiologically, although food-grade acetic acid derived petrochemically is also sometimes used as an alternative to vinegar. They can be an added ingredient in formulated products such as pickles and sauces, or they can be generated *in situ* in the large range of lactic-fermented products described

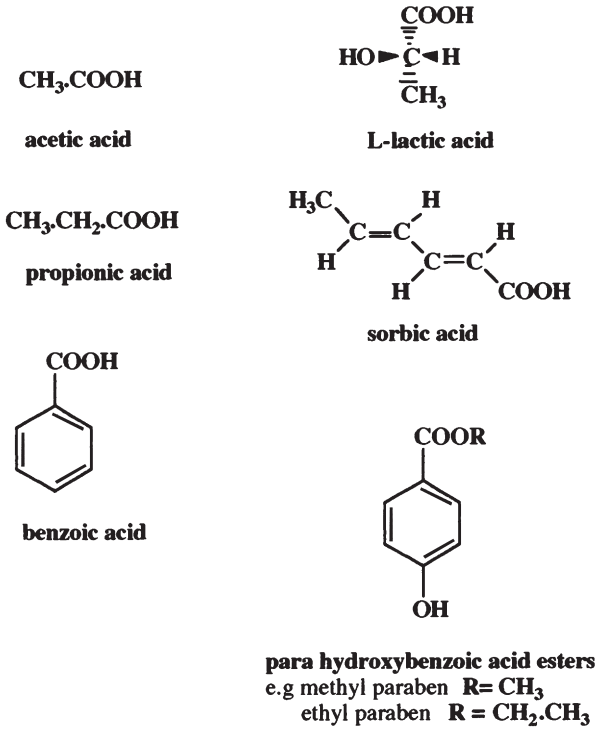

Figure 4.11 *Structures of organic acid food preservatives*

in Chapter 9. They differ from the other acids and esters described here in that they are usually present in amounts sufficient to exert an effect on flavour and on product pH, thus potentiating their own action by increasing the proportion of undissociated acid present.

Benzoic acid occurs naturally in cherry bark, cranberries, greengage plums, tea and anise but is prepared synthetically for food use. Its antimicrobial activity is principally in the undissociated form and since it is a relatively strong acid (pK_a 4.19) it is effective only in acid foods. As a consequence, its practical use is to inhibit the growth of spoilage yeasts and moulds. Activity against bacteria has been reported but they show greater variability in their sensitivity.

Inhibition by benzoic acid appears multifactorial. The ability of the undissociated molecule to interfere with membrane energetics and function appears to be of prime importance since growth inhibition has been shown to parallel closely the inhibition of amino acid uptake in whole cells and membrane vesicles. Some inhibition may also result from benzoic acid once it is inside the cell as a number of key enzyme activities have also been shown to be adversely affected.

Parabens (*para*-hydroxybenzoic acid esters) differ from the other organic acids described here in the respect that they are phenols rather

than carboxylic acids. They are much weaker acids with pK_a values of 8.5 and so are predominantly uncharged even at neutral pH. This means that they can be used effectively in non-acidic foods. Their antimicrobial activity increases with the length of the ester group carbon chain, although this also decreases their water solubility and may lead to poor performance in some foods where partition into the fatty phase may occur. Some Gram-negatives are resistant to the higher homologues and this has been ascribed to the cell's outer membrane acting as a barrier.

Parabens appear to act mainly at the cell membrane eliminating the ΔpH component of the protonmotive force and affecting energy transduction and substrate transport. In contrast to other weak acid preservatives, there is little evidence suggesting that parabens interfere directly with specific enzymic activities.

Sorbic acid is an unsaturated fatty acid, 2,4-hexadienoic acid, found naturally in the berries of the mountain ash. It has a pK_a of 4.8 and shows the same pH dependency of activity as other organic acids. It is active against yeasts, moulds and catalase-positive bacteria but, interestingly, is less active against catalase-negative bacteria. This has led to its use as a selective agent in media for clostridia and lactic acid bacteria and as a fungal inhibitor in lactic fermentations.

As with the other weak acids, the membrane is an important target for sorbic acid, although inhibition of a number of key enzymes of intermediary metabolism, such as enolase, lactate dehydrogenase and several Krebs cycle enzymes, has been shown. In contrast to its use as a selective agent for clostridia, some studies have shown that sorbic acid inhibits the germination and outgrowth of *C. botulinum* spores. At one time this attracted some interest in the possibility that sorbic acid could be used as an alternative or adjunct to nitrite in cured meats.

Propionic acid (pK_a 4.9) occurs in a number of plants and is also produced by the activity of propionibacteria in certain cheeses. It is used as a mould inhibitor in cheese and baked products where it also inhibits rope-forming bacilli. Objections to the use of preservatives led, in the late 1980s, to the increased use of acetic acid in the form of vinegar as an alternative to propionate but the complete omission of a rope inhibitor has had serious consequences for the public on at least one occasion (see Section 7.2.5).

4.5.2 Nitrite

The antibacterial action of nitrite was first described in the 1920s though it had long been employed unwittingly in the production of cured meats where it is also responsible for their characteristic colour and flavour. In early curing processes nitrite was produced by the bacterial

reduction of nitrate present as an impurity in the crude salt used, but now nitrate, or more commonly nitrite itself, is added as the sodium or potassium salt.

