The stability and shelf-life of food

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Preface

The food industry faces the task of satisfying the many varied requirements of an increasingly demanding consumer population. Food must be safe, of consistently good quality, healthy and inexpensive; food must satisfy the palates of adventurous variety-seekers, but most not alienate traditionalists; food should be perceived as natural and fresh; and food should keep as long as possible whilst maintaining the required qualities. It is easy to identify the potential conflicts in these requirements, but less easy to understand how to implement effective commercial scientific and commercial strategies.

One of the major problems facing food manufacturers is responding rapidly to the demands of the major retailers who supply a large proportion of the market. Rapid response can be driven by a number of factors, such as intense commercial competition and media-driven food issues, with the consequence that new or modified products need to be introduced to the shelves as quickly as possible. Manufacturers have an increasing range of technologies and ingredients with which to design these qualities into their products, but then face the enormous difficulty of trying to assess how these qualities will be maintained over the intended shelf-life. Commercial pressures are such that the development timescale for a new product can be so short that there is little or no opportunity to establish how the product quality is maintained on storage. Practical experience at the Leatherhead Food RA has shown that establishing reliable product shelf-lives in short development timescales is one of the greatest sources of confusion and frustration in the food manufacturing sector. How many companies can identify with this problem:

> We've been developing a new product concept for a few months, and a buyer from one of the major multiples has become very excited by one

> of the variants, and wants it on the shelves in four weeks. We're confident that we have a packaging system, and we have spare production capacity, but the supermarket wants a nine-month shelf-life. We only developed this variant three weeks ago, so what can we do to get a best before date?

The enormous difficulties inherent in this problem are compounded by the surprisingly sparse amount of helpful literature that is easily accessible.

The contributions to this book address important issues associated with the nature of shelf-life and the shelf-life characteristics of some important food types. Much of the content relates to the key issues of the microbial stability of foods and of the sensory changes that occur in foods on storage. One essential requirement for any product developer is to understand how these factors are themselves influenced by the chemical and physical changes that can occur during storage, and how these are influenced by the internal environment created by the packaging system and the external environment in which the food is stored. Methods for measuring changes relevant to product quality are under active development, and the industry must maintain awareness of innovations in this area. Even if the techniques appear to be inaccessible to the manufacturer, technological developments will inevitably bring the more useful techniques into general uses.

One of the most desirable but elusive goals of the industry is the construction of predictive shelf-life models. Much progress has been made in microbial modelling, but little in modelling other changes, including sensory changes. Such models should ideally be applicable to the even more difficult problem of the shelf-life of complex composite products such as prepared foods, which represent the fastest-growing food sector.

In editing this book we would like to thank all the contributors for their willingness to share their expert knowledge. We would particularly like to thank our colleagues at the Leatherhead Food RA for the years of invaluable discussion and advice. Finally, we need to thank all those workers in the food industry for coming to us with their problems; we hope that we have been able to help many, and that this book will give the remainder further insights.

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Introduction

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1.1 What is shelf-life?

Consumers are increasingly demanding consistently high food quality, and have corresponding expectations that such quality will be maintained at a high level during the period between purchase and consumption. These expectations are a consequence not only of the primary requirement that the food should remain safe, but also of the need to minimise unwanted changes in sensory quality. The quality needs are reflected in the labelling requirements to which food manufacturers must conform. In the UK, the date coding to be used is determined by the total life of the product: for microbiologically highly perishable foods, a 'use by' date is needed, while for other foods, including foods with more than 18 months' shelf-life, a 'best before' or a 'best before end' date is needed. In general, microbiological changes are of primary importance for short-life products, and chemical and sensory changes for medium- to long-life products; all three types of change can be important for short- to medium-life products (McGinn, 1982).

However, manufacturers must have the means available to predict the end-point of storage life under a given set of storage conditions. Criteria based on the measured numbers of spoilage and pathogenic microorganisms and their growth pattern are capable of relatively clear definition. Non-microbiological criteria are more difficult to define, although criteria based on well-defined chemical composition, such as vitamin content, are addressable. Defining desired sensory characteristics is a particular problem area for many companies even when dealing with fresh product; defining desired sensory characteristics following storage is even more difficult. The sensory characteristics of most foods deteriorate throughout storage (with important exceptions such as wine and

cheese), and yet, provided they remain safe, a large degree of change is evidently tolerable to consumers. Acceptable sensory characteristics are consequently often defined by company policy, but nonetheless it is important to understand how these change on storage and to use these data in helping define shelf-life.

This difficulty can be seen in the IFT (1974) definition of shelf-life, as:

> The period between manufacture and retail purchase of a food product during which the product is of satisfactory quality.

The use of the words 'of satisfactory quality' is too loose to be of much practical help, especially in situations in which microbial safety is not an issue. The more recent IFST Guidelines (1993) provide a more workable definition of shelf-life:

> Shelf-life is defined as the time during which the food product will:
>
> (i) remain safe;
> (ii) be certain to retain desired sensory, chemical, physical and microbiological characteristics;
> (iii) comply with any label declaration of nutritional data,
>
> when stored under the recommended conditions.

This definition succeeds in identifying the key factors that must be considered when assessing shelf-life, but again leaves interpretation of the words 'desired … characteristics' highly ambiguous. This ambiguity perhaps reflects an important consideration. Except in situations in which microbiological safety is an issue, the definition of shelf-life is related to the positioning of the product in the market in terms of quality and customer perceptions of that quality. For example, an economy product that, following manufacture, has a lower quality index than a premium product, does not necessarily have a shorter shelf-life, even if the deterioration rate is the same. Consumers of a premium product will have a higher expectation of quality over the entire shelf-life period. Alternatively, it is possible to picture a situation in which a premium product at the end of its shelf-life has a higher perceived quality than an economy product at the start of its storage life.

The IFST definition also raises the important issue of storage conditions on product shelf-life. Measurement of storage characteristics takes place under carefully controlled environmental conditions that are rarely met in practice, especially once the product has left the retail environment. Thermal abuse in the distribution chain is common, but becomes almost routine in a domestic environment. Ambient temperature conditions in the kitchen vary widely, and temperature control in domestic refrigerators and freezers is frequently poor. It is therefore important for the food manufacturer to have an understanding of the storage characteristics of the product under a wide range of storage conditions, and even under the fluctuating or cyclical conditions that are commonly encountered in practice in the supply chain. If the behaviour of the product on storage is to be understood, it is equally important for the manufacturer to have a thorough understanding of the mechanism of the deterioration process(es),

which can be complex in many foods, especially those with composite structures.

1.2 Factors influencing shelf-life

Many factors can influence shelf-life, and can be categorised into intrinsic and extrinsic factors (IFST, 1993). Intrinsic factors are the properties of the final product. They include the following:

- Water activity (a_w) (available water).
- pH value and total acidity; type of acid.
- Redox potential (E_h).
- Available oxygen.
- Nutrients.
- Natural microflora and surviving microbiological counts.
- Natural biochemistry of the product formulation (enzymes, chemical reactants).
- Use of preservatives in product formulation (e.g. salt).

Intrinsic factors are influenced by such variables as raw material type and quality, and product formulation and structure. Extrinsic factors are those factors the final product encounters as it moves through the food chain. They include the following:

- Time–temperature profile during processing; pressure in the headspace.
- Temperature control during storage and distribution.
- Relative humidity (RH) during processing, storage and distribution.
- Exposure to light (UV and IR) during processing, storage and distribution.
- Environmental microbial counts during processing, storage and distribution.
- Composition of atmosphere within packaging.
- Subsequent heat treatment (e.g. reheating or cooking before consumption).
- Consumer handling.

All these factors can operate in an interactive and often unpredictable way, and the possibility of interactions must be investigated. A particularly useful type of interaction occurs when factors such as reduced temperature, mild heat treatment, antioxidant action and controlled atmosphere packaging operate in concert to restrict microbial growth, the so-called ‘hurdle effect’. This way of combining factors which, individually, are unable to prevent microbial growth but, in combination, provide a series of hurdles which do so, allows manufacturers to use milder processing techniques which retain more of a product’s sensory and nutritional properties.

The interaction of such intrinsic and extrinsic factors as these either inhibits or stimulates a number of processes which limit shelf-life. These processes can be conveniently classified as:

- Microbiological.
- Chemical.
- Physical.
- Temperature related.

1.2.1 Microbiological changes

Growth of a specific microorganism during storage depends on several factors, the most important being: the initial microbial loading at the start of storage; the physicochemical properties of the food, such as moisture content, pH, presence of preservatives; the processing method used in the production of the food; and the external environment of the food, such as the surrounding gas composition and storage temperature. A number of key intrinsic and extrinsic factors affecting the growth of some key pathogens and spoilage organisms are shown in Table 1.1. It is important to note that this table lists approximate growth limits

Table 1.1 Minimum growth conditions for selected microorganisms

Type of microorganism	Minimum pH for growth	Minimum A_w for growth	Anaerobic growth[a]	Minimum growth temp.[b] (°C)
Pathogens[c]				
Salmonella	4.0	0.94	Yes	7
Staphylococcus aureus	4.0 (4.5 for toxin)	0.83 (0.90 for toxin)	Yes	6 (10 for toxin)
Bacillus cereus (psychrotrophic)	4.4	0.91	Yes	< 4
Clostridium botulinum				
Proteolytic A, B, F	4.6	0.93	Yes	10
Non-proteolytic B, E, F	5.0	0.97	Yes	3.3
Listeria monocytogenes	4.3	0.92	Yes	0
Escherichia coli	4.4	0.95	Yes	7.0
Vibrio parahaemolyticus	4.8	0.94	Yes	5
Yersinia enterocolitica	4.2	0.96	Yes	−2
E. coli 0157	4.5	0.95	Yes	−6.5
Spoilage organisms[d]				
Pseudomonas	5.5	0.97	No	< 0
Enterobacter aerogenes	4.4	0.94	Yes	2
Lactic acid bacteria	3.8	0.94	Yes	4
Micrococci	5.6	0.9	No	4
Yeasts	1−5	0.8	Yes	−5
Moulds	< 2.0	0.6	No	< 0

[a] Survival without oxygen, for example in vacuum pack.
[b] Minimum growth temperatures are for growth in typical neutral pH. High water activity, chilled foods.
[c] Data for pathogens taken from Anon., *Harmonisation of Safety Criteria for Minimally Processed Foods*. Inventory Report, Fair Concerted Action FAIR CT96-1020, 1997.
[d] Data for spoilage organisms taken from Brown, H.M., *Evaluation of the Shelf-life of Chilled Foods*. Campden and Chorleywood FRA Technical Manual No. 28, 1991.

with the various factors acting alone. Interactions between these factors may alter these limits considerably.

The growth of food-poisoning organisms such as *Salmonella* species and *Listeria monocytogenes* will not necessarily be accompanied by changes in appearance, odour, flavour or texture that could be detected by the human senses, and consequently pose serious health concerns. Growth of spoilage organisms is often readily identified by sensory changes, for example visual mould growth, generation of off-odours and flavours and changes in texture, frequently from the action of enzymes produced by microorganisms.

1.2.2 Chemical deteriorative changes

Many important deteriorative changes can occur arising from reactions within the food or from reactions of food components with external species, for example oxygen. Rancidity development is an important factor in fat-containing foods, and can occur via different mechanisms, for example lipolytic/hydrolytic reactions, oxidative reactions and flavour reversion reactions. Enzymic processes limit the shelf-life of fruits and vegetables, and oxidation reactions limit the shelf-life of meat. Chemical hydrolysis can occur in products containing intense sweeteners, reducing sweetness, and non-enzymic browning can occur in many foods from Maillard reactions. Changes can also occur on exposure to light, including colour loss in natural food colours and rancidity and off flavour development in milk and in snack foods.

1.2.3 Physical deteriorative changes

Moisture migration is a major cause of deteriorative physical changes in food. This is easily seen in fresh produce through moisture loss, and dry products such as breakfast cereals and biscuits can lose their crispness through moisture uptake. Delicatessen salads can also deteriorate from migration of water from the vegetable component into the dressing. Freezer burn is also a consequence of moisture migration from the surface of frozen foods. Other migration phenomena can limit shelf-life, particularly of more complex composite foods, such as migration of fat from one component to another (described in more detail in Chapter 10), and the bleeding of colours in composite products such as chilled desserts. Physical changes in packaging materials, sometimes coupled with subsequent chemical reactions, can also limit sensory shelf-life. As an example, permeability changes with time can change the in-pack equilibrium atmosphere, giving rise to both microbiological and chemical effects. Such changes may also allow migration of external volatiles into the food, resulting in the development of taint. Migration of chemical components from the packaging material can also produce taints, and this can be particularly serious in products with a long shelf-life.

1.2.4 Temperature-related deteriorative changes

Deterioration can occur at both elevated and depressed temperatures. The minimum growth temperatures for a range of pathogens and spoilage organisms outlined earlier illustrates the importance of effective temperature control in preventing microbial contamination and spoilage. Increasing the temperature generally increases the rate of chemical reactions that may result in deterioration. In foods containing fats, more solid fat will become liquid and act as a solvent for reactions in the oil phase, and changes in fat crystallinity can occur, for example producing bloom in chocolate. Increased temperature can also change the crystallisation characteristics of foods containing sugar syrups. Destabilisation of emulsion systems can also occur under conditions of fluctuating temperature or mechanical agitation. Fluctuating temperatures can cause ice crystal formation in frozen foods such as ice-cream. In contrast, increased temperatures can reduce the development of staling in bread, although the situation with other baked foods can be complex and unpredictable.

1.3 Types of deterioration

The factors described in the previous sections can result in a wide range of deteriorative changes, and these will depend on the food type. The Appendix at the end of this chapter shows examples of the main deteriorative changes in a variety of food classes, and the consequential factors limiting shelf-life. In composite foods the factors limiting shelf-life can be quite different from those that limit the shelf-life of the individual components. For example, an important factor limiting shelf-life in breakfast cereals containing a mixture of cereal and dried fruit is the hardening of the fruit from moisture migration into the cereal. In contrast, the limiting factors for the individual fruit and cereal components would be flavour changes arising from chemical reactions, and moisture uptake and softening of the cereal.

1.4 Measuring shelf-life

1.4.1 Sensory panels

Measurement of the changes in eating quality on storage requires the use of sensory techniques. These are usually quantitative quality measures from trained panels, but can also involve assessment of liking using naïve consumers, and are covered in more detail in Chapter 4. Sensory techniques, whilst powerful and of high direct validity, are expensive and time-consuming, especially for the repeated measures needed for shelf-life assessment. There are substantial difficulties in ensuring high quality sensory data over long test periods, and instrumental methods can be an important back-up to sensory methods, provided that their limitations are recognised.

Use of any sensory testing requires an appropriate set of ethical procedures that are designed to protect the health of the assessors. This is particularly

important in carrying out sensory shelf-life testing, where special care should be taken to ensure that microbiological risks are minimised. Testing chilled foods, in situations in which there is little information on the end of shelf-life, and carrying out accelerated testing at elevated temperatures, both require specific precautions. Microbiological testing should be carried out in advance of sensory testing if there are any doubts regarding safety, and if there are any residual doubts, sensory testing should be restricted to appearance and odour evaluation.

1.4.2 Instrumental methods

Sensory measures of quality changes on storage are an essential measure of perceived quality, but are expensive and time-consuming to operate. They also suffer from high variability when carried out over long time periods, requiring regular panel calibration. If valid instrumental methods are available, they can be of great value in augmenting sensory data.

Many attempts have been made to use instrumental techniques to measure sensory quality factors, but these can only be seen as reliable if the measurement has been validated against sensory measurements. Powerful instruments for measuring physical properties, such as computerised texture analysers and rheometers, and for measuring flavour properties, such as the volatile detectors misleadingly named ‘electronic noses’ are of value only if the measured parameters can be correlated with relevant sensory attributes. Instrumental methods can, however, be an important complement to sensory methods, provided that their limitations are recognised.

One example of how the two methods are used together is in the detection of off-flavours. The first stage in the analysis of an off-flavour is usually sensory evaluation of the product concerned by a trained panel. The ability of panellists to describe accurately the sensory properties of off-flavours and to relate them to known standards is particularly useful since it can provide vital information about the chemical nature of the off-flavour. After sensory evaluation, a concentrated flavour extract is prepared, and the extract fractionated into individual components. A wide variety of techniques may be used to extract the flavour (and off-flavour) from the product, but steam distillation solvent extraction (SDE) using Likens–Nickerson apparatus is particularly useful and is frequently used as the method of choice. The flavour extracts can then be fractionated by high resolution gas chromatography, sometimes coupled with sniffing port olfactometry. The chemical identification of the individual chromatographic peaks is usually carried out by mass spectrometry and descriptive information from the sensory panel can often allow selected ion monitoring of specific species to increase sensitivity. It is important that, whatever detection method is used, any tentative identifications are confirmed by sensory techniques. In addition, such techniques usually rely on comparison of the analytical and sensory data with those from a suitable control sample in order to identify chemical components that can give rise to off-flavours at low concentrations.

The on-line or rapid at-line measurement of variables relevant to shelf-life is of increasing importance to the food industry. An outline of some of these variables, their relationship to key aspects of shelf-life, and the kind of instrumentation available to measure these variables is shown in Table 1.2. One example of the way instrumentation can assist in the measurement and control of shelf-life is the measurement of water activity. Water activity has already been identified as an intrinsic factor in determining shelf-life. A very important function of water is in supporting the way enzymes interact within cells. A reduction in water activity thus affects the reproduction, metabolic activity, resistance and survival of microorganisms in food. Measuring and controlling water activity provides a means of monitoring and controlling pathogenic and spoilage bacteria using, for example, conductivity humidity meters or hygrometers. Such instrumentation has been used, for example, in computer-controlled air-conditioned curing chambers controlling the ripening of raw sausage (Rodel, 1993).

A more targeted approach is provided by the so-called 'marker' concept. This concept depends on identifying a chemical or physical property which is closely linked to the process of deterioration, and then designing a sensor able to measure some aspect of this property and thus track product deterioration. This can be done, for example, with biosensors: devices that incorporate a biologically active material which reacts with target chemicals related to the property being measured. The range of applications of marker devices includes biosensors used to measure meat freshness using levels of glucose concentration, and mechanical resonance probes for frying oil which measure the viscosity increase of the oil which accompanies the oxidation and polymerisation on frying-induced deterioration. A range of chemical markers which biosensors and other types of sensor can measure in tracking and analysing shelf-life deterioration is shown in Table 1.3. Recent developments in biosensors are summarised in Kress-Rogers (1997).

Chapter 4 reviews some instrumental methods used in detecting deteriorative changes in appearance, aroma and flavour, and texture. Chapter 6 describes the use of advanced instrumental methods, in this case nuclear magnetic resonance (NMR), to measure and analyse factors influencing shelf-life, and Chapter 11 provides a case study of the use of instrumental methods in tracking deterioration in fruit and vegetables.

1.4.3 Physical measurements

The most commonly used physical tests measure the changes in the texture of products. These changes may be the result of chemical reactions occurring in the product, such as those caused by interaction of ingredients or by environmental influences, such as moisture migration through the packaging. Methods of measurement for texture have to be chosen carefully so that the results correlate well with the textural changes as perceived by the use of sensory panels. Various instruments are available for texture measurement and instrumental methods of

Table 1.2 Selected measurement variables and instrumental types

	Food safety and stability				Food quality				Instrument type
	Microbial aspects	Chemical aspects	Physical aspects	Nutritional aspects	Appearance	Texture consistency	Aroma	Taste	
Colour					x				Ultraviolet, visible, near infrared
Sorting by colour	x	x	x	x	x	x	x	x	light detector; optical imaging
Temperature	x	x	x						Thermocouples; resistance thermometers;
Temperature–time integral	x	x	x	x	x	x	x	x	near infrared detector; fibre-optic probe with fluorescent tip
Particle, droplet or bubble size			x		x	x			Radiowave detector; ultrasound
Solid/liquid ratio and crystal size			x		x	x			Nuclear magnetic resonance (NMR); ultrasound
Bulk density			x		x	x			Mechanical resonance dipstick, gamma-rays; ultrasound
Rheology			x		x	x			Capillary viscometers; rotary viscometers; rheometers
Texture						x			Puncture/penetration devices; shearing and cutting devices; compression devices; flow and mixing devices; tenderometer, rheometer
Water activity/content/quality	x	x	x	x	x	x		x	Near infrared detector; microwaves; electrical conductivity
Proximates: fat, protein, carbohydrates, ash				x	x	x	x	x	Near infrared detector; microvaves
pH	x	x					x	x	Electrometric devices; biosensors; immunosensors
Acidity				x			x	x	Biosensors; immunosensors
Sodium, potassium, calcium				x				x	Radiowave detector
Humidity	x		x		x	x			Hygrometer, capacitance

Source: E. Kress-Rogers, *Instrumentation and Sensors for the Food Industry*. Cambridge: Woodhead Publishing 1993; P. Fellows, *Food Processing Technology*, Second

Table 1.3 Some applications of the marker approach

Indicator	Condition to be assessed	Instrument type
Viscosity	Frying oil quality *in situ* in hot oil	Mechanical resonance probe
Glucose profile	Meat freshness (pre-spoilage stage) Browning potential (Maillard reaction)	Biosensor in an aqueous phase
Amines:		
Trimethylamine	Loss of freshness (fish)	
Histamine, tyramine	Past microbial activity (meat, fish, cheese)	
Cadaverine, putrescine	Microbial spoilage (advanced)	
Purines: ATP, inosine, hypoxanthine	Fish freshness (for very fresh fish)	
Aldehydes: pentanal, hexanal	Oxidative rancidity	Sensor for gases and volatiles (in headspace or through membrane)
Hydrogen, a range of volatiles	Microbial spoilage of CAP and vacuum-packed meat	
Ethylene	Fruit ripening	

Source: E. Kress-Rogers, *Instrumentation and Sensors for the Food Industry*. Cambridge: Woodhead Publishing, 1993.

measuring attributes such as hardness, crispness and snap are commonly used during shelf-life testing. Some attributes, such as hardness, can be measured relatively easily by measuring the force required to penetrate a particular distance into the product. However, even in simple cases, the details of the tests, such as type of probe, cross-head speed, sample position and alignment, distance of penetration need to be chosen carefully to obtain the best possible correlation with sensory measurements. More sophisticated methods are also being developed, such as non-destructive tests for on-line texture measurement, the measurement of sound as a measure of textural attributes and methods for measuring difficult attributes such as stickiness.

1.4.4 Chemical measurements

Chemical analyses play a vital role in shelf-life testing as they can be used either to measure the end points of chemical reactions occurring in food during storage, or to confirm the results obtained by the sensory panels. Some examples of product deterioration caused by chemical reactions within the food are given in section 1.2.2. For any given product, many different chemical reactions occur simultaneously during storage. However, only the key reactions influencing changes in product quality need be measured during shelf-life testing. Some chemical tests determining changes in a particular quality characteristic can be applicable to different types of products. One such example is the measurement

of peroxide value and free fatty acid content as markers for the level of rancidity of products. Other tests may be product specific and sometimes exploratory, such as those used to measure off-flavour development in products. Particular attention to methodology is required to ensure that the tests are accurate, and as with any other tests, the greater the accuracy of measurement, the more accurate will be the estimation of shelf-life.

1.4.5 Microbiological measurements

There are two important aspects to be considered in determining the microbiological stability of a product;

1. microbial growth, which leads to the spoilage of a food product
2. the growth of microbial pathogens that affect the safety of the product.

The water activity, storage temperature, time and pH can be used to predict to a large extent the micro-organisms that are likely to grow in the product.

The 'time to spoilage' can be determined by storing the product at the appropriate temperature and measuring the microbial load at staged intervals. The time to reach a pre-determined level of microbial count (total count and level of individual microbes) will be considered to be the end-point. Since it is advisable to leave a safety margin in setting the shelf-life, generally 70% of the time to spoilage is taken to be the storage life (Patrick, 2000).

Challenge testing can be used to determine the likelihood of the growth of particular microorganisms, such as those causing food poisoning. In this case selected microorganisms are inoculated into products and the growth of these monitored through a storage test. Challenge testing can also be applied to study the possibility of the growth of selected resistant spoilage organisms that may contaminate the food from the factory or production environment. Mathematical modelling, such as Food MicroModel, can be used to identify specific organisms that could cause food safety problems and to predict their rate of growth in the products.

1.5 Predicting shelf-life

1.5.1 Accelerated shelf-life testing

Food manufacturers are under increasing pressures to introduce attractive new products into retail outlets with minimum delay, and legislation in many countries demands some form of sell by or use by labelling. While this is feasible for short shelf-life products, the introduction of new long shelf-life products requires knowledge of the storage characteristics over the intended shelf-life period, and can introduce unacceptable delays. Consequently, accelerated shelf-life procedures are often attempted in order to circumvent this problem. Such procedures can only be used if there is a known and validated relationship between the storage characteristics under an ambient storage

condition and the storage characteristics under an accelerated condition. The use of such methods has been addressed in detail (e.g. Labuza and Schmidl, 1985), and is covered in detail in Chapter 5, but some general aspects are now described briefly.

The basic premise of an accelerated test is that by changing a storage condition, the chemical or physical process that leads to deterioration is accelerated, and that a predictive shelf-life relationship related to ambient conditions can be defined. The key to this premise is the assumption that the deteriorative process limiting shelf-life remains the same under the two conditions. If this is not the case, and another deteriorative process dominates at the abuse condition, then a valid relationship is not attainable. It is also often (erroneously) assumed that accelerated deterioration can be achieved by raising the storage temperature, using an Arrhenius model (Labuza and Schmidl, 1985). This model is only appropriate for simple chemical systems, however, and often fails for complex foods, for example in bread, where an increase in temperature decreases the rate of staling reactions. Some of the processes that can take place at elevated temperatures and that change the deteriorative processes are as follows (Labuza and Schmidl, 1985):

- Phase changes from the melting of fats, and change in solvent properties.
- Crystallisation of amorphous carbohydrates.
- Change in the relative rate of chemical reactions with different activation energies.
- Increased water activity.
- Denaturation of proteins.
- Decreased solubility of gases.

The overall effects of such processes on quality is often not predictable, and can lead to either under- or overestimated shelf-life predictions.

It is therefore important to test the validity of any accelerated conditions against the known deterioration characteristics under ambient storage conditions, and to establish the limits of the reliability of any relationship found. If time pressures do not allow the identification of the ambient storage characteristics of a product, then comparisons can sometimes be made between the test product and an equivalent product of similar structure and for which a shelf-life has previously been established. Although there any many questions regarding the reliability of accelerated tests, when carefully designed these can be used as a valid measure of storage performance under the abuse conditions that can be encountered in the distribution chain and in the domestic environment.

1.5.2 Predictive models

The food industry has long been interested in ways of predicting rates of deteriorative change resulting from differing combinations of intrinsic and

extrinsic factors. With the increasing capabilities and availability of personal computers, predictive modelling, particularly of microbiological behaviour, has become a major area of research. Such models look for statistical and mathematical relationships between three sets of variables: intrinsic (product-related) factors; extrinsic (environmental) factors; and implicit factors, the characteristics of the microorganism itself and how it behaves in the presence of combinations of intrinsic and extrinsic factors. Such models need to be based on good experimental data mapping rates of change within given combinations of factors. The design of shelf-life experiments is discussed in section 1.6.

The data from these shelf-life experiments are analysed for statistical patterns and mathematical relationships from which a model can be built. For kinetic models, for example, this involves the fitting of growth or death curves to the data, followed by the use of an equation to define how the controlling factors affect the kinetics. The model then needs to be validated to determine how well it describes the original data. Shelf-life modelling is discussed in detail in Chapter 3.

1.6 The design of shelf-life experiments

The experimental determination of shelf-life can require a considerable amount of experimentation, with consequent costs and demands on time. Efficient design of such experiments is important if such tests are to be cost-effective. A statistical approach has been outlined by Gacula (1975), which describes a number of options for controlling the number of necessary measurements. In the most commonly operated type of test (called a partially staggered design by Gacula), a single batch of product (or replicate batches) is put on test at time zero, and samples are taken off for testing at intervals determined by the expectation of the probable shelf-life (Fig. 1.1). If there is no prior knowledge of the shelf-life, it may be necessary to take sufficient samples at each time point, therefore requiring extensive experimentation. In a variant of this procedure (called a staggered design by Gacula), the number of samples tested is increased up to the acceleration point, at which failure is expected, and after which a constant number of samples is tested. A further variant, the completely staggered design, uses an expansion in sample numbers determined by the number of failed units.

This basic type of design has the clear advantage that data related to shelf-life are generated at intervals and build up to give a moving picture of deteriorative change. While this carries few problems in circumstances in which instrumental measurements are the primary information source, problems are frequently encountered when sensory analysis techniques are being used to assess shelf-life (Chapter 4). This is related to the difficulties in generating consistent panel responses over time, these difficulties increasing over long storage times and if the test periods are infrequent. Several factors can contribute, mainly inconsistent use of scoring scales, changeover in panel composition and

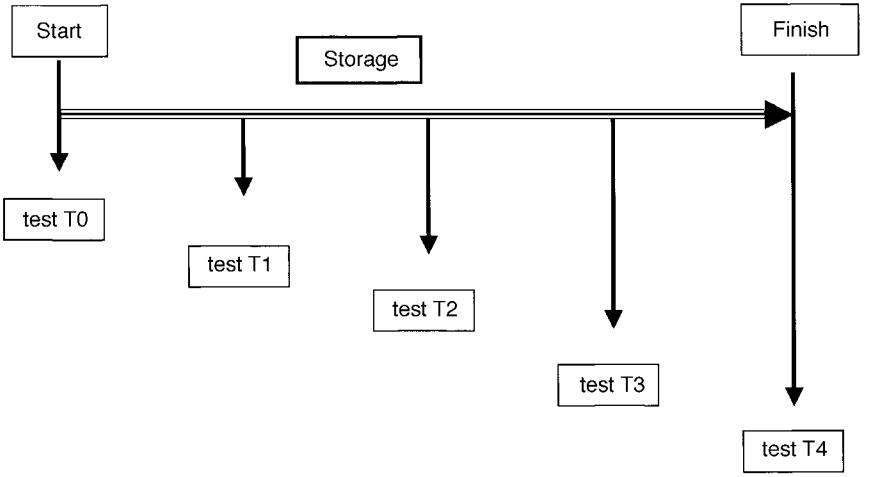

Fig. 1.1 A partially staggered design for shelf-life testing.

learning effects. In addition, the sensory nature of each set of samples will change over the storage periods, with consequent contextual effects. If sensory profiling is the method of choice, the appearance of new attributes not present at the initial panel training stage (e.g. off-flavours) can give rise to difficulties. The ideal design for sensory testing would involve having all samples from all storage treatments and from all time points tested together in a balanced design. In principle, this can be achieved in three different ways.

Firstly, samples can be drawn from successive production batches and put into storage for an appropriate time. At the end of shelf-life, all samples can be tested in an appropriate design. This design is of course susceptible to fluctuations in production quality, and can only be used in situations in which production consistency can be assured. A variant of this design is shown in Fig. 1.2, in which a single large batch is held under conditions under which quality changes are effectively zero, for example frozen storage. Samples are removed at appropriate intervals and stored under the desired conditions. Another variant is shown in Fig. 1.3. A large batch is put into storage, and samples are drawn at appropriate intervals and held under non-changing conditions (e.g. frozen) until the required storage time has been reached. The major difficulty in the last two designs is identifying appropriate non-changing storage conditions, as few foods can be stored in such a way without changes in some important quality attribute.

While these three designs all have the important advantage of delivering an internally consistent picture of changes in sensory characteristics, they suffer from a number of disadvantages. Firstly, no information is generated on stability and shelf-life until the very end of the storage trials, a situation that is unlikely to be tolerated in most industrial environments. Secondly, the setting up of the trials requires a prior knowledge of expected shelf-life. Thirdly, a global test can impose severe logistical problems for instrumental laboratory measurements.

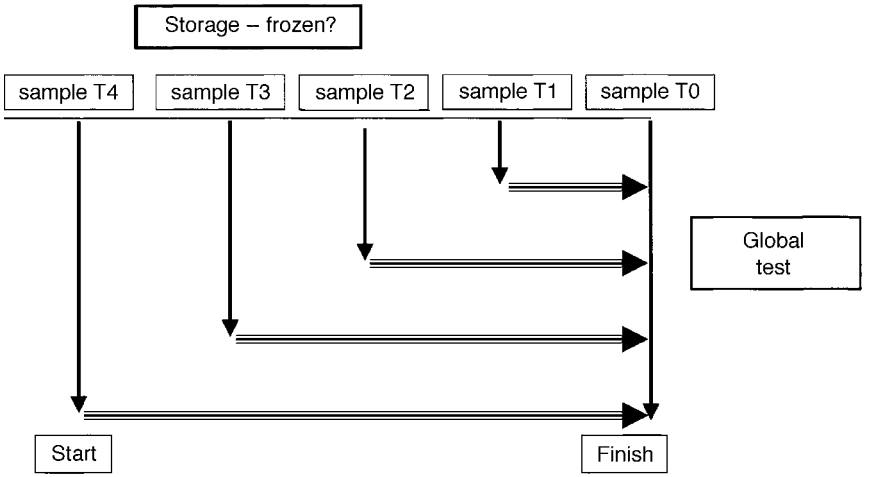

Fig. 1.2 Drawn sample design for shelf-life testing.

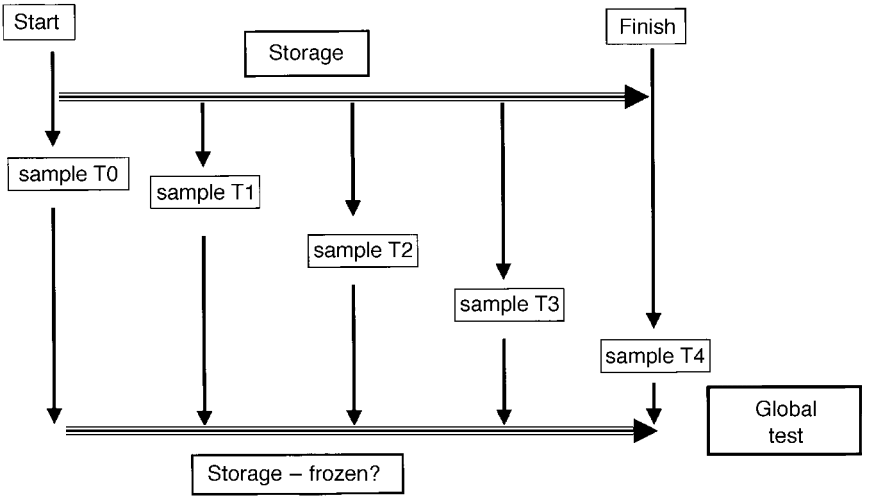

Fig. 1.3 Stored sample design for shelf-life testing.

1.7 Extending of shelf-life

There are a range of points in the food chain where manufacturers can influence the mix of intrinsic and extrinsic factors which affect shelf-life. These include:

- Raw material selection and quality.
- Product formulation and assembly.
- The processing environment.
- Processing and preservation techniques.

- Packaging.
- Storage and distribution.
- Consumer handling.

While all of these points are important, two of the most dynamic areas of research are in new processing methods and packaging techniques.

1.7.1 Influence of processing

The initial quality of a food product is determined by the quality of the raw materials and the processing methods used during the manufacture of the product. A wide range of processing techniques is used in the food industry to achieve the required level of sensory and microbiological quality. In the case of a perishable product, the extent to which microbial growth can be controlled after processing and packaging determines the final shelf-life. In some products with relatively low water activity (a_w) the shelf-life is determined by changes in the physical sensory characteristics of the product. The shelf-life of products can be extended by the use of processing treatments which kill the microorganisms (e.g. heat, radiation) or through the control of microbial growth by controlling temperature (chilling and freezing), reducing the a_w (drying and pickling) and by the addition of preservatives.

Long shelf-lives and shelf-stability at ambient temperatures commonly require the use of harsh treatments (e.g. canning) which often compromise the overall sensory quality of food products. Therefore, a combination of different processing methods can be useful in retaining the sensory quality, while achieving the same level of microbial stability. This is the principle of the hurdle technique for control of microbial growth.

Consumers often associate ambient storage and long shelf-lives with poor quality of products. Therefore, more recently there has been a move towards increased use of minimal processing methods, which result in higher quality but with a need for refrigerated storage. The many options available include mild treatments using heat, microwaves and radiation and the relatively new technologies such as high pressure processing, treating with electric fields and high intensity light. The shelf-life of a processed product can only be preserved by avoiding post-process contamination. The following in-pack processing techniques offer a way of achieving this aim.

Sous-vide processing

Heat processing of vacuum-packed products at low temperatures for long times is called the ‘sous vide’ process. The principle of the process is to avoid the use of high temperatures, which lead to irreversible damage (e.g. loss of succulence in meats and loss of crispness in vegetables). The pasteurised products are rapidly cooled and stored under chilled conditions. The shelf-lives achieved by this process are the longest for any chilled product. However, this process has raised many questions regarding food safety as discussed in Chapter 8.

Microwave processing
Microwave processing also involves in-pack pasteurisation and is used in Europe to process sliced bread. In this case the purpose of the process is to eliminate mould growth and to extend shelf-life without the need for traditional preservatives. The procedure involves heating the product to about 75–90ºC using microwaves. The process is often combined with conventional heating to make the process more economical. The advantage of the process is that it gives very rapid heating, which preserves the quality of the products.

High-pressure processing
High pressure processing involves pressurising the foods to pressures of up to 6 000 atmospheres to pasteurise and 10 000–12 000 atmospheres (with 60–80ºC) to achieve sterilisation. Therefore, as in the case of heat processing, the higher the pressure the greater the level of inactivation of microorganisms. High pressures can lead to interesting changes in texture, and can be used to preserve the colour and flavour associated with fresh products. However, there is still much to be understood about the effects on specific microorganisms and how to overcome the problem of inactivating enzymes present in the products. The combination of this process with mild heat treatments is likely to be more successful than the use of high pressure alone.

Irradiation
The irradiation treatment involves exposing packaged food to gamma rays, electron beams or X-rays. Since this is a cold process the food product does not become cooked. The optimal radiation dose varies with product types and application. Typically the medium range of doses of 1–10 kGy is suitable for extending the shelf-life of cooked and raw foods. Legislative restrictions apply to the use of this process in different countries. Since the irradiation process can alter the characteristics of certain packaging films the FDA has drawn up an approved list of packaging materials which can be used.

1.7.2 Packaging

There are many factors to be considered in choosing the optimal packaging form and material for any particular product, including the product characteristics, processing considerations, shelf-life required and overall cost. In many cases, packaging is an integral part of the processing stage. These factors are discussed in greater detail in Chapter 7.

Advances in packaging materials and techniques have increased the options available for maintaining quality and for improving the shelf-life of foods. Modified-atmosphere packaging, which involves replacing the air in the headspace of a packaged product with a single or mixture of special gases, has made the greatest impact on the shelf-life of chilled products. The gases commonly used are carbon dioxide and nitrogen. Carbon dioxide is used to suppress microbial growth, but its effectiveness is very much dependent on the

sensitivity of different classes of microorganisms to this gas. Nitrogen is used as an inert filler, where oxygen is to be excluded to prevent aerobic spoilage. One special case however is that of fresh fruit and vegetables, where a delicate balance of carbon dioxide and oxygen is necessary to allow aerobic respiration to continue at a very low rate and thereby extend shelf-life.

The consumer has also benefited from advances made in improving the convenience and safety aspects of food products. Microwaveable and dual ovenable packaging have played a vital role in the growth of the ready meals sector. Tamper evidence and reclosability features are seen as important factors in improving the performance of packaging. The approach of using oxygen scavengers within packs to reduce headspace oxygen levels to marginal levels has allowed the improvement of shelf-life of products very sensitive to oxygen.

One of the requirements for food packaging was that it should play a passive role, remaining inert and not interacting with the food it contains. However, the development of active packaging now makes it acceptable for the packaging to have a more interactive role in extending the shelf-life of foods. Oxygen absorbers, ethylene absorbers, carbon dioxide emitters and anti-microbial agents can be built into the packaging to actively improve the shelf-life.

The food manufacturer has an ever widening range of options available both in terms of processing and packaging to improve the quality and shelf-life of products. It is important that the manufacturers consider issues relating to food safety and consumer acceptance in the choice made for their products.

1.8 The structure of this book

The various chapters in this collection investigate key steps in extending shelf-life. As has already been described, the chapters in Part 1 concentrate on ways of analysing and measuring shelf-life, including the role of the glass transition as an intrinsic factor (Chapter 2), modelling shelf-life (Chapter 3), sensory evaluation methods (Chapter 4), accelerated tests (Chapter 5) and the use of advanced instrumental methods (Chapter 6).

Part 2 looks at ways of understanding and extending shelf-life, primarily from the point of view of specific foods. Given its particular importance and application to most foods, Chapter 7 considers the key role of packaging in extending shelf-life. Chapter 8 considers *sous vide* products and the range of factors involved in determining shelf-life, from raw materials and product formulation to packaging, thermal processing and cooling, chilled storage and distribution and reheating for use. It also discusses such ways of extending shelf-life as effective recipe development (including formulations of products with physiochemical and microbially-based hurdles to control growth of spoilage and pathogenic microorganisms), and the use of good manufacturing practice (GMP) and hazard analysis critical control point (HACCP) to ensure effective temperature control. Chapter 9 discusses milk and milk products and the role in extending shelf-life of such factors as raw material quality, heat treatment,

temperature control and the removal of oxygen in packaging and storage. Chapter 10 analyses the particular issues facing confectionery, while Chapter 11 discusses the unique problems of fruit and vegetables as living tissues, addressing such issues as respiration, the role of genetic make-up, stage of development (for example, ripening) and post-harvest handling. It analyses the range of options in extending shelf-life, including cooling technologies, surface coating, dehydration, the chemical control of fungi and pathogens, sprouting suppressants, and the role of packaging and storage in the regulation of temperature, ethylene synthesis and respiration. Chapter 12 addresses the range of issues connected with fats and oils. Chapter 13 looks at the shelf-life of sauces and dressings.

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Appendix

Table A1.1 Deterioration of fruit and vegetable products

Product	Deterioration mechanisms	Limiting changes
Soft fruit	Enzymic breakdown Mould growth Moisture loss	Textural softening Visible mould Dry appearance
Hard fruit	Enzymic action Moisture loss	Textural softening, bruising Dry texture
Potatoes	Enzymic action Sprouting	Softening, poor cooking Sprouting, toxin production
Cucumber	Enzymic action	Loss of crispness, gross structure breakdown
Coleslaw	Moisture loss from vegetables Fat oxidation	Loss of viscosity in dressing, appearance changes, microbial growth Rancidity
Prepared salads	Moisture loss Oxidation	Loss of crispness, drying Browning
Fruit preserves	Syneresis Oxidation	Serum separation, mould growth Flavour loss
Dried fruit	Enzymic action Chemical reactions	Browning Flavour changes

Table A1.2 Deterioration of meat and meat products

Product	Deterioration mechanisms	Limiting changes
Fresh red meat	Oxidation Microbial growth	Loss of red colour, rancidity Off-odours and flavours
Frozen meat	Oxidation Ice sublimation	Rancidity Freezer burn
Fresh fish	Microbial growth Chemical reactions	Microbial Off-odours Appearance changes
Fresh poultry	Microbial growth	Microbial Off-odours
Fresh sausages	Microbial growth Oxidation	Microbial Rancidity
Fresh bacon	Microbial growth Oxidation	Microbial Rancidity, colour change
Canned ham	Chemical reactions Can deterioration	Flavour loss Gas generation

Table A1.3 Deterioration of cereal and other dry products

Product	Deterioration mechanisms	Limiting changes
Bread	Starch retrogradation Moisture migration	Stale texture and flavour Dry texture, mould growth
Snack foods	Moisture uptake Oxidation	Loss of crispness Rancidity
Cakes	Moisture loss Starch changes Microbial growth	Drying and hardening Stale flavour and texture Mould formation
Dried pasta	Starch changes Protein changes	Texture changes, breakage Staling
Breakfast cereals	Moisture migration Starch retrogradation Oxidation	Softening (cereal), hardening (fruit) Stale flavour and texture Rancidity
Dry mixes	Moisture uptake	Caking Non-enzymic browning
Spices	Microbial growth Volatile loss Chemical reactions	Mould and bacterial growth Flavour changes Colour loss
Chocolate confectionery	Fat migration Oxidation	Fat crystallisation (bloom) Texture changes Staling, rancidity
Sugar confectionery	Moisture uptake Oxidation	Texture changes Rancidity

Table A1.4 Deterioration of beverages

Product	Deterioration mechanisms	Limiting changes
Carbonated beverages	Gas evolution Hydrolysis/oxidation	Carbonation loss Flavour loss, off-flavours, rancidity
Beer	Oxidation Microbial growth	Off-flavours Turbidity
Coffee	Volatile loss Oxidation	Flavour change Rancidity
Fruit juices	Oxidation Enzymic reactions	Flavour and nutrient loss Cloud instability
Tea	Volatile loss Volatile absorption	Flavour loss Off-flavours
Wine	Oxidation	Off-flavours Colour change
Low-calorie soft drinks	Hydrolysis	Sweetness loss

Table A1.5 Deterioration of dairy products

Product	Deterioration mechanisms	Limiting changes
Ice-cream	Moisture migration Oxidation	Ice crystal formation Rancidity
Fluid milk	Oxidation, hydrolytic reactions Microbial growth	Rancidity and other off-flavours
Dried milk powder	Moisture uptake Oxidation	Caking Flavour change, rancidity
Butter	Oxidation	Rancidity
Cheese	Oxidation Lactose crystallisation Microbial growth	Rancidity Gritty texture Mould production
Low-fat spreads	Microbial growth Oxidation	Mould Rancidity
Yoghurt	Syneresis Oxidation	Serum separation Rancidity
Fruit yoghurt	Syneresis Oxidation Microbial growth	Serum separation Rancidity Mould

Table A1.6 Examples of accelerated treatments

Product	Treatment	Deterioration accelerated
Canned food	37 °C storage 55 °C storage	Tin pick-up Indicator for thermophilic organisms
Beer	27 °C storage	General spoilage
Cakes and pastry products	25 °C or 30 °C/75% RH storage	Mould growth
Emulsion products	30 °C storage 55 °C storage Mechanical agitation	Sedimentation Rapid stability test Coalescence
Thickeners	Repeated cooling to 0 °C	Syneresis
Frozen foods	Freeze–thaw cycling	Starch suitability
Packaged foods	High humidity	Moisture transfer and uptake

Part 1:

Analysing shelf-life

2

The glass transition and microbial stability

M. T. Kalichevsky-Dong, Consultant

2.1 Introduction

This chapter introduces the concepts of water activity (a_w) and the glass transition temperature (T_g) for predicting and controlling the stability of food systems. It subsequently focuses on literature concerning the potential relevance of T_g to microbial growth and stability and to the rates of reactions in intermediate or low moisture foods. These include:

- Dehydrated foods (e.g. fruits, vegetables, meats, etc., including freeze-dried foods).
- Amorphous or partially amorphous food powders (e.g. powdered milk).
- Foods of high (amorphous) sugar or maltodextrin content (e.g. confectionery products).
- Cereal products (e.g. flour, breakfast cereals, pasta and baked products).