Nitrite is inhibitory to a range of bacteria. Early workers showed that a level of $200\,mg\,kg^{-1}$ at pH 6.0 was sufficient to inhibit strains of *Escherichia, Flavobacterium, Micrococcus, Pseudomonas* and others, although *Salmonella* and *Lactobacillus* species were more resistant. Of most practical importance though is the ability of nitrite to inhibit spore-forming bacteria such as *Clostridium botulinum* which will survive the heat process applied to many cured meats. To achieve this commercially, initial levels of nitrite greater than $100\,mg\,kg^{-1}$ are used. The mechanism of its action is poorly understood partly due to the complexity of the interaction of several factors such as pH, salt content, presence of nitrate or nitrite and the heat process applied to the cured meat. Descriptive mathematical models of these interactions have however been produced which quantify the precise contribution of nitrite to safety (see Section 3.5).

Bacterial inhibition by nitrite increases with decreasing pH, suggesting that nitrous acid (HNO_2, pK_a 3.4) is the active agent. In the case of spores, it appears that nitrite acts by inhibiting the germination and outgrowth of heated spores and by reacting with components in the product to form other inhibitory compounds. The latter effect was first noted in the 1960s by Perigo who observed that when nitrite was heated in certain bacteriological media, the resulting medium proved more inhibitory to clostridia than when filter-sterilized nitrite was added after heating. Clostridia are very sensitive to these 'Perigo factors' which differ from nitrite in displaying activity that is independent of pH. However, they do not seem to be formed in meat and their effect in bacteriological media could be removed if meat was added. The presence of 'Perigo-type factors' has been reported in heated cured meats but these are only produced by severe heating and have minor antibacterial activity.

Studies into the nature of Perigo and Perigo-type factors have looked particularly at the production of Roussin's salts; complex salts of iron, nitrosyl and sulfydryl groups. Although these compounds have not been shown to be present in cured meats in sufficient quantity to cause the inhibition observed, their formation may give an indication of the way nitrite itself interferes with bacterial metabolism. It has been proposed that the biochemical mechanism of inhibition involves nitrite reacting with iron and sulfhydryl groups of key cell constituents. Iron-containing proteins such as ferredoxins are very important in electron transport and energy production in clostridia. For example the phosphoroclastic system is used by clostridia to generate additional ATP by substrate-level phosphorylation. Pyruvate, produced by glycolysis, is oxidized to acetate *via* acetyl-CoA and acetyl phosphate which phosphorylates ADP to

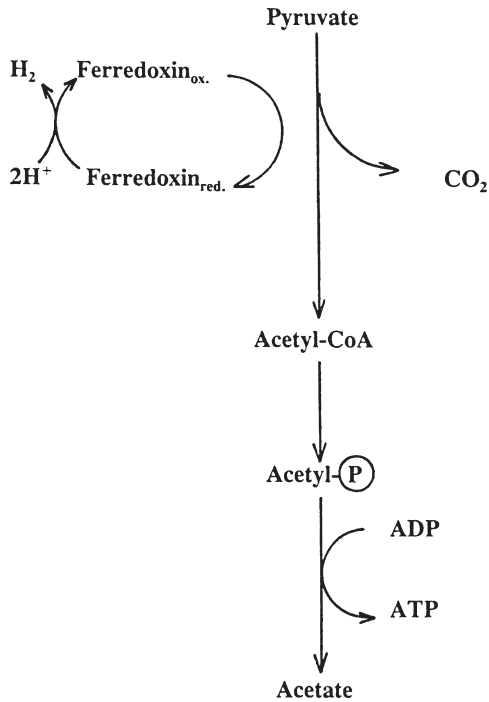

Figure 4.12 *The phosphoroclastic system*

produce ATP (Figure 4.12). Ferredoxin acts as a carrier for the electrons removed in the oxidation step and which are ultimately used to reduce hydrogen ions to hydrogen gas. Support for this hypothesis has come from the observation that nitrite addition leads to an accumulation of pyruvate in *C. sporogenes* and *C. botulinum*.

The use of nitrite and nitrate in food has attracted scrutiny since it was discovered in the 1950s that *N*-nitrosamines, formed by the reaction of nitrite with secondary amines, especially at low pH, can be carcinogenic. Concern developed that they may be present in food or formed in the body as a result of ingestion of nitrate or nitrite with food. Surveys have indicated that cured meats, particularly fried bacon, beer and, in some countries, fish, make the most significant contribution to dietary intakes of nitrosamines, although a US survey made the point that a smoker inhaled about 100 times the amount of volatile nitrosamines per day as were provided by cooked bacon. Dietary intake of nitrite is low, generally less than 2 mg $NaNO_2$ day^{-1}, and comes mainly from cured meats, although it is also present in fish, cheese, cereals, and vegetable products. Nitrate is also of concern since it can be reduced to nitrite by the body's own microflora. Cured meats are not a significant source; vegetables contribute more than 75% of the dietary intake of nitrate, although water can be an important source in some areas.

Awareness of the problem has led to changes in production practices for cured meats such as the use of low levels of nitrite in preference to nitrate and the increased use of ascorbic acid which inhibits the nitrosation reaction. These measures have produced significant reductions in nitrosamine levels.

Mention should also be made here of the other contributions made by nitrite to the quality of cured meats. Reduction of nitrite to nitric oxide produces the characteristic red colour of cured meats. The nitric oxide co-ordinates to the haem ferrous ion in the muscle pigment myoglobin converting it to nitrosomyoglobin (Figure 4.13). When raw cured meats such as bacon are cooked this pigment decomposes to produce nitrosylhaemochrome which has the pink colour also seen in cooked cured hams. Only small quantities of nitrite are required to produce the cured meat colour: theoretically $3\,mg\,kg^{-1}$ is sufficient to convert half the myoglobin present in fresh meat, but because of competing reactions, $25\,mg\,kg^{-1}$ are required to give a stable colour.

Nitrite also contributes to the typical cured meat flavour. Taste panels can distinguish cured meats where nitrite has not been used but the

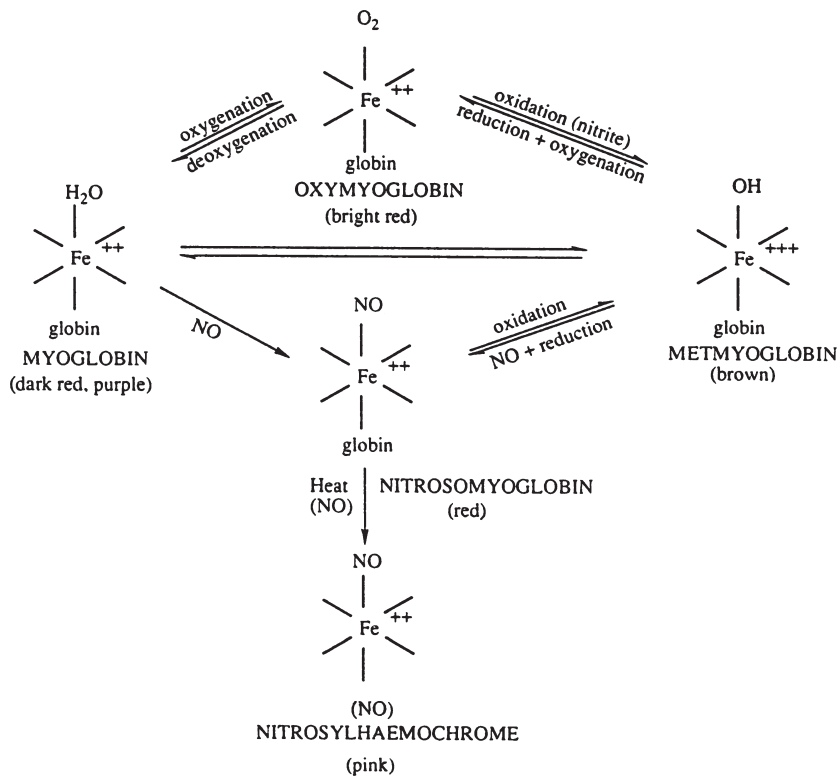

Figure 4.13 *Reactions of nitrite with meat myoglobin*

precise reasons for this are not known. It is thought that nitrite acts as an antioxidant to inhibit lipid degradation in the meat although this may only be part of the story.

4.5.3 Sulfur Dioxide

Sulfur dioxide (SO_2) has long enjoyed a reputation for its disinfecting properties and its earliest use in the food industry was when sulfur candles were burnt to disinfect the vessels used to produce and store wine. Nowadays, it is also used as an antioxidant to inhibit enzymic and non-enzymic browning reactions in some products.

Sulfur dioxide is a colourless gas that readily dissolves in water to establish a pH-dependent equilibrium similar to CO_2 (see Section 3.3.3).

$$SO_{2(gas)} \rightleftharpoons SO_2 + H_2O \rightleftharpoons H_2SO_3 \rightleftharpoons H^+ + HSO_3^- \rightleftharpoons 2H^+ + SO_3^{2-} \qquad (4.15)$$

Sulfurous acid (H_2SO_3) is a dibasic acid with pK_a values of 1.86 and 6.91.

The unionized forms of SO_2 which can readily penetrate the cell have the greatest antimicrobial activity. It has been reported that they are between 100 and 1000 times more active than the bisulfite anion. Since the unionized forms predominate at low pH values, it follows that SO_2 is used to best effect in acidic foods. At neutral pH, SO_2 is present as a mixture of the relatively inactive bisulfite (HSO_3^-) and sulfite (SO_3^{2-}) ions, although salts of these anions prove the most convenient way of handling the preservative in the food industry.

SO_2 is a reactive molecule and can disrupt microbial metabolism in a number of ways. As a reducing agent, it can break disulfide linkages in proteins and interfere with redox processes. It can also form addition compounds with pyrimidine bases in nucleic acids, sugars and a host of key metabolic intermediates. One disadvantageous consequence of this reactivity is its ability to destroy the vitamin thiamine in foods and the once widespread practice of using it in meat and meat products has now been prohibited, with the exception of British fresh sausage.