Frozen foods are also briefly considered.

Water activity has, with temperature, become the standard parameter for the prediction of food stability, particularly in terms of microbiological stability, but also other aspects of food quality. The particular usefulness of a_w results from its provision of a measure of the osmotic stress experienced by microorganisms and also from its ability to take into account water–solute interactions to some extent. These factors affect the availability of water to bacteria for growth or for other degradation processes. Consequently, a_w has been found to be of more use in predicting microbial stability than water content or solute concentration.

There are limitations and weaknesses in the use of water activity as, ideally, the use of the term ‘water activity’ should be restricted to systems in true

thermodynamic equilibrium. Many of the limitations to using a_w arise from the non-equilibrium nature of many foods, particularly at intermediate moisture contents and also from observed differences in the effects of different solutes at the same a_w. Water activity must always be used with an awareness of these limitations, but it still seems to be the most reliable and straightforward method for defining food microbial stability.

In recent years, the glass transition temperature has been proposed as an additional and potentially better parameter for predicting and understanding food stability (Slade and Levine, 1991). A glass is an amorphous (non-crystalline) solid, in which molecular mobility is inhibited by high viscosity; pure glassy materials are brittle and generally transparent. As a glass is warmed above its T_g its mobility increases and, if the material is polymeric (e.g. starch), it becomes rubbery – smaller molecules (e.g. sugars) may exhibit viscous flow. Many food components and food products exhibit glassy/rubbery behaviour, e.g. boiled sweets, dried foods and frozen foods. Water content is an extremely important parameter in glass-forming foods, because water acts as a plasticiser, reducing T_g. T_g is very moisture-sensitive and small amounts of water can decrease T_g enormously, particularly at low moisture contents. Food materials can therefore be rendered glassy by removing water, as well as by reducing temperature. This is why frozen foods can also be in a glassy state (freezing is effectively a means of removing liquid water, as well as reducing temperature).

Foods can be regarded as stable below T_g. At temperatures above T_g, the rates of degradation processes have been found to be critically dependent on $T - T_g$ (i.e. how far above T_g the storage temperature T is). This is particularly so if T is close to T_g. A knowledge of T_g has generally been accepted as being useful in understanding and predicting the physical stability of foods in relation to crystallisation rates, collapse and related non-equilibrium phenomena (Slade and Levine, 1993), but there has been considerable debate concerning its relevance to microbial stability.

As well as highlighting areas where T_g may be useful, this chapter points out limitations of the T_g approach, including the difficulties in defining and measuring T_g in multicomponent systems. Biological systems are complex, and other factors (apart from a_w or T_g), such as pH, specific antimicrobial activity and the permeability of membranes to solutes also play a role in determining microbial stability. However, the importance of T_g-related solute or substrate mobility to microbial activity is an aspect that has been insufficiently studied and should prove to be a useful concept, in addition to a_w, in the future. The relationship between T_g and a_w is, in fact, often linear at intermediate moisture contents, and foods can be defined as having a critical a_w, where T_g falls below ambient or storage temperatures. It is important to realise, therefore, that the concepts of a_w and T_g are not in opposition, but are rather complementary, together enhancing our understanding and prediction of food stability.

2.2 Methods used to predict microbial stability

2.2.1 Water activity – its uses and limitations

Initially, water content was used as a control parameter to predict microbial stability of foods, but it was found to be inadequate, as it does not take account of the state of water in the food. Under the same conditions of relative humidity (RH in %) of the surrounding air, different food materials can have very different equilibrium moisture contents, whereas, at the same moisture content, they may have quite different microbiological stabilities. The water in a food may be closely associated with the food molecules and relatively uniformly distributed, or 'clustered' in water-rich regions or capillaries within the food matrix. The state of the water affects its availability to microorganisms for their growth and metabolism. In an attempt to overcome these difficulties, Scott (1953) introduced a_w as a parameter giving a measure of water mobility and availability at a particular temperature:

$$a_w = \frac{P\,(\text{equilibrium partial vapour pressure of water above the sample})}{P_0\,(\text{equilibrium partial vapour pressure of pure liquid water})} \quad [2.1]$$

Real food systems are unlikely to be in true equilibrium with their surroundings (Peleg, 1988), but, under steady state conditions, where the relative vapour pressure (RVP) of water in a food material and in the surrounding atmosphere are equal, water activity can be obtained from the RH of the air, where RVP = $P/P_0 = a_w$ = RH/100 (Roos, 1995a, b).

Water activity control has become the traditional approach for optimising food quality and the chief means of predicting and controlling the shelf-life and safety of food products (e.g. the general findings that pathogenic bacteria do not grow below an a_w of 0.86 whereas osmophilic yeasts and xerophilic moulds can grow at a_w values down to 0.60; Chirife and del Pilar Buera, 1994; Troller, 1980). Minimum a_w values for the growth of various microorganisms have been tabulated (Troller and Christian, 1978) and 'food stability maps', e.g. that of Labuza *et al.* (1972) (Fig. 3.1) have been widely used.

Food stability maps (e.g. Fig. 3.1) indicate qualitatively that, for a given product, at very low a_w and moisture contents, lipid oxidation and other free radical reactions occur more rapidly than at higher a_w (water has been shown to shorten the lifetime of free radicals; Rockland, 1969), whereas, at high RH and moisture contents, biological reactions (enzyme activity, microbial growth) occur with increasing rates. Lipid oxidation rates were also found to increase with increasing a_w, after going through a minimum, owing to increasing diffusion rates of reactants and increasing exposure of catalytic sites as the product swells during hydration (Labuza *et al.*, 1972). These findings imply that there is an optimum a_w range for maximum product shelf-life. This type of plot of kinetically determined (time-dependent) deterioration rates against water activity (ideally an equilibrium thermodynamic quantity) has been strongly criticised (Slade and Levine, 1991; van den Berg, 1986) and, while useful, should be used with caution. These

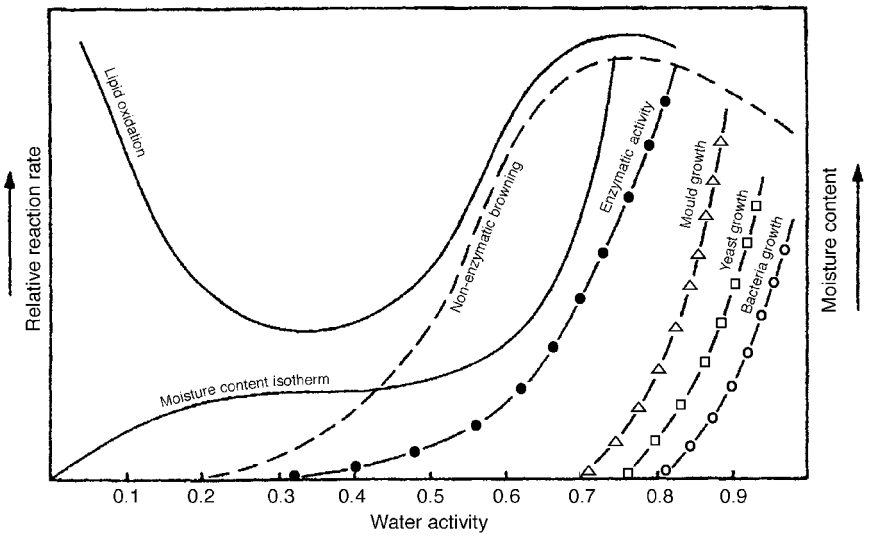

Fig. 2.1 Food stability map as a function of water activity, from Labuza *et al.* (1972).

diagrams show general trends which often vary significantly from product to product; the same microorganism may respond very differently to different solutes at the same water activity (van den Berg, 1986), and the minimum a_w for growth can be quite different on different substrates.

Strictly speaking, the use of the term 'water activity' should be restricted to systems in true thermodynamic equilibrium, which is rarely, if ever, the case in intermediate moisture foods (Reid, 1976; Peleg, 1988). A food will be at equilibrium when it is uniformly at the a_w corresponding to the surrounding relative (water) vapour pressure. If different components of a food have different a_w values, then moisture will migrate from the regions of high water activity to the regions of low water activity, at a rate depending on the difference in a_w between the regions. In intermediate moisture foods, equilibration can be very slow and may never be reached within the lifetime of the product; such products are often regarded as being in a state of 'pseudo' stability (Chirife and del Pilar Buera, 1994). Non-equilibrium effects may be observed at a_w values as high as ~0.9 (Slade and Levine, 1991); consequently, it has been stated that decisions regarding the safety and economic specifications for the dehydration and storage of a specific product should only be drawn up after careful consideration and conducting shelf-life studies (van den Berg, 1986). Water activity is a very useful parameter for determining microbial growth, as long as users are aware of its limitations.

2.2.2 Sorption isotherms

Sorption isotherms illustrate the relationship between water content and a_w for a particular food or ingredient, as illustrated schematically in Fig. 2.2. Sorption isotherms are generally sigmoidal in shape. Water absorbed at low a_w is

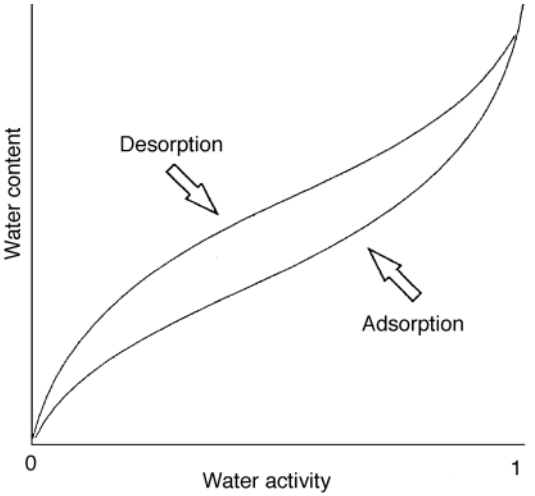

Fig. 2.2 Schematic diagram of a sorption isotherm, illustrating sorption/desorption hysteresis.

generally regarded as having restricted mobility and is often referred to as 'monolayer' water, following the Brunauer, Emmett and Teller (BET) isotherm analysis (Brunauer *et al.*, 1938) or comparable isotherm equations (e.g. Guggenheim–Anderson–de Boer (GAB), which fits data over a wider a_w range; Roos, 1995a; Barbosa-Cánovas and Vega-Mercado, 1996). The BET monolayer value gives an estimate of the number of primary sites for water sorption and is often considered as the optimum moisture content for stability of low-moisture foods (Labuza, 1980). In the case of starches it is found to correspond to approximately one water molecule per anhydroglucose monomer (van den Berg, 1986). However, the BET approach, in theory, assumes only non-specific surface sorption and an absence of specific interactions between sorbed molecules, which is clearly not the case for water sorption in food systems.

Standard isotherms and equations for their calculation for various foodstuffs are available in the literature (e.g. Iglesias and Chirife, 1982); however they are not invariant, but are sensitive to temperature, sample history and time-dependent effects (van den Berg, 1986). Chirife and del Pilar Buera (1995) have reviewed work carried out on equilibration times of various foodstuffs and concluded that 'sorption determinations performed with the usual precautions regarding ''practical weight constancy'' are not likely to be too far from equilibrium, and the differences are probably within uncertainties associated with the experimental determination of isotherms'. However, the non-equilibrium nature of many food systems is illustrated by the frequently observed difference between isotherms obtained by sorption or desorption of water, called sorption hysteresis, as illustrated in Fig. 2.2. There is also often a difference between the first desorption isotherm of a newly harvested product and one measured after some time (van den Berg, 1986). Microbial growth

(Plitman *et al.*, 1973) and lipid oxidation rates (Labuza *et al.*, 1972) have been found to vary depending on whether the product was prepared by desorption or adsorption to the same a_w; products produced by sorption are generally more stable than those produced by desorption, owing to their lower moisture content under the same RVP. Sorption properties are also affected by structural transformations (e.g. collapse) or phase transitions (e.g. crystallisation). This again indicates the kinetic, non-equilibrium nature of most foodstuffs and that, while useful in understanding and predicting the behaviour of foods, sorption isotherms must be used with an awareness of their limitations.

2.3 The glass transition approach

2.3.1 State diagrams and viscosity

In recent years, the application of polymer science to food science has led to the glass transition temperature being put forward as an alternative, potentially preferable, parameter for defining food stability (Slade and Levine, 1991), where the rates of degradation processes are critically dependent on $T - T_g$ (how far above T_g storage conditions are), particularly at temperatures close to T_g. A substance in the glassy state may be defined as 'a material formed by cooling from the normal, liquid state, which shows no discontinuous change (e.g. crystallisation or phase separation) at any temperature, but has become rigid through a progressive increase in viscosity' (Allen, 1993). This can easily be visualised by considering the formation of a boiled sweet, which is a sugar glass; however, polymeric materials, such as starch and gluten, can also form glasses. Owing to its small molecular size and high miscibility with biological materials, water acts as an excellent plasticiser, even a few per cent greatly reducing the T_g of food materials. Thus consideration of the glass transition (as with a_w) highlights the importance of controlling water content. In fact, the relationship between a_w and T_g is often linear over the practical a_w range of intermediate moisture foods (a_w = 0.1 to 0.8), particularly for foods where the T_g is basically that of a sugar or maltodextrin (Fig. 2.3a), but is sigmoidal over the whole a_w range. (The T_g/a_w relationship may be less linear for higher molecular weight materials as shown in Fig. 2.3b for amylopectin.) For a given food material, critical moisture and a_w values can be defined as those that depress T_g below ambient temperature (Roos, 1995b).

The viscosity in the glassy state is in the region of 10^{13} poise (Allen, 1993) and a material below its T_g is thus relatively inert, as the diffusion necessary for crystallisation or other degradation processes is very slow (negligible in practical timescales). The viscosity at T_g (measured using a particular method) varies depending on the material, some materials having more free volume at T_g than others (this may be related to the T_m/T_g value, as discussed later). The glass transition of any material measured using a particular method can be regarded at an iso-relaxation time point, the value of this relaxation time (and the measured T_g) depending only on the method used to measure T_g.

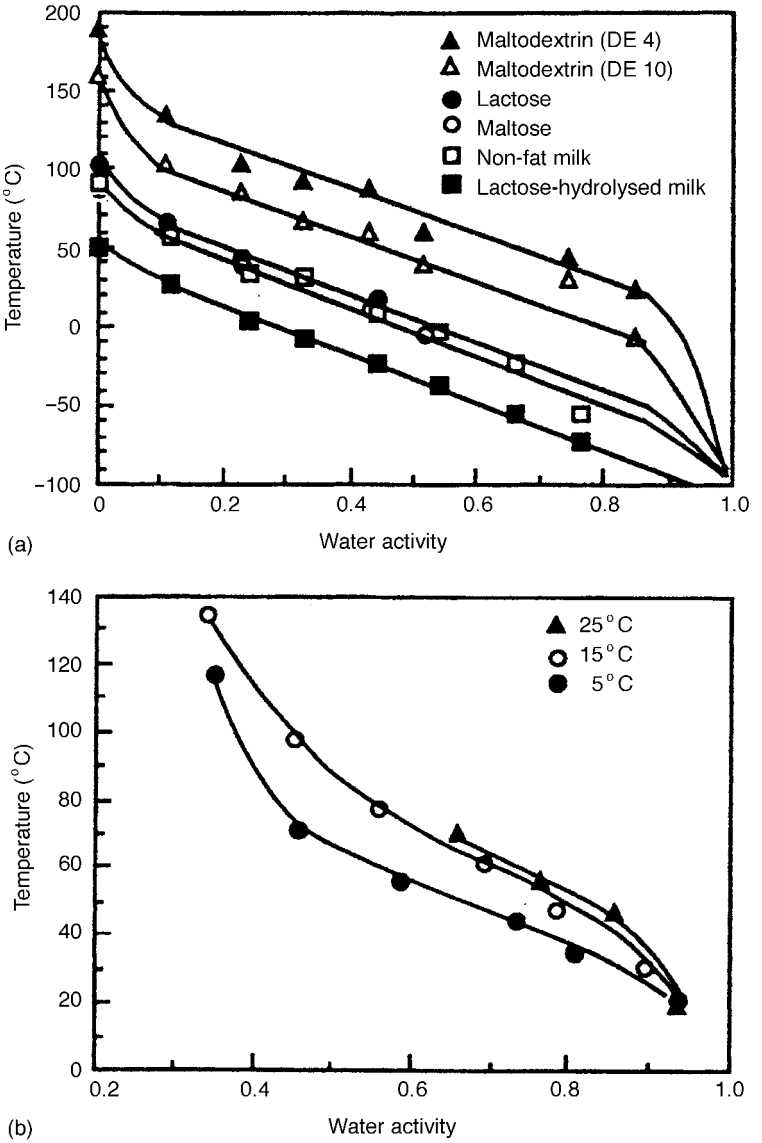

Fig. 2.3 Glass transition temperature as a function of water activity: (a) T_g of various food materials at 25 °C; and (b) T_g of amylopectin (the high molecular weight branched component of starch) at 5, 15 and 25 °C. Diagram reproduced from Roos, 1995a, with permission.

The rate of decrease in viscosity as temperature is increased above T_g varies for different materials, but for sugars and amorphous polymers has been found to correspond to the 'WLF' equation (Williams, Landel and Ferry, 1955; Soesanto and Williams, 1981) between T_g and T_m, or between T_g and $T_g + 100$ °C

(Sperling, 1986). Outside this range an Arrhenius temperature dependence is generally observed. The simplified WLF equation is given below:

$$= -\frac{C_1(T - T_9)}{C_2 + T - T_9} \qquad [2.2]$$

where a_T is the ratio of viscosity at temperature T to the viscosity at T_g (η/η_g) or of the corresponding ratio of values using another mechanical parameter. C_1 and C_2 are constants for which values of -17.44 and 51.6 are often used, although they are not always applicable. In general, because it is difficult to measure the viscosity at T_g, it is more accurate to use T_g + 50 °C as a reference temperature (in place of T_g) and to determine constants C_1 and C_2 graphically from experimental data (Peleg, 1992). Nelson and Labuza (1994) have discussed methods of determining WLF coefficients for assessing the applicability of the WLF equation to the temperature dependence of chemical reactions in foods.

The significance of a WLF dependence of viscosity above T_g is that, for properties in this temperature range, this equation predicts a dependence on $T - T_g$ far greater than that predicted by the Arrhenius equation (typical Arrhenius rates for aqueous systems above T_m might increase fourfold over 20 °C, whereas WLF rates near T_g would increase by 4 or 5 orders of magnitude; Slade and Levine, 1991). This difference is particularly important at temperatures just above T_g. T_g is itself very moisture sensitive and small amounts of water can decrease T_g enormously (at very low moisture contents an increase of 1% water can reduce the T_g of sugars by more than 20 °C; Roos and Karel, 1990). The glass transition temperature of dry materials depends on molecular weight, so that polymers (e.g. starch) have a much higher T_g than sugars. Disaccharides, such as sucrose or maltose, also have higher T_g values than monosaccharides, e.g. glucose or fructose.

At high moisture contents the glass transition temperature falls below 0 °C, and in the presence of sufficient water, freezing occurs on cooling. Freezing is a form of dehydration, as water is removed from the product to form ice. Frozen systems also exhibit a glass transition, which tends towards a constant temperature (T_g', independent of initial moisture content) for a particular product, when it has undergone maximal freeze-concentration. If maximal freeze-concentration has not occurred then the effective T_g will be lower than T_g' (as there will be excess water in the substrate); ice recrystallisation will occur on warming (below the T_m of ice) until W_g' (the composition of the maximally freeze-concentrated glass in g unfrozen water/g solute at T_g') is attained. It has been emphasised that the constant composition of the freeze-concentrated phase and the existence of 'unfreezable water' is not due to water binding to the substrate, but is rather to the high viscosity in the glassy state. This has the result that water does not freeze further within appreciable timescales (Slade and Levine, 1991; Franks, 1985). (Even at -40 °C, the homogeneous nucleation temperature, at least ~200 water molecules must associate within a domain of 40 Å to grow spontaneously into an ice nucleus; Franks, 1985.) As with T_g, T_g' has been found to increase with increasing molecular weight of the solute for a homologous series of samples.

W_g' (as measured by Slade and Levine, 1991) tends to decrease with increasing molecular weight, but this is not always the case, e.g. for sugars, W_g' varies considerably (0.23 g ufw*/g solute for sorbitol, to 0.41 for glucose, 0.77 for galactose, 0.96 for fructose, 0.56 for sucrose and 0.25 for maltose). These values have been the subject of considerable debate (Izzard *et al.*, 1991; Ablett *et al.*, 1993a, b; Simatos and Blond, 1991), but if these differences are real, they must reflect differences in the water relations of the different sugars. A large W_g' has been related to increased cryoprotective effects of the solute, resulting in reduced damage due to freezing, but generally a low stability due to the generally corresponding low T_g' value (Slade and Levine, 1991). However, Roos (1993) found that the calculated composition of the glassy solute at the measured T_g' did not vary greatly from about 80% solute for a wide range of different sugars; this corresponds to a W_g' value of 0.25 g/g. These results imply that the differences observed by Slade and Levine (1991) may not be real, and could arise from weaknesses in their method of determining W_g'.

With a minimum knowledge of the dry T_g, T_g', W_g' and the T_g of water (usually taken as approximately -135 to $-139\,^{\circ}C$; Roos, 1995a; Sugisaki *et al.*, 1968) a rough state diagram can be constructed and this can be supplemented with information on ice melting (e.g. Fig. 2.4). Ideally, owing to the difficulty in obtaining accurate T_g' and particularly W_g' data, it is preferable to obtain T_g values at several moisture contents and to obtain W_g' from the moisture content at which T_g is observed at T_g' (Hatley *et al.*, 1991; Izzard *et al.*, 1991, Ablett *et al.*, 1993b). A variety of other methods for determining T_g' and W_g' have been proposed, including mechanical (MacInnes, 1993), dielectric (Kerr and Reid, 1996) and nuclear magnetic resonance (Ablett *et al.*, 1993a) methods.

State diagrams (an example of which is given in Fig. 2.4) can be used as an alternative stability map for a given product, with the magnitude of $T - T_g$ (corresponding to iso-viscosity contours for a particular material), giving a measure of the probability of such degradation mechanisms as collapse, caking of powders, crystallisation (graining, staling of breads), loss of crispness, stickiness and even chemical reactions.

In frozen foods T_g' (rather than T_g) is the reference temperature, below which products may be regarded as stable and above which degradation may occur at rates increasing according to WLF kinetics, or possibly even faster, owing to the onset of ice melting (Roos, 1995a). Many of these viscosity-related changes have been studied in some detail in relation to the glass transition, but relatively little work has been carried out on the relationship between T_g and microbial stability, which is the main subject of this review.

2.3.2 Equations used to fit and predict T_g

It is useful to be able to fit data on the moisture content dependence of T_g to a model to enable predictions of T_g values to be made. Various theoretical and empirical equations have been developed and those most commonly used for

* ufw = unfrozen water.

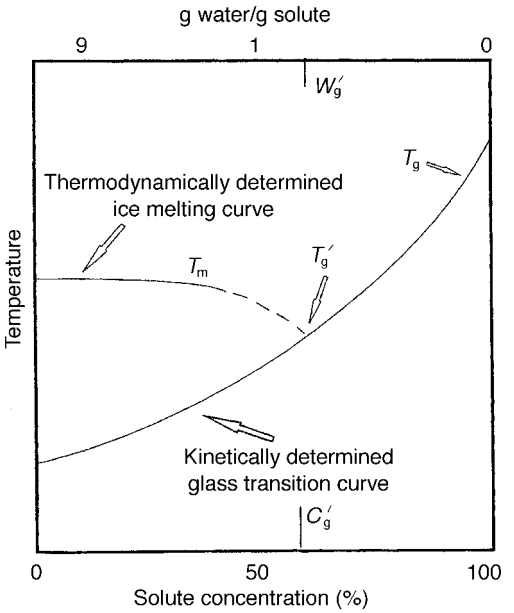

Fig. 2.4 Schematic solid–liquid state diagram, showing T_g as a function of water content and T_m of ice as a function of solute content, illustrating the following transitions: T_g', W_g', C_g' (after Levine and Slade, 1986).

food systems are given here. The glass transition of two-component systems have been fitted using the Couchman–Karasz equation (equation 2.3):

$$T_g = \frac{W_1 \Delta C_{p1} T_{g1} + W_2 \Delta C_{p2} T_{g2}}{W_1 \Delta C_{p1} + W_2 \Delta C_{p2}} \quad [2.3]$$

where the subscripts 1 and 2 refer to the component molecules (e.g. sugar and water), W is the weight fraction of one component of the system and ΔC_p is the difference in heat capacity between the liquid (or rubbery) and glassy states. ΔC_p for amorphous water is difficult to measure, and reported values range from 0.089 to 1.94 J/g °C, but the use of the latter value (Sugisaki *et al.*, 1968) in equation 2.3 gives the best fit with experimental data (Kalichevsky and Blanshard, 1993; Kalichevsky *et al.*, 1992). This equation can be extended to model three-component systems:

$$T_g = \frac{W_1 \Delta C_{p1} T_{g1} + W_2 \Delta C_{p2} T_{g2} + W_3 \Delta C_{p3}}{W_1 \Delta C_{p1} + W_2 \Delta C_{p2} + W_3 \Delta C_{p3}} \quad [2.4]$$

or simplified to the Gordon–Taylor equation by inserting a constant $K = \Delta C_{p2} / \Delta C_{p1}$:

$$T_g = \frac{W_1 T_{g1} + K W_2 T_{g2}}{W_1 + K W_2} \quad [2.5]$$

Values of K have been determined for several food systems, and for sugars appear to be proportional to T_g (Roos, 1993, 1995a; Roos and Karel, 1991).

2.3.3 The possible significance of T_m/T_g

According to the free volume theory of the glassy state, the glass transition was originally thought to arise when the free volume was reduced below a certain critical limiting value, and the glassy state was considered to be an iso-viscosity or iso-free volume state. However, the free volume (unoccupied volume) was subsequently found to vary with molecular weight, degree of molecular cross-linking and other structural features (Shen and Eisenberg, 1966). The amount of free volume in a glass has been related to the T_m/T_g value of a material, with typical T_m/T_g values for polymers being around 1.5. As a WLF viscosity dependence is expected to be observed between T_g and T_m (and Arrhenius kinetics outside this region), the size of this region is also of interest, and is generally of the order of 100 ºC, but can be much smaller. A large T_m/T_g ($>> 1.5$) implies a readily crystallisable material, with a large region of WLF-dependent behaviour (e.g. water $T_m/T_g = 2.04$), while a lower T_m/T_g value indicates a narrower WLF region and generally arises from a large free volume requirement for rotational diffusion, such as in some asymmetrical polymers (e.g. bisphenol polycarbonate ($T_m/T_g = 1.18$); Slade and Levine, 1991). Such materials will have a larger free volume in the glassy state, which may allow greater diffusion of small molecules, resulting in reduced stability compared with materials with higher T_m/T_g values. This implies that at the same $T - T_g$, materials with lower T_m/T_g values may be less stable.

2.3.4 Measuring T_g

Before we consider the work reported in the literature in relation to T_g and microbial stability, it is important to be aware of how the glass transition temperature is defined and measured, because (as for a_w) this is not altogether straightforward, especially for non-homogeneous, complex food materials. In fact one of the major difficulties in establishing the importance of T_g arises from difficulties in measuring it accurately, as the transition occurs over a range of temperatures and is not as well defined as a melting point, for example. The measured T_g will depend on the moisture content and the method (heating rate or frequency) used to detect it. The variation in the literature of T_g values for sugars and other food materials must arise, to a large extent, from these two variables. Ideally the measurement method details and moisture content (there may be moisture even in some ‘dry’ sugars) should be quoted with any published T_g data.

By far the most commonly used method for determining T_g is differential scanning calorimetry (DSC). Using this method the sample and reference (generally an empty aluminium pan) are heated at a controlled rate. At the glass transition there is a step increase in the heat capacity. This is generally easy to detect for sugars or maltodextrins, but becomes increasingly difficult to detect with increasing molecular weight and complexity of the material, as the

transition becomes broader and can be spread over about 40 °C. It is easier to detect the glass transition in materials which are amorphous, as partial crystallisation or other forms of ordering (as exist in native proteins) can also contribute to the broadening of the transition.

The glass transition is a kinetically determined (time-dependent) transition, and thus the temperature at which it is detected depends on the heating rate used to measure it. There are also several methods of determining the glass transition temperature from a DSC trace. Generally the onset value might be perceived as most satisfactory for stability considerations, also because this value tends to change less owing to sample history effects. However, the midpoint or inflection point of the transition is also often used, as this is easier to define. Ideally, one would obtain T_g at various heating rates and extrapolate to zero heating rate (e.g. Izzard *et al.*, 1991), but for most purposes it is sufficient to choose one method and stick to it, so that data on different materials can be directly compared. Most data in the literature use 5 or 10 °C/min heating rates (e.g. Roos, 1993; Kalichevsky and Blanshard, 1993), although a recent study has used a heating rate of 3 °C/min (using a different type of calorimeter, Setaram, which takes larger samples), obtaining consequently higher T_g values (Bizot *et al.*, 1997).

As previously mentioned, there has also been significant debate about the measurement of T_g' and W_g'. The method of Levine and Slade (1986), using DSC of 20% solutions to determine these parameters, has suffered from criticism about the definition of the T_g' transition and difficulties in determining W_g' accurately from a single DSC scan. The onset and baseline of the ice melting peak is difficult to determine, and the melting peak itself contains contributions from the heat of solution of the solute, as well as the temperature variation of the heat of fusion of ice, which are not taken into account (Williams and Hirsch, 1986; Izzard *et al.*, 1991; Hatley *et al.*, 1991). Thus the many T_g', W_g' data published by Slade and Levine must be used with caution. Their T_g' values for sugars are likely to be high relative to a 'true' T_g' value obtained by other methods, but as molecular weight increases, the difference becomes insignificant (Ablett, 1993a). Their W_g' value for sucrose (0.56 g/g = 64.1% sucrose) appears to be clearly too high as ice crystal growth has been observed in a 65% and even a 73% sucrose solution (Izzard *et al.*, 1991), which, according to the Slade and Levine value of W_g', should not be possible.

Dynamic mechanical thermal analysis (called DMTA or DMA) has also frequently been used to study the glass transition in food materials (e.g. Kalichevsky *et al.*, 1993). This method measures the effect of a sinusoidally varying stress on dynamic moduli and has the advantage of being more sensitive than DSC. DMTA can detect sub-T_g transitions, which are not normally detected by DSC and the frequency dependence of the transitions can be studied at the same time. Weaknesses of this method are the necessity for having solid samples, large and uniform enough to be clamped into the machine and the difficulty in controlling moisture loss during the experiment.

Spectroscopic techniques such as nuclear magnetic resonance (NMR) and electron spin resonance (ESR) are also of interest, because they provide

complementary information, enabling the mobility of different components within the glass to be probed. These and other methods used to study glassy states are discussed more fully in the literature (e.g. Roos, 1995a; Blanshard and Lillford 1993).

2.4 Current research on the glass transition

2.4.1 Microbial growth in the glass transition region

The debate in this area began when Slade and Levine (1988, 1991) extended their 'water dynamics' approach from the prediction and modelling of the physical stability of foods (e.g. against crystallisation, loss of crispness, collapse) to that of microbial stability. They presented some data on *Aspergillus parasiticus* mould growth in intermediate moisture systems and argued that an understanding of the glass transition could explain differences in mould growth in different solutes of the same a_w, which could not be explained using a conventional approach. This system was chosen because germination is a readily visible all-or-nothing process, and, near room temperature, the initial germination of *Aspergillus* depends only on water availability and not on the presence of nutrients.

Apart from the data themselves, there are questions about the methods used by Slade and Levine (1988, 1991) to interpret these mould growth data. Rather than using comparative glass transition temperatures ($T - T_g$, at each moisture content) to compare data on different solutes, they concentrated on using the T_m/T_g values of the solutes as a measure of mobility, and therefore stability in the glassy and rubbery state. The systems studied were various water-plasticised aqueous solutions at temperatures considerably above their T_g. Slade and Levine (1988) explained the reduced stability of systems containing fructose, using a relaxation observed, in dry fructose, at 100 °C, which they interpreted to be the effective T_g in these systems, rather than the conventionally accepted (lower) T_g in the region of 10 °C (e.g. Orford *et al.*, 1989; Finegold *et al.*, 1989, confirmed by the viscosity measurements of Ollett and Parker, 1990). The use of the higher T_g value, resulted in an anomalously low T_m/T_g value (1.09 rather than 1.40), to which Slade and Levine attributed the relatively low stability of systems containing fructose. An edited presentation of Slade and Levine's (1988) results is given in Table 2.1.

From these data (Table 2.1) it can be seen that at similar RVPs fructose showed faster spore germination than glucose or sucrose, but this could have been predicted from the lower T_g of fructose, without using T_m/T_g values. The fructose data are rather unusual, with constantly rapid germination occurring for almost every sample; however, Chirife and del Pilar Buera (1996) could not reproduce these results. Data on the germination rates of spores in PVP of different molecular weights were ambiguous; the long germination times may be due to the high T_g or additional antimicrobial effects. The long germination time at an RVP of 0.83 in the presence of glycerol was unexpected, as the T_g of

Table 3.1 Germination of mould spores of *Aspergillus parasiticus* in aqueous solutions

RVP (30 °C)	T_g (dry) (K)	T_m (K)	T_m/T_g	Conc. (w% H_2O)	Solute type	Days to germinate at 30 °C
1.0	134	273	2.04	100	None (water)	1
~1				99	Glucose (α–D)	1
~1				99	Fructose	1
~1				99	Glycerol	2
~1				99	PVP-40	21
0.99	316	402	1.27	60	Maltose	2
0.99	339			60	PVP-10	11
0.98	339			50	PVP-10	11
0.97	325	465	1.43	60	Sucrose	4
0.95	373			60	PVP-40	9
0.95	349	406.5	1.16	50	Maltotriose	8
0.93	339			40	PVP-10	11
0.98 (0.93)	373 (283)	397	1.06 (1.40)	60	Fructose	2
0.93	303	412.5	1.36	50	Mannose	4
0.92	373			50	PVP-40	21
0.92	302	444.5	1.47	60	α-Methyl glucoside	1
0.87 (0.91)	373 (283)	397	1.06 (1.40)	54	Fructose	2
0.83 (0.89)	373 (283)	397	1.06 (1.40)	50	Fructose	2 (~12)
0.82 (0.89)	293			40	1:1 Fructose/glucose	5
0.85	304	431	1.42	50	Glucose	6
0.83	180	291	1.62	60	Glycerol	11
0.70 (0.75)	373 (283)	397	1.06 (1.40)	30	Fructose	2 (> 70)

PVP, poly(vinyl)pyrrolidone. The Slade and Levine (1988) value for T_m/T_g of fructose = 1.06 (because their T_g = 373 K), the value of 1.40, in brackets, was calculated using the more conventionally accepted T_g value of 283 K. RVP and germination time values in brackets are values from Chirife and del Pilar Buera (1994, 1996).
Source: after Slade and Levine (1988).

glycerol is the lowest of the solutes studied. This behaviour was attributed (by Slade and Levine, 1988) to the relatively high T_m/T_g value for glycerol, resulting in a higher viscosity in the rubbery state. However, in the cases of *Penicillium implicatum*, *P. islandicum* and *Rhizopus* sp., glycerol proved less inhibitory than glucose or a glucose/fructose mixture (Chirife and del Pilar Buera, 1994), as might be predicted from the T_g values of the solutes.

These data illustrate the difficulties in dealing with biological systems, which do not simply follow physical rules. In fact, unlike the other solutes, the cell membrane of many bacteria is porous to glycerol, so glycerol might be expected to have a qualitatively different effect from that of other solutes (Chirife, 1994). It has been observed that the permeation of bacterial cells by solutes like glycerol often results in reduced inhibition of growth relative to salts, which do not enter cells (Chirife, 1994); however, the opposite effect has also been observed (Gould and Christian, 1988). Other data again contradict what would

be expected from T_g data, with growth rates of *Penicillium funiculosum* being somewhat greater in the presence of glucose than fructose. Also, for yeasts, the minimum a_w for growth in fructose tends to be generally higher than that in glucose, indicating that antimicrobial stabilisation is somewhat greater in fructose (Chirife and del Pilar Buera, 1994). It is possible that factors other than water availability and diffusion are important in these systems, e.g. glucose may actually provide a better substrate for *P. funiculosum* and yeasts than fructose.

Chirife and del Pilar Buera (1994, 1995, 1996) have reviewed research on the glass transition and microbial stability, and have carried out some work in this area. They generally doubt the relevance of the glass transition approach to microbial stability and have disputed some of the findings of Slade and Levine. For example, Slade and Levine (1991) quoted a commercial intermediate moisture pet food, which was microbiologically safe and stable below an a_w of 0.92. When the glucose/glycerol solute formulation was replaced by a fructose/propylene glycol combination they found that the product spoiled under the same a_w conditions, attributed to the low T_m/T_g of fructose, and hence the reduced microbial stabilisation of fructose compared with glucose (Slade and Levine, 1991). However, when Chirife and del Pilar Buera (1994) tested this finding, they obtained the opposite result, which they expected, because propylene glycol is known to have strong antimicrobial effects independent of effects on a_w.

Chirife (1994) studied the growth behaviour of *Staphylococcus aureus* in solutions of controlled a_w using 16 different solutes and reported that, although the minimal a_w for growth was sometimes solute-dependent (e.g. ethanol and polyethylene glycol were observed to have an antibacterial effect), *S. aureus* still did not grow below the current widely accepted minimum a_w of 0.86. Thus, in these systems, solute dependence of the growth rate did not constitute a safety risk. It has also been pointed out that the minimum a_w for microbial growth increases if growth factors are less than ideal (Troller and Christian, 1978), so that maximal growth rates will not always be observed in foods, if additional 'hurdles' are put in place (Leistner and Rödel, 1976).

Chirife and del Pilar Buera (1994) considered the stability of dried fruits, where sugars are a major component, lowering the RVP. The sugars are usually in the rubbery state, where they could theoretically crystallise, but they generally do not, which was taken as evidence of the glass transition approach not being applicable in this case. However, the presence of biopolymers and mixed rather than single sugars, which would be present in this system, are known to delay sugar crystallisation; so in this case T_g is not the only factor controlling the rate of crystallisation, but sample composition is also an important factor. As a result, dried fruits may be regarded as being in a 'pseudo' stable state, which may last beyond the product's normal lifetime.

The sorption isotherms of dried strawberries and prunes were shown to be similar, with strawberries having a $T - T_g$ of $> 59\,^{\circ}C$ at a moisture content of 20% (the $T - T_g$ of prunes at this moisture content was assumed to be similar). Prunes are known to be resistant to microbial growth at this moisture content, which might not be expected at such a large $T - T_g$ value, if T_g were the only

factor controlling stability. As a result, Chirife and del Pilar Buera (1994) concluded that '"mobility" effects have little influence on the observed growth inhibition'. Maillard browning reactions, the rate of which increases with increasing temperature, should also be considered when assessing the stability of dried fruit. These reactions result in a reduction in sugar content and an increase in water content, giving an increase in a_w on storage under unfavourable conditions (11 and 23 °C, but not 4 °C, in raisins of initial RVP 0.61; Cañellas *et al.*, 1993), which may allow further degradation of the product to occur. The relationship of T_g and reaction kinetics will be discussed briefly in a later section.

On turning their attention to biopolymer systems, which can be glassy in the a_w range of microbial growth, Chirife and del Pilar Buera (1994) draw attention to the work of Bothast *et al.* (1981), on the effects of moisture content and temperature on the microbial stability of wheat flour (and corn meal) during storage. Bothast *et al.* (1981) found mould growth in wheat flour at 17.6% moisture at 25 and 34 °C. Chirife and del Pilar Buera (1994) pointed out that, at this moisture content, the T_g values of gluten and wheat starch are 63 and 21 °C, respectively. This means that the gluten may be in the rubbery state while the starch is in the glassy state. Chirife and del Pilar Buera (1994) assumed that the operative T_g in this system must be that of starch, as it is by far the major constituent of flour (80–82%), therefore implying that mould growth can occur in glassy systems. Several factors may be relevant here. First, flour is more likely to resemble a phase-separated system (rather than a homogeneous molecular mixture), containing starch granules and gluten separately; therefore the T_g of gluten may be the important T_g in this case. In a study of a 1:1 gluten/maize starch mixture, two glass transitions were observed, related to the T_g values of gluten and maize starch (Kalichevsky and Blanshard, 1992). However, in such a system, moisture may not be evenly distributed; the starch may absorb more moisture than gluten, increasing the T_g of the gluten and reducing the T_g of the starch; water partitioning has been observed in other mixed systems (Hartley *et al.*, 1995; Farhat *et al.*, 1996). A further factor that should be considered is that mould growth is a surface phenomenon and itself influences the a_w of the substrate because of respiration and the production of heat and metabolic water (Richard-Molard *et al.*, 1985), enhancing any inhomogeneities in moisture distribution, perhaps locally plasticising the substrate and thus allowing mould growth. It would be interesting to study such systems more carefully to establish whether mould growth can occur in truly glassy systems, as theoretically this should not be possible.

Chirife (1995) quotes work on a variety of food products, showing that the activity of *Clostridium botulinum* in fresh pasta (Glass and Doyle, 1991) and the activity of *Staphylococcus aureus* in dehydrated milk, beef and pork appear to be controlled purely by a_w and do not appear to be influenced by compositional changes. He pointed out that dehydrated milk is stable (to growth of *S. aureus*) at a_w = 0.84, where $T - T_g > 82$ °C and where, from mobility considerations, microbial activity might be expected. However, in this system the measured T_g corresponds closely to that of lactose, which was (not surprisingly) found to be

the important parameter for the control of lactose crystallisation in milk powders (Jouppila and Roos, 1994). It is possible that milk protein mobility (the T_g of proteins is generally difficult to detect) may also be a factor in determining microbial stability. Alternatively, in this case, it is likely that a_w is the limiting factor as far as microbial growth is concerned.