Sulfur dioxide is active against bacteria, yeasts and moulds, although some yeasts and moulds are more resistant. Gram-negative bacteria are most susceptible and in British fresh sausage where sulfite is permitted up to a level of $450\,mg\,kg^{-1}$, the Gram-negative spoilage flora normally associated with chilled meats is replaced by one dominated by Gram-positive bacteria and yeasts. In winemaking the tolerance of the wine yeast *Saccharomyces cerevisiae* to SO_2 levels around $100\,mg\,l^{-1}$ is exploited to control the growth of wild yeasts and acetifying bacteria. Seasonal surpluses of soft fruits are also preserved by the addition of high levels of SO_2 to permit jam production throughout the year.

4.5.4 Natamycin

Natamycin (Figure 4.14), formerly know as pimaricin, is a polyene macrolide antibiotic produced by the bacterium *Streptomyces natalensis*. It is a very effective antifungal agent as it binds irreversibly to the fungal sterol, ergosterol, disrupting the fungal cell membrane leading to a loss of solutes from the cytoplasm and cell lysis. Natamycin is poorly soluble in water and is used as an aqueous suspension for the surface treatment of cheeses and sausages to control yeast and mould growth. It has some advantages over sorbate in this respect since it remains localised on the surface of the product, is not dependent on a low pH for its activity and has no effect on bacteria important in the fermentation and maturation of such products.

4.5.5 'Natural' Food Preservatives

The uncertainty voiced by consumer organisations and pressure groups over the use of food additives including preservatives has already been referred to. One approach to reassuring the consumer has been recourse to methods of preservation that can be described as 'natural'. The whole area though is riddled with inconsistency and contradiction; it can be argued that any form of preservation which prevents or delays the recycling of the elements in plant and animal materials is unnatural.

On the other hand there is nothing more natural than strychnine or botulinum toxin. Smoking of foods might be viewed as a natural method of preservation. Its antimicrobial effect is a result of drying and the activity of woodsmoke components such as phenols and formaldehyde which would probably not be allowed were they to be proposed as chemical preservatives in their own right.

The use of natural food components possessing antimicrobial activity such as essential oils and the lactoperoxidase system in milk (see Section

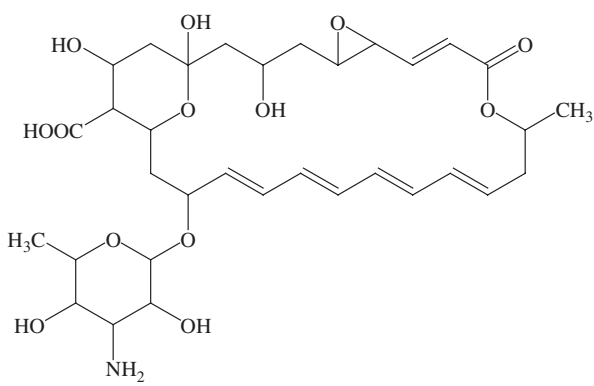

Figure 4.14 *Natamycin*

3.2.4) have attracted some attention in this respect. Attention has also been paid to the bacteriocins produced by food-grade micro-organisms such as the lactic acid bacteria. Nisin (see Section 9.4.1) is an already well established example and its use can be extended by expedients such as inclusion of whey fermented by a nisin-producing strain of *Lactococcus lactis* as an ingredient in formulated products like prepared sauces.

4.6 MODIFICATION OF ATMOSPHERE

At the start of the 19th century it was believed that contact with air caused putrefaction and that food preservation techniques worked by excluding air. We have already seen (Section 4.1) how this misapprehension applied in the early days of canning and it was thought that drying operated in a similar way, expelling air from the interior of food. Some preservation techniques, such as covering a product with melted fat and allowing it to set, did in fact rely on the exclusion of air but it is only in the last 30 years or so that shelf-life extension techniques based on changing the gaseous environment of a food have really come to be widely used.

Modified atmospheres exert their effect principally through the inhibition of fast-growing aerobes that would otherwise quickly spoil perishable products. Obligate and facultative anaerobes such as clostridia and the Enterobacteriaceae are less affected. Thus keeping quality is improved but there is generally little effect on pathogens, if present, and the technique is invariably applied in conjunction with refrigerated storage.

In practice three different procedures are used to modify the atmosphere surrounding a product: vacuum packing, modified-atmosphere packing or gas flushing, and controlled atmospheres. Here we will discuss some of their important characteristics although other aspects will be dealt with under specific commodities in Chapter 5.

An essential feature of all three techniques is that the product is packed in a material which helps exclude atmospheric oxygen and retain moisture. This requires that it should have good barrier properties towards oxygen and water and be easily sealed. The packaging materials used are usually plastic laminates in which the innermost layer is a plastic such as polyethylene which has good heat sealing properties. Mechanical closures on packs are far less effective as they often leave channels through which high rates of gas exchange can occur. Overlying the layer of polythene is usually another layer with much better gas barrier properties. No plastics are completely impermeable to gases, although the extent of gas transmission across a plastic film will depend on the the type of plastic, its temperature, the film thickness and the partial pressure difference across the film. In some cases, it can also be affected by factors

such as humidity and the presence of fat. Polyvinylidene chloride, PVDC, is a material commonly used as a gas barrier; the oxygen permeability of a 25 μm thick film is 10 $cm^3\,m^{-2}\,(24h)^{-1}\,atm^{-1}$ compared with values of 8500 and 1840 for low density and high density polythene respectively. Higher rates of transfer occur with CO_2 for which the permeability values are about five times those for oxygen. If a film is required to exclude oxygen transfer completely, then a non-plastic material such as aluminium foil must be included. This is seen for example in the bags used to pack wines. In addition to the sealing- and gas barrier-layers, laminates may also contain an outer layer such as nylon which gives the pack greater resistance to damage.

In *vacuum packing* the product is placed in a bag from which the air is evacuated, causing the bag to collapse around the product before it is sealed. Residual oxygen in the pack is absorbed through chemical reactions with components in the product and any residual respiratory activity in the product and its microflora. To achieve the best results, it is important that the material to be packed has a shape that allows the packaging film to collapse on to the product surface entirely – without pockets and without the product puncturing the film.

Vacuum packing has been used for some years for primal cuts of red meats. At chill temperatures, good quality meat in a vacuum pack will keep up to five times longer than aerobically stored meats. The aerobic microflora normally associated with the spoilage of conventionally stored meats is prevented from growing by the high levels of CO_2 which develop in the pack after sealing (see Section 3.3.3) and the low oxygen tension. The microflora that develops is dominated by lactic acid bacteria which are metabolically less versatile than the Gram-negative aerobes, grow more slowly and reach a lower ultimate population (see Section 5.3).

In recent years vacuum packing has been increasingly used for retail packs of products such as cooked meats, fish and prepared salads. It has been used less often for retail packs of red meats since the meat acquires the purple colour of myoglobin in its unoxygenated form. This does not appeal to consumers even though oxygenation occurs very rapidly on opening a vacuum pack and the meat assumes the more familiar bright red, fresh meat appearance of oxymyoglobin. Cured meats, on the other hand, are often vacuum packed for display since the cured meat pigment nitrosomyoglobin is protected from oxidation by vacuum packing.

The expanding range of chilled foods stored under vacuum and the availability of vacuum packing equipment for small-scale catering and domestic use has prompted concern about increasing the risk from psychrotrophic *Clostridium botulinum*. A number of surveys have been conducted to determine the natural incidence of *C. botulinum* in these products and the concensus is that it is very low. In one recent example,

workers failed to isolate *C. botulinum* or detect toxin in more than 500 samples analysed. When they deliberately inoculated these products with *C. botulinum* spores and incubated at the abuse temperature of 10 °C, only in the case of vacuum packed whole trout was toxin produced within the declared shelf-life of the product. Nevertheless, misuse of the technique does have the potential for increasing risk and a Government committee has recommended that all manufacturers of vacuum packing machinery should include instructions alerting the user to the risks from organisms such as *C. botulinum*.

In a variant of vacuum packing, known as *cuisine sous-vide* processing, food is vacuum packed before being given a pasteurization treatment which gives it a longer shelf-life under chill storage. The technique was developed in the 1970s in France and is said to give an improved flavour, aroma and appearance. It is used for the manufacture of chilled ready meals for various branches of the catering industry and *sous-vide* meals are also available in the retail market in some European countries. They have been slow to appear on the UK market due to the lack of appropriate UK regulations and concern over their microbiological safety with respect to psychrotrophic *C. botulinum*. It has been recommended that *sous-vide* products with an intended shelf-life of longer than 10 days at <3 °C should receive a minimum heat process equivalent to 90 °C for 10 minutes; 70 °C for 100 minutes should be sufficient for products with shorter shelf-lives.

For other types of chilled, vacuum or modified atmosphere packed foods, where there are no other controlling factors such as preliminary heat treatment, low pH or high salt, an advisory 10 day rule is sometimes applied. This states that such foods should have a designated shelf life of ≤ 10 days at storage temperatures ≤ 8 °C. The same rule can also be applied to foods packed in air as anaerobic niches suitable for *C. botulinum* growth can exist in these products too.

In *modified atmosphere packing, MAP*, a bulk or retail pack is flushed through with a gas mixture usually containing some combination of carbon dioxide, oxygen and nitrogen. The composition of the gas atmosphere changes during storage as a result of product and microbial respiration, dissolution of CO_2 into the aqueous phase, and the different rates of gas exchange across the packing membrane. These changes can be reduced by increasing the ratio of pack volume to product mass although this is not often practicable for other reasons.

The initial gas composition is chosen so that the changes which occur do not have a profound effect on product stability. Some examples of MAP gas mixtures used in different products are presented in Table 4.13. Carbon dioxide is included for its inhibitory effect, nitrogen is non-inhibitory but has low water solubility and can therefore prevent pack collapse when high concentrations of CO_2 are used. By displacing oxygen

Table 4.13 *MAP gas mixtures used with foods*

Product	*% CO_2*	*% O_2*	*% N_2*
Fresh meat	30	30	40
	15–40	60–85	–
Cured meat	20–50	0	50–80
Sliced cooked roast beef	75	10	15
Eggs	20	0	80
	0	0	100
Poultry	25–30	0	70–75
	60–75	5–10	>20
	100	0	0
	20–40	60–80	0
Pork	20	80	0
Processed meats	0	0	100
Fish (white)	40	30	30
Fish (oily)	40	0	60
	60	0	40
Cheese (hard)	0–70		30–100
Cheese	0	0	100
Cheese; grated/sliced	30	0	70
Sanwiches	20–100	0–10	0–100
Pasta	0	0	100
	70–80	0	20–30
Bakery	0	0	100
	100	0	0

From *J. Food Protection*, 1991, **54**, 58–70, with permission

it can also delay the development of oxidative rancidity. Oxygen is included in the gas flush mixtures for the retail display of red meats to maintain the bright red appearance of oxymyoglobin. This avoids the acceptability problem associated with vacuum packs of red meats, although the high oxygen concentration (typically 60–80%) helps offset the inhibitory effect of he CO_2 (around 30%) so that the growth of aerobes is slowed rather than suppressed entirely.