The question 'which T_g is the important T_g in a mixed system?' is of wider interest. Lower molecular weight species, such as sugars (not fats), generally lower the T_g of polymers (e.g. starch) in multicomponent systems, but multiple glass transitions have also often been observed in starch–sugar and starch–protein mixtures (Kalichevsky and Blanshard, 1993, and Kalichevsky *et al.*, 1992, respectively). This suggests that the onset of mobility of the sugar component may also be an important factor (in addition to the polymer T_g) for product stability or physical properties. Several studies have also already shown substantial mobility of lower molecular weight species in a polymer matrix below the polymer T_g, including water and various probe materials (e.g. Le Meste, 1995; Voilley and Le Meste, 1985; Ablett *et al.*, 1993a). NMR studies have shown that water has a high degree of translational mobility in the glassy state and also that water mobility (rotational, from NMR T_2 data) at the solute T_g is greater the greater the molecular weight of the solute (Ablett *et al.*, 1993a). Using ESR, the rotational mobility of tempol was shown to increase 100-fold above the glass transition temperature of a maltodextrin matrix material (Roozen and Hemminga, 1991), indicating co-operative relaxation behaviour in this case.

There seems to be no easy answer to the question 'which T_g is the important T_g in a mixed system?' The answer will depend on the deterioration process in question, e.g. in the case of microbial growth the rate-determining step could be the rate of oxygen diffusion, water diffusion, sugar diffusion or the mobility of starch, within a starch–sugar mixture. The question of whether a_w is the most important factor in all cases is still open to debate and further experimental investigation in this area would certainly be useful to clarify the situation.

2.4.2 Stabilising effects of the glassy state

It is well known that microorganisms, enzymes and proteins are actually stabilised against temperature denaturation (hot or cold) by lowering their moisture content; the glassy state appears to be used in nature as a mechanism for survival of extreme conditions (e.g. freezing or desiccation), sometimes by means of the production of sugars or oligosaccharides (Aguilera and Karel, 1997). It has even been speculated that spores may owe their stability to their being in the glassy state (Sapru and Labuza, 1993a). Although the glass transition of spores could not be detected, the temperature dependence of thermal inactivation rate constants of spores could be described by the WLF equation, and therefore appear to be consistent with this hypothesis (Sapru and Labuza, 1993b).

Bell and Hageman (1996) studied the effects of added polyhydroxy compounds on the denaturation of dehydrated β-lactoglobulin, ovalbumin and

ribonuclease A using DSC. The thermal stability of the dehydrated proteins seemed to correlate with the glass transition temperature of the polyhydroxy component. It was concluded that T_g could be used to predict the effect of a component on the denaturation of dehydrated proteins at elevated temperatures.

On looking again at literature data, many of the differences in the effects of different solutes on the stability of microorganisms could also be explained or predicted by considering the glass transition temperature of the solute. For example *Salmonella* and *E. coli* were found to be generally more heat resistant at the same a_w when the solute was sucrose (dry $T_g \sim 70\,^{o}C$) rather than glycerol ($T_g \sim -93\,^{o}C$) (Goepfert *et al.*, 1970). At the same a_w, glucose ($T_g \sim 38\,^{o}C$) was found to have a destabilising effect on the thermal stability of *Salmonellae* relative to sucrose, but a stabilising effect relative to glycerol (Corry, 1974). These differences (including the effects of different compositions) are again generally consistent with solutes of a higher T_g resulting in greater thermal stabilisation (the T_g of multicomponent systems can be estimated using equation 2.4).

A study of the survival of *Salmonellae* and *Staphylococcus aureus* in glassy and rubbery states of gelatin (containing nutrient) at Nottingham University (Blissett *et al.*, 1994; Bolton *et al.*, 1996), showed that some cells could survive the production of glassy sheets, but no growth occurred in the glassy state and very little in the rubbery state. Survival of the *Salmonellae* was reduced in gelatin sheets at intermediate moisture contents relative to those in the glassy state. Unlike the *Salmonellae*, *Staphylococcus aureus* was not stressed by gelatin sheet formation as it has a greater a_w tolerance. Growth of *S. aureus* occurred at high a_w values, but survival was again greater in and close to the glassy state (a_w = 0.25 and 0.43) than at intermediate a_w (= 0.62 and 0.92). In the rubbery state, where a_w remained above the minimum a_w reported for both anaerobic (0.90) and aerobic (0.86) growth, there was little evidence for growth, although in the absence of gelatin, under the same conditions of a_w, pH and temperature, growth was observed. Incubation at a higher temperature (26 instead of 22 °C) resulted in growth occurring in the rubbery state. In the samples studied no synthesis of new enterotoxin A was detected, but there was also no loss in pre-formed toxins. These results show that gelatin has some effect on stability, in addition to a_w; growth is not observed in the glassy state, but the stabilising effect of low a_w or glassy states on microorganisms should be taken into account when considering the safety of foods after rehydration.

The survival of microorganisms under stressed conditions (e.g. the conditions necessary for the production of glassy gelatin, in the experiments above) depends on their history and their ability to adapt to stresses. It is well known that when bacteria are starved of nutrients a number of physiological changes take place, which can lead to greater general resistance to a range of stresses. Some stages in this process can now be studied. One *E. coli* strain (W3110) did not survive the formation process into a glassy gelatin sheet, whereas another (BJ4) which is *rpo*S positive (*rpo*S regulates stationary phase gene expression) had a greatly improved survival, and a link was shown between *rpo*S expression (as measured using bioluminescence methods) and bacterial survival during

processing (Stewart *et al.*, 1997). It should therefore be borne in mind that subjecting microorganisms to stress conditions can result in increased resistance to further stresses. Consequently, the prior history of bacteria in raw materials can influence the level of contamination after processing.

The thermal stabilities of lactase (Schebor *et al.*, 1996; Cardona *et al.*, 1997) and invertase (Mazzobre *et al.*, 1997a, b) have been studied in relation to T_g in maltodextrin, poly(vinyl)pyrrolidone (PVP) and trehalose. Enzyme inactivation could be observed below T_g in the maltodextrin and PVP samples, and there appeared to be no discontinuity in inactivation kinetics at T_g. However, the rate of inactivation below T_g was T_g dependent, being greater in samples of lower T_g at a given temperature (Schebor *et al.*, 1996; Mazzobre *et al.*, 1997b). The exception in all cases was trehalose (a disaccharide of glucose), which gave greater protection, even above its T_g as long as trehalose crystallisation did not occur (Mazzobre *et al.*, 1997b; Cardona *et al.*, 1997). The occurrence of enzyme inactivation below T_g may possibly be due to insolubility of the enzyme in the matrix, resulting in phase separation and the possibility of the enzyme being mobile (depending on its own T_g) below the T_g of the matrix (Cardona *et al.*, 1997). The unusual stabilising effect of trehalose has been observed in many systems, and has been attributed to specific hydrogen bonding interactions in the case of biological membranes (Crowe and Crowe, 1984), as well as glass formation (Green and Angell, 1989).

It is therefore concluded that, while T_g is relevant to thermal and other forms of stabilisation of biological systems, other factors also play a role, and a knowledge of T_g is not sufficient in isolation to characterise biological stability fully.

2.4.3 Rates of reactions in relation to the glass transition

The relative importance of T_g and a_w in determining rates of reactions depends on the type of reaction, with diffusion-controlled reactions, such as browning, tending to be T_g-dependent. This is complicated by the fact that reaction rates are temperature-dependent and also increase with increasing reactant concentration (as occurs on dehydration or freezing) until the mobility of reactants is reduced to a level where translational diffusion is minimal. A further complicating factor is that pH is concentration- and temperature-dependent, which is often not taken into account. In the absence of selective precipitation of buffer salts, pH decreases with decreasing a_w and is also solute-dependent (Bell and Labuza, 1994). pH may be the determining factor for certain reactions, rather than T_g or a_w. This may be the case for aspartame degradation, which appeared to be a_w-dependent and also occurred below T_g (Bell and Hageman, 1994). Oxidation rates of orange oil encapsulated in maltodextrin increased with increasing moisture content within the glassy state, but the orange oil was found to be most stable to oxidation at an RH of 75%, in the rubbery state. This was attributed to structural collapse of the matrix occurring above T_g, inhibiting oxygen diffusion (Nelson and Labuza, 1994).

Studies on the effects of structural changes on lipid oxidation rates have generally shown that non-encapsulated lipids are susceptible to oxidation in low moisture foods (even below T_g), but are protected when they are encapsulated (even above T_g). However, crystallisation of the matrix results in the release of encapsulated lipids or volatiles, which are then subject to chemical changes, such as lipid oxidation (Roos, 1995a). Changes in structure can have a significant effect on reaction kinetics. Collapse, occurring above T_g, results in decreased diffusion and can result in an initial partial release of encapsulated lipids; however, most of the lipids appear to remain encapsulated and thus protected (Labrousse *et al.*, 1992). Crystallisation, which only occurs above T_g and the rate of which is strongly dependent on $T - T_g$, results in the release of free fat then available for oxidation (Shimada *et al.*, 1991; Labrousse *et al.*, 1992). Similar release behaviour was observed for encapsulated volatiles (Flink and Karel, 1972). Crystallisation, e.g. of sucrose or lactose, can also result in the release of absorbed water, resulting in an increase in a_w, which can result in accelerated reactions in the remaining material, e.g. browning reactions in dairy powders (Roos, 1995a).

Karmas *et al.* (1992) studied non-enzymatic browning in food systems and found that browning reactions could occur only very slowly below T_g. Changes in activation energy occurred above T_g in dehydrated vegetables and model systems, and reaction rates were found to increase exponentially with increasing $T - T_g$. T_g-dependent physical changes (collapse or crystallisation), moisture content and reactant concentration also affected reaction rates. Bell and Hageman (1995) developed a model polyvinylpyrrolidone system where T_g and a_w could be varied independently at constant reactant concentration, enabling the T_g and a_w dependence of reactions to be separated. In the case of non-enzymatic browning, pigment formation was shown to be more dependent on the state of the system (glassy or rubbery) than on its a_w (Bell, 1996). Several other studies have also suggested that browning reactions are T_g-dependent, but these were not able to distinguish so clearly between T_g and a_w. When the experimental reactant concentration is not kept constant, dilution effects may result in decreased rates of reaction at high a_w values or water contents.

The extent and rate of enzyme-catalysed reactions has been related to a_w (Acker, 1969) and particularly to the availability of 'solvent water' (Drapon, 1985). Water is often required for enzymes to be stabilised in their active conformation, as a solvent facilitating the diffusion of reagents and possibly as a reagent itself (in hydrolysis reactions); in some cases the products from a less hydrated medium may be different from those obtained in solution (Drapon, 1985). The mobility of the substrate (which may or may not be related to T_g) is decisive in propagating enzyme reactions (Acker, 1969). Some enzyme-catalysed reactions (e.g. lipolysis) in the non-aqueous phase may occur at very low moisture contents (in the glassy state) as long as the lipid substrate is in the liquid state. Although the relationship of enzyme activity to T_g has not been studied directly, T_g must have some relation to substrate mobility in many systems, e.g. it has been observed that the quantity of solvent (platicising) water

required for mobility depends on the molecular size of the reagents (more water being required for larger reagents; Drapon, 1985).

In conclusion, the matrix T_g is important to reaction kinetics, particularly where diffusion of reactants is a limiting factor; however, other factors, such as reactant concentration, sample pH and the relative sizes of the matrix and reactant molecules, also need to be taken into account. The glass transition temperature of importance may be that of the matrix, or that of the reactants or other components of a multicomponent system. In cases where the reactants are much smaller (and have a lower T_g) than the sample matrix material, they may be mobile below the matrix T_g, and therefore the T_g of the matrix may not be the most important factor in determining reaction rates, but rather the mobility of the reactants within that matrix. A knowledge of T_g is also clearly important in controlling structural changes which themselves affect diffusion, encapsulation and release of reactants.

2.4.4 The glass transition and the stability of frozen foods

As discussed earlier, T_g', the glass transition temperature in a maximally frozen system, depends only on the solute and not on the moisture content. Freezing is akin to dehydration, as moisture is removed by freezing, leaving behind a glassy solute below T_g', which has the composition W_g' (Levine and Slade, 1986). If maximal freezing does not occur, then the T_g of the solute will be below T_g', because its water content will be higher than W_g'. Slade and Levine (1988, 1991) have quoted sensory work on ice-cream and frozen novelties that indicate a WLF dependence of 'iciness score' on $T - T_g'$, illustrating the potential usefulness to T_g' in predicting and manipulating the stability of frozen foods. Simatos and Blond (1991) summarised data on various deterioration processes in frozen systems and illustrated that, while WLF behaviour is approached in some cases (although still being less dependent on $T - T_g'$ than predicted), in other cases the behaviour is quite different. They hypothesised that, while increasing $T - T_g'$ results in a reduction in viscosity of the solute, which increases reaction rates, T_g' is generally closely followed by ice melting (at higher temperatures), which results in dilution of the reactants; this can have the opposite effect and reduce reaction rates. This illustrates the complexity of frozen systems.

Lim and Reid (1991) studied the effect of the presence of glassy states in frozen systems, on the rates of three diffusion-controlled model reaction systems, using maltodextrins, carboxymethylcellulose (CMC) and sucrose as solutes. Rates of enzyme hydrolysis (of disodium-*p*-dinitrophenyl phosphate), protein aggregation and non-enzymatic oxidation were studied as a function of temperature. Rates of all three reactions were found to be negligible below T_g' and increased with increasing temperature above T_g' when maltodextrins were used as the solute. Sucrose did not show a stabilising effect in the non-enzymic oxidation reaction, where rates may be limited by oxygen diffusion, but tests were carried out above the T_g' of sucrose ($-33\,^{\circ}C$), so the absence of a glassy

state could be a reason for this observation. However, sucrose proved to be an excellent stabiliser in protecting actomyosin from aggregation. This could be related to the relatively high W_g' value of sucrose and also to solute exclusion from the protein surface (Carpenter and Crowe, 1988), but does not appear to be related to T_g'. A 1% solution of CMC gave no protection against protein aggregation or non-enzymatic oxidation, even though it had a high T_g' value. This could be due to its low concentration and may also result from CMC being a branched polymer, possibly resulting in a low-density glassy state (Lim and Reid, 1991).

Further studies by Kerr and Reid (1996) confirmed the $T - T_g'$ dependence of rates of enzymatic hydrolysis in frozen carbohydrate systems. However, the data revealed distinctly different behaviour for maltodextrins (polymers of glucose), and glucose and sucrose, the rates of reactions being higher in the polymeric materials at the same $T - T_g'$. The viscosity of solutions in equilibrium with ice at different temperatures was found to have a WLF-type dependence on $T - T_g$ (i.e. the T_g of the solution) rather than $T - T_g'$, as might be expected, as these systems varied in solute concentration and were not *maximally* freeze concentrated. These results indicate that while T_g' is an important parameter in understanding and manipulating the stability of frozen systems, different types of solutes can behave very differently at the same values of $T - T_g'$. The effects of ice melting and subsequent solute dilution above T_g' also need to be taken into account.

There appears to be no published work on the relationship of microbial activity to T_g' at sub-zero temperatures. Freezing reduces the a_w of the system, but microbial growth does not appear to be a_w-dependent in frozen systems. Freezing can kill or injure some microorganisms, while others can survive months of frozen storage. Microbial growth, being temperature-dependent, is rare below $-10\,^{\circ}C$, but relatively common at -5 to $-7\,^{\circ}C$ (Golden and Arroyo-Gallyoun, 1997). Solutes generally have a cryoprotective effect, which may be T_g'-related in many cases. Freeze-thaw tolerance of microorganisms can be of commercial value, as in frozen bread doughs and yoghurts. Slade and Levine (1991) have made a distinction between cryoprotecting and cryostabilising solutes, with high T_g', low W_g' solutes (e.g. maltodextrin) being cryostabilisers, whereas cryoprotection arises from the high W_g' (unfrozen water content) of many monomeric solutes. Cryoprotection is a huge area of research and it is not appropriate to go into it here; further details can be found in the literature (e.g. the review of MacDonald and Lanier, 1997).

2.5 Conclusions

Clearly the question of whether there is any relationship between microbial growth or stability and the glass transition of a system is a current subject of debate. The published data are contradictory and more work is required before firm conclusions can be drawn. Water activity continues to be a very useful

general measure for predicting microbial activity and other spoilage mechanisms in foods; however, the non-equilibrium nature of many food systems needs to be taken into account when considering their stability. The effects of any change in formulation on microbial stability should not be assumed, based on a_w measurements alone, but shelf-life tests must be carried out. Further work is needed before it becomes clear how helpful a knowledge of T_g would be in such cases. Certainly, T_g gives an indication of physical stability against collapse, agglomeration or crystallisation, which can also have implications for microbial stability.

In general, the thermal stability of microorganisms appears to increase with increasing glass transition temperature of the solute/solvent system, even where a_w is constant. A knowledge of T_g could therefore be useful in predicting the effects of changes in formulation on microbial stability, where food processing is expected to reduce microbial load.

It has been shown that the use of the glass transition itself is not straightforward in complex food systems, as they may be phase separated with more than one glass transition. This is particularly the case in polymer (e.g. starch or protein)–sugar mixtures, where storing the system above the lower T_g may be sufficient to allow mould growth or enzyme activity. Further studies are needed to determine whether truly glassy systems are stable against mould growth, as is theoretically expected. Measurement of the glass transition temperatures of real food systems can also be problematical; however, in many systems the phase diagram for a major component (e.g. starch or sugar) may already be known, and could be used as a first approximation.

It is important to realise that the concepts of a_w and T_g are not in opposition, but are rather complementary, the use of both having the potential to enhance our understanding and prediction of food stability. For example, where different solutes at the same a_w have different effects on microbial activity or stability, the T_g values of the solutes may explain the differences. Alternatively, in systems at high a_w values, far above T_g and close to equilibrium, T_g may have little relevance, and a_w will be the major factor determining microbial stability.

Clearly, further work is required on the relationships between T_g, a_w and microbial stability. It appears that the interrelationships can be very complex, depending on the complexity of the food system and on the type of microbial system being studied. There is a need to clear up some of the confusion in the literature and to establish where the use of a glass transition approach will be helpful, providing new insights into food and microbial stability.

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3

Modelling shelf-life

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3.1 Introduction

Food is inherently perishable and, depending on its physical and chemical properties and the storage conditions, there will come a point when either its quality will be unacceptable or it will become harmful to the consumer. At this point it has reached the end of its shelf-life and the ability to predict this is of great value to the food industry when defining storage and distribution conditions and limits, formulating products, assessing manufacturing processes and doing quantitative risk assessment. It is important to identify which factors determine the shelf-life of the product: these may be microbiological, chemical or physical depending on the product, the process, the packaging and the storage conditions.

Physical changes can be caused by the mishandling of foods during harvesting, processing and distribution.[1] Examples include the loss of water of leafy vegetables leading to wilt, increase in moisture of dry foods in a humid atmosphere, freezer burn and recrystallisation due to fluctuating temperature of frozen foods. Chemical changes as a result of enzymic action, oxidative reactions and non-enzymic browning can all lead to spoilage of a product. The use of chemical kinetics, the study of the rates and mechanisms by which one chemical species converts to another, and the Arrhenius relationship that describes the influence of temperature on the reaction rate constants have been used to model changes in food quality.[1] For example, mathematical models have been developed to describe the changes in aseptically packaged orange juice quality as affected by storage temperature and initial concentration of dissolved oxygen.[2] However, most of the effort in terms of mathematical modelling has focused on microbiological safety and spoilage and the rest of this chapter will be devoted to this application.

Depending on the product, process and storage conditions the microbiological shelf-life may be determined by either the growth of spoilage or pathogenic microorganisms. Traditional methods for the determination of shelf-life include storage of the product at different temperatures and determining spoilage by sensory evaluation or microbial count. This will involve the natural flora of the product, which may vary between batches. For products where the shelf-life may be set by the growth of pathogenic microorganisms (e.g. *Listeria monocytogenes*, *Clostridium botulinum*, *Staphylococcus aureus* and *Bacillus cereus*) this may involve challenge testing the product with the organism prior to storage and microbial analysis at intervals. For some processes, such as fermentations and heat treatments, the elimination of particular microorganisms is required and it may be appropriate to assess this using inoculated food studies.

Although the term 'predictive microbiology' is relatively new, the concept of mathematically describing microbial responses to the environment is not. For more than 75 years, the safe production of canned food has been based on thermal destruction models for *Cl. botulinum*.[3] In recent years, with the increasing capabilities and widespread availability of personal computers, predictive microbiology has become an abundant area for research and software development and application. Multifunctional models, which enable the quantification of the interactions between two or more factors and allow the interpolation of combinations of factors not explicitly tested, can now be used easily by food microbiologists.

In generic terms there are two categories of predictive models. Mechanistic models describe the theoretical basis of the microbial response, but owing to the complexity of microbial physiology and our current level of understanding, these types of models are rare. In contrast, there is a plethora of empirical models that mathematically describe the data, but do not give insight into the underlying process. Empirical models can be further subdivided into probabilistic and kinetic models. Probabilistic models describe the probability of a microbiological event occurring that is independent of time (e.g. the probability of growth or toxin formation ever occurring) or that is time-dependent (probability at a given time of an event occurring). Probabilistic models are most relevant for determining whether certain microorganisms will grow when they are close to their growth boundaries. This type of model is commonly used to predict the growth or toxin formation by *Cl. botulinum*.[4] Kinetic models describe the rate and extent of growth or inactivation. In practice the different types of kinetic models have included growth, survival (conditions at non-lethal temperatures that will not support growth) and thermal inactivation.

The use of mathematical models can help to reduce the need for storage trials, challenge tests, product reformulations and process modifications, which are labour intensive, time consuming and expensive.

3.2 Development of predictive models

The development of empirical predictive microbiology models involves a series of stages.

3.2.1 Identifying the key controlling factors

The many factors that can affect the growth and survival of microorganisms in food can be grouped into three categories:

1 Intrinsic factors – characteristics of the food itself, e.g. pH, water activity (a_W), oxidative–reduction potential (E_h), preservatives.
2 Extrinsic factors – characteristics of the environment in which the food is stored, e.g. temperature, gaseous atmosphere, humidity.
3 Implicit factors – the characteristics of the microorganism itself and how it behaves in the presence of combinations of the intrinsic and extrinsic factors.

Although a large number of factors may affect the growth or survival of a given microorganism, in most foods it is usual that only a few have the majority of the effect and it is important that these are included in the model. The intended use of the model is the prime consideration when determining the controlling factors to be included. There have been cases where models have been developed without much prior thought to the scope of the subsequent applications resulting in inappropriate choice of controlling factors and limitation of its use. A better strategy is to decide on the food or range of foods to be targeted and ensure that the controlling factors are selected to reflect this.

3.2.2 Experimental design

The range of conditions over which the model is to operate should be defined because empirical models should not be applied beyond the area defined by the conditions used to generate the model. An experimental system is required in which these factors can be altered easily. Although the heterogeneity of foods makes their use difficult for the generation of data for modelling,[5] foods, particularly homogeneous ones such as milk,[6] have been used. In most cases microbiological media are used because they are of consistent composition and can be easily and reproducibly modified to the required conditions. In some cases there may be different methods of applying factors, e.g. the choice of acidulant and humectant for adjusting pH and a_W, respectively. If the model is to be applied across a wide range of foods, then the use of less inhibitory chemicals, e.g. hydrochloric acid, are more likely to avoid fail-hazardous predictions (where slower microbial growth is predicted than actually happens). However, if the model is intended for specific foods then the choice of factors may need to be more focused, e.g. specific organic acid in order to include the effects related to the undissociated molecule. This approach allows inclusion of

additional inhibitory factors that may be the difference between a safe or stable formulation and a potentially hazardous or unstable formulation.

The choice of strain(s), size of inoculum and culturing conditions of the microorganism used in the model will all affect the outcome of the data and subsequent predictions. Different strains have different phenotypic responses and so the inclusion of mixtures of strains or some form of strain selection or screening needs to be carried out. The size of the inoculum has to ensure that the expected microbial response can be measured rather than necessarily actually reflecting the numbers commonly present. It should be noted that large inocula generally require more severe preservation systems. The pre-history (growth or storage conditions including temperature and growth medium) can affect the microorganism's response to the controlling factors and it should be carefully selected to reflect as far as possible the likely conditions of naturally contaminating microorganisms.

Sampling times are an important consideration for planning experiments and as far as possible these should be concentrated around the regions of most rapid change, e.g. end of lag phase for growth models. The same is true of the choice of levels of inhibitory factors, e.g. it may be more appropriate to study the effects of hydrogen ion concentration rather than the log of the hydrogen ion concentration. The choice of combinations of conditions should also be considered. It may be the case that a central composite design[7] is most suitable for generating particular models, such as those for thermal inactivation. However, for other models, such as growth models, the region of interest may be in the area where the response that is being measured is more variable (e.g. near a boundary of growth/no growth) and more measurements may need to be made in these areas. The use of screening or siting experiments may be helpful in elucidating the choice of experimental conditions and the use of optical density measurement can be particularly useful in this regard.[8]

3.2.3 Data generation

The most labour-intensive stage is the generation of growth, survival or thermal inactivation data of the organism in the model system. Quantification of microorganisms at selected time points is usually by standard colony count methods, but optical density and conductance measurement have also been used. When the target microorganism is in pure culture, methodology for enumeration is usually straightforward, but for survival and inactivation models provision to enumerate sub-lethally injured cells may need to be made. In the case of time-to-toxin formation models the use of a toxin assay is obviously used.

3.2.4 Modelling and mathematical validation

The next stage involves mathematical analysis of the data to produce a model and mathematical validation to determine the quality of the data and the goodness of fit of the data to the model. There are a number of different

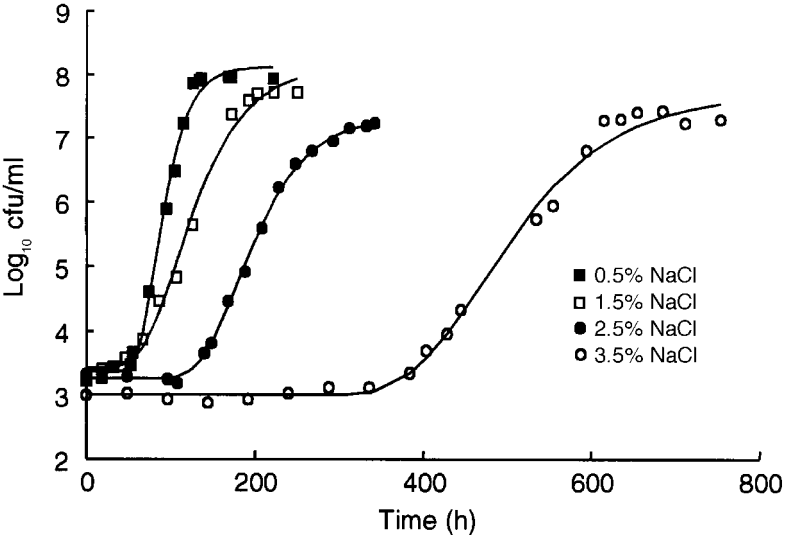

Fig. 3.1 *Aeromonas hydrophila* growth data modelled with the Gompertz equation.

modelling techniques for growth, survival and thermal inactivation.[9–12] Models have been described as being at the primary, secondary or tertiary level.[13] Primary level models describe changes in microbial numbers or other microbial responses with time (Fig. 3.1). Secondary level models describe the responses of parameters of primary models to changes in environmental conditions such as temperature, pH or a_W (Fig. 3.2). Tertiary level models are computer programs that enable users to 'interrogate' primary and secondary level models in order to obtain predictions. Whichever modelling technique is used, the model should describe the data as accurately as possible without being overly complicated. For kinetic models this involves the fitting of growth or death curves to the data followed by the use of an equation to define how the controlling factors affect the kinetics.

Mathematical validation is the process of quantifying how well the model describes the data and one approach has been described by McClure *et al.*[14] It is also important that the model predictions make biological sense. There are a number of sources of variability that may either be the inherent variability of the microorganism, systematic errors due to analytical laboratory methods or bias due to inappropriate modelling techniques not adequately describing the data. It has been estimated that for models generated in laboratory media the relative error in the prediction of specific growth rates is 7–10% for primary models and 20–50% for secondary models.[15] There is a degree of acceptance or rejection at this stage and any requirement for additional or repeated microbiological data, or the use of a more appropriate modelling technique, can be highlighted. Ross[16] introduced two indices: accuracy and bias, later modified and generalised by Baranyi *et al.*[17] to quantify the confidence in the model predictions.

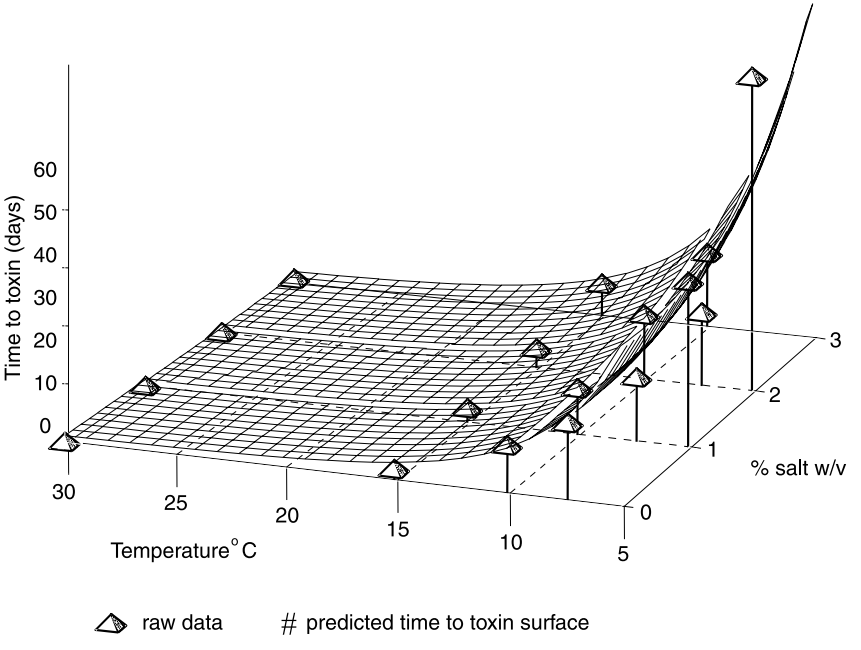

Fig. 3.2 *Clostridium botulinum* time-to-toxin model with temperature and salt concentration as controlling factors.

3.2.5 Product validation

Put simply, product validation involves the comparison of predictions from a model with growth, survival or death data of the relevant organism in food. The most rapid and inexpensive way of acquiring these data is the use of scientific publications, although the amount of data can be limited and is often incomplete with no measurement of some of the necessary physicochemical factors such as pH, sodium chloride concentration or a_W. These problems can be overcome by the use of challenge tests specifically designed for the purpose of product validation. In this way, the data are often more accurate, reliable and complete. In the past, because specific challenge tests can be time consuming and are relatively expensive, they have been used to supplement published data.[14]

Obtaining quantitative microbial growth, survival or thermal inactivation data can be problematic if the target organism or group of organisms is outnumbered by the natural food microflora. This may require the use of selective agars, which in themselves may not completely prevent overgrowth by competitor organisms, but may also lead to an underestimate of any injured cells that are present. One way of eliminating the problem of the natural flora in the food is simply by purchasing sterile or commercially sterile foods,[18] or using a heat, filtration or irradiation process. While this approach enables non-selective agars to be used, it can be criticised for not reflecting the ecology of most foods. The use of antibiotic-resistant strains of the target organism and the incorporation of the antibiotics in

non-selective or minimally selective agar or impedance media has enabled specific enumeration in the presence of outnumbering background flora.[19]

Ideally, the validation should include the foods in which the organism is considered a hazard or the cause of spoilage and the physicochemical properties of the foods and storage/heating temperatures should, as far as possible, cover the range of the controlling factors of the model. Physicochemical analysis of the food and monitoring of the storage conditions are required and as a minimum these must include the controlling factors of the model (e.g. temperature, pH, aqueous sodium chloride, a_W). Measurement of other factors that are not in the model that may affect growth/survival (e.g. preservatives) can be useful to help explain any deviations between predictions and challenge test data.

When comparing with the model, the criteria for comparison (e.g. growth rate and time for a defined $\log_{10}$ increase for growth models, time for a defined $\log_{10}$ decrease for survival models and *D*-value (time required for a 10-fold reduction in cell nembers) for thermal inactivation models) are determined and the challenge test data calculated accordingly. Predictions are obtained from the model with due consideration to physicochemical data. The results of product validation studies using literature and challenge test data have often compared well with predictions from models.[12, 14, 18, 20] In an attempt to quantify product validation, bias and accuracy indices have been used.[16, 17] Bias is a check for systematic over- or underprediction by the model and accuracy provides a measure of the average difference between the observed and predicted values. Both factors are based on geometric means and are expressed as ratios. A bias factor of 1.0 would indicate the lack of systematic error, whereas values of 1.1 and 0.9 would indicate over- and underprediction, respectively, by an average of 10%. An accuracy factor of 1.1 would indicate that the observed and published values differ by 10% on average.

There are a number of reasons why significant deviation between predictions and observed data may be seen. Published data are usually not designed for validation purposes and are, therefore, often incomplete. There can be considerable variation between species and strains, particularly in terms of heat resistance. There may be growth-inhibitory or heat-protective factors in the food that are not accounted for in the model, e.g. the presence of an organic acid or different humectant. This tends to lead to fail-safe predictions for growth models and can lead to fail-hazardous predictions for thermal inactivation models. The history of the inoculum can affect the subsequent lag phase or heat resistance of the population. The natural food microflora can affect the physicochemical properties of the food when they reach spoilage levels. The inappropriate use of physicochemical data (e.g. the use of an aqueous salt measurement for a food in which a_W is affected by other humectants) or the use of the model outside its working area can account for some of the differences between predictions and experimental data (Fig. 3.3). In the case of growth models, regions where no growth was observed may further limit this working area. Obtaining predictions outside these regions is usually not possible with commercially available

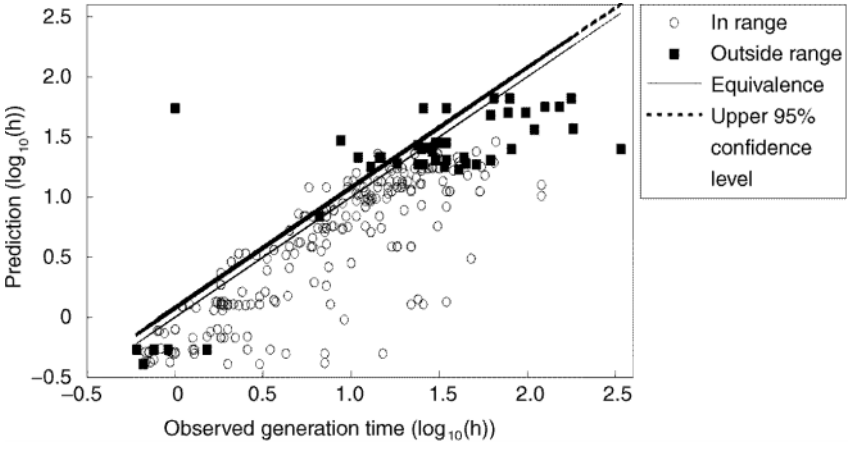

Fig. 3.3 Validation of a *Listeria monocytogenes* growth model with published data.

software. An understanding of all these factors greatly enhances the interpretation and application of predictions.

In the past, there has been considerable scepticism towards the application of predictions from models developed in laboratory media to foods. Product validation has gone some way to redress the balance and demonstrate the value of predictive models. More specifically it determines the applicability of a model for use with different foods and can highlight foods or conditions where care is needed in applying predictions. In this way the data can be used as a means of accepting, rejecting or modifying the model.

When models were first undergoing product validation they were screened against a large number of samples from a standard range of food groups. Recent studies have involved a limited number of samples and greater consideration to the applicability of the foods and the extent to which they cover the ranges of the model. Ironically, it is often when conducting product validation that any limitations of a model, in terms of the choice of controlling factors and their ranges, are realised. In fact, an initial, limited product validation study is useful as an integral part of experimental design.

3.3 Software systems

In addition to the numerous predictive microbiology models that have been published, several software systems incorporating microbiology models have been produced, some of which are commercially or freely available.

3.3.1 Food MicroModel

Food MicroModel is a Windows™ software package consisting of mathematical models that enables users to predict the safety of foods using a personal

computer.[21] In the UK, a multicentre research programme, initiated and funded by the Ministry of Agriculture, Fisheries and Food (MAFF), was undertaken to produce mathematical models that would predict the growth, survival and thermal inactivation of foodborne pathogenic bacteria.[14] The other main objective was to incorporate the models into a software programme, which became known as Food MicroModel. Predictions from Food MicroModel were originally available to the food industry via a bureau service, which was run from 1992 to 1994 by all the laboratories participating in the research programme. In 1994, MAFF granted a licence to Food MicroModel Ltd, a company jointly owned by the Leatherhead Food RA and the software company ICS and STD, to develop and market Food MicroModel software for the personal computer. The product was launched at the end of 1994 and is available as single user and network versions. The software contains growth models for all the major foodborne pathogenic bacteria and a survival model for *Campylobacter jejuni*. There are also growth models for spoilage organisms including *Lactobacillus plantarum, Brochothrix thermosphacta*, *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii*. For some organisms there is more than one model, with different controlling factors to reflect different food formulations. There are also thermal death models for *Salmonella*, *L. monocytogenes*, *Escherichia coli* O157, *Yersinia enterocolitica*, non-proteolytic *Cl. botulinum* and *S. cerevisiae*. All the models are extensively validated for use with foods using published data and/or challenge test data prior to inclusion in the software.[14] This has highlighted where these growth,[14, 18, 22] survival[20] and thermal inactivation models[12] are particularly relevant and where they have certain limitations.

Most of the models have temperature, pH (adjusted with hydrochloric acid) and a_W (adjusted with sodium chloride) as controlling factors. However, there are some models where controlling factors have included the concentration of a specific organic acid, a sugar as the humectant, gaseous atmosphere and preservatives such as sodium nitrite.

Food MicroModel is easy to use, although one-day training courses are available, and predictions can be obtained in a number of different formats, both tabular and graphical (Fig. 3.4). The software is available as a yearly licence, which is quite expensive but does cover upgrades and support. Since the Food MicroModel software has become available, the Leatherhead Food RA has also launched the Food MicroModel Prediction Service enabling anyone without the software to obtain predictions.

3.3.2 Pathogen Modeling Program

The Pathogen Modeling Program was developed by the United States Department of Agriculture (USDA) Eastern Regional Research Centre as a result of research on predictive microbiology.[23] The program contains growth models for *Aeromonas hydrophila*, *B. cereus*, *Clostridium perfringens*, *E. coli* O157:H7, *L. monocytogenes*, *Salmonella*, *Shigella flexneri*, *Staph. aureus* and *Y.*

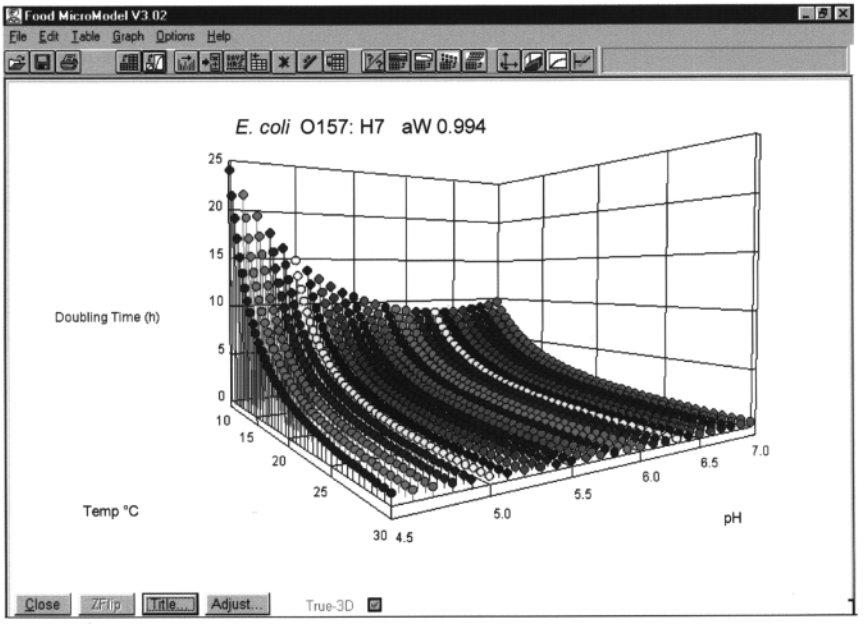

Fig. 3.4 Example of predictions from Food MicroModel showing the effect of temperature and pH on the growth of *E. coli* O157:H7 (kindly supplied by Food MicroModel Ltd).

enterocolitica. These models all predict increase in numbers over time, but there are also time-to-visible growth models for proteolytic and non-proteolytic *Cl. botulinum* and a time to detectable toxin production model for a mixture of *Cl. botulinum* E and non-proteolytic B and F strains. In addition there are non-thermal inactivation/survival models for *E. coli* O157:H7, *L. monocytogenes*, *Salmonella* and *Staph. aureus*. Recently, a thermal inactivation model for non-proteolytic *Cl. botulinum* and gamma irradiation models for *Salmonella typhimurium*, *E. coli* O157:H7 and the 'normal' flora in meats have been added. The models have not been formally validated prior to inclusion in the software, but subsequent assessment has shown that there can be good agreement or certain limitations depending on the application.[18, 22]

The software has the advantage of being available free of charge, but the users are asked to register in order that they can be contacted with news of changes or upgrades to the program.

3.3.3 *Pseudomonas* Predictor

Pseudomonas Predictor is temperature function integration software, developed at the University of Tasmania with Orion/Gemini dataloggers in the UK, based on a model for the growth of psychrotolerant pseudomonads.[24] The software, which is applicable to a wide range of proteinaceous foods stored in

different atmospheres, can read and evaluate temperature profiles as collected by temperature loggers. The developers state that it requires an operator with some degree of computer literacy and that it is a research and development tool enabling simulation of the effect of modified formulation or storage conditions. *Pseudomonas* Predictor has been commercialised and is marketed in Australia by Hasting Data Loggers under the name Food Spoilage Predictor, but there are plans to make the software more widely available in the future.

3.3.4 Seafood Spoilage Predictor

The Seafood Spoilage Predictor (SSP) was developed to facilitate the practical use of mathematical seafood spoilage models.[25] The effect of both constant and fluctuating temperatures can be predicted and the software is compatible with different types of temperature loggers. SSP was developed as a time–temperature integration device that uses different mathematical spoilage models to calculate remaining shelf-life from temperature profiles.

There are two types of models within the software. The first type comprises the relative rate of spoilage (RRS) models, developed on the basis of RRS values of seafood stored at different temperatures. The user needs to know only a product's shelf-life at a constant temperature of storage in order to use the RRS model for prediction of shelf-life at different temperatures. Different RRS models are required for different seafoods, e.g. fresh seafood from temperate and from tropical waters, and lightly preserved products (marinated shrimps in MAP). RRS models are developed on the basis of shelf-life data obtained directly from storage trials with naturally contaminated seafoods.

The second type of models comprise the microbial spoilage models that have been developed based on the specific spoilage organisms (SSOs), which are defined as the part of the total microflora responsible for spoilage of a given product. In some seafoods the SSO hypothesis seems to apply, whereas in others the SSOs have not yet been determined and may not even exist. As a consequence of the simple SSO hypothesis, shelf-life can be predicted from the initial numbers of the SSOs, the growth rate of the SSOs and the numbers of SSOs corresponding to the minimum spoilage level.

This software is available on the Internet together with an extensive help function with explanations, references and validation studies of the different models.

3.3.5 Decision Support System

The Decision Support System is an example of an expert system, which is a software package containing a large set of data and a set of rules to enable a conclusion to be reached. The Decision Support System was developed for the prediction of food safety and quality and the effect of constant and fluctuating temperatures on the growth of several microorganisms can be predicted.[26]

Predictions are made by pattern matching the physical characteristics of the food, contained in one database, with the physiological characteristics of spoilage organisms, including growth ranges, optima and fastest growth rates, contained in a second database.

3.3.6 Forecast

Forecast is a shelf-life prediction service, run by Campden and Chorleywood Food Research Association, consisting of a suite of spoilage models. Kinetic growth models for *Pseudomonas* spp., *Enterobacteriaceae*, *Bacillus* spp., yeasts and lactic acid bacteria as affected by temperature, pH and sodium chloride concentration are included. There is also a growth/no growth model for yeasts with sugar as the humectant. It is advertised that predictions from Forecast can aid in the establishment of shelf-life of chilled foods.

3.3.7 ERH CALC™

ERH CALC™ is part of a computer-based 'Cake Expert System' for the baking industry produced by the then UK Flour Milling and Baking Research Association. It enables the calculation of the theoretical equilibrium relative humidity (ERH) of formulation simulations and hence an estimate of their mould-free shelf-life at 21 and 27 ºC.[27] ERH CALC™ is applicable for a wide range of perishable bakery products including baked products, unbaked toppings and fillings, baked fillings, high fruit products and composite products. The system comes with its own ingredient lists, but there is the option for customisation to allow the user to input ingredient details manually. There are also advice sections on adjusting formulations to achieve ERH targets and considering the effects of mould inhibitors. ERH CALC™ is available from the Campden and Chorleywood Food Research Association and there is a demonstration of the software via the Internet.