In *controlled-atmosphere storage, CAP*, the product environment is maintained constant throughout storage. It is used mainly for bulk storage and transport of foods, particularly fruits and vegetables, such as the hard cabbages used for coleslaw manufacture. CAP is used for shipment of chilled lamb carcasses and primal cuts which are packed in an aluminium foil laminate bag under an atmosphere of 100% CO_2. It is more commonly encountered though with fruits such as apples and pears which are often stored at sub-ambient temperatures in atmospheres containing around 10% CO_2. This has the effect of retarding mould spoilage of the product through a combination of the inhibitory effect of CO_2 on moulds and its ability to act as an antagonist to ethylene, delaying fruit senescence and thus maintaining the fruit's own ability to resist fungal infection.

4.7 CONTROL OF WATER ACTIVITY

The water activity of a product can be reduced by physical removal of liquid water either as vapour in drying, or as a solid during freezing. It is also lowered by the addition of solutes such as salt and sugar. Freezing has already been discussed in this chapter (Section 4.4) and so here we will confine ourselves to drying and solute addition. The primal role of these techniques in food preservation has been alluded to in a number of places. It was the earliest food preservation technique and, until the 19th century, water activity reduction played some part in almost all the known procedures for food preservation.

Nature provided early humans with an object lesson in the preservative value of high solute concentrations in the form of honey produced by bees from the nectar of plants. The role of salt in decreasing a_w accounts for its extreme importance in the ancient economy as evidenced today in the etymology of the word, salary, and of place names such as Salzburg, Nantwich, Moselle and Malaga. It can also be seen in the extraordinary hardship people were prepared to endure (or inflict on others) to ensure its availability; to this day the salt mine remains a byword for arduous and uncomfortable labour.

Solar drying, while perhaps easy and cheap, is subject to the vagaries of climate. Drying indoors over a fire was one way to avoid this problem and one which had the incidental effect of imparting a smoked flavour to the food as well as the preservative effect of chemical components of the smoke.

Salting and drying in combination have played a central role in the human diet until very recently. One instance of this is the access it gave the population of Europe to the huge catches of cod available off Newfoundland. From the end of the 15th century, salted dried cod was an important item in trans-Atlantic trade and up until the 18th century accounted for 60% of all the fish eaten in Europe. It remains popular today in Portugal and in the Caribbean islands where it was originally imported to feed the slave population. Other traditional dried and salted products persist in the modern diet such as dried hams and hard dry cheeses but the more recent development and application of techniques such as refrigeration, MAP, and heat processing and the preference for 'fresh' foods has meant that their popularity has declined. Nevertheless this should not obscure the important role that low a_w foods still play in our diet in the form of grains, pulses, jams, bakery products, dried pasta, dried milk, instant snacks, desserts, soups, *etc*.

Among the main features of the effect of a_w on the growth and survival of micro-organisms discussed in Section 3.2.5 it was noted that microbial growth does not occur below an a_w of 0.6. This applies to a number of

food products (Figure 3.9), but the fact that microbial spoilage is not possible given proper storage conditions, does not mean that they do not pose any microbiological problems. Micro-organisms that were in the product before drying or were introduced during processing can survive for extended periods. This is most important with respect to pathogens if they were present in hazardous numbers before drying or if time and temperature allow them to resume growth in a product that is rehydrated before consumption. There have been a number of instances where the survival of pathogens or their toxins has caused problems in products such as chocolate, pasta, dried milk and eggs. Generally *Salmonella* and *Staphylococcus aureus* have been the principal pathogens involved – there have been about 20 major outbreaks associated with these organisms and dried milk since 1955, but spore formers are particularly associated with some other dried products such as herbs or rice.

Intermediate moisture foods, IMFs, are commonly defined as those foods with an a_w between 0.85 and 0.6. This range, which corresponds roughly to a moisture content of 15–50%, prohibits the growth of Gram-negative bacteria as well as a large number of Gram-positives, yeasts and moulds, giving the products an extended shelf-life at ambient temperature. When spoilage does occur, it is often a result of incorrect storage in a high relative humidity environment. In correctly stored products growth of xerophilic moulds, osmophilic yeasts or halophilic bacteria may occur, depending on the product, and in many IMFs the shelf-life is further protected by the inclusion of antifungal agents such as sulfur dioxide or sorbic acid.

At the a_w of IMFs, pathogens are also prevented from growing. Although *Staph. aureus* is capable of growing down to an a_w of 0.83, it cannot produce toxin and is often effectively inhibited by the combination of a_w with other antimicrobial hurdles.