3.3.8 ChefCad software

A prototype system was developed to evaluate changes in food composition, process steps and process parameters on microbiological safety and textural quality of foods.[28] In this system, which was later named ChefCad,[29] models for heat transfer calculation routines, microbial growth and inactivation, and texture kinetics are combined with food composition data, thermophysical properties, process steps and expert knowledge on type and quantity of microbial contamination. By means of a sophisticated graphical interface, the user can define recipes and visualise and evaluate the effects of changes in food composition, shape, process steps and parameters on the microbial load, food texture and centre temperature.

3.3.9 MIRINZ software

A program that predicts the number of generations of bacterial growth from time/temperature history data has been developed by the Meat Industry Research Institute of New Zealand (MIRINZ). The program uses response surface models for *L. monocytogenes*, *Aeromonas hydrophila*, *Yersinia enterocolitica* and *Pseudomonas fragi* to predict growth as affected by pH, sodium chloride, sodium nitrite and varying temperature.[30] Initially MIRINZ produced models using optical density-derived growth data and these were converted to a form from which the effects of fluctuating temperature could be predicted. To obtain time/temperature histories, the program could either receive data manually or be set up to read time/temperature data loggers.

Based on an *E. coli* growth model, MIRINZ developed software for use with Delphi temperature loggers that provided a Process Hygiene Index (PHI) and enabled an assessment of hygiene to be made.[31] Subsequently, a program running under Microsoft Windows called Dlog32 was developed for down-loading data from the Delphi loggers.[32]

Food Product Modeller (FPM), which aids the design and evaluation of chilling, freezing, thawing and heating processes for most food products, has also been developed. The software, which runs under Microsoft® Windows®, was developed from the DOS-based package MirTherm. Having simulated temperature profiles, FPM can be used to calculate aerobic and anaerobic PHI values or provide predictions from other microbial growth models.[32]

3.3.10 Quantitative risk assessment

Software is being developed to enable the use of predictive microbiology models to be used to perform quantitative risk assessments (QRA). One such general QRA program is '@Risk', which uses statistical simulation techniques to repeatedly solve models, and this has been applied to generate a predicted distribution of the risks associated with food processes.[33] Predictive models have also been combined in software for the QRA of salmonellosis from frozen poultry products.[34] This model-based QRA takes into account three types of information: occurrence and distribution of *Salmonella*, sensitivity of populations to infection and the effect of cooling (in the factory and home) and concentration of the agent and hence risks of infection after product consumption.

3.3.11 MicroFit

MicroFit, developed by the Institute of Food Research in the UK as part of a MAFF LINK project, enables the determination of parameters from microbial growth data. As well as calculating growth rate, doubling time, lag time, initial cell number and final cell concentration, MicroFit also fits and displays growth curves. The software is a helpful tool for analysing and storing challenge test data and it facilitates the comparisons to be made with predictions from software

packages such as Food MicroModel and the Pathogen Modeling Program. MicroFit can be downloaded free of charge from the Internet.

3.4 Applying predictive models to particular foods

Models are valuable tools for making predictions, but they do not completely negate the need for microbial testing nor do they replace the judgement of a trained and experienced microbiologist. Predictive models have the potential for a range of safety and spoilage applications including shelf-life determination and extension, distribution and storage condition assessment, product formulation and reformulation, process design, hazard analysis critical control point (HACCP) and risk assessment.[28, 33, 35–39] Owing to the widespread availability of predictive microbiology software systems and the increased interest and knowledge of predictive microbiology within the food industry, predictive models are being applied in practical situations. By necessity, the following examples cover only published uses of predictive microbiology for shelf-life assessment, but they give an indication of the range of applications that exist.

3.4.1 Dairy products

One of the most critical factors affecting the shelf-life of pasteurised dairy products is the temperature of storage. The relationship between bacterial growth and storage temperature of pasteurised milks of varying hygienic quality has been modelled.[6] At refrigeration temperatures spoilage was mainly due to the growth of *Pseudomonas* spp., while above 10 °C the growth of *Enterobacteriaceae* and Gram-positive bacteria became more important. The resulting models demonstrated that the main factors affecting shelf-life were temperature, initial level of contamination and the length of the lag phase. This emphasised the importance of good hygienic processing to reduce post-pasteurisation contamination, resulting in products with a predominantly Gram-positive flora and hence a longer lag phase at low temperatures.

More recently, predictive models describing the growth and toxin production for a number of bacteria of concern to dairy microbiologists have been developed.[40] In addition, a more mathematical approach has also been adopted for determining effective pasteurisation conditions for organisms present in milk.[40]

3.4.2 Meat and meat products

Modelling has found applications in the meat industry from the slaughterhouse through to meat products stored in the consumers' refrigerator. Temperature function integration (TFI) provides a means of predicting the microbiology status of meat, as well as other products, from a record of its storage temperature.[36] The microbiological effects of temperature regimes can be

evaluated by collecting temperature histories from the products and integrating the histories with respect to models that describe the dependencies on temperature for the growth of bacteria of concern.[38] A variety of conveniently small, battery-powered, electronic temperature data loggers are now commercially available. Temperature function integration techniques can be most obviously applied to processes where microbiologically unstable foods can be expected to experience varying temperatures that could permit the growth of both pathogenic and spoilage bacteria, e.g. cooling, storage, transportation and display of chilled foods.

Following slaughter, carcass cooling should be well controlled to limit the opportunity for microbial growth. The proliferation of *E. coli* and psychrotrophic pseudomonads has been shown to be suitably indicative for the behaviour of enteric mesophilic pathogens and spoilage bacteria on raw meats. Determination of temperature histories and integration with respect to growth models for *E. coli* and/or *Pseudomonas* spp., produced in microbiology media, has enabled the assessment of different carcass cooling[41] and transportation processes.[42] A similar approach has also been taken to assess the display of meat products in retail cabinets.[38] In order to generate meaningful predictions it is important that temperature measurements are made in the warmest region of the product and that the models of appropriate pathogenic and spoilage bacteria are applied.

Predictive models have also been applied to meat products. To predict microbial growth during chill storage of a traditional Greek raw sausage, a kinetic model was developed and validated.[43] The specific growth rates of populations of lactic acid bacteria, pseudomonads, *Enterobacteriaceae*, yeasts and *Micrococcaceae* naturally present in batches of sausage were calculated at two temperatures (3 and 12 ºC) and the model was used to predict microbial growth in other batches at both storage temperatures. In order to predict the growth and interaction of the different microbial populations, the initial microbial numbers are required together with the chemical factors (pH, moisture, sodium chloride concentration). It was stated that the model could be used to set the shelf-life provided that the batches of sausage were similar to those used to generate the model.

In order to be able to predict the growth of *L. monocytogenes* and *Staph. aureus* in different foods, predictions from Food MicroModel and the Pathogen Modeling Program were compared with challenge tests using commercially available sterile baby food.[18, 22] The product contained chicken and vegetables and was modified to different pHs and levels of sodium chloride. In general there was good agreement between the results of the challenge tests and predictions from both modelling software. However, in the case of *Staph. aureus* there were some cases where there were quite large differences, particularly in the lag phase between predictions and challenge test data.[22] It was emphasised that predictive models can offer advantages of quickly and easily determining the likelihood of bacterial growth and enabling a focus on which challenge tests are most appropriate, but they should not be relied upon as the sole determinant of a product's safety.

Quantitative microbial risk assessments of food processing and preparation operations are becoming more common. There is often a need to account for the changes in bacterial populations as a result of food environments and processing and predictive food microbiology models can be used for this application. In this way it is possible to estimate how changes in unit operations are likely to affect the overall safety of a food. Hypothetical examples of how these techniques could be applied to both single-step and multiple-step food processing and preparation operations have been documented.[33] Models have been combined in software that provides a quantitative risk assessment of salmonellosis from frozen poultry products.[34] This model-based QRA takes into account three types of information: occurrence and distribution of *Salmonella*, sensitivity of populations to infection and the effect of cooling (in the factory and home) and concentration of the agent and hence risks of infection after product consumption. The software demonstrates the impact of a thermal process step (using a thermal inactivation model for *Salmonella*) and the effects of changes in population sensitivity, raw material quality and cooling regime on the final risk. It would be possible to extend the scope of this QRA to other infectious pathogens (e.g. *L. monocytogenes* and *E. coli* O157) provided that dose–response relationships and thermal inactivation models are available. The risk of microbial toxin formation during the processing, distribution and storage of the product could be estimated by incorporating appropriate growth and survival models.

3.4.3 Fish

Kinetic modelling was found to be valuable for evaluation and prediction of microbial fish spoilage. Dalgaard[25] attempted to evaluate the possibility of predicting the shelf-life of packed cod from the growth and activity of specific spoilage organisms in model substrates. Different growth models for estimation of kinetic parameters were compared and the effect of CO_2 on the maximum specific growth rates of *Pseudomonas phosphoreum* and *Serratia putrefaciens* were quantified and modelled. The predicted shelf-lives confirmed that *Ps. phosphoreum* was the organism responsible for spoilage of packed cod. The model predictions clearly showed that an organism as CO_2 sensitive as *S. putrefaciens* could not possibly be responsible for spoilage of product with the short shelf-life extensions found in fresh fish products.

There is a risk of type E botulism associated with commercially manufactured, vacuum-packaged fish products. The variety of vacuum-packaged, lightly processed novel types of fish products with long shelf-lives has expanded rapidly. It is to the benefit of industry, inspecting officials and consumers to develop mathematical microbiology growth models that could be used to predict how changes in formulations and storage conditions may affect microbial growth. Food MicroModel and Pathogen Modeling Program were evaluated for their ability to determine the safety of different types of vacuum-packed fish products with respect to *Cl. botulinum* type E.[44] Predictions from the models were most accurate when all the controlling factors were close to the midpoint of

their overall ranges. As any of the factors moved towards their limits there was greater variation in the predictions. Deviations from predictions do not necessarily imply that the models are defective, but more likely that knowledge of some food ecosystems is incomplete, factors other that those used in the model have an affect on microbial behaviour, or the food is outside the domain of validity or minimum convex polyhedron of the model.[45]

A model for predicting the lag time of *Cl. botulinum* in raw fish has been used to predict the safe refrigerated shelf-life of *sous vide*-type food products.[4] Inoculated pack studies using a variety of different food products in addition to fish were included and in general the model was found to be an accurate guide upon which to base a safe refrigerated shelf-life and highlighted the importance of achieving storage temperatures of 4 °C or below.

3.4.4 Vegetable products

In common with most rapidly respiring vegetables, the edible spears of asparagus are a highly perishable commodity. Predictive modelling has been used to establish a theoretical shelf-life as a function of temperature for the microbial spoilage of packaged green asparagus.[46] It was found that a level of 10^8 colony-forming units per gram (cfu/g) of aerobic psychrotrophic flora was an indicator of the beginning of spoilage. The authors modelled the growth of the aerobic psychrotrophic flora and lactic acid bacteria in packaged fresh green asparagus stored at various temperatures between 2 and 20 °C. As a result, the shelf-life of packaged green asparagus could be predicted and this demonstrated the importance of storage temperature, with a predicted shelf-life of 18.5 days at 2 °C reduced to 9 days at 8 °C.

During the refrigeration of minimally-processed fresh vegetables, changes related to enzymic browning, elevated respiration/transpiration rate and the metabolic activities of spoilage microorganisms shorten their shelf-life. A model was developed to predict the effect of CO_2 concentration, temperature and *Lactobacillus casei* inoculum size on the growth of *Aeromonas hydrophila* in ready-to-use mixed salad vegetables packed under modified atmosphere.[47] The model emphasised the role of *L. casei* inoculum size in controlling *A. hydrophila* and allowed identification of combinations of variables to increase the shelf-life and microbiological safety of the product.

The shelf-life of ready-to-eat vegetable salads estimated by the manufacturer is usually 7–14 days depending on the vegetable. The predominant microbiological populations in ready-to-eat salads comprise the psychrotrophs *Pseudomonas* spp. and *Erwinia* spp. in addition to lactic acid bacteria including *Leuconostoc mesenteroides*. The growth of spoilage organisms in a mixed salad of red cabbage, lettuce and carrot stored at 4, 10 and 15 °C and changes in CO_2 concentrations and pH were measured. Predictive modelling was then used to establish a theoretical shelf-life as a function of temperature.[48] It was possible to set a maximum lactic acid bacteria level of 10^6 cfu/g as an indicator of the beginning of spoilage. The predictions of a product's shelf-life indicated that at

4 ºC storage could be as long as 8.7 days, which was substantially longer than the 6 days estimated by the manufacturer.

3.4.5 Considerations when applying models

Obtaining and utilising predictions requires a considerable amount of care, expertise and knowledge of the food and the microorganism(s) of concern as well as the models being used and their regions of validity.

The intrinsic and extrinsic factors of the food of relevance to the microorganisms of concern should be correctly identified and accurately measured. For factors that are constant there will still be a range of values obtained because of analytical limitations and/or batch variations and it is usually appropriate to take the 'worst case' measurement. In addition, some foods (e.g. emulsions) are inherently complex or heterogeneous and if the microenvironments are not well understood this can lead to inappropriate data or models being used for predictions. In general, growth models tend to predict more rapid growth of the particular organism than is actually seen in the food. In most cases this can probably be explained by other growth-limiting factors in the food that are not taken into account by the model. This may be because the factor (e.g. preservative) has not been included or that a 'worse case' choice, e.g. sodium chloride or hydrochloric acid for adjusting a_W and acidity, respectively, has been included. When a particular food is not accurately described it suggests that additional factors, or a consideration of microenvironment, need to be included in the model to increase its capability. The presence of additional inhibitory or protective factors in a food that were not present in the model invalidate the model or require cautious interpretation of the predictions. Most models do not include factors such as anion effects from the acidulant used, phosphates, sorbates and bacteriocins and humectants other than sodium chloride.

Foods, to a greater or lesser extent, are dynamic environments and factors may fluctuate over time. This is particularly true of temperature, but may also include factors such as pH and a_W (e.g. during a fermentation) and gaseous atmosphere (e.g. storage of modified-atmosphere-packed products). Models derived from experiments under static conditions may be of limited use for these applications, although there are modelling techniques that can describe bacterial growth in an environment where factors change with time.[49]

Knowledge of the microorganism(s) of concern and the microbial ecosystem of the food is required. To obtain predictions from most models a starting concentration of microorganisms is required; however, in practice this information is not likely to be available and predictions are usually based on an assumed starting level from past experience, a good manufacturing practice (GMP) level or a worst-case scenario. The physiological state of the microorganisms in the food compared with those in the model system can have a dramatic effect on the resulting predictions. The physiology of microorganisms can change owing to adaptation or injury and this should be considered when modelling or predicting growth in foods for determining product shelf-life

or safety. As well as differences in physiological responses due to intrinsic and extrinsic factors there is inherent variability among genera, species and strains of microorganisms, particularly when close to their growth boundaries. Knowledge of the strain(s) used for the model and the confidence limits of the model will help to interpret and apply predictions.

Microorganisms do not greatly affect the growth of one another, except when population densities are very high, e.g. after spoilage levels have been reached. At least one exception to this is the production of bacteriocins by *Lactobacillus* strains.

3.5 Future trends

Predictive microbiology modelling is still an active area of research and future developments and improvements of models and associated software should be expected. In the past, most models were targeted towards pathogenic bacteria in order to help ensure the microbiological safety of food products. Although safety is of paramount importance to the food industry, it is spoilage that often provides the day-to-day challenges and economic considerations for food microbiologists. As a result there is likely to be increased research in the understanding of food spoilage and the applicability of predictive models.

When poor correlation between predictions from a model and observed data in foods have been obtained, it is often due to a factor not included in the model (e.g. a specific preservative) or differences in the means of modifying that factor (e.g. organic acid in the food and inorganic acid in the model). Increasingly, models are being developed to address these shortfalls with controlling factors being more food-specific.

The physiological state of microorganisms in food, particularly if injured or preconditioned, can have a dramatic effect on their fate and growth/survival kinetics. For most current models, inocula cultured in optimal media at favourable temperatures are used and other than removal of the lag phase there is usually no scope for predicting the effect of preconditioning or injury. At least one exception to this is the work of Baranyi *et al*.[50] where the effect of the state of the inoculum culture on the observed lag period of the growth model was included. In the future, more consideration will have to be paid to microbial physiology, both in the model system and the food.

There is a relative plethora of growth models, but as foods become less preserved and alternative decontamination processes are sought, the need will increase for survival and inactivation models and models that cover the boundaries for growth. The variability of survivor curves together with the dramatic effects that microbial history can have, as well as the gaps in our understanding of injury and death, have meant that survival models are somewhat scarce. The fact that conditions, such as low temperature, that inhibit bacterial growth can often favour survival, highlights the importance of increasing our understanding and predictive capabilities in this area.

We are beginning to see predictive microbiology models being used to validate HACCP systems, set critical control points (CCPs) and become incorporated into quantitative risk assessments. This trend is likely to continue, and there is the scope for incorporating models in neural network expert systems that can learn from previous decisions. It may be possible to include models into the algorithms of microprocessors that monitor and control food production processes. In this way process deviations can be evaluated and corrected for during the actual process. Fuzzy logic, a branch of artificial intelligence developed as a way of dealing with uncertainty, may be a way to deal with the uncertainties associated with predictive models and food safety decision-making in general.

Most predictive microbiology models are empirical and describe a set of observations rather than helping to elucidate underlying processes. Ultimately, mechanistic models will enable us to have the greatest control over the microbiological safety and quality of food. Until that time, empirical models still have value in helping the day-to-day decision-making of the food microbiologist.

3.6 Sources of further information and advice

Food MicroModel software and enquiry service are available from Food MicroModel Ltd, Leatherhead Food RA, Randalls Road, Leatherhead KT22 7RY, UK. Tel. +44 (0)1372 376761; Fax. +44 (0)1372 386228; http://www.lfra.co.uk

Pathogen Modeling Program is available free of charge via the internet at: http://www.arserrc.gov/internet/mfs/pathogen.htm, or on floppy disk by sending a request to: ATTN: PMP51, USDA, ARS, ERRC, MFSRU, 600 East Mermaid Lane, Glenside, PA 19038, USA. Tel. +1 215 233 6616

Food Spoilage Predictor is available from Hasting Data Loggers, PO Box 5112, 1/8-12 Acacia Avenue, Port Macquarie, NSW 2444, Australia. Tel. +61 (02) 6581 3900; Fax. +61 (02) 6581 3988; http://www.hdl.com.au/html/body_fsp.htm

Seafood Spoilage Predictor is available free of charge via the Internet at: http://www.dfu.min.dk/micro/ssp

Forecast and ERH CALC™ are available from Campden and Chorleywood Research Association, Chipping Campden, Gloucestershire, GL55 6LD, UK. Tel. +44 (0)1386 842000; Fax. +44 (0)1386 842100; http://www.campden.co.uk

Food Product Modeller details are available on the Internet at: http://www.dever.com.au/fpm/food.htm and a demo can be downloaded from http://www.mirinz.org.nz/pref/download.htm

'@Risk' is available from Palisade Corp., Newfield, NY, USA; http://www.palisade.com

MicroFit can be downloaded from the Internet at: http://www.ifr.bbsrc.ac.uk

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4

Sensory evaluation methods for shelf-life assessment

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4.1 Introduction

The various definitions of shelf-life, discussed in Chapter 1, present some difficulties to the food industry when investigating the shelf-life of microbiologically stable foods, in which the factors limiting shelf-life are changes in sensory characteristics. The Institute of Food Technologists' definition[1] is particularly unhelpful, as the phrase 'of acceptable quality' can be open to many interpretations. The more specific Institute of Food Science and Technology definition[2] 'be certain to retain desired sensory ... characteristics' is an improvement, but requires definition and measurement of desired sensory characteristics. This definition also implies that sensory characteristics should not change over the shelf-life of the product. While this might be seen as desirable, in practical terms most foods undergo deterioration following production, and this must be recognised by defining bands of desired characteristics. Further, some foods, notably cheese and wine, undergo sensory changes on storage that generate the desired product characteristics.

When considering sensory quality issues, it must be remembered that many factors other than sensory characteristics can influence consumer purchase decisions. For some years, psychology researchers have been developing models to understand consumer behaviour.[3] There are many possible circumstances under which non-sensory factors such as price and nutritional image can have dominant effects, and there is evidence for changing consumer understanding of the concept of freshness that may influence attitudes to issues related to shelf-life. Although the sensory characteristics of foods are central to continued purchase of foods, care should be taken not to overlook these extrinsic factors.

The sensory evaluation of food is frequently defined by the term 'tasting', but this term is clearly inadequate to describe all the perceptual processes involved in eating food. When we eat food, we perceive a whole range of different characteristics relating to the appearance, flavour and texture of the food. Physiological differences between individuals result in a range of responses to these stimuli, and we must expect these differential responses to be encountered within a given consumer population. Further, differences in ethnic and cultural backgrounds and in experiences of foods will further broaden the response of consumers to foods. In using sensory methods, we must be prepared not only to encounter and work within this wide response, but also to interpret data generated by sensory measurements in the context of the target consumer population.

Changes in all the different sensory modalities can occur throughout the shelf-life of foods. Appearance changes are commonly seen on storage of, for example, red meat (browning), fruit juices (darkening), dairy gels (syneresis) and emulsions (separation). Odour loss is a particular problem in products such as bread and coffee, whereas the development of off-odours is particularly important as an index of deterioration in many products. Odour changes are frequently accompanied by flavour changes, but flavour is a complex characteristic that is perceived in different ways (see section 4.2), and consequently flavour changes can occur independently of odour changes. Textural changes can be seen as positive (for example maturation and softening of fruit), but are more frequently deteriorative, for example staling of bread and loss of crispness in snack foods.

There is often a temptation to interpret measured sensory changes in terms of perceived quality, but this must be given careful consideration. In general, we dislike extremes, preferring intermediate levels of a sensory characteristic, leading to the inverted-U relationship shown in Fig. 4.1, and simple linear relationships are not often seen within a typical consumer population, although different relationships can be seen in segmented populations.

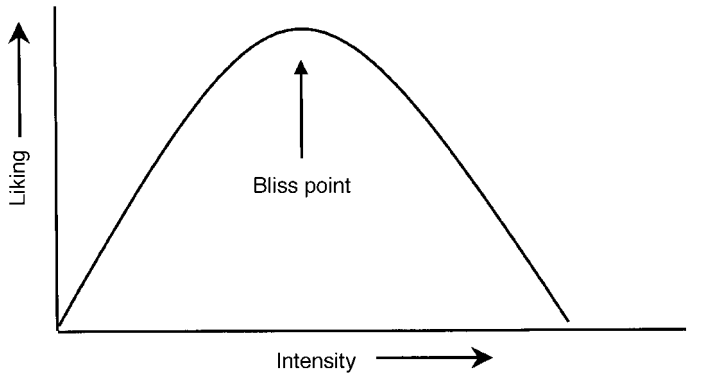

Fig. 4.1 Typical relationship between consumer liking and analytical measures.

Assessment of sensory shelf-life can therefore be approached in one of two ways: from measurement of sensory characteristics, or from measurement of consumer liking. In this chapter, the principles underlying the measurement of sensory characteristics will be described, together with practical measurement systems and the interpretation of the measured data in terms of sensory shelf-life.

4.2 Principles of sensory evaluation

Human beings employ a range of senses in perceiving food quality (Fig. 4.2). The discussion below summarises these senses briefly. Fuller descriptions can be found in the following references: *appearance* in Hutchings;[4] *odour* in Maruniak;[5] *taste* in Plattig;[6] *texture* in Bourne,[7] Brennan[8] and Rosenthal.[9]

4.2.1 The human senses

The visual senses are of particular importance in generating an initial impression of food quality that often precedes the input from the remaining senses. Indeed, if the appearance of the food creates a negative impact, then the other senses might not come into play at all. The visual sense is often equated only with colour, but provides input on many more appearance attributes that can influence food choice, for example size, shape, surface gloss and clarity. In particular, the visual senses can provide an early, and strong, expectation of the flavour and textural properties of foods.

Taste (gustation) is strictly defined as the response by the tongue to soluble, involatile materials. These have classically been defined as four primary basic taste sensations – salt, sweet, sour and bitter – although in some countries this list is extended to include sensations such as metallic, astringency and umami, this last sensation associated with monosodium glutamate. The taste receptors are organised groups of cells, known as taste buds, located within specialised structures called papillae. These are located mainly on the tip, sides and rear upper surface of the tongue. Sweetness is detected primarily on the tip of the tongue, salt and sour on the sides of the tongue and bitter on the rear of the

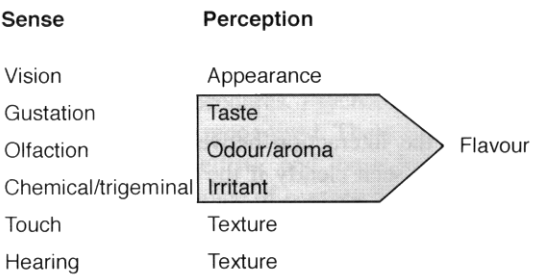

Fig. 4.2 The human senses.

tongue. Taste stimuli are characterised by the relatively narrow range between the weakest and the strongest stimulants (*ca* 10^4), and are strongly influenced by factors such as temperature and pH.[10]

The odour response is much more complex, and odours are detected as volatiles entering the nasal passage, either directly via the nose or indirectly through the retronasal path via the mouth. The odorants are sensed by the olfactory epithelium, which is located in the roof of the nasal cavity. Some 150–200 odour qualities have been recognised, and there is a very wide range (*ca* 10^{12}) between the weakest and the strongest stimulants.[10] The odour receptors are easily saturated, and specific anosmia (blindness to specific odours) is common. It is thought that the wide range of possible odour responses contributes to variety in flavour perception. Both taste and odour stimuli can be detected only if they are released effectively from the food matrix during the course of mastication.

The chemical sense corresponds to a pain response through stimulation of the trigeminal nerve. This is produced by chemical irritants such as ginger and capsaicin (from chilli), both of which give a heat response, and chemicals such as menthol and sorbitol, which give a cooling response. With the exception of capsaicin, these stimulants are characterised by high thresholds. The combined effect of the taste, odour and chemical responses gives rise to the sensation generally perceived as flavour, although these terms are often used loosely.

Texture is perceived by the sense of touch and comprises two components: somesthesis, a tactile, surface response from skin; and kinesthesis (or proprioception), which is a deep response from muscles and tendons. For many foods, visual stimuli will generate an expectation of textural properties. The touch stimuli themselves can arise from tactile manipulation of the food with the hands and fingers, either directly or through the intermediary of utensils such as a knife or spoon. Oral contact with food can occur through the lips, tongue, palate and teeth, all of which provide textural information.[11]

The descriptions given above, while appropriate for the individual sensing modalities, fail to take into account their interactive nature, shown schematically in Fig. 4.3. These interactions have been extensively reviewed by Cardello.[12] Colour, which is obviously an important appearance characteristic, can be shown to have an influence on flavour perception. For example, Dubose *et al.*[13] found significant increases in perceived flavour intensity in beverages with increasing colour intensity. Textural properties of foods have substantial effects on the perception of flavour, and sound emission from crisp and crunchy foods has been shown to be of great importance in the perception of their texture (e.g. Vickers[14]).

The importance of the interaction between the texture of foods and their perceived flavour can be seen clearly if the time course of events during food consumption is considered. As already indicated, strong expectations of the flavour and texture characteristics can be generated before the food is introduced into the mouth. As food enters the mouth, and is either bitten or manipulated between tongue and palate, catastrophic changes occur to the structure of the

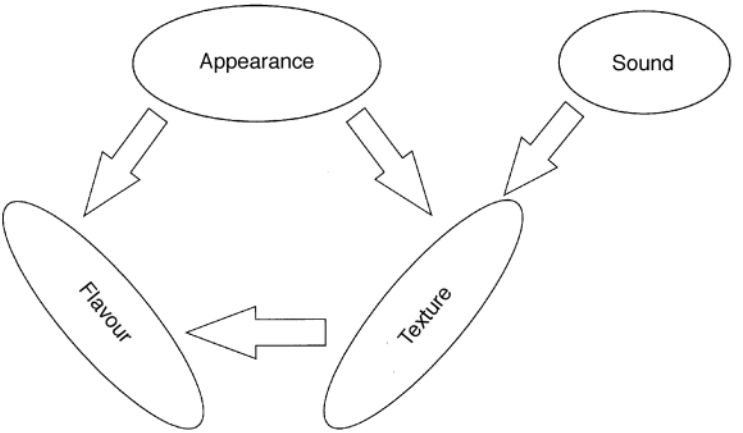

Fig. 4.3 Interactive processes operating for the human senses.

food that strongly influence the way in which tastants and odorants are released from the food. These processes can result in important effects on perceived flavour, and can produce substantial changes in flavour and texture quality if changes to food structure occur on storage.

4.2.2 Factors influencing the quality of sensory data

The complex nature of food quality perception creates many difficulties for the sensory analyst, whose primary task is to use human subjects as an instrument to measure the sensory quality of foods. The factors that should be considered in assessing the performance of human subjects in this way are accuracy, precision and validity.[15] Sensory measurements are a direct measure of human response, and have an inherently higher validity than instrumental measures, which are nonetheless of value as a complement to sensory data in shelf-life assessment. In measuring human responses, low precision must be expected, but variation can be reduced by careful selection of a range of human subjects who can produce a response with lower variability, and by extensive training.

Improving accuracy (giving the correct answer without systematic error or bias) can be achieved by recognising the various sources of physiological and psychological biases that can influence human subjects.[10] The effect of physiological differences among individuals can be reduced, but not completely eliminated, by careful selection procedures. Psychological factors can introduce systematic biases that might not be recognised. These include those arising from unwanted interaction between panellists, and those from more subtle sources. These can be greatly reduced by choice of sensory test procedure and by careful experimental design and operation of sensory test procedure. Such factors play a major role in generating sensory data that can be interpreted in terms of shelf-life.

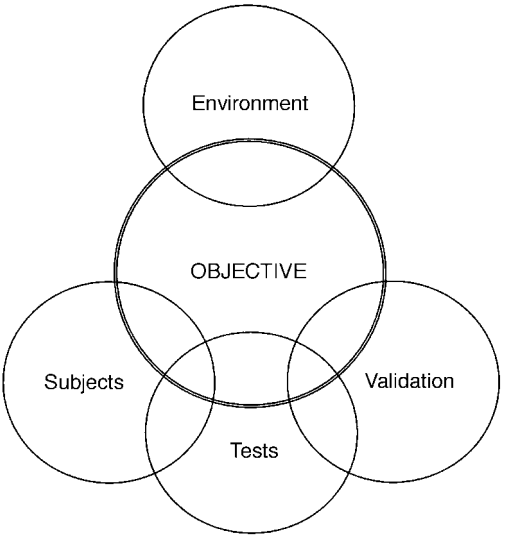

Fig. 4.4 Requirements for formal sensory analysis.

4.3 Basic requirements for sensory analysis

In developing and implementing a high-quality sensory evaluation system a number of inter-related requirements can be defined; these are discussed below, and more detailed discussions can be found in standard texts (e.g. Piggott;[16] Meilgaard *et al.*;[10] Muñoz *et al.*;[17] Stone and Sidel;[18] Lawless and Heymann[19]). The requirements are shown schematically in Fig. 4.4.

4.3.1 Clear definition of the objectives of the sensory evaluation system

Clear objectives are central to the establishment of any system that will be sufficiently accurate to measure the required sensory characteristics with the required precision and that will be cost-effective. This is particularly important in shelf-life assessments, in which repeated measurements over a period of time demand substantial resources and commitment. Large amounts of sensory data can be generated over the test period, and careful planning must be given to how these data are produced and handled if a meaningful interpretation is to be achieved. Problems commonly seen in industry include: underestimation of panellist requirements, including enforced changes in personnel over the test period; ambiguity in the type of sensory information to be generated; and absence of guidelines on the interpretation of storage changes in terms of shelf-life.

4.3.2 Provision of a dedicated sensory testing environment

A suitable environment is essential for generating high-quality sensory data with minimal bias. The environment is important not only in providing standardised

working conditions for the assessors, but also in providing a work area for sample preparation and for data analysis. Detailed advice is given in a number of publications (e.g. Stone and Sidel;[18] BS 7183, 1989/ISO 8589[20]). The three main components of a sensory evaluation environment are:

- A preparation area of adequate size and appropriately equipped.
- A testing environment, adjacent to, but separated from, the preparation area.
- Individual booths to eliminate assessor interaction.

4.3.3 Selection of suitable test procedures

Many sensory test methodologies are available, but fall into two main classes, shown schematically in Fig. 4.5:

- *Analytical tests.* These tests are used to measure sensory characteristics of products by providing answers to the questions:

  (a) Is there a difference?
  (b) What is the nature of the difference(s)?
  (c) How big is (are) the difference(s)?

- *Hedonic/affective tests.* These tests are used to measure consumer response to sensory characteristics of the products by providing answers to the questions:

  (a) Which product is preferred?
  (b) How much is it liked?

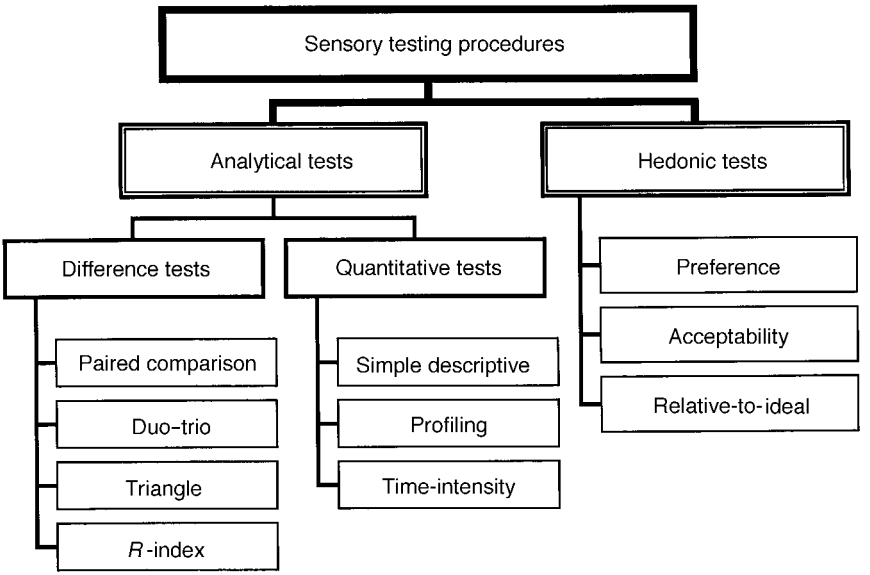

Fig. 4.5 Classification of the main types of sensory test procedures.

The two classes comprise tests that satisfy completely different objectives, and that are subject to different operating principles. Analytical tests use human subjects as a form of instrument to measure properties of the food. Hedonic tests measure the response of consumer populations to the food in terms of likes or dislikes. Different psychological processes are used for each type of test, and in general there is no simple linear relationship between the two types of data, with the relationship shown in Fig. 4.1 being typical. Of great practical importance, the type and numbers of subjects used for the analytical and hedonic tests are quite different. Use of each test type for shelf-life determination is described in more detail in subsequent sections.

4.3.4 Selection and training of suitable test subjects

The subjects to be used are defined by the objective of the test and by the consequential choice of test. The numbers of subjects to be used depends on the level of expertise and training of the assessors. Recommended numbers are give in BS 5929 Part 1, 1986 (ISO 6658),[21] which also discriminates between assessors, selected assessors and experts.

Analytical tests

Both discriminative and descriptive tests use small panels of assessors chosen for their abilities to carry out the tests. Guidelines for establishing such assessors are given in BS 7667 Part 1, 1993 (ISO 8586-1).[22] A general scheme for establishing a panel requires the following steps:

- *Recruitment.* Panellists can be recruited from within the company, or dedicated part-time panellists can be recruited from the local population (company employees should not be compelled to participate).
- *Screening.* These preliminary tests are used to establish that sensory impairment is absent, to establish sensitivity to appropriate stimuli and to evaluate the ability to verbalise and communicate responses. These tests will depend mainly on the defined objectives of the sensory testing, but will typically consist of the following:

  (a) the ability to detect and describe the four basic tastes: sweet, sour, salt and bitter; these may be extended to cover metallic, umami and astringent;
  (b) the ability to detect and recognise common odorants, together with those characteristic of the product range of interest;
  (c) the ability to order increasing intensities of a specific stimulus correctly, for example increasing sweetness or increasing firmness;
  (d) the ability to describe textural terms characteristic of relevant food types;
  (e) absence of colour vision deficiencies. Approximately 8% men, but only 0.4% women, suffer colour vision deficiencies.[4] Tests can be carried out using Ishihara charts (available from opticians or booksellers).

Selection of suitable panellists is usually made on the basis of a good performance across the entire range of tests, rather than excellence in some and poor response to others. If the panel is to be used for a specific purpose, then the tests relevant to that purpose can be weighted appropriately.

- *Training.* In the initial stages, training is limited to the basic principles and operations, following which further selection can be made. More closely targeted training can then be carried out using the products of interest and aimed towards the specific tests to be used in practice.
- *Monitoring.* Close monitoring of panel performance is essential, and any drift that is identified must be corrected by retraining procedures.

Hedonic tests

Subjects (respondents) for hedonic tests are chosen to represent the target consumer population, and to reflect any inhomogeneity in that population. Consequently, they need to be used in sufficient numbers to give statistical confidence that they are representative, and they must be given the opportunity to behave as they would in a real consumption environment. In particular, they must not be selected on the basis of sensory ability and must not be given any training. More than 100 respondents are normally used. For the early stages of concept development, qualitative studies using focus groups with small numbers of respondents can be used, but the data generated should be treated carefully and conclusions must not be generalised.

The same subjects must not be used for both types of test and, in particular, in-house staff must not be used to generate hedonic data that may be viewed as consumer-related.

4.3.5 Data handling, analysis and presentation

Sensory experiments can generate large amounts of data, and reliable conclusions require validation using statistical techniques. Details of suitable statistical methods can be found in a number of texts, e.g. O'Mahony,[23] Smith,[24] Meilgaard *et al.*[10] and Lahiff and Leland.[25] Different types of sensory test procedures generally utilise specific analysis procedures but, in the case of the more sophisticated profiling techniques, a wide range of options is available, both univariate and multivariate. Many statistical software packages are now available. The most sophisticated require a sound understanding of statistical principles, but more user-friendly packages are available that satisfy most requirements. However, it is usually found that no single package can cover the entire range of basic requirements.

Clear and effective presentation of sensory data, including the results of statistical tests, is essential. Most standard spreadsheets are now able to offer a wide range of presentation possibilities for both univariate and multivariate data.

4.4 Discrimination tests

Discrimination tests are perceived as one of the easiest classes of sensory testing to apply in an industrial environment, and are consequently heavily used. The tests can be used in two ways: to determine whether there is an overall difference between two samples, or to determine whether one sample has more or less of a specific attribute than another. However, there are inherent limitations of such tests, for example the restricted information content and the difficulty in determining whether the absence of a difference can be interpreted as the samples being the same. Consequently, such tests are often overused in circumstances in which alternative methods such as profiling would be superior (see section 4.5). As used at the time of writing, difference tests are almost universally used to ascertain whether two samples are different, not to ascertain whether two samples are the same. However, future revisions of ISO standards will advise sensory analysts on how to use the tests for the latter purpose. Alternative types of difference tests such as the '*R*-index' test[23] are available, but are less well-validated. In this section, the main types of test with practical value for shelf-life assessment will be described.

4.4.1 Paired comparison test

In the most common form of the test (less commonly referred to as the 2-AFC, alternative forced choice, test), two coded samples are presented either sequentially or simultaneously in a balanced presentation order (i.e. AB and BA). There are two variations on the test. In the directional difference variant, the panellists are asked to choose the sample with the greater or lesser amount of a specified characteristic. The panellists are usually instructed to make a choice (forced-choice procedure), even if they have to make a guess, or they may be allowed to record a 'no-difference' response. In the directional form, it is important that the panellists clearly comprehend the nature of the attribute of interest. It has been pointed out that, if time is needed to train panellists to recognise a specific characteristic, a descriptive test should have been selected.[18] The non-directional variant is the paired preference test used in consumer testing.

4.4.2 Duo–trio test

In the most common variant of the duo–trio test, the panellists are presented with a sample that is identified as a reference followed by two coded samples, one of which is the same as the reference and the other different. These coded samples are presented in a balanced presentation order, i.e.

A	(reference)	A	B
A	(reference)	B	A

The panellists are asked to identify which sample is the same as the reference. The duo–trio test is particularly useful when testing foods that are difficult to prepare in identical portions. Testing such heterogeneous foods using the

triangle test, which relies on identical portions (see section 4.4.3), can give rise to difficulties, but in the duo–trio test there are no inherent difficulties in asking the question: *Which sample is most similar to the reference?*

4.4.3 Triangle test

Three coded samples are presented to the panellists, two of which are identical, using all possible sample permutations, i.e.

ABB	AAB
BAB	ABA
BBA	BAA

The panellists are asked to select the odd sample in either fixed-choice or no-difference procedures. The increased number of samples compared with a paired comparison test can result in problems with flavour carry-over when using strongly flavoured samples, making identification of the odd sample more difficult. Difficulties can also be encountered in ensuring presentation of identical samples of some foods.

4.4.4 Difference from control test

The panellists are presented with an identified control and a range of test samples. They are asked to rate the samples on suitable scales anchored by the points 'not different from control' to 'very different from control'. The test results are usually analysed as scaled data.

4.4.5 Analysis of discrimination tests

The basic principle underlying the analysis of difference is to test the actual response obtained against the response that would have occurred purely by chance; for the paired comparison and duo–trio tests this is 1 in 2; for the triangle test this is 1 in 3. One consequence of the different probabilities is that the statistical power of the tests differs, together with the numbers of responses that are needed in order to give a meaningful and reliable result. These numbers are related to the levels of risk that are deemed acceptable. These are the Type 1 risk (incorrectly concluding that there is a difference that does not exist) and the Type 2 risk (not identifying a difference that is present). Table 4.1 shows the minimum numbers of panellists recommended in BS 5929 Part 1 (1986); ISO 6658.[21] It is possible to generate the required number of judgements by replicated tests with a smaller number of panellists. Such a procedure should be used with care (for example, generating 15 responses by using 3 panellists in 5 replicates is not recommended), and each replicate should be set up as a separate test. This table also illustrates the principle that the number of panellists required decreases with increasing expertise. However, these numbers should be used for

Table 4.1 Minimum numbers and experience of assessors (BS 5929 Part 1; ISO 6658)

Test	Experts	Trained assessors	Assessors
Paired comparison	7	20	30
Triangular	5	15	25
Duo–trio			20
Two out of five		10	
'A' – 'not A'		20	30
Ranking	2	5	10 (100[a])
Simple descriptive	5	5	
Profile (QDA)	5	5	

[a] Consumer tests.

guidance only, and it is probable that future revisions of ISO standards will recommend the use of higher numbers of panellists.

The test results are usually analysed using tables of the binomial expansion, although other distributions have been used. The 5% level of significance is frequently used in sensory tests, but an increasingly common procedure is to calculate exact probability levels. If a strict statistical interpretation is required, a forced-choice response must be used. Similarly, if relatively inexperienced panellists, or consumers, are being used, then a forced-choice test must be used to prevent 'fence-sitting'. However, if highly experienced panellists are used, a no difference response can be highly informative in specific circumstances.

Other useful information can be acquired from discrimination tests, although some authors warn that the tests should be strictly limited to establishing a difference.[10, 18] Descriptions of the nature of any difference can provide useful guidance for further testing. A simple scaled assessment of the degree of confidence in the decision (absolutely sure/fairly sure/not very sure/only guessed) is very useful, especially when using forced-choice procedures. Assessment of the degree of difference is only likely to be of value if panellists have been trained in scaling procedures. More controversially, panellists can be asked which samples they prefer, but this type of procedure is of value only for crude guidance; preference tests should be set up separately as consumer tests. There is potential value in acquiring this information in shelf-life assessments, but the hedonic information should be used with great care. All such information is supportive in nature only, and can only be used from panellists who have given the correct response.

4.5 Quantitative descriptive tests

The major advantages of discrimination tests are their relative simplicity to set up and operate, and their high sensitivity. However, they have two important

limitations. Firstly, only two sample treatments are compared. Secondly, the information content of discrimination tests is limited, even when operated in an extended format, incorporating a range of questions. More informative tests can produce more quantitative data, which can be subjected to a wider range of statistical treatments.

4.5.1 Scaling procedures

Quantification of sensory data is needed in many applications, and the recording of perceived intensity of attributes or liking requires some form of scaling procedure. These procedures should be distinguished from quality grading systems, which are used to sort products into classes defined by a combination of sensory characteristics. Such systems are not open to quantitative numerical analysis. Scaling procedures are mainly used to generate numeric data that can be manipulated and analysed statistically. Before this can be carried out, however, thought must be given to how the scales used are seen and interpreted by the assessors, and how this may influence the type of analysis that can be safely applied. The different types of scale used are described below.

- *Category scales* use a defined number of boxes or categories (often 5, 7 or 9, although other numbers are often used). The scale ends are defined by verbal anchors, and intermediate scale points are often given verbal descriptions.
- *Graphic scales (line scales)* consist of a horizontal or vertical line with a minimum number of verbal anchors, usually at the ends. Other anchors can be used, for example to define a central point, or to denote the position of a reference sample.
- *Unipolar scales* have a zero at one end, and are most commonly used in profiling, especially for flavour attributes.
- *Bipolar scales* have opposite attributes at either end. Definition of the central point can often give rise to logical difficulties, as can ensuring that the extreme anchors are true opposites. This can be a particular problem for textural attributes, for example when using *soft . . . hard* type scales. Bipolar scales are frequently used for consumer acceptability testing, especially using the *like extremely . . . dislike extremely* format.
- *Hedonic scales* are used to measure consumer liking or acceptability. Category scales are usually used.
- *Relative to ideal scales* are a type of hedonic scale which measures deviation from a personal ideal point.

The type of scale used and its construction depend on a number of factors:

- *Purpose of test*. Both category and graphic scales are commonly used with trained panels. In consumer testing, category scaling methods are usually used.
- *Expertise of assessors*. Untrained assessors are generally poor discriminators, and can discriminate only over a small number of scale points. Consequently,

5- or even 3-point category scales are often used with consumers. Trained panels can start with 5- or 7-point category scales, but as their discrimination ability increases, they can use effectively more scale points or graphic scales. When using inexperienced assessors or consumers, scales incorporating a 'neutral point', such as the central point in an odd-numbered category scale, are sometimes avoided in order to minimise the risk of 'fence-sitting'.

- *Number of assessors.* Using small assessor numbers with a low number of category scale points will limit statistical analysis options.
- *Data-handling facilities.* Category scaling responses can be entered relatively quickly onto a spreadsheet, whereas data from line scales must be measured, and this can be a time-consuming procedure. Computerised data acquisition, either directly from a terminal or indirectly from optical readers, can avoid this problem.

In practice, establishing a trained sensory panel can often proceed from a category scale with a small number of scale points (e.g. 5), through a category scale with more points (e.g. 9) to a line scale. Sensory analysts should be aware of difficulties that panellists have in using scales, and careful training is needed to ensure that scales are unambiguous and can measure the intended response.

4.5.2 Simple descriptive procedures

Scaling may often be needed in order to quantify a single, well-defined attribute. However, it should be established that there is no ambiguity in the attribute of interest. This is particularly relevant during product development or modification, when the assumption that a process or ingredient modification will change only a single attribute is frequently violated. Such changes are especially common when textural changes are a consequence of process or ingredient modifications. If it is suspected that several attributes might be of interest, then the profiling procedures described in the subsequent sections should be considered.

4.5.3 Quantitative descriptive analysis (QDA)

QDA is a total system covering sample selection, panellist screening, vocabulary development, testing and data analysis.[18] Variants of the original QDA procedures are probably used more than any other profiling procedure. The QDA technique uses small numbers of highly trained panellists. Typically, 6 to 12 people are screened for sensory acuity and trained to perform the descriptive task and evaluate the product. Three major steps are required: development of a standardised vocabulary, quantification of selected sensory characteristics and analysis of the results by parametric statistics.

Vocabulary development

Development of the vocabulary is a group process for creating a complete list of descriptors for the products under study. Panellists freely describe the flavour,

appearance, odour, mouthfeel, texture and aftertaste characteristics of different samples. No hedonic (*good* or *balanced*), general (*full* or *typical*) or intensity-based (*strong* or *weak*) terms are permitted. Terminology should be consistent from product to product and tied to reference materials. The references decrease panellist variability, reduce the amount of time necessary to train sensory panellists, and allow calibration of the panel in the use of intensity scales. References should be simple, reproducible and clear to the assessors, and illustrate only a single sensory descriptor. They can be single chemical substances or finished products, and are made available during both the training and the testing phase, at various concentrations or intensity. One requirement for the use of QDA in shelf-life testing is the use of training samples that illustrate quality changes that occur on storage. This is often difficult to achieve in practice, especially for long shelf-life foods.

The attributes are collected and compiled into a master list. This individual preliminary evaluation of the samples may be revised during an open discussion to eliminate any redundant or synonymous descriptors. New terms might be added and physical references proposed. The panel leader condenses and formats the information into a proposal for standardised vocabulary. This vocabulary is then modified and improved in several interactive sessions. Multivariate statistical methods (e.g. factor analysis) are sometimes used to reduce the number of descriptors. Finally, definitions for the attributes are agreed.

Intensity measurement

Once panellists feel comfortable with the vocabulary, further training is performed. The number of training sessions is dependent on the subject's performance, product and attribute difficulties and the time allowed for QDA testing. Panel training increases panellist sensitivity and memory and helps panellists to make valid, reliable judgements independent of personal preferences.

Once the training sessions have established satisfactory panel performance, and removal of ambiguities and misunderstandings, the test samples can be evaluated. This is usually carried out in replicated (commonly three) sessions, using experimental designs that minimise biases.

4.5.4 The Spectrum™ method

This more recent method provides a tool with which to design a descriptive procedure for a given product category.[10] The method resembles QDA in many respects; for example the panel must be trained to define all product sensory attributes, to rate the intensity of each and to include other relevant characterising aspects such as change over time, difference in the order of appearance of attributes, and integrated total aroma and/or flavour impact.

Panellists develop their lists of descriptors by first evaluating a broad array of products that define the product category. The process includes using references to determine the best choice of term and to define that term so that it is understood in the same way by all panellists. Words such as vanilla, chocolate or

orange must describe an authentic vanilla, chocolate and orange character for which clear references are supplied. All terms from all panellists are then compiled into a list that is comprehensive yet not overlapping.

The Spectrum™ method is based on an extensive use of reference points. The choice of scaling technique may depend on the available facilities for computer manipulation of data and on the need for sophisticated data analysis. Whatever the scale chosen, it must have at least two, and preferably three or five, reference points distributed across the range.

4.5.5 Free choice profiling (FCP)

Free choice profiling is a very different concept, which removes the need to generate a compromise consensus vocabulary,[26] and which can also be used in consumer research.[27,28] Assessors are allowed to develop their own individual vocabularies to describe sensory perceptions and to use these to score sets of samples. As a consequence of removing the need to agree vocabularies, free choice profiling requires little training – only instruction in the use of the chosen scale. Assessors merely have to be objective, capable of using line scales and able to use their developed vocabulary consistently. Thus, assessors can still be regarded as representing naïve consumers. Characteristics being judged can be restricted by the panel leader, but the number of descriptors produced is limited only by the perceptual and descriptive skills of the assessors. A range of sensory characteristics such as appearance, flavour, aroma or texture can be examined. One particular advantage of the technique for shelf-life assessment is that new attributes that develop on storage can readily be incorporated into the profile. The disadvantages include the need to use a complex statistical analysis technique (generalised Procrustes analysis) in order to generate an 'average' profile, and the absence of any agreed terminology.

4.5.6 Time-related methods

Sensory attributes are not perceived instantaneously, and can change in intensity with time in the mouth. Time–intensity methods are used to measure intensity of a specific attribute as a function of time in the mouth, and have been used extensively to investigate the temporal behaviour of tastants, such as sweet and bitter molecules, and the release of volatile flavour materials from foods.[29,30] Such studies are particularly important in the reformulation of foods that results in structural modifications, and in changes that can occur on storage. These structural modifications are often accompanied by textural changes, and these often result in complex perceptual phenomena that are direct consequences of the changes in texture with time producing different flavour release phenomena. Although the use of time–intensity for flavour measurement is relatively well established, textural changes can also be monitored using the method.[31,32]

A major limitation of the time–intensity method is that only a single attribute can be tracked with time, and, if a number of important attributes are thought to

be time-dependent, separate sessions are need for each attribute. Difficulties encountered in time–intensity profiling prompted the development of a hybrid technique, progressive profiling.[33] In this technique, assessors carried out a profile on a set of texture descriptors at each chew stroke over the mastication period. Such a method has a number of potential advantages: several attributes can be assessed in one session; scaling is reduced to a unidimensional process; and the most important aspects of the shape of a time–intensity curve are retained.

4.5.7 Univariate analysis of scaled data

Univariate procedures are the starting point for the analysis of any sensory data, regardless of the data complexity. The procedures can be used at different stages of a sensory programme, but are particularly useful in assessing the performance of panellists undergoing training for profile panels, and for exploratory investigation of scaled data. The techniques used can be found in numerous standard texts on statistics, but a number of useful publications specific to sensory data are available (e.g. O'Mahony;[23] Smith;[24] Meilgaard *et al.*;[10] Lahiff and Leland;[25] Lea *et al.*[34]).

An important consideration in selecting appropriate analysis techniques is the nature and distribution of the data. Prior to the use of any statistical procedure, the form of the data should be examined by visualisation techniques, such as the use of scatter plots. Data that are not normally distributed are analysed by non-parametric methods. It is frequently assumed that sensory data are normally distributed, and that parametric tests can be used. The distribution of all sensory data should be examined, however, especially when relatively small numbers of responses are being used. If in doubt, non-parametric tests can be employed.

The most commonly used procedures used to examine sensory data are *t*-tests, analysis of variance (ANOVA) and multiple comparison tests. The *t*-test procedure can be used to compare the mean scores from two samples, usually used in the paired format if the same panellists have assessed both samples. If more than two samples are to be compared, two-way ANOVA is used with the panellists and samples as factors. Panellist $\times$ sample interactions are also usually examined. If significant differences for a given attribute are identified by ANOVA, multiple comparison tests can be used to identify which samples differ. The various types of multiple comparison tests, and their use, have been described by O'Mahony.[23]

4.5.8 Multivariate analysis of scaled data

In most applications of any form of sensory testing, the intensities of many attributes are being measured, leading to highly complex data sets. Multivariate analysis (MVA) methods are increasingly being seen as essential in interpreting such data sets, and several different uses are evident:

- Assistance in panel training, including assessment of panellist performance and reduction of attribute lists in forming profile vocabularies.

- Simplifying the complexity of data presentation. Visualisation of the relationships between two attributes is easily accomplished, and visualisation of three attributes presents few difficulties, but greater numbers of attributes present substantial problems.
- Identification of redundancy in the use of descriptive attributes.
- Investigation of the underlying structure between products and between the attributes characterising them.
- Construction of 'maps' visualising the similarities and dissimilarities among products.

There are numerous MVA techniques available for the analysis of sensory data, and several valuable texts and papers describing their use (e.g. Martens and Russwurm;[35] Piggott;[16,36] Aishima and Nakai;[37] and Meilgaard *et al.*[10]).

4.6 Consumer acceptability testing

Consumer tests give a direct measure of liking that can be used more directly to estimate shelf-life. The most common procedure is to ask consumers representative of the target population to scale acceptability on a 9-point category scale, anchored from like extremely to dislike extremely. A minimum of 50 consumers should be used, and preferably 100, although lower numbers (32–40) have been reported.[38] Suitable experimental designs should be used, and appropriate statistical analysis. Other information on individual modalities (appearance, odour, flavour and texture) can also be obtained, together with attribute intensity information, but it is preferable to keep such tests simple and to focus on overall acceptability. The most common procedure for operating the tests is to recruit consumers from a convenient high street or mall location and to carry out the tests in a convenient hall. Alternatively, a mobile test laboratory can be used to increase the degree of control.

4.7 Operation of sensory shelf-life tests

4.7.1 Selection of tests for shelf-life assessment

The choice of tests for shelf-life assessments depends on the purpose of the assessment, and on the way in which the sensory storage changes are to be interpreted in terms of shelf-life (see Chapter 1). Quality grading schemes are available for some foods, for example fish,[39] but cannot be regarded as suitable systems for the shelf-life assessments of most foods. Difference tests can be used if the shelf-life criterion is defined in terms of the first detectable change, but in general difference tests will detect changes that are small and of little relevance to shelf-life. Consequently, most sensory tests employ quantitative measures of change that are more open to interpretation in consumer terms. It is also possible to use hedonic tests to generate consumer acceptability directly.[38] Such tests can

be expensive, and an alternative is to use quantitative sensory tests to measure change, and at critical change points to carry out consumer tests to evaluate the impact of the changes on consumer acceptability.

4.7.2 References for sensory shelf-life assessment

The variability of sensory data can be reduced substantially if a reference standard can be made available at each assessment session. Unless a very high level of panel training is feasible, memory of sensory quality is unreliable for most shelf-life testing, especially over medium/long-term storage periods, and reference samples should be provided for all tests.[40] Ideally, a reference standard should be used from the same batch of product under test that can be stored under conditions in which changes do not occur. This is rarely achieved in practice, and more frequently it must be assumed that a stored reference undergoes quality change. Care must be taken to choose conditions that minimise the change. An alternative procedure is to manufacture a new reference for each test point. This is a valid procedure only in circumstances in which batch-to-batch variation is minimal; substantial variations will prejudice data interpretation. An increasingly common alternative to physical reference standards is a written standard, generated by sensory techniques such as QDA. While considerably superior to reliance on memory, successful use of such a standard requires extensive panel training and maintenance of a stable panel performance over the storage period. The problems described above are inevitably more serious in the case of shelf-life tests carried out over long storage periods.

4.7.3 Ethical considerations

Any sensory testing of foods must be carried out under a defined ethical policy for the use of human subjects. This is particularly important in the case of storage testing, especially when the test protocol takes the products close to, or even past, the shelf-life of the products. In particular, it is essential to assess any microbiological hazards that might be associated with testing, especially near the end of shelf-life and under accelerated (elevated-temperature) storage conditions. If necessary, microbiological testing must be carried out prior to sensory testing, and, if appropriate, on the same samples to be used for sensory testing. Under no circumstances should samples of questionable microbiological quality be submitted for sensory testing. If there are any residual questions regarding microbiological quality, sensory testing should be limited to assessment of appearance and odour only.

4.8 The interpretation of sensory shelf-life data

The various sensory test procedures generate information on whether changes are occurring, the nature of the changes that are occurring and the magnitude of

the changes. Such information cannot be transformed into shelf-life information unless two criteria are satisfied. Firstly, the pattern of the changes must be understood, in terms of both the form and the direction of the change. Secondly, there must be a company policy on sensory quality that forms a framework within which the data can be interpreted. This is essential when interpreting analytical sensory data in terms of consumer response. These two issues are closely related, and are discussed subsequently.

Important quality changes on storage are often assumed to be linear, but this is rarely the case in practice. It is also often erroneously assumed that any change represents quality deterioration, but this is clearly not the case with foods such as cheese, and beverages such as wine. Changes in product attributes with forms such as those shown in Fig. 4.6 are not uncommon, especially in the period immediately following manufacture. Clearly, the form of such changes must be known before any reliable interpretation can be made.

The criteria that can be used for interpreting sensory shelf-life data have been reviewed by Dethmers,[41] and fall into three categories: first detectable change, measured attribute change and change in consumer acceptability. The first detectable change (or just noticeable difference) in product quality can be measured using difference tests, assuming that a suitable reference sample is available. While giving a sensitive measure of change, difference tests can be over-sensitive to changes that have little relevance to sensory quality as perceived by consumers, and give limited information on the nature of the change. An additional problem can be encountered when non-linear changes occur, as shown in Fig. 4.7. In this case, spot difference tests carried out at timepoints A, B and C would all identify the same level of difference. This illustrates an underlying problem with the use of difference tests, which is that a quantitative picture of change is rarely attainable.

If quantitative measures of relevant sensory attributes are made, a fixed level of change can be used as a criterion. This is illustrated in Fig. 4.8a for two

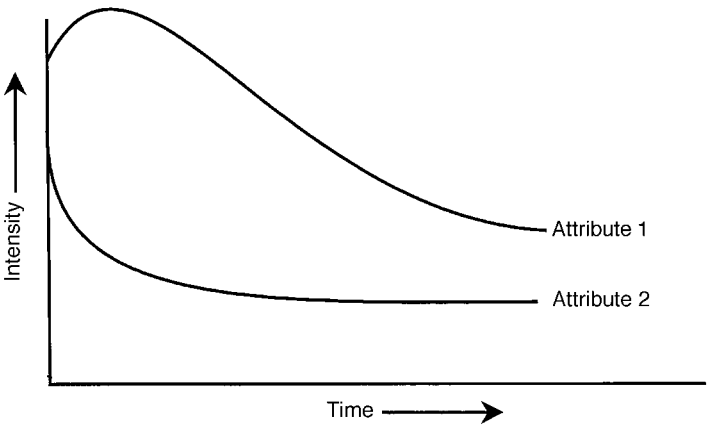

Fig. 4.6 Illustrative changes in sensory attributes following manufacture.

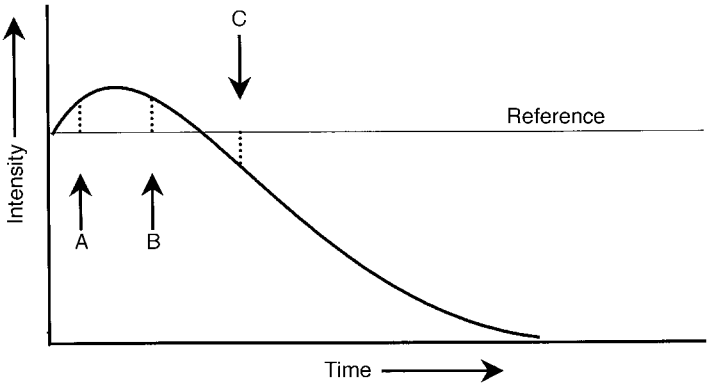

Fig. 4.7 First detectable change. Difference tests can give ambiguous result with non-linear attribute change: similar levels of difference will be found at each timepoint – A, B, C

products showing a decreasing attribute intensity. The decrease of this attribute is faster for product 1, reaching a critical limit at a shorter storage time. The critical limit needs to be agreed as representing the end of shelf-life. Figure 4.8b shows an analogous situation in which an attribute that is absent at the start of storage increases in intensity. This typifies the situation in which an off-flavour develops on storage. Growth of a non-characteristc attribute is often more easily detected than decrease of a characteristic attribute, and is likely to be of great importance to consumer acceptability.

The change in intensity level must be related to perceived quality if the sensory data are to be interpreted in terms of shelf-life, and an alternative approach is to measure consumer acceptability directly. Figure 4.9a shows how direct measurement of consumer acceptability can be used to compare the shelf-life of two products. Greater difficulty in interpretation is encountered, however, when the changes of acceptability of two products of different initial quality are measured. This is illustrated in Fig. 4.9b, in which product 1 represents an economy product, and product 2 a premium product. The use of a single critical acceptability level fails to recognise the different quality levels, and in these circumstances it may be preferable to define critical levels for each product that reflect its market.

4.9 Instrumental methods in sensory shelf-life testing

Sensory measures of quality changes on storage are essential as the only valid reflection of perceived quality, but are expensive and time-consuming to operate. In addition, they suffer from high variability when carried out over long time periods, and need regular panel calibration, especially if the panel composition changes. If valid instrumental measurement methods are available, these can be of great value in augmenting the sensory data, although they are

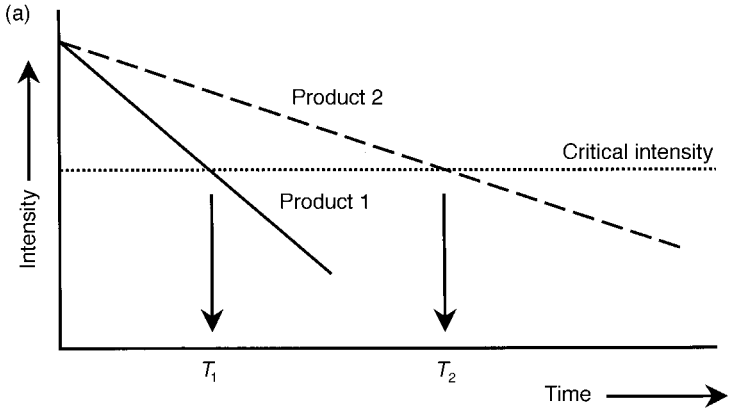

Fig. 4.8a Level of change of critical attribute. The decrease of this attribute is faster for product 1 than for product 2, and a critical lower intensity is reached more quickly.

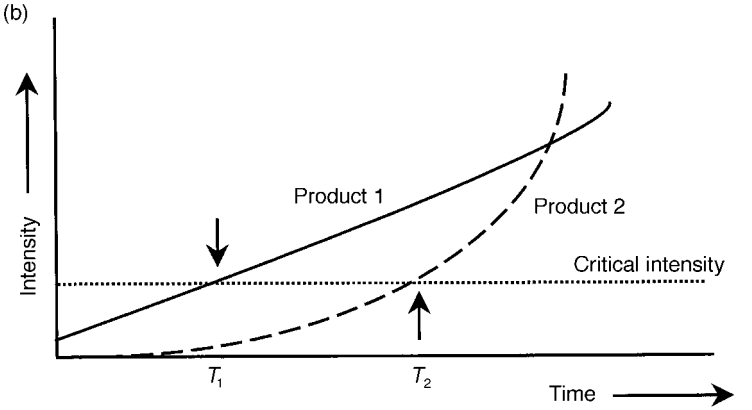

Fig. 4.8b Level of change of critical attribute. The increase of this attribute is faster for product 1 than for product 2, and a critical intensity is reached more quickly.

only rarely sufficiently reliable in replacing sensory data (e.g. Kress-Rogers[42]). Their value can most clearly be found in long-term measurement of shelf-life, which poses substantial challenges to the sensory analyst.

4.9.1 Appearance

Overall appearance changes on storage can readily be tracked using either conventional or digital still photographs. This is a particularly powerful means of monitoring change in form of a product, and can be used to monitor visual colour changes. However, accurate rendition of colour changes requires careful standardisation of lighting conditions and photographic technique, and ideally should be carried out by a professional photographer. Successful imaging of

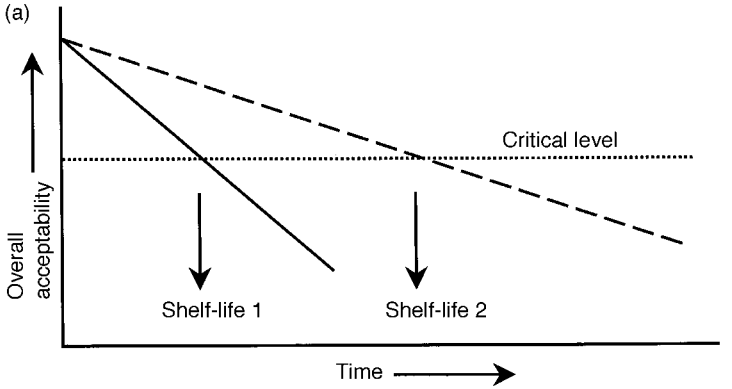

Fig. 4.9a Change in consumer acceptability. Product 1 has a shorter shelf-life than Product 2.

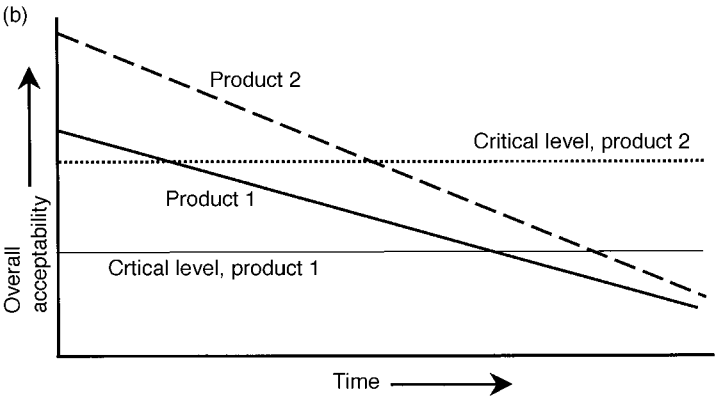

Fig. 4.9b Change in consumer acceptability. Estimation of shelf-life of products at different quality levels.

appearance has the benefit of providing accurate visual standards that are of great value in shelf-life measurement. For colour assessment alone, many instruments are available that can give relevant measurements of product colour characteristics. In addition, extensive use is made of standard colour atlases, although there are problems in applying these to wide ranges of foods. Consequently, many sectors of the food and drinks industry have devised colour matching charts specifically for there own products. Colour measurement and the use of colour atlases is discussed in detail in Hutchings.[4]

4.9.2 Aroma and flavour

The complexity of the flavour response presents enormous difficulties for those needing a rapid and simple assessment. Measurement of the wide range of

volatiles that contribute to food flavour is technically feasible, but even the most sophisticated techniques, such as gas chromatography–mass spectrometry, carry the risk of not identifying trace volatiles that have low detection thresholds. In principle, analysis of involatile tastants should pose a lesser problem, but even though there are few basic tastes, the taste response can be stimulated by a wide range of food components. As a consequence, generalised analysis plays a limited role in shelf-life assessment studies. If the deterioration mechanism is known, however, analysis for specific deterioration indicators, such as chemical compounds produced from rancidity development, can be highly effective.

4.9.3 Texture

Changes in physical properties that are perceived as textural changes can be measured using a range of techniques. Properties of fluid foods can be measured by a range of rheological techniques; properties of solid foods can be measured using mechanical techniques that typically measure force-deformation behaviour.[7–9] Many of the techniques are capable of measuring change, but not necessarily change that is relevant to perceived texture. If a valid relationship can be established, such measurements can be a valuable adjunct to sensory testing.

4.10 Future trends

Sensory techniques are the essential backbone of shelf-life assessments, but the practical difficulties in applying the most informative techniques will continue to spur efforts to find simpler, less time-consuming and less expensive alternatives. A potential adaptation of sensory methods, driven by the retail sector in the UK, is the development of sensory specifications for foods, and incorporates a simple assessment of product quality against specification. Although relatively crude, such systems offer the opportunity for low-cost sensory appraisal of shelf-life on a qualitative or semi-quantitative basis.

The development in instrumental methods is likely to follow the route exemplified by the ‘electronic nose’ systems, more correctly described as volatile sensors.[43] At present, these systems are detection instruments, and cannot easily identify specific volatiles. However, they are more usefully used as pattern recognition devices, using multivariate or neural network software systems. These can detect changes in volatile patterns that can potentially be related to changes occurring on storage. Recent reports have also indicated that similar sensing and pattern recognition systems could also be used for involatiles, although these may be less relevant to storage changes.[44]

Further investigations of physicochemical and spectroscopic techniques are also likely to reveal novel means of identifying deteriorative changes. Such investigations have been carried out at the Leatherhead Food Research Association and the Institute of Food Research, and have identified the way in which spectroscopic nuclear magnetic resonance and infrared techniques can

identify deterioration in chocolate products. The ideal techniques are those that are non-destructive and that can give warning of deterioration earlier than those detected by other methods.

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5

Accelerated shelf-life tests

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5.1 Introduction

The food industry has a great need to obtain, in a relatively short time, the necessary information for determining the shelf-life of its products. It has a very important impact on handling of the products' storage, distribution and shelf-life dating.[1] Moreover, it provides an essential tool to probe the possibilities of extending shelf-life through proper product formulation and processing techniques. For practical reasons, especially when the actual storage time is long, the industry resorts to accelerated test techniques that considerably shorten the process of obtaining the necessary experimental data. In the context of this chapter, therefore, accelerated shelf-life testing (ASLT) will refer to any method that is capable of evaluating product stability, based on data that is obtained in a significantly shorter period than the actual shelf-life of the product.

This chapter will discuss first the scientific basis of accelerated shelf-life testing. It will indicate what tools are available for carrying out the tests and explain the problems encountered when using them. At the end, an attempt is made to suggest where this important area of accelerated shelf-life testing is heading and what expectations one should have with regard to developing novel practical and reliable tools that the industry will find convenient to use.

5.2 Basic principles

ASLT is applicable to any deterioration process that has a valid kinetic model. That process may be chemical, physical, biochemical or microbial. The principles of the ASLT will be the same in all cases. However, most of the

studies on ASLT have been done on chemical deterioration of foods and therefore the examples in this chapter will be based on them.

There are a number of approaches to ASLT but all are concerned with how to get reliable deterioration data in a short period, what model to use and how eventually to predict the actual shelf-life of the product. All these questions will be dealt with relation to the different ASLT methods that are discussed in the following sections.

5.3 Initial rate approach

Conceptually, one of the simplest techniques for accelerating the shelf-life testing is the 'initial rate approach'.[2] It may be applicable to cases where the deterioration process can be monitored by an extremely accurate and sensitive analytical method. This method should be capable of measuring minute changes in the extent of deterioration after a relatively short storage time at actual conditions. In such a case, it is possible to get the kinetic data of the initial rate of the deterioration process at a very early stage of the process. To predict the actual shelf-life, one needs only to know or to evaluate how the deterioration process behaves as a function of time. In chemical reactions that information is provided by the order of reaction (n). In the case of monitoring the change in concentration c of a component of interest, the kinetic equation may be expressed as:

$$\frac{\mathrm{d}C}{\mathrm{d}t} = KC^n \qquad [5.1]$$

where K is the kinetic constant and t is time. For sake of simplicity, let us define an index of deterioration (D) that has the form:

$$\mathrm{d}D = \frac{\mathrm{d}C}{C^n} = K\mathrm{d}t \qquad [5.2]$$

By doing that, the index of deterioration will be always linear with time and will have the following form:

$$D - D_0 = Kt \qquad [5.3]$$

where D_0 is the initial level of the index of deterioration. Equation 5.3 is the only kinetic model that is required to employ this approach to ASLT and the extrapolation process, after evaluating the value of K from the initial rate, is obviously very simple. The product shelf-life (t_s) is therefore:

$$t_s = \frac{D - D_0}{K} \qquad [5.4]$$

Fortunately, information about the order of reactions in many food systems is available in the literature. Most of the chemical deterioration reactions in foods follow either a zero or a first order kinetics. The value of the index of deterioration will be in these cases:

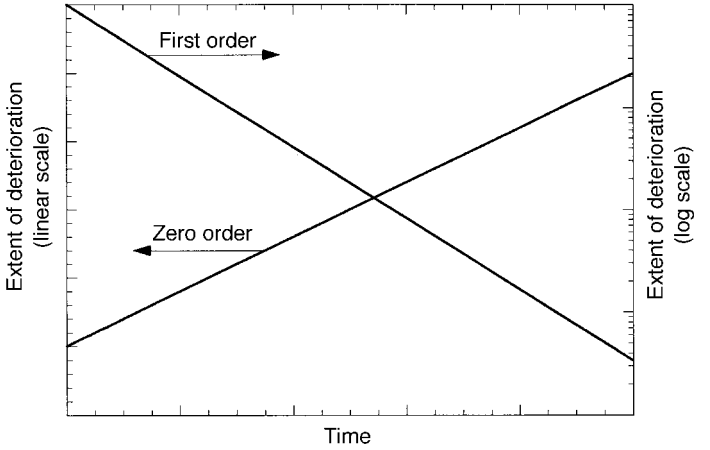

Fig. 5.1 Extent of deterioration as a function of time for zero and first order kinetics.

(a) Zero order ($n = 0$) $D = C$ [5.5]

(b) First order ($n = 1$) $D = C$ [5.6]

On a time scale it is translated to a linear or semi-logarithmic relationship, respectively (Fig. 5.1).

When the order of reaction is unknown, a simple accelerated test procedure may be used to evaluate it empirically. In that case the simplest version of the kinetic model approach, which is discussed in the following sections, may be used. Such a method uses any convenient kinetically active factor to accelerate the deterioration process.

The initial rate method, when applicable, can provide an ideal accelerated shelf-life testing technique. It has the advantage of obtaining, in a relatively short time, the kinetic data at the actual storage conditions and yet requires only the simplest kinetic model that relates solely to the order of reaction.

An example of using a relatively sensitive analytical method was attempted by Teixeira Neto *et al.* to determine the rate of oxygen uptake during oxidation of dehydrated foods.[3] The commonly used manometric techniques are notorious for being insensitive to minute changes in the relatively large mass of oxygen in the headspace.[4] Instead of using this method, Teixeira Neto *et al.*[3] determined the rate of oxygen uptake by analyzing the changes in the mass of the oxygen, which was adsorbed or entrapped in the product.[5] Since that mass is relatively much smaller than that of the manometric method, the data of the rate of oxygen uptake by the product was obtained in only a few days.

The discussion of the initial rate approach may serve also as an appropriate reminder to why there is a need to have other accelerated shelf-life testing methods. In the absence of a very sensitive and accurate analytical technique, the deterioration process should be allowed to progress for longer to enable the available method to detect the changes in a statistically significant way. The

minimal time required to obtain significant data is therefore dependent on the accuracy and sensitivity of the analytical method; the worse they are the longer, the time needed to obtain the data. In a way, accelerated shelf-life testing is required to overcome the shortcoming of the analytical methods that are used by the industry. Therefore, the selection of the proper analytical techniques for monitoring the deterioration process is of great importance to shorten the period of the accelerated shelf-life testing.

5.4 Kinetic model approach

The kinetic model approach is the most common method for accelerated shelf-life testing. The basic process involves the following steps:

- Selection of the desired kinetically active factors for acceleration of the deterioration process.
- Running a kinetic study of the deterioration process at such levels of the accelerating factors that the rate of deterioration is fast enough.
- By evaluating the parameters of the kinetic model, extrapolating the data to normal storage conditions (Fig. 5.2).
- Use the extrapolated data or the kinetic model to predict shelf-life at actual storage conditions.

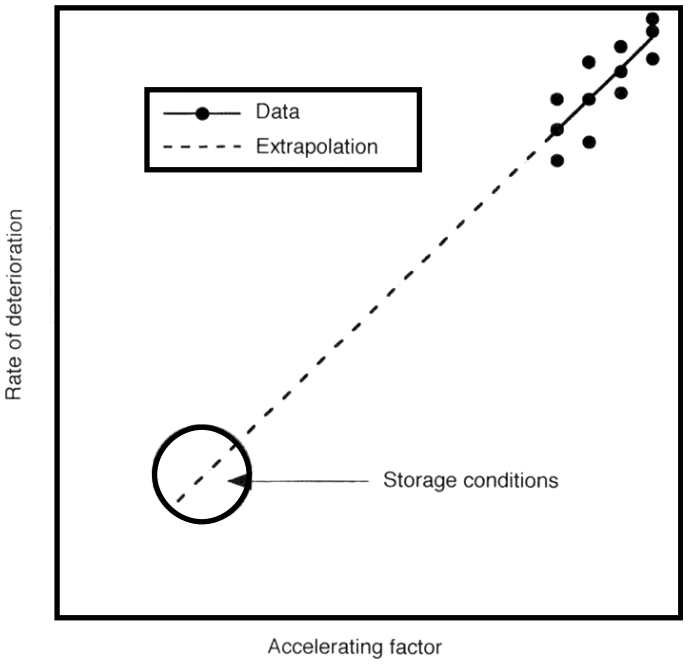

Fig. 5.2 Schematic diagram of data extrapolation in accelerated shelf-life testing.

The absolute requirement for using this procedure is to have a valid kinetic model for the deterioration process. The general and most comprehensive kinetic model for chemical reactions in foods includes all the factors that may affect their rate. These factors may be divided into two main groups, namely compositional (CF_i) and environmental factors (EF_j).[6] The model may be generally expressed as follows:

$$\frac{dD}{dt} = K(CF_i, EF_j) \quad [5.7]$$

This equation indicates that the kinetic constant K is a function of these factors. In practice, however, one does not need a comprehensive kinetic model. For prediction of shelf-life at actual storage conditions, the model should include only those factors that change during storage (SF_i). Therefore, the required model should look as follows:

$$\frac{dD}{dt} = K(SF_i) \quad [5.8]$$

The list of SF_i should include factors such as temperature, moisture content, light intensity, composition and others, but only if they change during storage. Obviously, when one is interested in predicting the shelf-life of a product at constant temperature, it is of no interest to have a kinetic model that includes this factor. Yet temperature can be used very effectively to accelerate the rate of the deterioration process. Therefore, the demands from a kinetic model for ASLT may be different from one that is used only to predict shelf-life. The model for accelerated shelf-life testing should contain two groups of factors. The first comprises those that are changing during storage (SF_i), as is in equation 5.8, and the second those that are used to accelerate the rate of reaction (AF_j). The kinetic model for ASLT therefore has the form:

$$\frac{dD}{dt} = K(SF_i, AF_j) \quad [5.9]$$

The kinetic model for accelerated shelf-life testing may therefore be different from the one usually used to predict product stability at normal storage conditions. Obviously, any of the factors that are changing during storage may be used to accelerate the rate of reaction.

Equation 5.9 expresses a concept of great practical importance for ASLT. It indicates that it is possible to use any desired factor to accelerate the process of deterioration regardless of whether it is active during normal storage conditions. Weissman *et al.*[7] have suggested that one might even use compositional factors to accelerate the rate of deterioration. This implies that the composition of a product may be altered just for the benefit of accelerating the deterioration rate. Clearly, the information obtained is useful only if a valid kinetic model is available for these compositional factors. Such a concept can open a large number of creative avenues for conducting accelerated shelf-life testing.

5.4.1 Single accelerating factor

When applying the kinetic model approach, the first question that has to be considered is whether to use a single or multiple factors for accelerating the deterioration reaction, as well as which ones to choose. The simplest and most commonly used method of ASLT is based on employing only a single factor to expedite the deterioration process. The simplicity of such a method is related to both the experimental procedure and the extrapolation of data. As already stated, such tests requires a valid kinetic model. It should be emphasized that in ASLT, the validity of the kinetic model is crucial to obtaining accurate prediction of the shelf-life. Unfortunately, the validity of the model cannot be fully verified by the ASLT procedure, because the levels used for the accelerating factor do not include those of actual storage conditions. This is in contrast to the situation where the kinetic model is established and verified for actual storage conditions. Therefore, the selection of a model for ASLT must be based on prior knowledge of its validity. The latter may rely either on available empirical data or on a sound physical and/or chemical theory, which has been extensively tested in a large number of similar cases. The Arrhenius model that relates the rate of a chemical reaction to the changes in temperature is the best example of such a validated model. This model is represented by:

$$K = K_0 \exp\left(-\frac{E_a}{RT}\right) \qquad [5.10]$$

where K_0 is a constant, E_a the energy of activation, R the gas constant and T the absolute temperature. Since this model has been used in many cases, a large database is available, mainly of the energy of activation of different reactions. One may conveniently use this information to get a reasonable estimate of the extent a change in temperature may affect the rate of reaction. To simplify the process further, one may get over the need to evaluate K_0 by using a ratio between the rates of reaction when the temperature is changed by any arbitrary value. The most commonly used value is 10 ºC and therefore the ratio between the rate of reactions is known as Q_{10}. The value of Q_{10} may be calculated using equation 5.8 to express the rate of reaction first for a temperature of $(T + 10)$ and then for T and divide the two, namely:

$$Q_{10} = \frac{\frac{dD_2}{dt}}{\frac{dD_1}{dt}} = \frac{\exp\left(\frac{E_a}{R(T+10)}\right)}{\exp\left(\frac{E_a}{RT}\right)} = \exp\left(\frac{10E_a}{RT(T+10)}\right) \qquad [5.11]$$

The simplicity of using Q_{10} has made it a very popular method for estimating shelf-life. If prior knowledge or estimates of the value of the energy of activation are relied on, the accelerated tests must be run only at one elevated temperature. When choosing the maximal possible temperature, for which the Arrhenius model is still valid, the data are obtained in the shortest possible time by minimal experimental efforts. To improve the accuracy of this version of tests further, the energy of activation may also be evaluated. In that case, the rate of

reaction must be obtained at a number of different temperatures below the maximal one in order to be within the range where the model is valid. Obviously, such a procedure takes a much longer time to run. The rule in accelerated stability tests is that to get more accurate data requires a longer experimental time.

The popularity of using the Arrhenius model has made it synonymous with ASLT. Most of the reported ASLTs are based on this model.[8–13] Owing to its popularity, the use of the Arrhenius model has received a lot of attention, especially with regard to two subjects. The most important one has to do with the validity of this model, especially when changes in the mechanism of reaction might take place due to phase transition, competitive reactions, glass transition, etc.[14] The second one is related to the evaluation of the statistical methods used to fit the model with the empirical data.[15, 16]

The most common way of accelerating the rate of reaction is by placing the product at elevated constant temperatures. However, non-isothermal procedures, using programmed changes in conditions, were also tested.[1, 17–19] This is an example of a dynamic test approach, which will be discussed later, where the accelerating factor changes with time. In a procedure where samples are withdrawn from the test for analysis, this type of approach has no advantage over the isothermal method. Moreover, it might have a severe drawback when the samples are not allowed to stay at the lower range of temperature for long enough. In such a case the data obtained for that range are not as accurate as the isothermal method where the sample is kept as long as necessary. The only possible practical advantage that the non-isothermal may have over the isothermal one is where the number of samples is very small and their deterioration process can be monitored continuously.