There are a number of traditional IMFs such as dried fruits, cakes, jams, fish sauce and some fermented meats. Sweetened condensed milk is one interesting example. Milk is homogenized, heated to 80 °C and sugar added before it is concentrated in a multi-effect vacuum evaporator at 50–60 °C. When the product emerges from the concentration stage it is cooled and seeded with lactose crystals to induce crystallization of the lactose. This gives sweetened condensed milk its characteristic gritty texture. Although the product is packed into cans and has an almost indefinite shelf-life, it is not an appertized food. Its stability is a result of its high sugar content (62.5% in the aqueous phase) and low a_w (<0.86). Spoilage may sometimes occur due to growth of osmophilic yeasts or, if the can is under-filled leaving a headspace, species of *Aspergillus* or *Penicillium* may develop on the surface.

Some years ago, our developing understanding of the stability of IMFs led to considerable interest in applying the same principles to the

development of new shelf stable foods. Novel humectants such as glycerol, sorbitol and propylene glycol were often used to adjust a_w in these products in addition to the solutes salt and sugar. They were not however well received in the market for human food because of acceptability problems, although a number of successful pet food products were developed. One interesting observation made during this work is that products with the same water activity differ in their keeping quality depending on how they are made. Traditional IMFs are generally made by a process of desorption whereby water is lost from the product during processing but a number of the new IMFs used an adsorption process in which the product is first dried and its moisture content readjusted to give the desired a_w. The hysteresis effect in water sorption isotherms (see Section 3.2.5 and Figure 3.11) means that although products made using the two techniques will have the same initial a_w they will have different moisture contents and so will eventually equilibrate to different a_w values. It was found that products made by desorption and having the higher water content were also more susceptible to microbial spoilage.

Solar drying is still widely practised in hot climates for products such as fruits, fish, coffee and grain. The traditional technique of spreading the product out in the sun with occasional turning often gives only rudimentary or, sometimes, no protection from contamination by birds, rodents, insects and dust. Rapid drying is essential to halt incipient spoilage; this is usually achievable in hot dry climates, though in tropical countries with high humidity drying is usually slower so that products such as fish are often pre-salted to inhibit microbial growth during drying.

There are a number of procedures for mechanical drying which are quicker, more reliable, albeit more expensive than solar drying. The drying regime must be as rapid as possible commensurate with a high-quality product so factors such as reconstitution quality must also be taken into account. With the exception of freeze-drying where the product is frozen and moisture sublimed from the product under vacuum, these techniques employ high temperatures. During drying a proportion of the microbial population will be killed and sub-lethally injured to an extent which depends on the drying technique and the temperature regime used. It is however no substitute for bactericidal treatments such as pasteurization. Although the air temperature employed in a drier may be very high, the temperature experienced by the organisms in the wet product is reduced due to evaporative cooling. As drying proceeds and the product temperature increases, so too does the heat resistance of the organisms due to the low water content. This can be seen for example in the differences between spray dried milk and drum dried milk. In spray drying, the milk is pre-concentrated to about 40–45% solids before being sprayed into a stream of air heated to

temperatures up to 260 °C at the top of a tower. The droplets dry very rapidly and fall to the base of the tower where they are collected. In drum drying, the milk is spread on the surface of slowly rotating metal drums which are heated inside by steam to a temperature of about 150 °C. The film dries as the drum rotates and is scraped off as a continuous sheet by a fixed blade close to the surface of the drum. Although it uses a lower temperature, drum drying gives greater lethality since the milk is not subject to the same degree of pre-concentration used with spray-dried milk and the product spends longer at high temperatures in a wet state. Spray drying is however now widely used for milk drying because it produces a whiter product which is easier to reconstitute and has less of a cooked flavour. Milk is pasteurized before drying although there are opportunities for contamination during intervening stages. Most of the organisms which survive drying are thermoduric but Gram-negatives may survive and have on occasion been the cause of food poisoning outbreaks.

The limited lethality of drying processes and the long storage life of dried products means that manufacturers are not exempt from the stringent hygiene requirements of other aspects of food processing. Good quality raw materials and hygienic handling prior to drying are essential. Outbreaks of *Staph. aureus* food poisoning have been caused by dried foods which were stored at growth temperatures for too long prior to drying allowing the production of heat resistant toxin which persisted through to the final product. The dried product must also be protected from moisture by correct packaging and storage in a suitable environment otherwise pockets of relatively high a_w may be created where microbial growth can occur.

4.8 COMPARTMENTALIZATION

Butter is an interesting example of a rather special form of food preservation where microbial growth is limited by compartmentalization within the product.

Essentially there are two types of butter: sweet cream butters, which are often salted, and ripened cream butters. In ripened-cream butters, the cream has been fermented by lactic acid bacteria to produce *inter alia* diacetyl from the fermentation of citrate which gives a characteristically buttery flavour to the product. They have a stronger flavour than sweet-cream butters but are subject to faster chemical deterioration. Sweet-cream butter is most popular in the United States, Ireland, the UK, Australia and New Zealand whereas the ripened cream variey is more popular in continental Europe.

Butter is an emulsion of water droplets in a continuous fat phase in contrast to milk which is an emulsion of fat globules in a continuous

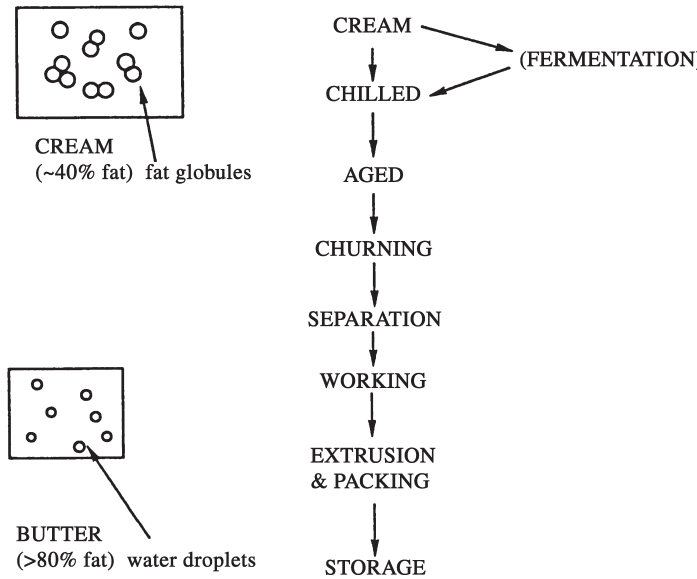

Figure 4.15 *Buttermaking*

water phase (Figure 4.15). It has a higher fat content than milk (80%) and uses pasteurized cream as its starting point. Typically, the cream is pasteurized using an HTST process of 85 °C for 15 s and held at 4–5 °C for a period to allow the fat globules to harden and cluster together. In making a conventional ripened cream butter, the starter culture is added at this stage and the cream incubated at around 20 °C to allow flavour production to take place. A more recent method developed at NIZO, the Dutch Dairy Research Institute, employs a concentrated starter added to sweet-cream butter after manufacture. Phase inversion, the conversion from a fat-in-water emulsion to a water-in-fat emulsion, is achieved by the process of churning. During this process fat globules coalesce, granules of butter separate out, and considerable amounts of water are lost from the product in the form of buttermilk. The buttermilk phase retains most of the micro-organisms from the cream and numbers may show an apparent increase due to the breaking of bacterial clumps.

Traditional farmhouse buttermaking used wooden butter churns and these were originally scaled up for the earliest commercial butter-making. However, the impossibility of effectively cleaning and sanitizing wood has led to its replacement by churns made of stainless steel or aluminium–magnesium alloys. After the butter has formed, the buttermilk is drained off, the butter grains washed with water and, in the case of sweet-cream butter, salt is added usually at a level of 1–2%. The butter is then

'worked' to ensure further removal of moisture and an even distribution of water and salt throughout the fat phase. In properly produced butter the water is distributed as numerous droplets ($>10^{10}\,g^{-1}$) mostly less than 10 μm in diameter. Since the butter should contain at most around 10^3 cfu g^{-1}, most of these droplets will be sterile. In those that do contain micro-organisms, the nutrient supply will be severely limited by the size of the droplet. If the butter is salted, the salt will concentrate in the aqueous phase along with the bacteria which will therefore experience a higher, more inhibitory salt level. For example, bacteria in a butter containing 1% salt and with a moisture content of 16% would experience an effective salt concentration of 6.25%.

Few micro-organisms survive pasteurization so the microbiological quality of butter depends primarily on the hygienic conditions during subsequent processing, particularly the quality of the water used to wash the butter. Good microbiological quality starting materials are essential though, as preformed lipases can survive pasteurization and rapidly spoil the product during storage. Butter spoilage is most often due to the development of chemical rancidity but microbiological problems do also occur in the form of cheesy, putrid or fruity odours or the rancid flavour of butyric acid produced by butterfat hydrolysis. Pseudomonads are the most frequently implicated cause and are thought to be introduced mainly in the wash water. Psychrotrophic yeasts and moulds can also cause lipolytic spoilage and these are best controlled by maintaining low humidity and good air quality in the production environment and by ensuring the good hygienic quality of packaging materials. In this respect aluminium foil wrappers are preferred to oxygen-permeable parchment wrappers as they will help discourage surface mould growth.

Butter is a relatively safe commodity from a microbiological standpoint, although there was an outbreak of listeriosis in Finland in 1998/9 which affected 18 people, four of whom died (see 7.9.5).

Margarine relies on a similar compartmentalization for its microbiological stability, but uses vegetable fat as its continuous phase. Although skim milk is often included in the formulation, it is possible to make the aqueous phase in margarine even more deficient nutritionally than in butter, thus increasing the microbiological stability further. With the move towards low fat spreads containing 40% fat, the efficacy of this system is more likely to breakdown. A higher moisture content means that the preservative effect of salt or lactic acid, which is often included, is diluted and that micro-organisms can grow to a greater extent in the larger aqueous droplets. In these cases the use of preservatives may be required to maintain stability. An approach developed at Unilever's laboratories in the Netherlands is based on a two stage approach where the composition of the aqueous phase is analysed to determine its

capacity to support the growth of different spoilage organisms. If there is some potential for microbial growth to occur, this is then calculated by working out which fraction of water droplets will be contaminated and then summing the growth in each of them. These models have been incorporated into an expert system to predict the stability of any proposed product formulation so that microbiological stability can be designed into the product.