The use of the Arrhenius model is questionable if it has to deal with changes in the reaction mechanism mentioned above. However, even if it is valid, its use, or rather any approach that is based on a single accelerating factor, may be problematic with regard to the accuracy of the extrapolated data. To demonstrate that problem, let us consider first a simple case where the kinetic constant of the reaction is linearly related to the accelerating factor (Fig. 5.3). In this figure, the solid line represents the true relationship between the kinetic constant (y) and the accelerating factor (x). The point at the top end of the line represents the true kinetic constant (Y_e) at the level (X_e), which may be estimated from the experimental data. To extrapolate the data, the slope (a) of the line must be evaluated by curve fitting of the accelerated test's kinetic data. That value of the slope is used to extrapolate the line to actual storage conditions (X_s) where the true rate of reaction is supposed to be (Y_s). However, the error in the slope (Δa) may cause the extrapolated line to produce a predicted kinetic constant (Y_p (high) or Y_p (low)) which deviates from that true value (Y_s) by ΔY (Fig. 5.3). For the line that has a slope of ($a-\Delta a$), which is symmetrical to the one with a slope of ($a+\Delta a$), the following expression should hold:

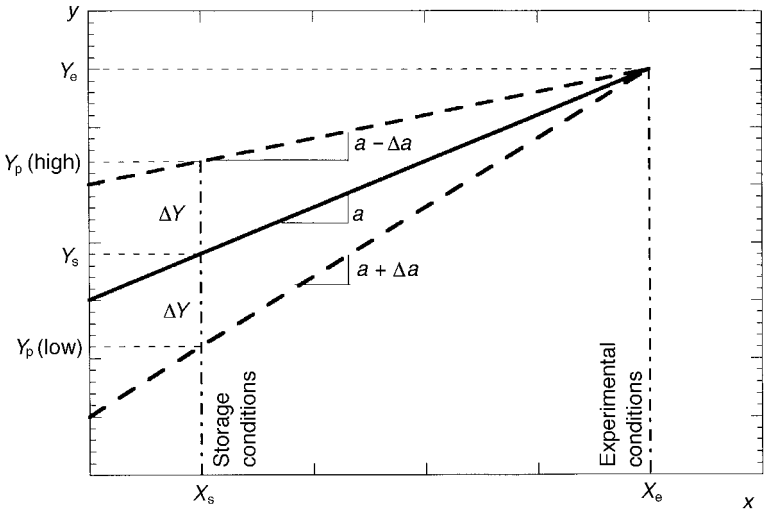

Fig. 5.3 Analysis of extrapolation error in linear plot.

$$\frac{Y_e - Y_p\,(\text{high})}{X_e - X_s} = \frac{Y_e - (Y_s + \Delta Y)}{X_e - X_s} = a - \Delta a \qquad [5.12]$$

For the true line:

$$\frac{Y_e - Y_s}{X_e - X_s} = a \qquad [5.13]$$

Subtracting equation 5.13 from equation 5.12 one obtains:

$$\frac{\Delta Y}{X_e - X_s} = \Delta a \qquad [5.14]$$

To find how the error in evaluating slope (a) affects the accuracy of the extrapolated value, equation 5.14 should be divided by equation 5.13, resulting in the following expression:

$$\frac{\Delta a}{a} = \frac{\Delta Y}{Y_e - Y_s} = \frac{\Delta Y}{Y_s\left(\frac{Y_e}{Y_s} - 1\right)} \qquad [5.15]$$

Therefore, the error in the extrapolated value is:

$$\frac{\Delta Y}{Y_s} = \frac{\Delta a}{a}\left(\frac{Y_e}{Y_s} - 1\right) \qquad [5.16]$$

Let us define the acceleration ratio (AR) as the rate of the accelerated reaction in reference to that at normal storage conditions. In case of the linear relationship between the kinetic constant and the accelerating factor, the value of that acceleration ratio is expressed as:

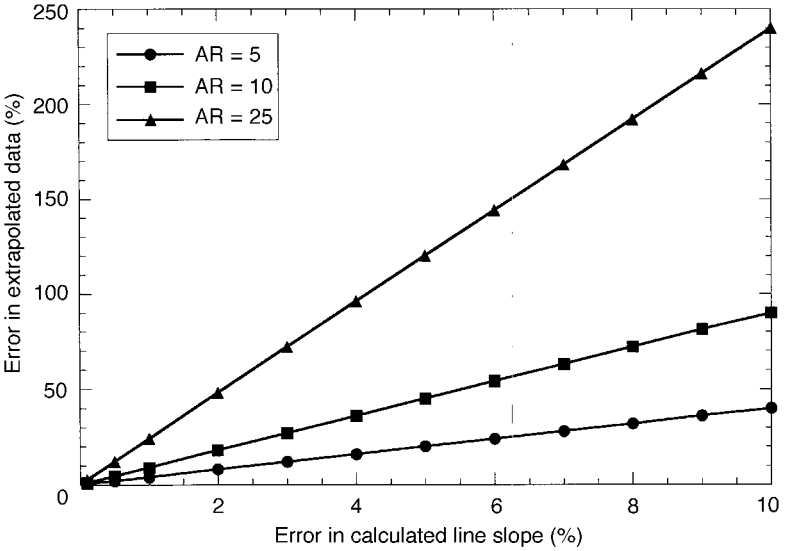

Fig. 5.4 Error in linear extrapolation.

$$\mathrm{AR} = \frac{Y_e}{Y_s} \qquad [5.17]$$

Therefore, the relative error of the predicted value of the kinetic constant is:

$$\frac{\Delta Y}{Y_s} = \frac{\Delta a}{a}(\mathrm{AR} - 1) \qquad [5.18]$$

The extrapolation process multiplies the experimental error of evaluating the slope of the line by the acceleration ratio minus one. The error of the predicted kinetic constant may be extremely high, especially when a very high acceleration ratio is used and if special care is not taken to reduce the experimental error to a very low value (Fig. 5.4). The magnitude of the error changes when the relationship between the kinetic constant and the accelerating factor is no longer linear. In the case, for example, when that relationship is exponential (Arrhenius model) or a power law, the extrapolation error may be different and it can be estimated by turning these models into their linear form and then using the above equations. The only step needed is to assign the *y*-axis the value of ln*K*. In such a case, equation 5.18 will read:

$$\frac{\Delta \ln K}{\ln K_s} = \frac{\ln K_p - \ln K_s}{\ln K_s} = \frac{\ln \dfrac{K_p}{K_s}}{\ln K_s} = \frac{\Delta a}{a}\left(\frac{\ln K_e}{\ln K_s} - 1\right) \qquad [5.19]$$

Therefore:

$$\ln \frac{K_p}{K_s} = \frac{\Delta a}{a}(\ln K_e - \ln K_s) = \frac{\Delta a}{a}\left(\ln \frac{K_e}{K_s}\right) = \frac{\Delta a}{a}\ln \mathrm{AR} \qquad [5.20]$$

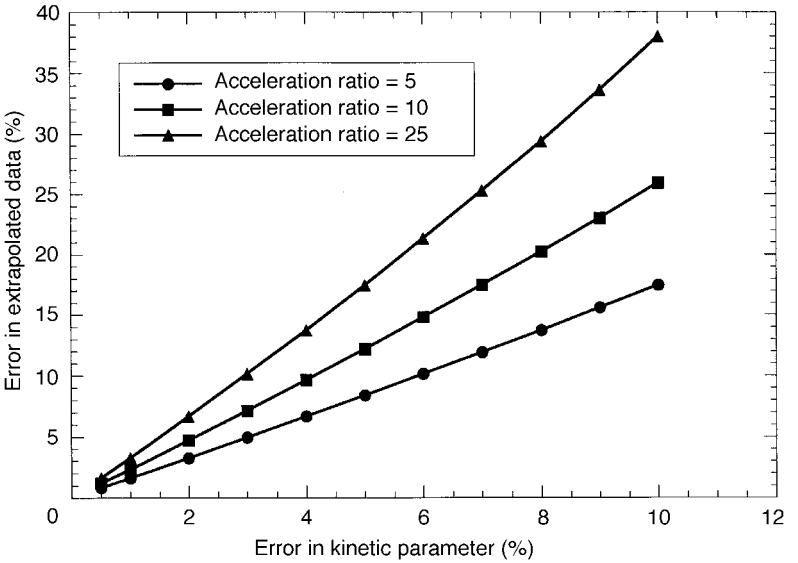

Fig. 5.5 Error in exponential or power law extrapolation.

That results in:

$$\frac{K_p}{K_s} = (AR)^{\Delta a/a} \qquad [5.21]$$

The error in the extrapolated data is:

$$\frac{K_p - K_s}{K_s} = \frac{K_s(AR^{\Delta a/a} - 1)}{K_s} = AR^{\Delta a/a} - 1 \qquad [5.22]$$

It appears, therefore, that using a model like the Arrhenius equation involves a lower error in extrapolating data (Fig. 5.5) than in the case of a simple linear model (Fig. 5.4).

5.4.2 Glass transition models

One of the most interesting approaches to kinetic studies and their use for ASLT is based on glass transition models, which were borrowed from polymer science. Clearly, this approach may be applicable only to products that are in the physical state for which such models are valid. These models, such as the Williams, Landel and Ferry (WLF) model, relate changes in the system properties, which are related to the polymer molecular mobility, to the temperature within the range of the transition of the product from its glassy to rubbery state.[20] Based on the assumption that the rate of the deterioration reactions should relate to molecular mobility in much the same way, this approach yielded valuable information about processes of recrystallization, and losses of flavor and desired textural attributes

caused by such structural changes.[21] When applicable, the glass transition models offer a number of very attractive features with regard to kinetic studies and ASLT. The first one is the fact that it combines both the effects of the temperature and the moisture content into one relatively simple equation.[22] The second one, which is even more interesting, is that the rate of the deterioration is related only to the physical state of the system, which can be independently determined in a very short time by readily available physical techniques. That considerably simplifies the experimental work since one needs only the kinetic data, at one high level of temperature or moisture content, and the physical characterization of the system. Unfortunately, that kind of interesting approach to ASLT has, so far, found very limited use. In general, the glass transition model was found to correspond closely to a stability limit with respect to physical processes, such as the ones mentioned above.[23] On the other hand, the glass transition model proved inadequate to account for different deterioration kinetics. [21, 24–28] In general, the glass transition model failed to account for diffusion of some small molecules, especially water. However, it has been proposed that the glass transition model may be applicable to predict changes in the rate of chemical reactions in food deterioration but only if proven to be diffusion limited.

5.4.3 Multiple accelerating factors

The use of multiple accelerating factors presents an effective approach to obtain a high acceleration ratio of the deterioration reaction at a minimal cost of prediction error. To demonstrate this fact, let us consider a simple theoretical case of a kinetic model that has the following form:

$$K = (c_1F_1)(c_2F_2) = c_1c_2F_1F_2 \qquad [5.23]$$

where c_1 and c_2 are the estimated parameters of the accelerating factors F_1 and F_2, respectively. In order to evaluate the error in the kinetic constant due to that of the estimated parameters, equation 5.23 is differentiated with regard to these parameters, resulting in:

$$\mathrm{d}K = c_2F_1F_2\mathrm{d}c_1 + c_1F_1F_2\mathrm{d}c_2 \qquad [5.24]$$

When dividing equation 5.24 by equation 5.23 and combining it with equation 5.18, the estimated error is found from the following expression:

$$\frac{\Delta K}{K} = \frac{\Delta c_1}{c_1} + \frac{\Delta c_2}{c_2} = (\mathrm{AR}_1 - 1)\mathrm{RE}_1 + (\mathrm{AR}_2 - 1)\mathrm{RE}_2 \qquad [5.25]$$

where RE_1 and RE_2 are the experimental relative errors for the factors F_1 and F_2, respectively. By using multiple factors, a 100-fold acceleration of the deterioration reaction, e.g. a single one may be replaced by two factors each having an acceleration ratio of only 10. This one order of magnitude reduction in the acceleration ratio decreases considerably the extrapolation error. If, for example, the error in estimating the model parameter for each of these factors is

only 1%, the extrapolated data might deviate from the real value by 99% (equation 5.18) for a single as compared to 18% (equation 5.25) for two accelerating factors. While the total acceleration effect of using two or more factors is a multiplication of their effect, the error is only the summation. Moreover, the required relatively low acceleration ratio is achieved by a much smaller change in the level of the kinetic factors and thus the system stays much closer to the actual storage conditions. Furthermore, when a narrower range of the accelerating factor is used, not only is the validity of the kinetic model better maintained but also the kinetic model may have a simpler form. The advantages of the multiple factors approach are obtained at a cost of running a more complicated experimental procedure. That is the result of the need to evaluate not only the effect of each factor on the reaction kinetics but also a possible interaction between them. The procedure, therefore, lacks the simplicity that makes such a technique more practical for the food industry.

A multiple factor acceleration of the deterioration reaction was carried out by Mizrahi *et al.* by combining the effect of temperature and moisture content (m).[8] It enabled a shelf-life that lasts for over one year to be predicted based on an experimental study that required only 10 days. The basic kinetic equation had the following general form:

$$K(m,T) = f(m)_{T_r} \exp[E_a/R\left(\frac{1}{T_r} - \frac{1}{T}\right)] \qquad [5.26]$$

where T_r is a reference temperature. Since moisture content in a food product is related to the water activity (a_w) by the sorption isotherm, the kinetic function at the constant reference temperature ($f(m)_{T_r}$) could be expressed also in terms of that water activity.

One form of such a function for non-enzymatic browning of cabbage is:[8]

$$K = K_0(a_w)^s \qquad [5.27]$$

The kinetic model shown in equation 5.26 indicates that the evaluation of the kinetic effect of moisture content is performed for a constant reference temperature (T_r). Theoretically, therefore, the evaluation of the kinetic model may be as simple as first running an experiment at an elevated constant temperature and changing only the moisture content and then keeping the latter constant at any desired level and varying the temperature. In many cases, especially when the range of temperature and moisture content changes are kept within a relatively narrow range, that procedure may be adequate. However, when that range is relatively large, a possible interaction between the two factors might play an important role in determining the accuracy of the shelf-life prediction. Such was the case in the study of the non-enzymatic browning of cabbage where the energy of activation happened to be affected by the moisture content.[8] The empirical expression that was used to describe the effect of the moisture content on the energy of activation was:

$$E_a = c_1 \exp(-c_2 m) \qquad [5.28]$$

where c_1 and c_2 are constants. That interaction between the factors greatly complicates the experimental procedure since the effect of the moisture content on the energy of activation should be tested by changing both factors at the same time. That requires much longer time and more experimental work, which may make this method very unattractive for practical use. However, as stated before, when a narrower range of the accelerating factors is used, that elaborate and cumbersome procedure may not be necessary.

5.4.4 Accelerated methods for establishing a kinetic model

The lack of well-proven general kinetic models often makes it necessary to establish or to validate a model for ASLT. Since the commonly used procedure to establish a reliable kinetic model may take a longer time than the actual shelf-life of the product, an accelerated method was developed to do it. Such a method is based on a dynamic testing procedure.[29–31] The product is subjected to conditions where the kinetically active factor is programmed to change with time in any desired way. That creates a situation where both the extent of deterioration and the value of the kinetic factor are changing with time (Fig. 5.6). At any given time, namely at a given level of the kinetic factor, the rate of reaction can be obtained by a numerical or graphical derivative of the deterioration curve. In that way one obtains the relationship between the value of the kinetic factor and the rate of reaction. The reason such a method requires a relatively short time is because most of the deterioration is taking place at the levels of the kinetic factor where the rates are very high. This casts a serious question on the accuracy of using the obtained data to establish or validate a model that should apply to those levels of the kinetic factor where the rate of

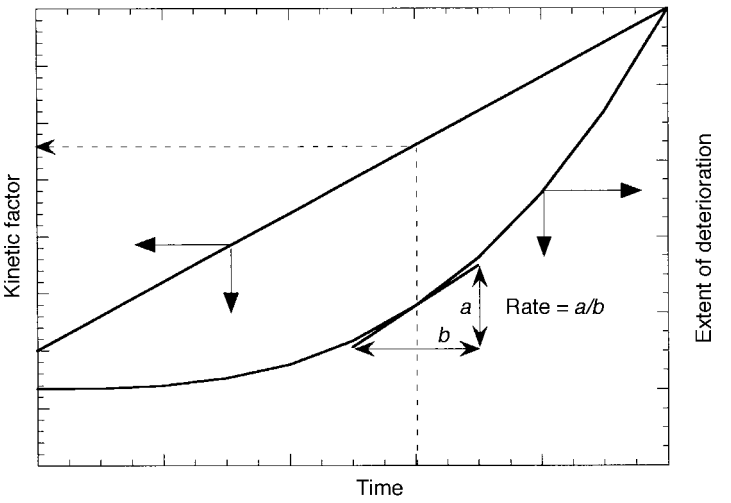

Fig. 5.6 Schematic diagram of dynamic testing of deterioration processes.

reaction is very low. Moreover, since the kinetic factor is programmed to change continuously, the system usually stays at the condition where the rate of reaction is very low for too short a time to develop any significant change in the extent of deterioration. The use of dynamic testing for accelerating the time for establishing a kinetic model is therefore not as accurate as the conventional process that takes a much longer time.

5.4.5 The 'no model' approach

The 'no model' approach is a term used for the accelerated shelf-life testing method that assumes that a valid kinetic model exists but does not require experiments to evaluate it. This approach may apply only to cases where the kinetically active factor (F) is changing during storage in a monotonically and continuous way. The ASLT technique is based on monitoring the extent of deterioration in the same product in which that factor is programmed to change in such a way that it goes through the 'storage' cycle in a shorter period. The obtained data are then converted into real storage conditions by a calculation that is based only on knowing how the kinetically active factor (F) is changing with time (t), namely on having the following function (g):

$$F = g(t) \qquad [5.29]$$

The inverse of that equation yields the function (f) of how time relates to the changing factor:

$$t = f(F) \qquad [5.30]$$

It should be noted that this equation might have an analytical expression, but may as well represent a numerical or graphical datum. Assuming that a valid kinetic model exists for the deterioration reaction, it will have the following form:

$$\mathrm{d}D = K(F)\mathrm{d}t \qquad [5.31]$$

The value of dt may be replaced in this equation by using the derivative of equation 5.30, namely:

$$\mathrm{d}t = f'(F)\mathrm{d}F \qquad [5.32]$$

Thus equation 5.31 changes into:

$$\mathrm{d}D = K(F)f'(K)\mathrm{d}F \qquad [5.33]$$

When we have two samples of the same product, one at actual storage conditions and the other at accelerated test conditions (denoted by subscript s and a, respectively), the ratio between their rate of deterioration is:

$$\frac{(\mathrm{d}D)_{\mathrm{s}}}{(\mathrm{d}D)_{\mathrm{a}}} = \frac{[K(F)f'(F)\mathrm{d}F]_{\mathrm{s}}}{[K(F)f'(F)\mathrm{d}F]_{\mathrm{a}}} \qquad [5.34]$$

Thus, the rate of deterioration at actual storage conditions is related to that at accelerated ones by:

$$(\mathrm{d}D)_\mathrm{s} = \frac{[K(F)f'(F)\mathrm{d}F]_\mathrm{s}}{[K(F)f'(F)\mathrm{d}F]_\mathrm{a}}(\mathrm{d}D)_\mathrm{a} \qquad [5.35]$$

Let us consider first a situation where the kinetic factor (F) is changing linearly with time both in storage and accelerated test conditions, thus having the following respective expressions:

$$F = F_0 + b_\mathrm{s}t \qquad [5.36]$$

$$F = F_0 + b_\mathrm{a}t \qquad [5.37]$$

where b is a constant. Using the inverse form of these equations, the ratio of their derivative is:

$$\frac{f'_\mathrm{s}(F)}{f'_\mathrm{a}(F)} = \frac{b_\mathrm{a}}{b_\mathrm{s}} \qquad [5.38]$$

Therefore, the ratio between the extent of deterioration in this case is:

$$(D - D_0)_\mathrm{s} = \frac{b_\mathrm{a}}{b_\mathrm{s}} \frac{\left[\int_{F_0}^{F} K(F)\mathrm{d}F\right]_\mathrm{s}}{\left[\int_{F_0}^{F} K(F)\mathrm{d}F\right]_\mathrm{a}}(D - D_0) = \frac{b_\mathrm{a}}{b_\mathrm{s}}(D - D_0)_\mathrm{a} \qquad [5.39]$$

Since both integrals in this equation are only functions of the factor F, they have the same value and therefore cancel out. The extent of deterioration at storage conditions is therefore obtained by accelerating the change in the kinetically active factor with time and multiplying the obtained data by the ratio of the rates of change.

So far, this method is applicable only to cases where the kinetic factor is changing linearly with time. The application of this approach may be extended also to the general situation, which is expressed by equation 5.35. In that case, it is possible to divide the whole range of these equations to n sections, each of which may be approximated by a straight line with a slope, which can be calculated from the derivative of this equation. The basic equation in this case will be:

$$(\Delta D_j)_\mathrm{s} = \frac{f'_\mathrm{s}(F_\mathrm{j})\mathrm{d}F}{f'_\mathrm{a}(F_j)\mathrm{d}F}(\Delta D_j)_\mathrm{a} = \frac{(b_j)_\mathrm{a}}{(b_j)_\mathrm{s}}(\Delta D_j)_\mathrm{a} \qquad [5.40]$$

The extent of deterioration is therefore:

$$(D - D_0)_\mathrm{s} = \sum_{j=1}^{n}(\Delta D_j)_\mathrm{s} = \sum_{j=1}^{n}\frac{f'_\mathrm{s}(F_\mathrm{j})}{f'_\mathrm{a}(F_\mathrm{j})}(\Delta D_j)_\mathrm{a} \qquad [5.41]$$

This 'no model' approach was developed and successfully tested for a moisture-sensitive dry product.[32] The product was packaged in a water vapour

permeable plastic film. Since the water activity in common storage conditions of such a product is higher than that of the packaged foods, the product will continuously absorb moisture through the film. The accelerated shelf-life testing in this case was carried out by packing the same product in a film that has significantly higher water vapour permeability than the original one. In both the actual storage and the accelerated test conditions, the change in moisture content with time is not linear. In fact, the derivative of the relationship between time and moisture content, for the samples that were kept at external constant water activity (a_e) can be expressed:[32]

$$f'(m) = [\mathrm{k}P(a_e - h)(m)]^{-1} \quad [5.42]$$

where h denotes a function of moisture content (m), k is a constant and P is the packaging film permeability to water vapour. If different films are used for storage and for accelerated tests having a permeability of P_s and P_a, respectively, then:

$$\frac{f'_s(\mathrm{m})}{f'_a(\mathrm{m})} = \frac{P_a}{P_s} \quad [5.43]$$

In that case the extent of deterioration is given by:

$$(D - D_0)_s = \frac{P_a}{P_s}(D - D_0)_a \quad [5.44]$$

This is the same solution as the linear case owing to the fact that the external water activity is the same for storage and accelerated tests. Such an accelerated shelf-life testing method is simple to perform, especially since it does not require the evaluation of the kinetic model. However, there is one important problem that should be considered. It has to do with the fact that the higher the rate that one programs the change of the kinetic factor, namely the moisture content in this example, the lower the extent of deterioration. That is simply the result of the fact that the deterioration reaction is given less time to develop. This approach is therefore more effective the better the accuracy and sensitivity of the analytical method used to monitor the deterioration process. In any case, the acceleration ratio in this approach is very dependent on how small a fraction of the total acceptable extent of deterioration may be significantly determined.

5.4.6 Combination of approaches

The application of a combination of methods to accelerated shelf-life testing has the same advantages as using multiple accelerating factors. Such a combination may provide an effective approach in obtaining a high acceleration ratio of the deterioration reaction at a minimal cost of prediction error by staying closer to actual storage conditions. Moreover, this approach provides potentially the largest number of avenues to ASLT. One may use a combination of multiple factors together with initial rate and ‘no model’ approaches. Mizrahi and Karel have used a combination of the ‘no model’ approach together with elevated

temperature for accelerated stability tests of moisture-sensitive products.[33] This combination presents an interesting case of how to link the effect of two methods where one requires evaluation of the kinetic model and the other one does not. The assumption was that the Arrhenius equation is a valid kinetic model for the rate of deterioration at different temperatures when the moisture content is kept constant. The procedure is based on packing the product in films of different permeability and placing them in an environment of the same, or different, water activity and elevated temperatures. The temperature changes not only the rate of reaction but also the moisture gain. Therefore, in order to evaluate the parameters of the Arrhenius equation one has to separate the two processes. The technique is based on the following steps:[33]

- Arbitrarily select a reference moisture gain curve. It may be, for example, the moisture gain of the product at actual storage conditions. For some cases, one may conveniently select a straight line.
- At each temperature, transform the extent of deterioration to the reference moisture gain line by using the procedure outlined in the 'no model' approach, namely by using equation 5.35 or 5.44 for the simple case where the ratio of the moisture gain is constant.
- Use the transformed data, which are now normalized to the same reference line, to obtain the parameters of the Arrhenius equation.
- Use the combination of the reference data and Arrhenius equation to extrapolate the data to actual storage conditions.

5.5 Problems in accelerated shelf-life tests

The problems that are related to ASLT may be classified into three main groups. The first has to do with those cases where no valid kinetic model is believed to exist for any accelerating kinetic factor. No accelerated test procedure is available for such a case. The second kind of problem is encountered when a model does exist but it is very complicated and requires the evaluation of too large a number of parameters. The experimental procedure in such a case may prove very cumbersome to a point where the ASLT procedure may not be practical. The third group of problems relates to the application of valid ASLT methods. These problems are discussed in the following section.

5.5.1 Absence of deterioration index

Food products may be judged on a basis of sensory evaluation that is influenced by the combined effect of a multitude of different reactions. In many cases, a measurable deterioration index, which correlates well with the sensory evaluation, is unavailable. The product may therefore be judged only on the basis of acceptable or unacceptable and not by a continuous scale, thus eliminating the possibility of using the 'initial rate' or the 'no model' approaches to accelerated stability tests. However, the kinetic model approach may be used

in such cases simply by assigning the kinetic constant (K), at constant conditions, a value of:

$$K = 1/t_c \qquad [5.45]$$

where t_c is the critical time that marks the end of the shelf-life of the product. This approach arbitrarily assigns the point of product failure a value of one. As in any other kinetic study, this kinetic constant is evaluated by an experimental procedure that is carried out at different constant storage conditions. The obtained data of the values of the kinetic constant as a function of these conditions provide the basis for evaluating the kinetic model and its parameters. That model can be used for predicting shelf-life by integrating the kinetic equation and finding the time it takes to reach a degree of deterioration of one. This approach is exactly the same as the time–temperature tolerance (TTT) that has been extensively used to predict shelf-life mainly in frozen products.[34, 35]

5.5.2 Time-dependent effects

All available methods for accelerating the product stability tests are based on the ability to predict the progress of the deterioration process based on the order of reaction. This order of reaction can be evaluated by the ASLT procedure. However, the situation becomes much more complicated when other time-dependent effects play a major role in the deterioration process, namely when the deterioration rate is affected by the history of the process.[4, 36, 37] The effect of any specific storage history may be evaluated by carrying out kinetic studies only at actual storage conditions. So far, there is no way to simulate a given storage history by accelerated tests.

5.5.3 Statistical problems

Statistics is an essential part of designing the experimental procedures and analyzing the data both in common kinetic studies as well as in ASLT. It is essential that the proper statistical methods be used in ASLT. One particular subject in that respect, which relates to the validation of kinetic models, should be especially noted. The validity of the model is best established when kinetic data are available for both the actual storage and the accelerated tests conditions. Obviously, the ASLT technique by itself lacks the capability of verifying the validity of the model, especially an empirical one, for actual storage. Moreover, when any model is used its parameters are evaluated only by using the data of the very high rate of reaction. That may produce a large deviation of the extrapolated data to normal conditions. One should therefore use statistical methods that test the sensitivity of the model by a cross-validation method. In principle, these methods are using part of the data to verify the validity of the model. This requires a wider range of accelerated storage conditions. The closer they are to the actual storage conditions the better. Such an approach costs more both in time and in experimental efforts.

5.6 Future trends

The main problem of accelerated shelf-life testing is the availability of general valid kinetic models that have been proved to apply to different types of deterioration reactions. That requires an in-depth understanding of the mechanism of the deterioration reactions not only in homogeneous systems but also in complex heterogeneous ones. Molecular mobility seems to be the key factor in determining the kinetics of these reactions. Of special importance is, therefore, the recent main thrust to understand molecular mobility better, especially in glassy systems. This is a subject of great interest not only in foods but also in polymers, where scientists pursue development of theories for the physics of glassy polymers. Their goal is to understand the mechanism of molecule motion and how it affects the physical, mechanical and transport properties of the system.[38–42] In foods, it is expected that better characterization of the different deterioration reactions in foods as well as the understanding of the molecular mobility of their reactants and products especially in complex glassy systems, may, in the long term, provide attractive options for accelerated shelf-life tests. In the shorter term, significant progress can be made by rationally employing newly available very sensitive analytical methods. Such methods will facilitate the obtaining of reliable data in a much shorter time than in many of the commonly used ones.

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6

Advanced instrumental methods: the use of ^{1}H relaxation NMR to monitor starch retrogradation

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6.1 Introduction: starch retrogradation

Retrogradation, the main molecular scale physical change taking place during staling, refers to the reassociation of the polysaccharides of gelatinised starch. This phenomenon, also referred to as gelation (not to be confused with gelatinisation), involves the reordering of the starch polysaccharides (amylose and amylopectin). A considerable amount of research effort has been invested into the understanding of starch retrogradation because of its impact on important textural and nutritional attributes of some starch-based foods. An obvious example would be some baked goods (bread, pizza, etc.) where loss of freshness on storage, especially in flavour, is paralleled by a hardening of the crumb and a loss of moistness, even when the conditions for moisture loss are strictly controlled, for example by packaging. Another example is the retrogradation of starch during the tempering of the high water content (*circa* $>$ 25% w/w wet basis) half-products of many snack products and breakfast cereals, producing textural changes such as increased hardness, reduced stickiness, etc. The decreased digestibility of retrograded starch can be both a drawback and, in some circumstances, an advantage, for example owing to the health benefits associated with non-digestible fibres. There has, therefore, been significant interest in analysing the process of starch retrogradation to limit or control its impact on final product quality.

As early as 1928, in an excellent review of his work published between 1912 and 1916, Katz[1] laid down the molecular foundations of the research into staling when he demonstrated using X-ray diffraction that starch recrystallisation was responsible for bread staling. Extensive work on the effects of storage temperature, water content, sugars, lipids, salts, etc., and relatively recently anti-staling enzymes have been described in hundreds of scientific and technical

papers.[2–7] A quantum leap in the understanding of the factors controlling the kinetics of starch retrogradation[4–7] was achieved through the application of the 'food polymer science' approach pioneered by Slade and Levine.[8] The cornerstone of this approach is the relationship between the temperature of the glass–rubber transition and the degree of molecular mobility and the subsequent rate of chemical and physical changes.

6.2 Instrumental methods available for the investigation of retrogradation

As mentioned earlier, retrogradation involves the reordering transition of the starch polysaccharides. The instrumental methods used to monitor starch retrogradation could therefore be broadly divided into two groups.

6.2.1 Techniques measuring directly the degree of molecular organisation

- X-ray diffraction where the increase in the degree of crystalline order is measured using often wide angle X-ray diffraction techniques.[1–3, 6–7] In addition to the degree of crystallinity, the technique yields valuable information on the type of crystalline packing obtained (A or B polymorphs).
- Molecular spectroscopy techniques such as solid state NMR, infrared and Raman have been used to study starch retrogradation in bread and related systems.[9] These techniques are sensitive to short range order and many authors[10] have emphasised the difference between degree of molecular order (amount of polysaccharides in the helix conformation) and the degree of crystallinity as measured by wide angle X-ray diffraction (XRD).
- Calorimetric measurements using for example differential scanning calorimetry (DSC) are by far the most widely used tool for the monitoring of starch retrogradation.[4–6, 8] The extent of retrogradation is obtained from the enthalpy of melting of the ordered structure formed on storage.
- Other techniques: the change in the density on crystallisation can be used to monitor the progress of starch recrystallisation. These measurements are often complicated by issues such as the structure of the product (e.g. aeration), water migration, etc. The use of turbidity measurements to monitor starch recrystallisation has also been reported. This, however, is readily feasible in dilute solutions but impractical in real systems such as bread and other baked systems.

6.2.2 Techniques monitoring the impact of reordering on molecular mobility and rheology

The effect of retrogradation on the visco-elastic properties of concentrated starch systems can be monitored by a range of mechanical spectroscopy techniques (e.g. DMTA, DMA), measurement of stress–relaxation response or the very widely documented measurement of firmness.

The reordering (gelation) of the amylose and amylopectin components of starch has a major effect on their solubility and pasting profile. Viscosity measurements, as for example the viscographs performed during a time/temperature profile carried out in a Barbender or a Rapid Visco Analyser, are sensitive to the degree of retrogradation.

In addition to the effect of the degree of molecular order on the NMR spectrum in terms of distribution of chemical shifts, a clear decrease in molecular mobility is recorded as molecules undergo ordering transitions. Furthermore, the involvement of the starch OH groups in intra- and intermolecular hydrogen bonding networks in the ordered structure makes such ^{1}H less likely to be involved in proton exchange with water. Finally, retrogradation does also alter the mobility of water in the system, an aspect that may relate to the dry mouthfeel of the crumb of staled products.

6.3 Advantages of the NMR approach

NMR is the technique of choice for the study of molecular dynamics over a range of time and distance scales. The relationship between the degree of molecular mobility both rotational and translational and the physical and mechanical properties of a food system is now well established. In addition to its unique insight into the changes in dynamics of the various constituents of starch-based systems during the retrogradation process, pulsed NMR relaxometry offers many practical advantages over the techniques usually employed to monitor the reordering of starch components.

The most important advantage is the non-destructive character of NMR. This means that the changes in exactly the same sample are monitored over storage time. Additionally, the changes in the bulk of the sample are monitored and not only at the surface as is the case of X-ray diffraction, reflectance infrared, etc. Furthermore NMR probes relatively large amounts of sample (typically between 1 and several 10s g) compared with DSC (typically between 5 and 50 mg). These aspects are particularly advantageous when the heterogeneity of the sample is inherent to its nature (wholemeal bread, fruitcakes, etc.) or to the preparation method.

The sample can be hermetically sealed in the NMR glass tube limiting the loss/uptake of moisture during measurement. Reliable temperature control, which is often difficult to implement on X-ray diffraction or texture analysis equipment, is routinely available on NMR spectrometers. Finally, study of retrogradation does not require any specific software/hardware modification of existing equipment such as benchtop spectrometers widely available in the food industry (often used for fat and moisture content measurements).

6.4 Principles of NMR

Since the first published work on NMR by the groups of Bloch (Stanford University) and Purcell (Harvard University) in 1946, NMR has become the

single most widely used type of spectroscopy as a consequence of the wealth of information that the technique provides at the molecular level. Although the elucidation of molecular structures constitutes the largest share of the use of the technique, the contribution of NMR to studies of molecular dynamics (relaxometry, MRI, flow studies, etc.) is increasing exponentially particularly as a consequence of developments in hardware, computing power and the ever-increasing number of pulse-sequences available.

The spin motion of the positively charged atomic nuclei generates a nuclear magnetic moment given by:

$$\mu = \gamma \frac{h}{2\pi} \sqrt{I(I+1)}$$

where I is the spin quantum number, γ is the magnetogyric ratio of the specific nucleus (e.g for ^{1}H $\gamma = 26.7519 \times 10^{7}$ rad T^{-1} s^{-1}) and h is Planck's constant (6.6262×10^{-34} J s). A nucleus with $I \neq 0$ can absorb and emit electromagnetic radiation and therefore be studied by NMR spectroscopy. For most elements, at least one isotope possesses this property, the most commonly studied in biological systems are: ^{1}H ($I = \frac{1}{2}$), ^{2}H ($I = 1$), ^{13}C ($I = \frac{1}{2}$), ^{15}N ($I = \frac{1}{2}$), ^{17}O ($I = \frac{5}{2}$), ^{19}F ($I = \frac{1}{2}$), ^{23}Na ($I = \frac{3}{2}$) and ^{31}P ($I = \frac{1}{2}$).

Since this chapter is concerned with ^{1}H NMR, the following discussion will focus on $I = \frac{1}{2}$ nuclei. In the presence of an applied magnetic field $\boldsymbol{B}_0$, the orientations assumed by the nuclear magnetic moment relative to $\boldsymbol{B}_0$ (given by the angle θ between $\boldsymbol{B}_0$ and μ) are quantised as described by:

$$\cos\theta = \frac{m_I}{\sqrt{I(I+1)}}$$

where the quantum number m_I is equal to $-\frac{1}{2}$ or $+\frac{1}{2}$. The motion of μ in the magnetic field $\boldsymbol{B}_0$ can be described using a simplified approach based on classical mechanics theory: the torque exerted on μ by $\boldsymbol{B}_0$ is perpendicular to the [$\boldsymbol{B}_0$, μ] plane resulting in the precession of μ on so-called precessional cones about $\boldsymbol{B}_0$ at a frequency ν_0 (Larmor frequency) given by:

$$\nu_0 = \frac{\gamma}{2\pi} \boldsymbol{B}_0$$

At a macroscopic level, the many nuclei present in the system are distributed randomly between the $m_I = +\frac{1}{2}$ and $m_I = -\frac{1}{2}$ states (Fig. 6.1) with a slight excess in the low energy $m_I = +\frac{1}{2}$ state. For a total nuclei population N, this excess is given by the Boltzmann equation:

$$n_{+1/2} - n_{-1/2} = N \frac{\gamma h \boldsymbol{B}_0}{4\pi kT}$$

($k = 1.38062 \times 10^{-23}$ J K^{-1}) is Boltzmann's constant) and is responsible for the measured macroscopic magnetisation M.

The use of short radio frequency (rf) pulses (in the form of rf magnetic field $\boldsymbol{B}_1$) enables the net magnetisation M to be placed in any chosen direction (Fig.

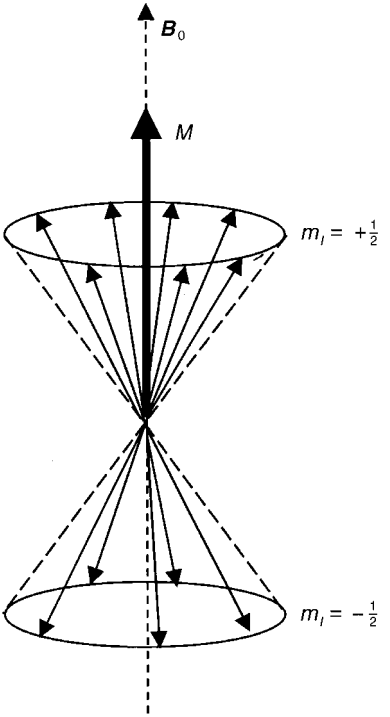

Fig. 6.1 The precessional cones of a spin $\frac{1}{2}$ in the magnetic field $\boldsymbol{B}_0$.

6.2). After the perturbation, the equilibrium is restored through relaxation mechanisms: (i) the spin–spin relaxation, also called transversal relaxation in the *xy* plane and characterised by the relaxation time T_2 and (ii) the spin–lattice relaxation, also referred to as longitudinal relaxation along the *z* direction and described by the relaxation time T_1.

Both T_1 and T_2 are influenced by the mobility of the molecules containing the resonant nuclei responsible for the recorded NMR signal. This is summarised in Fig. 6.3 describing the behaviour of the relaxation times in the case of isotropic motion.

Several techniques can be utilised to measure the spin relaxation times. The T_2 can be measured by fitting the free induction decay (FID) acquired directly after a 90° pulse to the following equation:

$$M_{xy}(t) = \sum_i M_{0i} \exp\left[-\left(\frac{t}{T_{2i}}\right)^{n_i}\right]$$

where M_{0i} and T_{2i} are the magnitude and the relaxation time of component *i* of the signal and n_i defines the lineshape (exponential: $n = 1$; gaussian: $n = 2$) (Fig. 6.4a).

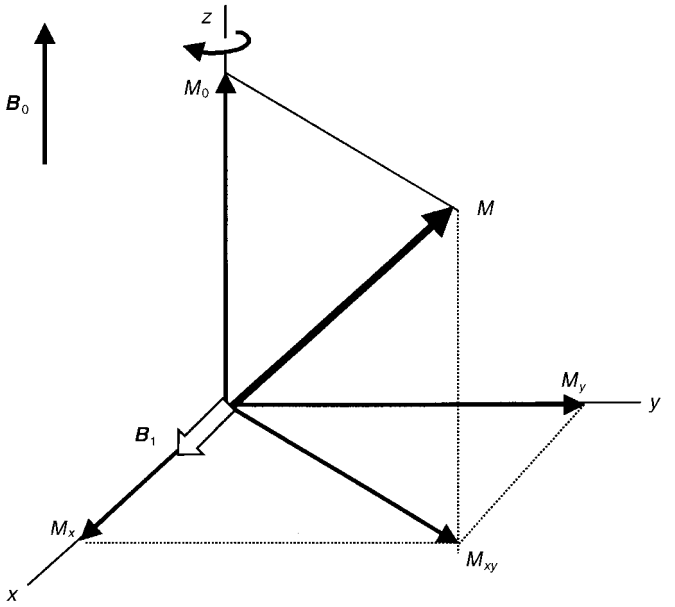

Fig. 6.2 The magnetisation *M* as described in the rotating frame.

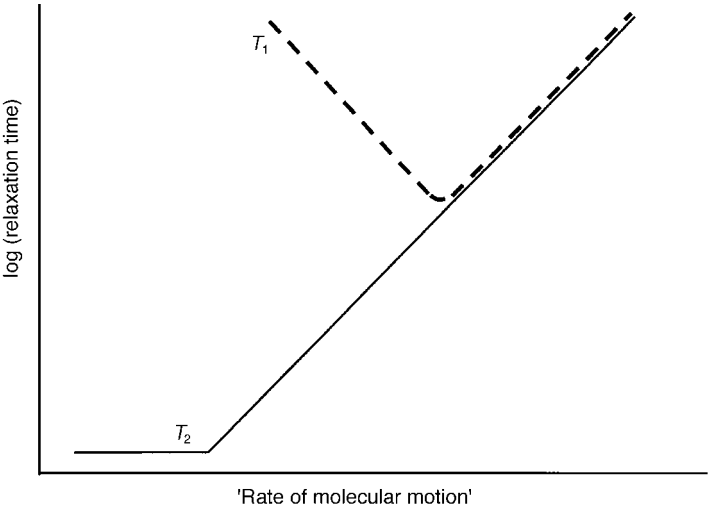

Fig. 6.3 Schematic representation of the dependence of the spin relaxation times on molecular mobility.

For systems with high molecular mobility (long T_2), in addition to the transverse relaxation process, the loss of magnetisation in the *xy* plane during the FID experiment is also due to the inhomogeneity of the magnetic field across the sample. This leads to a wider distribution of Larmor frequencies and therefore to a faster dephasing of the magnetisation yielding an FID relaxation time (also

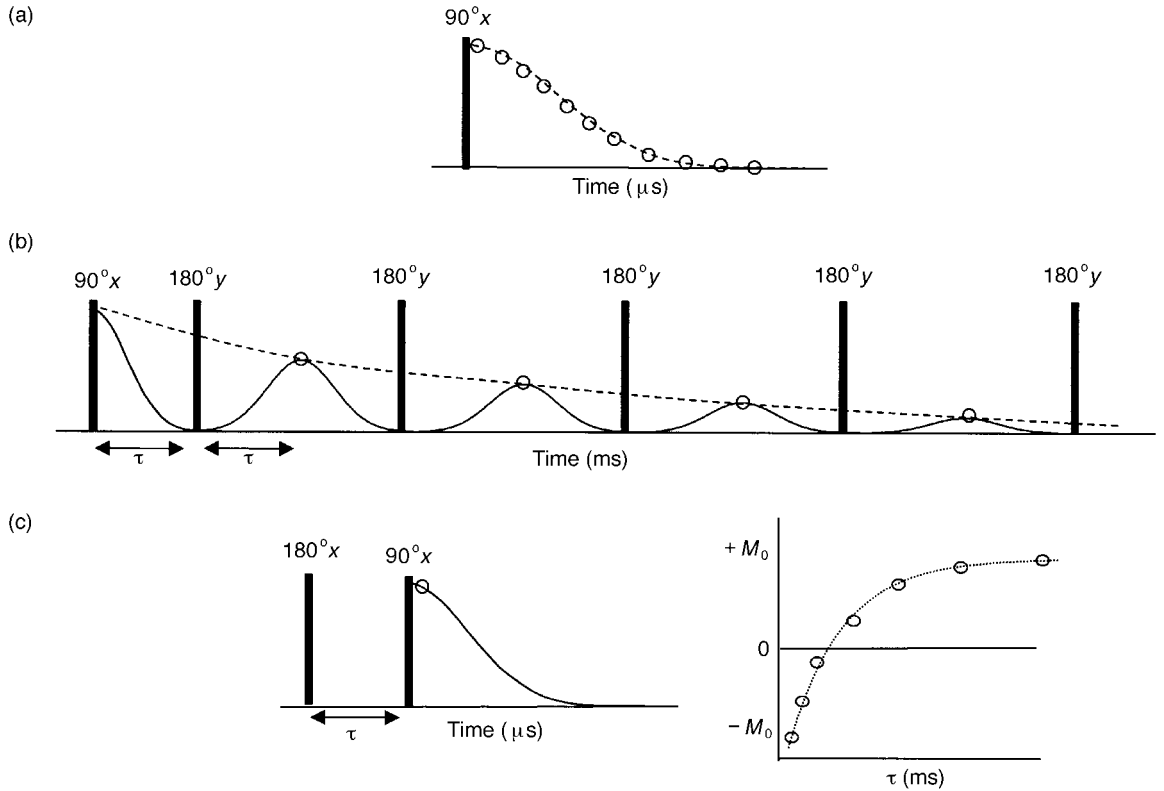

Fig. 6.4 Schematic representation of various NMR experiments. The diagrams depict for various RF pulses the digitised data points (o) and the fitted lines (– – –), allowing the extraction of the relaxation parameters for: (a) the FID, (b) the CPMG and (c) the inversion-recovery experiments.

referred to as T_2^*) much shorter than the 'true' T_2. This problem is often overcome using the spin–echo pulse sequence, also called CPMG after Carr, Purcell, Meiboom and Gill, where a train of refocusing 180 º pulses is employed to rephase the magnetisation vectors as shown in Fig. 6.4b. The maxima of the echoes are acquired and fitted to one or several exponentials to obtain T_2 values.

The measurement of T_1 is often performed using the inversion–recovery pulse sequence since the relaxation following a 180 º pulse occurs by the spin–lattice process. The signal can be monitored at any point in time by 'flipping' the magnetisation into the *xy* plane with a 90 º pulse where the FID can be recorded and analysed as previously described (Fig. 6.4c). The pulse sequence is typically 180 º–τ–90 º followed by the acquisition of all or selected points of the FID for several values of the spacing τ. The recorded amplitudes are modelled using the following equation to obtain the T_1 values:

$$M_{xy}(t) = \sum_i M_{0i}\left[1 - 2\exp\left(-\frac{\tau}{T_{1i}}\right)\right].$$

6.5 Case study: extruded starch

6.5.1 Materials and methods

Waxy maize starch (WMS) *circa* 98% amylopectin, was extruded at 120 ºC into non-expanded ribbons through the slit die (1×30 mm) of a twin screw extruder. Samples were extruded with different amounts of water, sealed in airtight bags to avoid moisture loss and stored in controlled temperature environments.

NMR experiments were performed using a 20 MHz Bruker benchtop Minispec PC120 at 40±0.1 ºC. Typically four scans were accumulated and the ^{1}H spin–spin relaxation parameters were obtained from the FID recorded directly after a 90 ºrf pulse, or from the CPMG decay with a τ spacing of 262 μs between the 90 º and the 180 º pulses. The FID was deconvoluted into two gaussian components while the spin–echo decay was described by a single exponential. Spin–lattice relaxation measurements were performed using the inversion–recovery pulse sequence; the T_1 values of the signal recorded at 11 and 70 μs on the FID were obtained by fitting the data (20 τ values) to a single exponential.

6.5.2 Results and discussion

The NMR signals acquired with all three types of ^{1}H relaxation experiments: FID, CPMG and inversion–recovery showed a strong dependence on the duration of storage. The implication is that the NMR properties were affected by the extent of reordering of the starch occurring during the retrogradation process. A typical example of the effect of storage on the FID and CPMG decays is shown in Figs 6.5 and 6.6 for a sample with a moisture content of 60% (w/w dry basis). As the retrogradation progressed, the rigid, 'solid-like' component of the FID showed shorter T_2 relaxation times and provided a larger contribution to the total NMR signal.

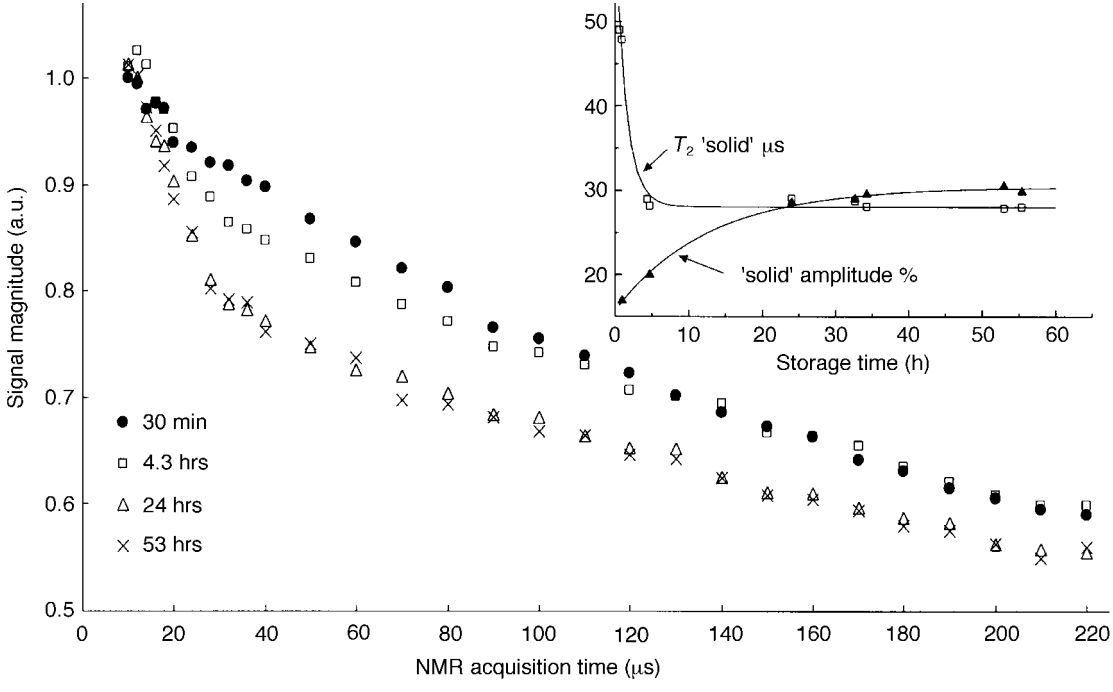

Fig. 6.5 Effect of amylopectin retrogradation on the FID of a 100:60 amylopectin–water extrudate. Results for different storage times are shown. The insert depicts the plots of the T_2 and the amplitude (fraction of total signal) of the rigid component versus storage time. The lines were obtained using Avrami-type kinetics.

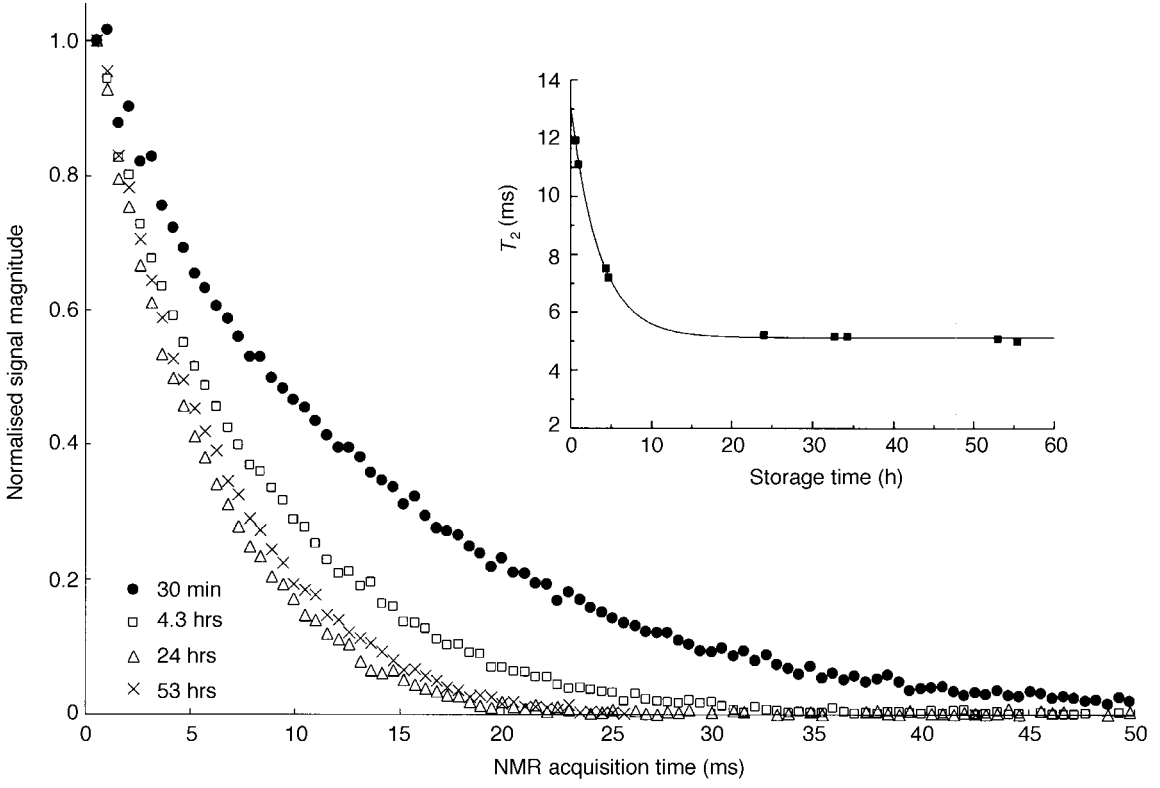

Fig. 6.6 The CPMG results for the same sample as in Fig. 6.5.

While considerable changes were observed between 0.5 and 4.3 h of storage, no significant difference was noticed on comparing the NMR relaxation results recorded at 24 and 53 h. The effect of the retrogradation upon the spin–echo decays recorded using the CPMG pulse sequence showed a similar shortening of the relaxation times. This suggests that at the water content (amylopectin–water 100:60) and the storage temperature (40 °C) of the experiment, the effect of the retrogradation process on the relaxation parameters reached equilibrium after approximately 24 h of storage.

These results are indicative of a decrease of the molecular mobility of the polymer as it goes through the reordering transition, leading to the conclusion that the gelatinised starch component is more mobile than the ordered crystalline fraction. The total amplitude of the NMR signal did not depend on the storage time, indicating that the water loss from the sealed NMR tube was insignificant throughout the whole duration of storage.

The spin–spin relaxation times derived from the CPMG decay representing mainly the water component in the system decreased considerably as the retrogradation of the starch evolved. The direct interpretation of this observation is a lower mobility of the water in the retrograded system compared with the freshly gelatinised gel as the 3D crystalline structure developed. This observation may be partly explained by the immobilisation of water molecules in the structure of the crystal cell unit. In addition, the T_2 of the water will be affected by the relaxation of the polymer owing to the enhanced proton exchange between water and the hydroxyl groups on the glucosyl units of mainly the amorphous component of amylopectin. A lower mobility of the polymer would lead to lower observed T_2 values for the water protons.

The rates at which these changes in the relaxation parameters describing the molecular dynamics properties of the different components were observed, were comparable and depended strongly on the water content present in the sample. The spin–echo relaxation times ($T_{2\,\mathrm{CPMG}}$) are usually more reliable than the parameters of the solid FID component as this fast-decaying component of the FID is normally difficult to measure reliably. Such difficulties are due to technical limitations such as the long dead time between the rf pulse excitation and the data acquisition ($\approx 10\,\mu$s) which leads to a considerable part of the signal not being recorded, the slow digitisation rate (this has been improved dramatically in modern hardware), etc.

While the retrogradation of amylopectin led to a decrease of the T_2 values of both the solid component of the FID yielding from the biopolymer and the CPMG decay describing the behaviour of water, a different behaviour was observed for the spin–lattice relaxation times of the rigid and mobile components of the NMR signal (Fig. 6.7). The two components had comparable T_1 values. This is in agreement with the cross-relaxation model by which the efficient relaxation of the spins of the mobile (water) component through those of the rigid component (polymer) leads to the equalisation of the T_1 of these two components. The increase of T_1 on storage is understood in the context of the non-monotonic change of T_1 with the rate of molecular motion (Fig. 6.3).

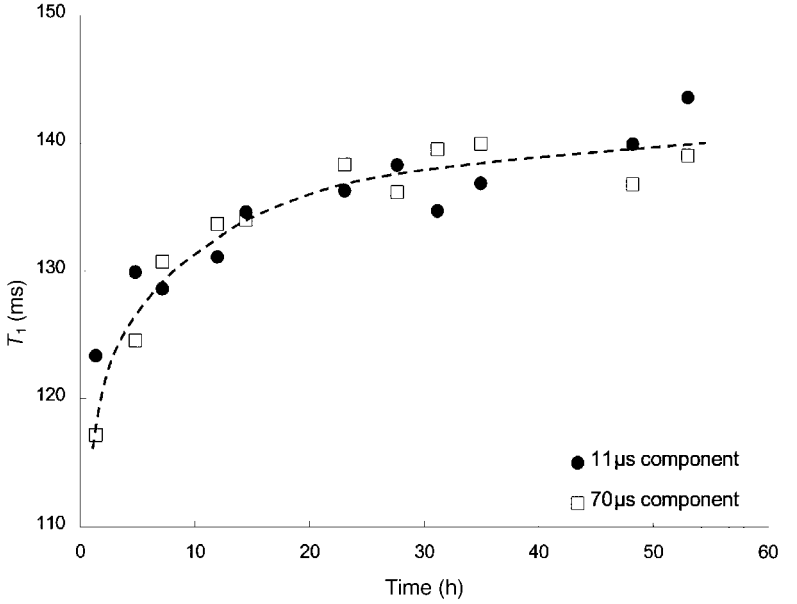

Fig. 6.7 The effect of retrogradation on T_1 of the 100:60 amylopectin–water extrudate.

Finally, a good correlation was found between the retrogradation kinetics derived from NMR, X-ray diffraction and stress–relaxation on similar samples (extruded 100:35 amylopectin–water) stored in similar conditions (approximately 20 °C) (Fig. 6.8). It is tempting to suggest that the effect is first detected by NMR followed by XRD and then by stress–relaxation. This is not unreasonable considering that NMR is sensitive to short distance scale while XRD only senses long range (several tens of nm) molecular organisation, which should in turn affect the mechanical properties. However, it is now clear[7] that small variations in water content and/or storage temperature could have a large effect on the kinetics of retrogradation particularly since in the water content/storage temperature conditions of this experiment the sample is approximately 20 K above its glass transition temperature (T_g). In this range the retrogradation is limited by the degree of molecular mobility of the amylopectin chains.

6.6 Future trends

The application of continuous distribution analysis of relaxation times based on the CONTIN algorithm (available from some NMR equipment manufacturers) as described for example in a series of excellent publications by Brian Hills and co-workers is likely to extend the amount of information accessible by NMR relaxation on the range of molecular mobilities in model and real food systems.

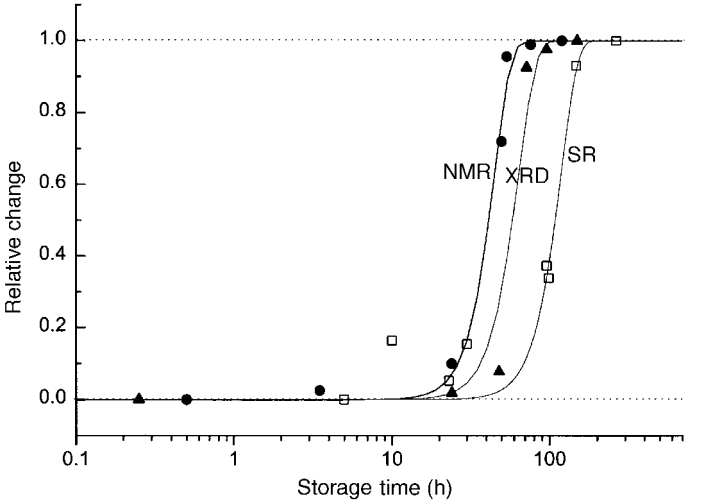

Fig. 6.8 The effect of retrogradation on NMR spin–spin relaxation rates $1/T_{2\,\mathrm{CPMG}}$ (molecular mobility), the XRD crystallinity index (molecular crystalline packing) and the initial stress–relaxation modulus (SR) (mechanical property) of a 100:35 amylopectin–water extrudate stored at approximately 20 °C. The solid lines illustrate the Avrami fit to the experimental results.

On the retrogradation front, progress is anticipated in three main areas: (i) the fundamental understanding of the phenomenon in the framework of the food material science approach, (ii) the use of various additives, particularly the so-called anti-staling enzymes and (iii) the use of chemical and genetic modifications to develop starches with well-defined composition and fine structure and with controlled susceptibility to retrogradation.

6.7 Sources of further information and advice

On retrogradation:

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9. WILSON, R H, GOODFELLOW, B J, BELTON, P S, OSBORNE, B G, OLIVER, G and RUSSELL, P L 1991. Comparison of Fourier-transform mid infrared-spectroscopy and near-infrared reflectance spectroscopy with differential scanning calorimetry for the study of the staling of bread. *J. Science of Food and Agriculture*, **54**: 471–83.
10. COOKE, D and GIDLEY, M J 1992. Loss of crystalline and molecular order during starch gelatinization – origin of the enthalpic transition. *Carbohydrate Research*, **227**: 103–12.

Part 2:

Case studies

7

Predicting packaging characteristics to improve shelf-life

A. Emblem, The Institute of Packaging

7.1 Introduction

7.1.1 The role of packaging

The use of packaging to extend the shelf-life of foods is not a new phenomenon. Our Stone Age ancestors' discovery of farming, raising animals and growing cereals brought with it the need to store food and keep it in edible condition until required. Wooden tubs and clay pots, grasses woven into baskets and, eventually, metal and glass containers were used to meet this need.

This role of packaging, what we call the preservation role, is a fundamental requirement of food packaging. With very few exceptions, the methods by which food is treated to maximise or extend its shelf-life rely also on packaging for their success. Without developments in packaging, our food harvesting, processing and distribution systems would not have developed to their present levels, we would not have easy access to the wide range of foods on offer today, and food wastage due to spoilage, damage and loss would be high.

Packaging, then, is an integral part of the food industry. As well as the preservation function, packaging has several other important roles to play in delivering safe, wholesome and attractive foods to the market, and to do this economically and with minimal environmental impact. These roles must be considered at the earliest stages of product development, and not left until the product is ready to launch, when it may be too late to obtain the optimum product/pack combination. It is worth reviewing these other roles now and bearing them in mind during the rest of this chapter.

Packaging must contain the product, which means preventing leakage, or keeping together a multi-part product, e.g. a pack of soup sachets, or a number of different items brought together as one meal in a pack. Leakage is not only

undesirable in that it almost certainly renders the product inedible but it can also cause irrevocable and costly damage to neighbouring packs and products. Gradual leakage due to poor pack sealing can potentially cause more problems than catastrophic failure, especially if the problem goes unnoticed at the point of sale. By this time even if the product remains wholesome and fit for consumption, the weight may be below the legally allowed tolerance.

During its journey from source to final consumer, the product will be exposed to a number of different hazards, and packaging must provide physical protection against these hazards. The complexity and cost of modern food processing has meant a greater tendency to fewer and larger processing and packaging operations, bringing with it greater journey distances and multiple handling for the final packed product.

Damage can result from mechanical and environmental hazards throughout this supply chain. Products can be jolted, subjected to vibration on vehicles and compressive loads during stacking in warehouses, all of which can potentially damage both the product and the pack. Damage effects include breakage of fragile products such as biscuits and weakening of pack closure systems such as heat seals and screw caps, owing to external forces, thereby destroying the preservation function. Environmental hazards include exposure to light and to high and low temperatures and humidity levels, dust and dirt, and insect and rodent infestation. The human hazard must not be forgotten here. The threat of tampering, whether malicious or just curious, has led to manufacturers having to consider the tamper evidence of their packed products and, where necessary, incorporating additional devices to deter tampering.

Another important role of packaging is to provide a convenient way of handling the product, all the way from the packaging line to final consumption and pack disposal. Good packaging line performance consistently achieves correctly formed and sealed packs, which is critical for maintaining the preservation function with which this chapter is concerned. Poorly sealed packs mean vastly reduced shelf-life. Good performance in what are usually very busy storage and handling environments means that packaging must provide ease of movement of the goods, with maximum efficiency and minimum effort.

Convenience in product use is one of the major benefits of packaging, and innovative solutions can provide major points of difference from one product to another. Convenience features abound in modern food packaging. The bottle of oil which allows for careful dispensing of the product is superior to that which permits the product to pour out uncontrollably. Ready-prepared meals which allow us to take the pack directly from the freezer and put it in the microwave or conventional oven save time and reduce the washing up. Most importantly for today's busy consumers, ease of opening and, if relevant, reclosing are essential considerations. If the contents of the pack cannot be removed without causing frustration to the person opening it, he/she will at the least register a silent protest by purchasing a different pack type in the future. If attempts to open the pack result in damage to the consumer, the protest may not be silent and may be a very costly and image-damaging lawsuit against the brand owner.

Packaging is the means by which manufacturers tell their customers about the product. Even if supplementary information is available at the point of sale, once the product is purchased packaging is usually the only way the customer has of finding out important information such as nutritional details, storage and usage instructions and ingredients. Also, in the competitive market of food retailing, the product that fails to draw the shopper's attention will remain on the shelf. Successful companies recognise the importance of using packaging to sell their products by means of distinctive features such as colour, shape, size and graphics to attract the purchaser.

Thus the role of packaging can be summarised as a means of containing, protecting and preserving, and providing a convenient way of handling the product, as well as providing information and selling the product. Finally, it must be emphasised that these requirements must be met at an acceptable economical level and within the bounds of environmental acceptability. The cost of packaging must be in line with what the product and the market demand and no amount of attractive packaging can sustain the sales of substandard products. However, an otherwise good quality product may fail in the market-place owing to inadequate packaging chosen solely on the basis of its attractive unit price.

With regard to environmental acceptability, it is worth giving consideration to carrying out a life cycle analysis (LCA) on any new product or packaging development, to compare with existing products. LCA is a technique that quantifies the environmental burden of a total product and pack, in terms of consumption of raw materials and energy, and the emissions to air, water and the solid waste stream during its production, distribution, storage and use. Also, products sold within the European Community are now subject to the requirements of the Packaging Waste Directive, which affects packaging selection; readers are advised to consult the references at the end of this chapter for further information on this subject.[1]

7.1.2 Packaging usage

We have come a long way since the packaging of our ancient ancestors and the World Packaging Organisation gives an estimated annual usage for 1995 of 1350 million tonnes of packaging per annum at a value of US$475 billion.[2] However, packaging value is not evenly spread worldwide but is roughly related to living standards as Table 7.1 shows.

Approximately 70% of all retail packaging is used for food and drink products. Changes in the nature, production, use and retailing of these products in the developed countries have directly affected packaging usage levels to the extent shown in Table 7.1. Lifestyle changes such as increased income levels, smaller family units, ownership of domestic appliances such as freezers and microwave ovens and an interest in greater variety have all stimulated demand for prepacked foods which can be safely and conveniently stored until required.

Changes in manufacturing and retailing operations have also influenced how goods are packed. The globalisation of manufacturing means more transit

Table 7.1 Packaging value per capita

Country	US$ per capita
Japan	602
USA	348
Western Europe	270
Eastern Europe	70
Developing countries	38

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packaging to protect goods in the supply chain. The modern supermarket demands a vast array of goods which are easy to display and which can be handled quickly through the checkout. Finally, public concern about health and hygiene has highlighted the use of packaging to assure the consumer of the cleanliness and authenticity of the product.

7.2 The role of packaging in extending shelf-life

In this section we will consider product deterioration due to abiotic spoilage and how packaging can be used to reduce this deterioration. In most cases it will be the packaging alone that is providing the preservation function. In the next section (7.3) we will concentrate on how foods are treated to reduce deterioration due to biotic (biological) spoilage, e.g. by sterilisation, and where the packaging then plays an integrated role with this treatment (and possibly storage conditions) to extend the product's shelf-life.

7.2.1 Abiotic spoilage of foods

Abiotic spoilage is defined as physical or chemical changes brought about by factors such as temperature, moisture, oxygen (air), light and volatile matter affecting odour and flavour.

The extent to which packaging can be successfully used to reduce spoilage depends on two considerations:

1. Understanding the properties of the product, i.e. just how sensitive it is to changes in these factors.
2. Knowing the conditions to which the packed product is likely to be exposed in the supply chain (hence the emphasis on the hazards in this area referred to in section 7.1.1).

Only when all of this information is available can packaging with an appropriate barrier to the relevant factors(s) be designed and selected. We will now look at these factors in turn.

7.2.2 Moisture

For the purposes of our discussion here we can say that all foods contain a certain percentage of water, even those that appear to be dry, such as potato crisps, instant coffee or granulated sugar. Moisture is by far the most likely factor to bring about undesirable changes in such products; when exposed to a moist atmosphere they will absorb moisture and when placed in a dry atmosphere they will give up moisture.

Two important factors must be known about a product with respect to its reaction to the ambient moisture level. The first is its equilibrium relative humidity (ERH) which is the humidity at which the moisture content of the product is in equilibrium with the relative humidity of the surroundings. At this level the product will neither absorb nor lose moisture. The second factor is the moisture range within which the product remains palatable and wholesome and this is largely determined by organoleptic and microbiological testing.

Typical moisture levels and ERH ranges for some common foods are shown in Table 7.2, which gives guidance in determining the moisture barrier characteristics of a suitable packaging material. Products such as instant coffee, with a low moisture content and an ERH well below the humidity level likely to be encountered in temperate climates such as ours, demand the use of packaging with a high moisture barrier to avoid caking. Breakfast cereals are less critical as far as moisture gain is concerned and thus demand lower barrier levels. The closer the ERH of the product comes to the ambient humidity level (40–65% in the UK), the lower the moisture barrier needed. Nuts and dried fruit listed in Table 7.2 may be stored with a minimal moisture barrier (although the oil content of nuts means they will require an oxygen barrier and the packaging solution to provide this may also provide a good moisture barrier anyway). Note also that some dried fruits are preserved not only by their natural sugar but also by adding sulphur dioxide to prevent discoloration, hence the packaging must provide a barrier to the loss of this gas.[3] Crystalline products such as white granulated sugar and salt require a moisture barrier only if the expected ambient humidity is above the ERH levels shown, which would be the case in tropical climates. The important point about these two products is that they show a step rather than a gradual change when exposed to high moisture levels, with a non-reversible change in the crystalline state. Repeated exposure to moist, followed

Table 7.2 ERH ranges for some common foods

Product	Typical moisture (%)	ERH (%)
Potato crisps, instant coffee	< 3	10–20
Breakfast cereals	3–7	20–30
Nuts, dried fruit	7–20	30–60
Salt		75
Sugar		85

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by dry, conditions will eventually result in caking if they are stored without any moisture barrier.

The examples given so far are concerned with moisture gain. Moisture loss can also be critical, e.g. for fresh produce which respires, generating moisture, and for some baked goods, especially if packed when still warm. If moisture is allowed to remain in the pack around these products, ideal conditions for microbiological growth soon develop and spoilage will occur. Thus there is a need for moisture permeable packaging materials as well as those that provide a moisture barrier. As a general rule, the moisture level at which microbiological growth becomes a potential problem is around 65%.

7.2.3 Oxygen

The main effect of oxygen gain on food products is to oxidise any fatty constituents, causing rancidity which can be readily detected by taste and smell even at fairly low levels. Whenever a product contains fat we can assume that an oxygen barrier is required, although the nature and content of the fat, and whether or not any antioxidants are used will determine the degree of barrier. Shortbread biscuits are a typical example of a product needing a good oxygen barrier.

For most foods the packaging technologist is concerned with keeping oxygen out of the pack. One exception is fresh meat, especially beef, where oxygen is needed to develop and maintain the bright red colour associated with freshness, certainly in the minds of the UK consumer. The colour is due to the presence of oxymyglobin, which develops whenever the meat is exposed to air. The deliberate introduction of oxygen into the pack, and the use of a good seal to ensure minimal oxygen loss, is a common way of achieving and maintaining this bright red colour (see section 7.3.5 on modified-atmosphere packaging).

7.2.4 Light

While food products may be exposed to daylight at various points in the supply chain, good working practices and the correct transit packaging should largely prevent this. However, we have to consider the effects of exposure to artificial light when products are on display on the retail shelf and, to a lesser extent, when the consumer takes them home. These effects include colour fade, or product degradation as happens in vitamin C and beer (hence beers packed in amber glass bottles will generally have a longer shelf-life than those packed in clear glass). It is usually the high-energy UV part of the light spectrum with which we are concerned, i.e. around 290–400 nm.

For fatty foods the effect of exposure to light becomes more complex and critical, since light accelerates the oxidation process and therefore the rate at which rancidity develops. For this reason potato crisps enjoy a considerable increase in shelf-life if packaging with a light barrier, e.g. a metallised film, is used.

7.2.5 Odours

The requirement to exclude undesirable odours from the pack is especially important for sugar and chocolate products, which can act as 'blotters' and soak up volatile matter, affecting both odour and taste. While packaging solutions will be examined in section 7.4, it is worth noting that keeping susceptible products odour- and taint-free is also very much down to good housekeeping and good stock control, ensuring that highly perfumed products, e.g. soaps and disinfectants, are not stored directly adjacent to them. Control of cleanliness of the vehicles used to transport foods, with measures in place to prevent contamination from previous usage, is also important e.g. a vehicle used to move a load of liquid chemical cleaner should not immediately be used to move a load of sugar, without thorough inspection and cleaning. The same controls must also apply to the use of pallets, especially wooden pallets which can readily absorb odours and liquid contaminants, often without any obvious visual effects. Inspection and cleaning regimes are essential for all pallet usage, especially when using multi-trip pallets from an external pallet pool.

Care must be taken that the packaging itself does not become the cause of an odour or taint problem. Inks and adhesives used in printing and laminating processes for food packaging can potentially result in unacceptable odour levels and product contamination. They must be formulated such that the pigments, resins, solvents, oils and any additives used are all of low odour and specified for food use. Drying conditions are critical and agreement on a regular test programme with the packaging supplier to evaluate taint and odour is recommended.

Preventing loss of volatile substances, i.e. keeping desirable odours and flavours inside the pack is an important requirement for products such as fruit flavoured teas, herbs and spices. Unless wrapped in a suitable odour barrier pack, these products will rapidly lose their distinctive taste and effectiveness, as well as contaminate adjacent products.

7.2.6 Temperature

The factors mentioned so far are temperature dependent to the extent that they become more effective as the ambient temperature increases. The mechanism of permeability of gases and vapours through a packaging material is largely that of diffusion until a steady state is reached, with the rate of permeation being proportional to the area and inversely proportional to the thickness of the material. As the temperature increases, the molecules of the gas or vapour move faster and the resulting increase in permeation is exponential. This is generally held to be true where there is no reaction between the gas or vapour and the packaging material.[4]

This highlights the importance of knowing the range of temperatures to which food products are likely to be exposed in the supply chain, before one can begin to evaluate the range of packaging options for an appropriate solution. Even for existing products, if any change to the temperature hazard is

anticipated, such as a decision to extend beyond the current domestic market into international markets, the packaging must be re-evaluated for its barrier performance under the new conditions anticipated.

Measurement and control of temperature throughout the supply chain is also important. This applies not just to the obvious categories of frozen and refrigerated products, but also to foods that are distributed under ambient conditions. The temperature inside a vehicle parked outside in the sun on a hot day in midsummer can rise far beyond that at which the packaging material barrier has been evaluated. Such exposure must therefore be kept to a minimum. Monitoring of conditions inside warehouses and retail stores and some means of controlling such conditions is also recommended.

Allowing products to be exposed to excessively low temperatures may be detrimental, both to the product and the pack. Product effects include emulsion breakdown on freezing and the formation of large ice crystals in soft fruits, destroying their texture. Some plastic packaging materials may become brittle and crack at low temperatures, thus destroying the preservation function of the pack.

7.3 Integrating packaging and other methods of extending shelf-life

In this section we will look at the role packaging plays in maintaining food products in a safe and wholesome condition, in combination with specific treatments carried out to reduce or eliminate biotic spoilage. The treatments considered here will be heating, cooling (including freezing) and packaging under modified atmospheres. While not exhaustive, it is felt that these treatments demand the most critical packaging requirements. However, as has already been said, all the methods by which food is treated to maximise or extend its shelf-life rely on packaging for their success and this applies equally to other methods such as drying, using chemical preservatives (including pickling) and irradiation.

We will review each treatment and then summarise the packaging requirements relevant to each type. Inherent in all of these requirements is that the packaging materials must also run efficiently on the designated packaging equipment, much of which will be fully automated and running at high speeds.

7.3.1 Biotic spoilage

This is defined as deterioration due to the effects of biological agents such as enzymes within the food itself, or microorganisms such as bacteria, moulds and yeasts which are naturally present on many foods.

Biotic spoilage manifests itself in food products variously as unsightly visible effects (green mould in bread or cheese), off-tastes or smells (sour milk, rotten

fish) and toxicity causing anything from mild sickness to death. Several treatments exist by which this type of deterioration can be reduced or eliminated and we will review each of the common treatments and look at how packaging is used to maintain and support them, to ensure the product remains acceptable to the end of its shelf-life.

However, before that it is worth mentioning that it is not only the microorganisms inherent in food with which we need to be concerned here. Microorganisms can also be introduced into food products by external factors such as poor hygiene in preparation, handling, packaging and storage areas, and these operations should be managed accordingly. Packaging materials used for foods should be manufactured, delivered and stored in premises where the importance of personal hygiene and general environmental cleanliness is clearly understood and integrated into company management and quality systems. Controls should be in place to prevent the introduction of unacceptable microbiological contaminants into the food-handling area via primary packaging materials and the outer wrappings and pallets used to protect and deliver them from the packaging supplier to the food producer or packer.

7.3.2 Controlling biotic spoilage

Treatments to control biotic spoilage are generally based on controlling one or more of the conditions required to support the growth of microorganisms, thus reducing or terminating their propagation in the food product. For the purposes of this section we can say that the conditions supporting microbiological growth and which can be controlled are temperature, oxygen, humidity and pH. As stated, we will be reviewing methods that change temperature (both heating and cooling) and oxygen level.

With this in mind, it is useful to list the preferred propagation conditions of microorganisms within the following broad classification:

- Mesophyles prefer ambient conditions, 20–45 ºC.
- Psychrophyles prefer cool conditions, 0–20 ºC.
- Thermophyles prefer warm conditions, 40–65 ºC.
- Aerobic organisms need oxygen to propagate.
- Anaerobic organisms propagate in the absence of oxygen.
- Additionally, very few organisms propagate below a humidity level of 65%.

7.3.3 Heat treatment as a means of extending shelf-life

While different types of microorganisms have their ideal propagation temperature, it is generally true to say that few survive beyond about 65 ºC. This fact forms the basis of all methods of extending shelf-life by the use of heat treatment. The amount of heat required depends on the characteristics of the most harmful microorganism present, the nature of the food in terms of how solid or liquid it is, the pH of the food, the shape of the pack and the shelf-life required.

In traditional food processing systems such as canning (dating back to the early nineteenth century) the food is filled into a container, which is then hermetically sealed and the food sterilised by autoclaving at 121 ºC or above to ensure that all microorganisms, especially *Clostridium botulinum*, are killed. The critical factor here is the time it takes for the coldest part of the product (usually that at the container's geometric centre) to reach the required temperature. The more liquid the food, the higher the thermal conductivity and thus the faster heat will be conducted to this point. Similarly, the higher the thermal conductivity of the container material, the shorter the autoclaving time required. Size and shape of the container will also have an effect, as exploited in the use of retort pouches, which are sachets formed from reels of laminate. Their flat shape means that processing time is reduced compared with conventional cylindrical cans, with a corresponding improvement in taste and texture of the food. For these reasons, we are now seeing an increase in the use of this packing format, beyond long-life rations for the armed forces, which until recently has been their main application.

Autoclaved foods normally have a long shelf-life, measured in months or years rather than days. This is an important feature as far as stock management is concerned and it is unlikely, although not impossible, that such goods will have to be written off in the supply chain because of being out of shelf-life. The negative aspect of this long-life feature is that the heating process changes the consistency, taste and/or colour of the product, compared with the fresh equivalent. For products such as milk, pasteurisation by heating to 72 ºC for 15 seconds is sufficient to kill off the most harmful bacteria with no significant change to the product's taste. However, the shelf-life is measured only in days and refrigerated transport and storage are essential to slow the growth of the remaining microorganisms to an acceptable level. Ultra high temperature (UHT) processing at around 135 ºC for 1 second will extend the shelf-life to several months, but will significantly change the taste and reduce the nutritional value of the milk.

Highly acidic products such as fruit juices require only short processing times and hence canned varieties show less marked differences from the fresh product. Such products also lend themselves to aseptic packaging where, in contrast to canning, the product and the packaging are sterilised separately and then brought together under clean conditions. This method is also used in the food industry for soups and sauces, as well as in the pharmaceutical industry for medicines.

Packaging requirements for heat-treated foods can be summarised as follows:

- The packaging has to withstand the rigours of the heat treatment (or in the case of aseptic packs, the method of sterilisation, which usually entails passing the material through a bath of hydrogen peroxide). For cans, glass jars and retort pouches this means the physical handling and pressure changes during the heating and subsequent cooling processes.
- Most importantly, the packaging is the only means available by which the product can be maintained in the state of sterility brought about by the process. This means the packaging must provide a total barrier to the ingress

of any agent likely to cause spoilage, both through the walls of the pack and through the pack seals. Failure to meet this requirement for a hermetic pack will invariably result in product spoilage, possibly with very serious consequences. The walls of metal cans and glass jars are totally impermeable, but the importance of checking the integrity of the pack seals throughout the process must be emphasised.
- Secondary packaging must adequately protect the primary packs from mechanical damage which could result in loss of pack integrity.

7.3.4 Reducing temperature to extend shelf-life

Reducing the temperature of a food product slows down both enzyme activity and the growth of microorganisms. Reducing to temperatures of $-18\,^{o}C$ and below virtually stops such activities and even kills some microorganisms, although not enough for the process to be commercially useful for this purpose.

When considering how packaging integrates with either chilling or freezing as a means of extending shelf-life, it is important to understand that in this case the main method of preservation is by maintaining the correct temperature. The packaging provides more of a secondary means of preserving the product. No matter how good the barrier or how effective the pack seals, if the temperature of the product is not maintained at the required level throughout all stages in the supply chain, spoilage will occur. Therefore, unlike the packaging of heat-treated foods discussed in section 7.3.3, for chilled and frozen foods we are not usually concerned with specifying and maintaining a hermetic pack. This does not mean that packaging requirements can be overlooked and they can be summarised as follows:

- The packaging must withstand the temperature changes brought about by the chilling/freezing processes by remaining dimensionally and physically stable. This applies equally to any printing inks and adhesives used.
- The packaging must provide a good moisture barrier, especially to protect frozen foods from excessive drying out, which manifests itself as 'freezer burn' and adversely affects both texture and taste. Also, limiting the amount of free air space in the pack by using materials that conform as closely as possible to the product shape will limit the amount of moisture trapped inside at the point of packing. Some meat and poultry products are vacuum packed before freezing, to avoid this problem.
- Fatty products will require an oxygen barrier as well as a light barrier (see section 7.2.4) for optimum shelf-life, to prevent oxidation and rancidity.
- A liquid-tight pack will be required, especially for frozen foods that are meant to be defrosted prior to cooking/eating, to contain any product drip.

7.3.5 Modified-atmosphere packaging (MAP)

This is a growing area of food preservation, now extended beyond its early uses for meat packaging, to fruit and vegetables, bread, fresh pasta, cheese and many

Table 7.3 Typical modified atmospheres for selected food products (%)

Product	Oxygen	Carbon dioxide	Nitrogen
Red meat	40	20	40
White meats/pasta	–	50	50
Fish	20	80	–
Produce	5	–	95
Baked goods	1	60	39

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more otherwise relatively short shelf-life products. Dry goods such as nuts, dried yeast and coffee have been supplied in gas-flushed packs for many years. The principle is the relatively simple one of altering the atmosphere around the product from the natural air of the packaging area (i.e. oxygen-rich) to one that does not favour the propagation of microorganisms. Typical modified atmospheres for a selection of food products are shown in Table 7.3.

The proportion of oxygen is reduced by the use of nitrogen and/or carbon dioxide, although total removal of oxygen is not always desirable. The special case of oxymyglobin in red meat has already been discussed (section 7.2.3) and fresh fruit and vegetables also need some oxygen to continue respiration. In some cases an oxygenless environment is undesirable as it would be conducive to the growth of anaerobic bacteria and thus for baked goods, pasta and dairy products the oxygen level is reduced to a minimum level rather than zero.

Nitrogen is inert and has no noticeable effect on the product or pack. Carbon dioxide is a natural bacteriostat and controls propagation of most microorganisms. However, over time carbon dioxide dissolves in water and the partial vacuum that forms as a result of this means that packs can collapse and become difficult to handle.

Vacuum packaging, in which air is removed from the pack may be regarded as a form of MAP. Owing to the vacuum created, the packaging is pulled in towards the product and hence this method cannot be used for delicate items such as soft fruit or potato crisps. It is used for dry goods such as salted nuts, which are robust enough to withstand the pressure differential between the inside of the pack and the surrounding atmosphere, and for compressible items such as ground coffee. Here, a brick-shaped pack which allows for efficient display on the retail shelf can be produced, although a disadvantage is that once the vacuum is released by cutting open the pack, the fine coffee grounds escape into the air. As has been mentioned, vacuum packaging also works well in combination with freezing for primal cuts of raw meat.

MAP has increased natural shelf-life by 2 to 10 times and is no doubt an important method of food preservation today and for the future. The benefits include reduced spoilage leading to less product wastage and the improved shelf-life allows products to be readily distributed over long distances, thus increasing the variety of foods available at any one time. MAP foods look attractive on display, thus enhancing the selling function of packaging, and the

additive-conscious consumer sees freshness without large quantities of chemical preservatives as a benefit. However, foods that normally require refrigeration to maintain freshness must still be transported and stored under refrigerated conditions, even when packed in modified atmospheres. Processing costs can be high and, along with this requirement for temperature control, mean that MAP is an expensive method of preservation and its costs must be carefully balanced against its benefits.

Packaging requirements, which also affect this economic equation, can be summarised as follows:

- Gas barrier packaging is required, to maintain the modified conditions inside the pack.
- Pack seal integrity is critical, owing to the extended shelf-life expected and displayed on the pack. There is a need for a high level of awareness among operators, with frequent monitoring of seals on the packaging line. Training, always important in any food processing and packaging operation, is perhaps even more vital when using MAP.
- Owing to the space taken up by the modifying gases, packs are often significantly larger in volume than the product, which means less product weight per palletised load. Also the visible effect of product *v.* pack size may adversely influence consumer perception of value for money.

7.3.6 Summary

For all of the methods of extending shelf-life described in this section, as well as the packaging requirements specifically covered, the role of packaging in providing the correct storage and handling information must also be emphasised. Shelf-life must be clearly visible and legible on all levels of packaging, which includes the palletised load, the secondary or outer packaging, and the pack displayed and purchased by the consumer. For transit packaging there is an increasing requirement for variable information such as shelf-life to be included in a bar code and thus automatically captured when the product is scanned at various points throughout the supply chain. Visibility and correct reading of coded data is essential. Where automated systems of reading this information are in place, there are guidelines for position and size.[5]

7.4 The range of packaging options available

In sections 7.2 and 7.3 we discussed the various roles that packaging plays in extending shelf-life and we identified a number of specific requirements. In this section we will look at some of the packaging options available and review how they perform against these requirements. While we will be looking primarily at barrier properties, economic factors related to material costs and total cost in use will also be considered. Packaging decisions in the food industry are often a

compromise between the ideal barrier and the affordable option for a particular product or market.

This section represents only a brief overview of the packaging materials available and is not a full treatise on packaging technology. The reader is referred to the sources of further information at the end of this chapter for texts giving more detailed information on material properties, uses and applications.

7.4.1 Barrier properties

As we have seen, the extent to which packaging provides a preservation role is dependent on the barrier of the material to the environmental factors that cause spoilage, and the total barrier of the pack, i.e. the effectiveness of the pack closure during the life of the product.

The barriers with which we are concerned are moisture vapour, gases (mainly oxygen, but we can also include odours here) and light. Maintaining protection of the product against temperature variations is not usually the role of packaging, as this relies primarily on good control of storage and transport conditions, although there is some use of insulated packaging using expanded polymeric foams, to limit temperature changes to the product. This is mainly for short-term use and for mail order shipment of speciality foods such as smoked salmon.

When considering the barrier properties of the common packaging materials, we can divide them into two types:

1. Total barriers such as glass, tin-plated and other coated steels and aluminium above 17 μm in thickness.
2. Partial barriers, which embrace all other packaging materials, i.e. aluminium foil below 17 μm, and paper-based and plastics materials.

7.4.2 Glass

Glass offers a total barrier to moisture vapour and all gases and is relatively inert, being attacked only by strong alkalis and hydrofluoric acid. It is dimensionally stable and maintains its rigidity under changes in environmental usage conditions, which means that it is not subject to deterioration due to climatic changes, or deformation due to stacking in storage. Its rigidity is also beneficial when filling, especially when using vacuum methods, as the containers do not distort. Contingent on the application of good design principles such as avoiding sharp corners which can lead to poor glass distribution (and therefore points of weakness), glass is resistant to the temperature changes encountered in well-controlled food preservation treatments, such as sterilising of containers, hot filling and autoclaving.

Clear glass, known as white flint, does not provide a barrier to light but where light protection is required the glass can be coloured. Amber glass is the universal option if the exact wavelength of the damaging radiation is not known,

as at 2 mm thick it provides almost a complete barrier up to 450 nm. However, the clarity and sparkle of clear glass packaging, which contribute a high-quality image to the product and enhance its sales appeal, are obviously lost when amber glass is used.

The main raw materials used in glassmaking (sand, soda ash and limestone) are readily available and relatively inexpensive. The factors affecting glass container cost are the high energy required to melt the ingredients to about 1500 ºC and the capital investment costs for what is a continuous process, operating every day throughout the year. Setting up to run a new bottle or jar design can take up to 24 hours before acceptable quality containers are being made and to offset the cost of this 'dead' time a production run of at least three days is needed. The number of containers produced in this minimum run may vastly exceed the food producer's immediate requirements and hence the cost of storage has to be taken into account. Also, the cost of tooling to produce different shapes of bottles and jars can be significant if quantities are modest, although most manufacturers offer a range of standard shapes.

A further factor affecting the cost in use of glass is the weight of the material compared with the weight of similarly sized metal and plastic containers. Although the glass industry has made, and continues to make, many innovative developments aimed at keeping glass weight to a minimum, the transport and storage costs of products packed in glass are likely to be higher than metal, and certainly plastic equivalents. The fragility of glass is another negative point, although here again the industry is engaged in developments in design, glass mixture and coatings to minimise the likelihood and the effects of breakage.

As there is clearly no interaction between the contents of a glass container and the surrounding atmosphere through the glass walls, the critical area affecting total pack integrity is the closure. Closures used on food products are usually metal or plastic and many different types are available. They all require a material which needs to be flexible enough to take up any imperfections in the sealing area at the top of the neck of the container (called the 'land') and which effectively forms a seal between closure and container. Metal closures give a particularly effective seal and the additional benefit of tamper evidence on products which are hot filled or autoclaved in the sealed container. The partial vacuum generated in the head space when the product cools pulls the closure and its liner firmly down onto the neck of the jar. If a small dimple has been incorporated into the top of the closure, this is also pulled down and when the closure is unscrewed the release in vacuum immediately causes the dimple to reform, giving an obvious visible and audible sign that the pack has been opened.

7.4.3 Metal containers

Like glass, metal containers in steel or aluminium offer a total barrier to water vapour and gases, subject to the integrity of the closure and the seams. They also offer a total light barrier. They cannot be described as inert, however, and need to be coated both externally and internally to prevent corrosion of the metal and

interaction with the contents. There have been and continue to be, many developments in metal coating technology, aimed at improving corrosion resistance.

Steel is one of the oldest packaging materials. The development of cylindrical tin-plated steel cans went hand in hand with the commercial development in food preservation, by sterilising the food in the sealed can and thus destroying the microorganisms that would cause spoilage. The can provides both the sterilisation vessel and the means of keeping the product free from subsequent contamination. Aluminium is now also used extensively for can making.

Metal containers are sufficiently rigid to maintain their stability during different climatic and processing conditions. They are less fragile than glass and will not shatter, but they can become damaged by denting when exposed to the physical hazards of dropping and puncturing in the distribution environment. If this damage is confined to small dents with no evidence of weakening of the integrity of the pack, the result, while aesthetically poor, is not injurious to health, although dented cans are likely to be avoided by the consumer unless marked down in price. Hence secondary packaging for canned goods should provide adequate protection against such damage.

Metal cans are generally produced in very large quantities of a fairly limited range of standard shapes and sizes, which accounts in some way for their cost effectiveness as packaging formats. Tooling for a new can shape is very expensive, although unique custom-made shapes are used, usually as a key part of the product's image in the market. Metal cans for food products are more cost-effective overall than glass containers, which has resulted in glass being used mainly for higher-value items.

The can closure for autoclaved food products is obviously a vital part of the pack. Food cans have either three seams or just one, dependent on the can shape and the method of manufacture. The older three-piece style, still commonly used, is made up of a body piece that is formed into shape around a mandrel and then sealed longitudinally by welding with copper wire. The can ends are made separately and one is seamed in place by the can maker using a double seaming method with a flowed-in liner which fills in the interstices and gives a hermetic seal. The second end is seamed in place by the canner after the product has been filled into the can.

The later development of making cans by drawing the base and body section from one piece of metal has reduced the number of seams, and therefore potential leakage sites, to only one. Drawn and redrawn cans are becoming more commonly used in food packaging, and drawn and wall ironed cans are the mainstay of the carbonated drinks market.

7.4.4 Aluminium foil

Aluminium foil is used both unsupported and, in combination with other materials such as paper and plastics in laminates, for a wide variety of packaging applications.

As already stated, aluminium foil above 17 μm in thickness is regarded as a total barrier to moisture, gases and light. It can be formed into containers that lend themselves to a number of food packaging applications, such as chilled and frozen foods, especially ready-made meals with solid and liquid constituents. The good thermal conductivity of aluminium allows for fast freezing and heating and, subject to following a few simple guidelines, foil containers can be used in microwave ovens. They are, however, easily damaged through physical handling and require extra protection in the form of cartons or sleeves and strong outer cases to survive the rigours of the supply chain.

Owing to the presence of small quantities of impurities in the metal structure, aluminium foil below 17 μm is subject to pinholing and thus, used on its own, cannot be guaranteed to provide a total barrier. Foil used for packaging heat-sterilised products (e.g. retort pouches, see section 7.3.3) where a long shelf-life is required, must always be above this minimum gauge. However, even down at 6–9 μm in gauge, while it is no longer a total barrier, aluminium is still an excellent barrier to moisture, gases and light and is far superior to most polymeric materials (although developments in these will be briefly reviewed in section 7.6). As an example, some 50 times less moisture vapour will pass through 9 μm foil *v*. 25 μm low-density polyethylene film of the same surface area, exposed to the same conditions. Coating and/or laminating low-gauge foil dramatically reduces the effect of the pinholes and thus the moisture vapour transmission rate. Aluminium foil is therefore an excellent contender when choosing laminated structures to provide high levels of protection against spoilage due to moisture or gas gain or loss. The cost of aluminium is generally perceived as high but true comparisons can only be made when taking the barrier properties into account.

7.4.5 Paper-based packaging materials

In its natural, untreated state paper cannot be said to present a barrier to either moisture vapour or gases, although it may provide a light barrier. Paper is made from cellulose, a natural fibre which is hygroscopic, absorbing moisture which makes the fibres swell. Dimensional stability under changes of humidity and temperature is therefore poor.

There are various treatments that enhance the moisture barrier performance of paper. These can either be internal chemical treatments at the pulp preparation stage before paper-making, or coatings applied to the paper surface once it is made. Coatings can be tailored to give extra properties such as heat sealability, one example being the use of polyethylene-coated board for frozen food cartons. The polyethylene contributes the moisture barrier required, while at the same time providing a means of sealing the cartons. The performance of such coatings is directly related to the extent to which they cover the paper surface, and the smoother the surface, the better the coating.[6]

With regard to gas barrier, the arrangement of the cellulose fibres in the paper sheet is such that there are gaps through which air and odours can readily

permeate. This can be improved at the pulp preparation stage by beating the fibres to the point where they become extended and almost gelatinous. When the paper surface is then calendered (a smoothing process carried out on the paper-making machine), the air gaps are reduced considerably and the resulting paper, known as glassine, provides a reasonable barrier to gases.[7]

In summary we can say that except for the example just mentioned, paper and board products alone are not suitable as barrier materials in packaging applications. However, they have a wide range of applications when combined with plastic films and foil in laminate structures, where they present an economical means of printing and decoration to aid the selling function, and greatly assist pack openability. An example is sachets for dried soups, which are printed with attractive full-colour graphic representations of the product and which can be easily opened by tearing across the top. Also, the use of board in liquid-proof packaging structures for products such as milk, fruit juices and ready meals must not be overlooked. Finally, a major use of paper and board products is in meeting the protection role of packaging, just some examples being folding cartons, corrugated cases and fibreboard drums.

7.4.6 Plastics

The use of plastics in packaging has been one of the most significant developments of the late twentieth century and now accounts for 30% of the total value of all packaging materials used. (See Table 7.4 for a breakdown of packaging usage by material type.) Plastics packaging is the fastest growing sector, replacing the traditional materials of glass, metal and paper and board. Examples of such replacements over the past two or three decades include:

- Polyethylene terephthalate (PET) bottles replacing glass for soft drinks.
- Film/foil laminate pouches replacing tinplate cans for soup and pet food.
- Polypropylene film replacing paper and aluminium foil for wrapping confectionery bars and replacing glassine paper for potato crisps.
- Polyethylene film bags replacing waxed paper for wrapping bread (although there is some use of waxed paper for the ‘retro’ image).

Table 7.4 World packaging expenditure by value and tonnage 1995

Material	Estimated value		Estimated quantity	
	US$ billion	%	Million tonnes	%
Paper/board	160	34	500	37
Plastics	140	30	300	22
Metal	120	25	150	11
Glass	30	6	400	30
Others	25	5	–	–
Total	475	100	1350	100

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In these examples the change to plastics has meant a reduction in the total overall cost of the product, taking into account the unit cost of packaging and the cost of storage and distribution. In some instances, e.g. crisps and bread, plastics have also brought increased shelf-life owing to their barrier properties.

For our purposes, all packaging plastics are made by the polymerisation of individual monomers and their barrier properties are a function both of the type of monomer(s) used and the way in which they are arranged within the polymeric chain structure. Where chains are highly ordered (crystalline) as opposed to random (amorphous), barrier properties are improved. Thus high-density polyethylene (HDPE) with its orderly molecular arrangement is a better barrier to both moisture vapour and oxygen than the more random structure of low-density polyethylene (LDPE).

Polymer chains can be deliberately orientated during the process of forming a plastic resin into a packaging component such as a film or a bottle. This is achieved by applying force to the softened material either in one direction (monoaxial orientation) or in two directions (biaxial orientation), and then cooling rapidly. The significance for material performance is that orientating the chains in this way increases barrier properties and tensile strength. Two common examples of orientation in packaging are PET bottles for carbonated soft drinks and biaxially orientated polypropylene (BOPP) film.

Barrier performance is reported as moisture vapour transmission rate (MVTR) and oxygen transmission rate (OTR) and these values for the common packaging plastics are shown in Table 7.5. Measurements are by ISO test

Table 7.5 Barrier properties of the common packaging plastics

Material	MVTR	OTR
LDPE Low-density polyethylene	15–20	6500–8500
HDPE High-density polyethylene	7–10	1600–2000
LLDPE Linear low-density polyethylene	15–20	6500–8500
Cast PP Cast polypropylene	10–12	3500–4500
OPP Orientated polypropylene	5–7	2000–2500
PET Polyethylene terephthalate	15–20	100–150
UPVC Unplasticised polyvinyl chloride	30–40	150–350
PVDC Polyvinylidene chloride	0.6–1.0	2–4
PA Polyamide (Nylon)	300–400	50–75
PS Polystyrene	70–15	4500–6000

methods and the results represent the amount of moisture vapour or oxygen which permeates through one square metre of the barrier material of a given thickness (25 μm in Table 7.5) over 24 hours. Because permeation generally increases with temperature (see section 7.2.6) the conditions under which the properties are measured must be specified. Two sets of conditions are commonly used: 25 ºC/75% RH, usually regarded as temperate and 38 ºC/90% RH, usually regarded as tropical conditions.

Plastics used for packaging vary from the glass-clear transparency of PET bottles for soft drinks to the milky white translucence of HDPE bottles used, for example, for milk. If a light barrier is needed for product preservation, pigments are easily incorporated into the mix prior to manufacture of the film or container. Polypropylene films used for wrapping chocolate confectionery, where a light barrier will help to delay rancidity, can be pigmented or cavitated. This process introduces small voids into one layer of the film, thereby obstructing light transmission. Vacuum deposition of a very thin layer of aluminium also provides a light barrier, as well as improving moisture and oxygen barriers.

7.5 Predicting packaging characteristics for particular foodstuffs

Provided the critical values of a food product are known, i.e. at what level the environmental spoilage-causing factor of gain or loss of moisture vapour, gas (or light) becomes unacceptable, it is possible to calculate shelf-life based on the relevant barrier properties of the packaging material. Conversely, knowing the desired shelf-life can dictate the barrier specification of the packaging material. This applies equally to abiotic spoilage discussed in section 7.2 and the methods of heat treatment, cooling and modified atmosphere packaging discussed in section 7.3. Assuming correct storage conditions are maintained, especially for chilled and MAP products, it is the pack's barrier to gain or loss with respect to these environmental factors which will determine shelf-life.

Knowing the spoilage mechanism of the product is thus the first step in predicting packaging requirements. A useful list of deterioration indices for different classes of foods is given in Table 7.6. Note that many foods are sensitive to more than one spoilage factor.

7.5.1 Calculating barrier requirements

The following is a very simple approach which can be used as an indicator and is demonstrated in relation to determining the required moisture barrier, although it can be applied to gas barriers.

First of all the maximum amount of moisture allowable in the product before spoilage starts to occur must be known. Assume this to be W grams. Next we need to measure the surface area of the pack as carefully as possible, allowing

Table 7.6 Approximate order of importance of specific deterioration indices for certain foods: 1 = most important, 7 = least important

Foods	Microbial changes	Inherent changes	Moisture changes	Oxidation changes	Taint, etc	Light	Physical damage
Baked goods	4	4	1	2	4	4	2
Raw & cooked meats	1	2	4	2	6	4	6
Fish	1	3	4	2	–	–	–
Shellfish	1	4	1	1	–	–	–
Potatoes	2	–	1	–	–	3	4
Green vegetables	–	–	1	2	–	–	3
Soft fruits	1	–	–	–	2	–	3
Salads	2	–	1	–	–	–	–
Breakfast cereals	–	–	1	4	3	–	2
Chocolate	5	–	1	2	3	–	4

Acknowledgement is made to Blackie Scientific and Technical for permission to reproduce this material from Paine, F., *A Handbook of Food Packaging*, London, Blackie, 1983, p. 197.

for changes due to handling, especially for flexible packs. Assume this to be A square metres. If the desired shelf-life is T days, then we must look for a pack with an MVTR of less than:

$$\frac{W}{T \times A} \text{ grams per square metre per 24 hours}$$

7.5.2 Choosing the right barrier

Having identified the moisture barrier required, Table 7.5 can be now be used as an initial guide to packaging material selection and to inform storage trials, thus providing a basis on which to start comparing potential alternatives and gain a preliminary idea of packaging material costs. Note that permeation is inversely proportional to thickness of the barrier and hence a 50 μm layer will be twice as good a barrier as a 25 μm layer. This process is useful at the start of a new product/pack development, or when packaging material cost savings are being sought.

Note that the temperature and humidity conditions to which the product is likely to be exposed in the supply chain are vital in calculating the required barrier. It is essential to specify these and check that the data being quoted are applicable to the conditions expected.

The data given in Table 7.5 are generic and transmission rates for specific grades within each type of plastic are quoted by individual manufacturers and can usually be obtained from data sheets. Actual measurements of MVTR, OTR and light transmission can be carried out using laboratory testing and there are a number of independent laboratories carrying out such work. It should be noted that the quoted data are for sheet materials in pristine condition and any creases or folds introduced during the packaging and handling processes will reduce the barrier performance. This is why the calculated barrier levels in section 7.5.1 should be regarded as minimum values.

Factors not mentioned so far, which have an affect on shelf-life and barrier requirements, are the size and geometry of the pack. Because moisture vapour and gas transmission rates are related to pack surface area, the smaller the pack (i.e. the higher the pack surface area to product ratio) the greater the permeation through the pack and the higher the barrier required per gram of product. This is important when developing different pack sizes and shapes and it cannot be assumed that a material that provides an acceptable barrier for one size already on the market will be suitable for another size or shape variant.

7.5.3 Confirming barrier performance

Having identified potentially suitable packaging solutions, it is essential to carry out storage trials to verify acceptability and methods of doing so have been covered elsewhere in this book.

While it is accepted that in the early stages of a development packs may have to be made up by hand in the laboratory, shelf-life testing should also be carried out on machine-made packs as soon as practicable. This gives a better simulation of actual, rather than ideal conditions.

7.5.4 Transit testing

In addition to shelf-life testing, filled packs must be evaluated for their total performance throughout the supply chain. This includes all levels of packaging, primary, secondary and palletised loads and all stages from the end of the packaging line to use and disposal by the final consumer.

Transit testing can be carried out by sending packed products into the existing storage and distribution operation, and observing the effects after a given period of time over a given set of conditions such as vehicle type, mileage covered, class of road, etc. There are many variables in this method and a control sample of known performance is essential.

Alternatively, the effects of the hazards in the supply chain can be measured using laboratory methods, simulating specific conditions such as vibration frequency, drop height and compressive load. A summary of the relative merits of actual *v.* laboratory testing is given in Table 7.7. A combination of both is an ideal solution, possibly using laboratory testing to inform selection and transit trials to verify choice. Of course, it must always be understood that test programmes will give only an indication of performance. It is not possible to cover all the eventualities that will occur once large quantities of goods are being despatched and it is therefore necessary to monitor performance via observations at as many points as possible in the supply chain.

7.5.5 Product and packaging development

Predicting packaging characteristics is clearly a fundamental part of the process of bringing a new product to the market and should be viewed alongside product

Table 7.7 A comparison of transit testing *v.* laboratory testing for evaluating packaging

	Transit testing	Laboratory testing
Samples needed	At least one pallet load	Can be small number of packs
Time taken	Depends on transport availability and journey time – may be very long, e.g. export shipments	Short time needed for results. Testing can be carried out any time
Reproducibility	Not possible to reproduce exact conditions	Can be reproduced accurately
Reliability of results	Damage is only observed in total, and after it has occurred	Damage observable throughout testing and exact cause established
Cost	Minimal if part of existing transport system	Can be expensive

Table 7.8 Sources of information in the development process

Information	Source
Product characteristics	Research and development
Competitive products	Market-place
Consumer expectations	Marketing
Mode of product use	Marketing Research and development
Product sales volume	Marketing/sales
Packaging machine requirements	Engineering/production Planning/forecasting
Storage, handling, distribution hazards	Warehousing and Distribution Retail/wholesale customer Customer/consumer complaints
Display, selling requirements	Retail/wholesale customer
Suitable suppliers	Purchasing Quality assurance
Legal requirements	Specialist legal advice

development. Given the importance of the role of packaging in determining and maintaining shelf-life, the stepwise approach of developing an exciting new product and only then considering how to pack it for sale is unlikely to succeed. Either the packaging will not be ideal in terms of performance and/or cost, or the process will take so long that a competitor may pre-empt your launch.

Successful packaging development relies on being able to access information from a number of different sources and a guide to this is given in Table 7.8. This information-gathering stage should be built in to a development schedule and any likely costs incurred also taken into account when estimating the total cost of development. Also, adequate time and resources must be allowed for carrying out storage trials and evaluating their results.

The same methodology of predicting packaging characteristics applies to changes to existing products as well as to new product development. This is the

case even for what may be perceived as simple line extensions such as a size change, or a flavour variant. In many instances the time schedule may be reduced, but failing to follow the process could lead to disastrous results.

7.6 Future trends

Some of the future trends in packaging materials have been alluded to throughout this chapter and will be included in this summary. Obviously, many are instigated by commercial pressures, as manufacturers seek out ever more cost-effective solutions without compromising shelf-life performance. In parallel, the requirement to meet environmental legislation, with particular regard to minimising total packaging usage, is also influencing developments.

- Light-weight and surface strengthening of glass containers will continue to be developed by the glass industry, in an attempt to minimise the disadvantages of this otherwise excellent material for food use.
- Continuation of the development of coatings for metal containers, especially polymeric laminations, to provide improved corrosion resistance.
- The superior barrier properties provided by aluminium foil will continue to be challenged by the plastics industry, using coating technology designed to provide high levels of moisture and oxygen barriers, while at the same time maintaining transparency.
- There will be greater use of oxygen scavengers as part of a pack, in combination with high barrier materials, to enhance the shelf-life of oxygen-sensitive products.
- Temperature indicators on packs will be used, both to measure storage temperatures in the supply chain, and as easy indicators to the consumer that a food product has been heated to a safe temperature.

7.7 Acknowledgement

Thanks are expressed to Professor Frank Paine, consultant and author of several works on packaging technology, for reviewing this chapter and providing most helpful comments and suggestions for improvement. While every effort has been made in writing this chapter to ensure that the material is accurate, no legal liability is accepted for any errors or omissions, or any conclusions drawn from the information. Information is given on the understanding that it must be verified by means of trials, before being implemented. No responsibility is accepted for such trials.

7.8 Sources of further information and advice

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8

Sous vide products

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8.1 Introduction

In recent years one particular form of enhanced cook–chill technology, '*sous vide*', has created immense interest in the foodservice and retail sectors. The term *sous vide* is used to describe the process of vacuum packaging food before application of low temperature (65–95 °C) thermal processing and storage under chill conditions (0–3 °C).[1,2] *Sous vide* products range from portioned raw materials to complete 'gourmet' meals. Typical meat products include beef bourguignon, lamb cassoulet and coq au vin, which are traditionally braised and stewed 'classical French dishes'.[3] Typical fish and vegetable products include fish stews, fillet of salmon, ratatouille and gratin dauphinoise. In addition, sauce, soup and dessert variations are being developed.[4]

The *sous vide* process is more than a catering technique[5] and involves a precise, carefully designed and extensive manufacturing procedure, as shown in Fig. 8.1. Despite being hailed as a 'revolutionary technique',[6,7] *sous vide* technology originally evolved from *Cuisson en papillote* (food cooked in oiled paper bags), which incorporated aspects of the Nacka, AGS and Capkold systems. The unique benefits of *sous vide* in comparison with these earlier enhanced cook–chill technologies include a substantial increase in shelf-life (up to 42 days) and improvements in sensory and nutritional quality.[8] The process produces these benefits by controlling causes of negative changes in quality such as exposure to oxygen and extreme temperatures.[4]

Over the past two decades the application of *sous vide* technology has developed from a craft-based to a manufacturing approach. The main factor responsible for this development has been the need by the food service industry to become more efficient and to satisfy customer demands for higher-quality

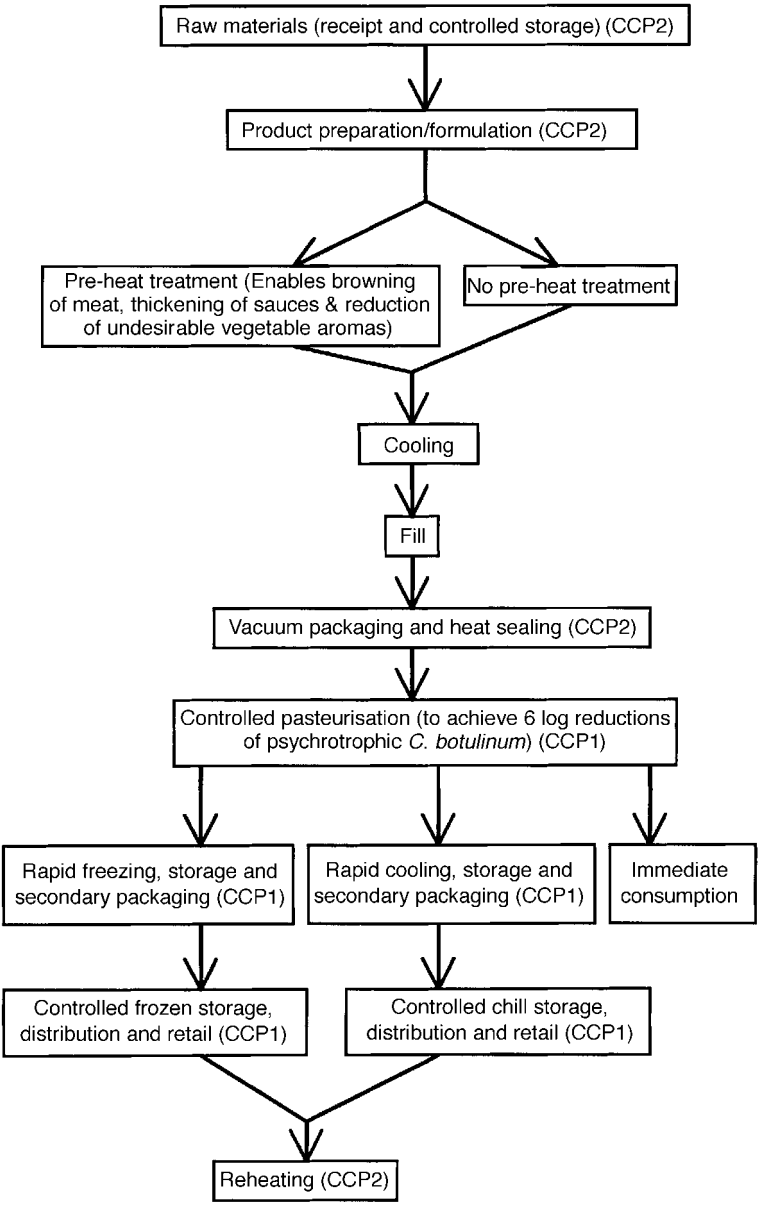

Fig. 8.1 Flow diagram of the *sous vide* process and its critical control points.[1,9–12]
Note: CCP = critical control point.

foodservice.[13] A secondary factor has been the increasing demand for foods that require minimal preparation time, are of high quality, contain low levels of preservatives and are only minimally processed to achieve a fresh-cooked taste (i.e. refrigerated processed foods of extended durability, REPFEDs).[2] Factors

such as these have contributed to sales of *sous vide* foods and other REPFEDs expanding at a considerable rate. The UK foodservice industry reported volumes of *sous vide* products having substantially increased over the last four years with well over four million packs being manufactured and consumed in 1998.[14] The European chilled prepared food sector showed a 50% increase in the period 1991–1996 and is currently estimated to be worth 9.1 billion Euros, while a further 60% increase is predicted by the year 2003, worth 15.1 billion Euros.[15]

Despite the high market uptake of *sous vide* products in many European retail markets, the UK retail market has been less buoyant. The limited uptake in this particular sector is due to the microbiological risks associated with the *sous vide* process and a lack of specific legislation and guidance in the UK.[16] The UK Government does not currently provide specific legislation for the *sous vide* process and *sous vide* processing is grouped under conventional cook–chill processing, falling under the general requirement that products must be consumed within five days of production. Concerns about the microbiological safety of the *sous vide* process have been reported.[9, 17, 18] These concerns are largely due to the risks associated with inadequately controlled pasteurisation and storage implications arising from the *sous vide* process.[19, 20] Numerous microorganisms can survive and grow in *sous vide* products which have received insufficient heat treatment during primary processing and temperature abuse during chilled storage. However, the organism that poses the greatest threat to *sous vide* products is *Clostridium botulinum* (*C. botulinum*), types A, B, E and F.[2, 21, 22] It is reported that as little as 0.1 g of food containing *C. botulinum* toxins, can cause botulism, which is potentially fatal.[1] *Clostridium botulinum* is a particular hazard in *sous vide* processing as it can withstand mild heat processing (a 10^6 reduction in numbers of *C. botulinum* = 70 °C for 1675 min) and storage temperatures as low as 3.5 °C.[20] *Clostridium botulinum* has been reported to inactivate normal spoilage microflora.[18, 21, 23] Hence, growth and toxin production of *C. botulinum* may have occurred before the food is perceived to be spoiled.[24]

As a result of the microbiological risks associated with the *sous vide* process, most research activity has been directed to this aspect. In contrast, research activity on the sensory quality of *sous vide* products has been considerably less, despite the fact that the perceived sensory quality of *sous vide* products initially ensured international support and acclaim.[25] An almost evangelical attitude towards the improved sensory quality, particularly flavour and texture quality of *sous vide* foods compared with conventionally cooked foods is evident in the technical press.[26] However, the validity of research on *sous vide* sensory quality is limited. Sensory analysis is rarely the major objective in published studies, but rather forms part of a ‘greater experimental package’. Problems also include a lack of standardisation in definition of systems, in process parameters applied, objectives and, particularly, in the experimental methodology used.[27, 28] Hence, a meaningful comparison of data from different sources is impossible. This lack of reliable and consistent information on sensory quality may further explain the relatively slow market penetration and uptake of *sous vide* in the UK retail sector.[25]

8.1.1 Categorisation

Sous vide products can be classified into several categories, depending on temperature of storage, duration of storage and the scale of production. However, categorisation of *sous vide* products is somewhat blurred owing to a wide range of variations in practice. The categories given below all involve the generic *sous vide* process shown in Fig. 8.1.

- *Sous vide* cook–chill – stored between 0 and 3 °C.
- *Sous vide* cook–freeze – stored at −18 °C.
- In-house, small-scale production.
- Food manufacturing, large-scale production.

For the purposes of this chapter, *sous vide* cook–chill will be reviewed, considering the challenges that chilled storage poses to the product stability and shelf-life of *sous vide* products.

8.2 Factors affecting the shelf-life of *sous vide* products

The shelf-life of *sous vide* products ranges from 5 to 42 days, depending on several factors. These factors have different degrees of influence, but all must be taken into consideration in the achievement of a maximum quality shelf-life for each *sous vide* product. The application of HACCP to the *sous vide* process (see Fig. 8.1) provides an effective basis to identify critical factors affecting the shelf-life of *sous vide* products.[20, 29, 30]

8.2.1 Raw materials

The microbiological quality, storage and handling of raw materials constitute the first stage in the *sous vide* manufacturing process which is critical to the safety, sensory quality and subsequent shelf-life of *sous vide* products. To control undesirable microorganisms and ensure desirable levels of product quality, purchasing specifications are recommended for all raw materials. Conditions for raw material storage and the duration of storage should also be controlled so that the production of all bacterial toxins is prevented.[9]

8.2.2 Product formulation

As research into the quality of *sous vide* products during storage increases, the formulation of such products has been reported to have an effect on product shelf-life.[28] For example, the formulation of products with a pH of 5 or below, a minimum salt content of 3.5% and available water of 0.97% or less has been recommended (in addition to the heat treatment of 90 °C for 10 min or equivalent lethality) to control the microbiological hazards associated with *sous vide* and ensure a safe shelf-life.[9, 24] Following these recommendations, research efforts

have focused on the formulation of products with low pH, salt and water activity.[31,32] Simpson *et al*.[33] demonstrated the benefits of such product formulation in a *sous vide* spaghetti and meat sauce product, inoculated with *C. botulinum* (types A and B spores). For example, the spaghetti and meat sauce product was formulated to ensure a pH value < 5.25 or a salt content of $< 1.5\%$ and was reported to inhibit *C. botulinum* toxin production throughout a 42-day storage period. Meng and Genigeorgis[34] reported that the addition of sodium lactate significantly delayed toxigenesis of *C. botulinum* (types B and E spores) in (commercially produced and inoculated) beef, chicken and salmon *sous vide* products. However, the effect of recommended hurdles[24] on the sensory quality of *sous vide* products is relatively unknown.[28] Research on these effects is therefore required to ensure a maximum quality shelf-life for *sous vide* products.

The composition of *sous vide* products has been reported to have a considerable effect on sensory quality. The maximum sensory shelf-life for *sous vide* products would appear to be product-dependent and related to the level of recipe development applied to the *sous vide* process.[35,36] There is general agreement[8,27] that meat-based products have a sensory shelf-life of 21–40 days, fish products a shelf-life of 7 days and vegetable products a shelf-life of 7–20 days, under ideal (0–3 ºC) storage conditions.

Beef

In a roast beef product, no deterioration in sensory quality was observed until after 23 days' storage.[37] In a spaghetti and meat sauce product, negative changes in appearance and odour were reported[38] not to be significant until 35 days' storage. In this study, however, the influence of storage on the flavour and texture of the product was not reported. Despite this limitation in sensory methodology, the findings of the Simpson *et al*.[38] study are supported in a recent sensory study. The sensory quality and consumer acceptance of a bolognese meat sauce product was reported[36] to be maintained for 40 days, using a range of quantitative descriptive and affective sensory techniques.

Poultry

Results from a range of studies evaluating poultry products have indicated acceptable sensory quality in poultry products during storage up to 6 days,[39,40] 14 days,[35] 21 days,[41,42] 30 days[43] and 40 days.[36]

Measures reflecting the colour and sauce consistency of poultry-based products have been highlighted in several studies as undergoing change during storage. Changes in 'pea colour' and 'sauce consistency' in a chicken *à la king* product, were reported[35] to have occurred more rapidly during the first 7 days of storage, than during the subsequent week (7–14 days). In a chicken tikka masala product, changes in 'caramel colour' and 'sauce viscosity' were reported[36] to occur during a 40-day storage period. As part of a study on the sensory quality of freshly prepared conventionally cooked product compared to a *sous vide* equivalent (stored up to 12 days), changes in the colour of the *sous vide* sauce component was reported.[26]

Fish

Limited research[42, 44] has been published on the influence of the duration of storage on the sensory quality and acceptability of *sous vide* fish products. The methods in these studies included subjective observations of selected sensory attributes, rather than objective evaluation of all sensory attributes. The results of such studies revealed acceptable sensory quality up to 7 days' storage for salmon products processed either at 65 °C for 10 min or 70 °C for 12.5 min.[42] Acceptable sensory quality up to 28 days was reported for salmon processed under more severe conditions (75 °C for 20 min). Another study reported acceptable sensory quality of a salmon product stored up to 84 days.[44] The temperature/time combination in thermal processing, however, was not reported. In this study, overall acceptability was reported to be negatively correlated with fish odour.

Vegetables

Results from studies evaluating vegetable products have indicated acceptable sensory quality during storage up to 7 days,[35, 45] 8 days,[46] 20 days[36] and 21 days.[47] On review of changes in product characteristics during storage, measures relating to aroma, colour and texture have been reported to limit product shelf-life. Changes in product characteristics of cooked green beans have been reported[46] to include a reduction in green colour during storage. In a courgette provençale product, storage above 7 days (> 21days) was reported[35] to result in a 'soggy, poorly coloured and unpleasant' product.

8.2.3 Filling and vacuum packaging

The level of vacuum applied to *sous vide* products can determine product shelf-life through inconsistent pasteurisation values and the development of aerobic organisms. To ensure a high level of vacuum intensity, product fill temperatures need to be specified and seal integrity of packs monitored. In practice, high product fill temperatures causing condensation in packs can limit vacuum intensity. An insufficient vacuum may also occur if the manufacturer is trying to retain appearance in delicate *sous vide* products. An unacceptable level of aerobic microorganisms was reported[35] in a rolled stuffed fish product which had received an insufficient vacuum to maintain high appearance quality.

8.2.4 Thermal processing

It is recommended that *sous vide* products receive a heat treatment sufficient to achieve a 6-log reduction in the numbers of psychrotrophic *C. botulinum*.[1] A number of regulatory bodies have specified a range of temperature–time combinations to achieve the 6-log reduction, as shown in Table 8.1. The more intense the thermal process the longer the shelf-life and vice versa.[48] Generally, reviews on the risk of *C. botulinum* to *sous vide* foods (stored < 3 °C) suggest shelf-life maxima of <10 days in products processed at 70 °C for 100 min and

Table 8.1 Recommended process parameters and shelf-life for *sous vide* products[1]

Reference	Pasteurisation Temperature (ºC)	Minutes	Recommended shelf-life (days)
Advisory Committee on the Microbiological Safety of Foods (ACMSF), 1992	70	1675	10
	75	464	10
	80	129	10
	85	36	10
	90	10	10
Department of Health Guidelines (DOH), 1989	70	2	5
French Regulations, 1974, 1988	70	40	6
	70	100	21
	70	1000	42
Sous vide Advisory Committee (SVAC), 1991	80	26	8
	85	11	8
	90	4.5	8
	95	2	8
Syndicat National des Fabricants de Plats Prépares (SYNAFAP), 1989	70	40	6
	70	100	21
	70	1000	42

$<$ 42 days in products processed at 90 ºC for 10 min.[1,9] However, in practice thermal processing for a 6-log reduction of *C. botulinum* may be avoided in the production of 'gourmet' type *sous vide* products as a means of retaining desirable sensory attributes.[22,49] Genigeorgis[48] reported that the difference between *sous vide* products processed at 60 ºC for 12 min at the centre and those processed for a 6-log reduction was like 'day and night'. Different heat treatments have been reported to produce different texture quality and acceptance scores in poultry and fish-based *sous vide* products. Chicken breast products processed at 77 ºC have been reported to be more tender and juicy than those processed at 94 ºC.[50] Salmon processed at 75 ºC was reported as being 'dry in taste' and subsequently scored lower in relation to acceptance than other samples processed at lower temperatures (65 and 70 ºC).[42] Hence, the shelf-life of *sous vide* products can depend on the intensity of thermal processing applied and on the level of sensory quality required.

8.2.5 Cooling

Cooling within the *sous vide* process is recommended[1,9] within 30 min of completion of thermal processing and aims to result in a core temperature of 0–3 ºC within a further 90 min. This processing parameter aims to prevent negative quality changes and the germination/growth of surviving *C. botulinum* spores,

which tend to be associated with a slow rate of temperature change after thermal processing. However, in practice difficulties have been reported with cooling large packs designed for the foodservice market, such as large joints of meat, within 90 min. To overcome some of the difficulty, the application of iced water or liquid nitrogen is being used, as opposed to using a blast chiller.[41] If the recommended cooling rate still cannot be achieved, reducing the temperature range 50–10 ºC within 4 h is unlikely to increase the risks from *C. botulinum*.[9] However, despite the fact that cooling rates can vary depending on pack size and method of cooling, rapid cooling is still essential to control *C. botulinum* and to maximise product shelf-life.

8.2.6 Chilled storage and distribution

Different time–temperature combinations during storage have been reported to have a critical effect on the microbiological and sensory quality of *sous vide* products.[21,38] Numerous microorganisms can survive and grow in *sous vide* products that have received insufficient heat treatment during primary processing and temperature abuse during chilled storage.[51] Microorganisms that can occur in *sous vide* products and their lowest growth temperatures are included in Table 8.2. Obviously, psychrotrophic *C. botulinum* presents the greatest microbiological hazard during *sous vide* chilled storage, with a minimum growth temperature of 3.3 ºC. *Clostridium botulinum* spore formation and toxin production have been reported in *sous vide* potato and beef with gravy products, within 6 days at 10 ºC and within 31 days at 4 ºC.[52–54] In relation to sensory quality, higher storage temperatures have been reported to produce

Table 8.2 Minimum growth temperatures of common pathogenic bacteria recovered from *sous vide* products[4,21,24]

Organisms	Minimum growth temperature (ºC)
Clostridium botulinum (mesophilic)	10
(psychrotrophic)	3.3
Listeria monocytogenes	0
Clostridium perfringens	15
Yersinia enterocolitica	−1
Salmonella	6
Bacillus cereus	4
Escherichia coli	7
Staphylococcus aureus	6
Vibrio parahaemolyticus	5
Aeromonas hydrophila	0

negative changes in sensory quality in a shorter period, in contrast to lower temperatures over a longer period. In the evaluation of a spaghetti and meat sauce product stored at 5 and 15 ºC for 35 days, negative changes in sensory quality ('fruity' odour) were only detected in products stored at the higher temperature (day 14).[38] Hence, one of the most critical determinants of the shelf-life of *sous vide* products is the temperature used for chilled storage.

8.2.7 Reheating

The times and temperatures established for reheating *sous vide* products are designed to minimise the risk of microbial growth associated with minimal processing and extended chilled storage. Reheating is recommended[1] to begin within 30 min of the product being removed from storage and to include a core temperature of 70 ºC for 2 min. Once heated, *sous vide* products should be served within 15 min, during which time the centre temperature should not drop below 63 ºC. Such strict control of temperature at the reheating stage and throughout the *sous vide* process is another critical factor in the determination of *sous vide* shelf-life.

8.3 How shelf-life is measured

Product stability is a measure of a product's ability to retain its desirable sensory, chemical and microbiological characteristics during storage.[55] From the information given above it is evident that much is known about the microbiological characteristics of *sous vide* products during storage, but much less is known about the sensory characteristics. In addition, the effect of recommended product formulation on the sensory quality of *sous vide* products is relatively unknown. Sensory evaluation is thus a much-needed tool in the measuring and monitoring of *sous vide* product stability.[56]

The value of sensory evaluation in the measurement of product stability is not primarily in its ability to identify a consumer rejection point, but rather in its ability to model the loss of sensory quality over time and the effect this may have on acceptance.[57] Thus, descriptive and affective techniques have been widely used in the assessment of product stability. In particular, quantitative descriptive analysis (QDA), a descriptive technique, and the degree of acceptance measured on a hedonic scale, have been widely adopted and reported.[58–63] These particular techniques are not only compatible with the test objectives, but have the ability to provide quantitative and reproducible measures of product stability.[62]

Within these two general categories a number of modifications of the standard descriptive and acceptance techniques have been previously reported in stability studies. Meilgaard *et al.*[61] recommend that certain applications of descriptive analysis, such as QDA, be conducted in a reduced or modified form for the purposes of stability testing. For example, the evaluation of a few

detailed attributes is recommended, rather than conducting a full analysis of all product characteristics. In the estimation of consumer acceptance, various workers consider the use of employee panels (conducted in a laboratory) to be more sensitive to subtle product changes, than the use of larger numbers of consumers from a wider population.[58, 64, 65]

To ensure panel safety during sensory assessment in stability studies it is usually necessary to subject three or more pre-production runs/replicates to microbiological analysis before any actual sensory evaluation is conducted and during sensory studies.[55,66] The product sampling regime for sensory evaluation depends upon the anticipated microbiological status of the product during storage. It is generally recommended, however, that sampling is carried out at the start of the storage period, at the maximum expected shelf-life and on three occasions in between.[62, 66]

As descriptive and affective techniques have been widely used in stability studies and are compatible with test objectives, the following sections describe a transferable methodology (using these techniques) on how to produce a reliable and reproducible measure of *sous vide* sensory quality and consumer acceptance during storage.

8.3.1 A transferable methodology

The test product was a *sous vide* bolognese meat sauce recipe dish, processed at 70 ºC for 900 min and stored under ideal storage conditions (0–3 ºC) for 40 days. The storage period of 40 days was selected after completion of three microbiological trials that were conducted to establish the product's maximum microbiologically safe shelf-life. The sensory techniques used included QDA, 9-point hedonic scales and a paired preference test. These techniques were all carried out in a six-booth sensory evaluation laboratory[67] and assessor responses were collected using a computerised sensory system (PSA System 3, version 2.05).

8.3.2 Quantitative descriptive analysis – sensory profiling

A trained QDA[62, 68] panel ($n = 13$) was used to quantify the sensory characteristics of *sous vide* bolognese meat sauce during storage.

Screening subjects for QDA

Individuals willing to participate in a trained sensory panel were recruited within a university, following a publicity campaign, i.e. posters and information sessions. The information sessions explained the focus of the research project, its duration and rewards (participants would be entitled to gourmet meal vouchers at the university training restaurant). Details of the individuals and information about any food aversions were collected in a product attitude survey, adapted from Stone and Sidel.[62] Analysis of the data allowed the

Table 8.3 Products used in screening tests and sensory characteristics studied

Sensory characteristics	Test products	Product/process variations
Texture	1. Carbonnade of beef	Slow *v* quick cooking
	2. Bolognese sauce	Slow *v* quick cooking
	3. Brown stew	Slow *v* quick cooking
Colour	4. Savoury loaf	100% mince *v*. 75% mince + 25% bacon
Odour	5. Beef ragout	Fresh *v* dried garlic
Flavour	6. Beef mince	Strong *v* weak beef stock
	7. Carbonnade of beef	Strong *v* weak beer
	8. Chilli con carne	Fresh *v* canned tomatoes
	9. Beef mince	Olive *v* vegetable oil
Appearance	10. Beef mince	Minced in two different sizes

exclusion of individuals with medical conditions or habits that affected their sense of smell, or those who disliked the foods under investigation.

Prospective panel members were screened using ten discrimination tests (five triangle and five duo–trio)[61] carried out in duplicate, to produce a total of 20 responses from each individual.[69] These screening procedures were specific to the product category under investigation (red meat-based products). They also covered most of the major sensory characteristics reported[11] to be relevant to red meat *sous vide* products, i.e. meat tenderness, colour changes, strength of aroma, flavour intensities and changes in appearance. The product variations used within this discrimination testing and the sensory characteristics on which the products were studied are shown in Table 8.3. The screening procedure enabled the identification of subjects with more than 65% correct responses.[62, 70, 71] This was taken as the threshold level for inclusion on the QDA training programme.

A generic QDA training programme

The generic QDA training programme was an adaptation of the methods of Zook and Pearce,[71] Pizzimenti[72] and Stone and Sidel.[62] Descriptors ($n = 3-4$) relating to quality were included in this storage study, to enable an overall, absolute quality measure to be available for products during storage. The descriptors were defined during training, to minimise the possibility that individual panellists would use them in a subjective/hedonic manner. QDA training consisted of ten 1 h sessions and consisted of six distinct phases:

1 'Orientation phase' (1×1 h) – used to explain the objectives of training, the scoring method (placing a mark on a line scale) and methods of standardising testing procedures. This phase concluded with panellists independently sampling the *sous vide* product, at day 0 and beginning to generate descriptors to describe the whole product.

2 'Finding the word' (2×1 h) – in which panellists sampled the *sous vide* product, at days 0, 20, 40 and selected product raw materials. During sampling, each panel member independently generated descriptors (found words to describe sensory sensations) under the headings of aroma, appearance, flavour and texture.
3 'Developing a workable score sheet' (2×1 h) – in which panellists concentrated on all suggested descriptors being presented to the panel (under the headings of aroma, appearance, flavour and texture). Those that were synonymous for individual descriptors were grouped. Descriptors and word anchors (i.e. weak – strong) to be used on the score sheet were agreed and defined by the panel.
4 'Quantification of line scales' (2×1 h) – in which panellists sampled reference products developed in-house to demonstrate extreme intensities (word anchors) of each descriptor. The *sous vide* product at day 0 was evaluated using the developed score sheet, individually and by the panel during a group discussion. This process established a sensory profile of the perceived characteristics of the *sous vide* product, at day 0.
5 'Line scale scoring practice' (1×1 h) – in which panellists individually scored three different products, i.e. the *sous vide* product at day 0, and commercially available high- and low-quality bolognese products. A quadrant rating technique[71] was used to ensure that panellists understood each descriptor and the associated sensory perception being described. In this procedure panellists were asked to allocate products in the first, second, third or fourth quarter of the line scale.
6 'Familiarisation with the computerised QDA system' (2×1 h) – in which panellists achieved further familiarisation with using line scales and the computerised sensory system. The *sous vide* product at days 0, 10, 20, 30 and 40 (products to be tested in the storage study) were examined in this phase to check that the developed score sheet had sufficient descriptors to enable all product sensory perceptions (during a 40-day storage period) to be scored. On completion of this phase, the panel was given the opportunity to agree on new descriptors and/or eliminate descriptors that were consistently causing confusion or not being used to discriminate between products.

Evaluation of panellists and descriptors

An evaluation of the discriminatory ability, consistency and panel agreement of panellists and descriptors, was carried out immediately after QDA training and periodically to monitor and develop a profile on panellist and descriptor performance. After QDA training, a three-product balanced block design[62] was conducted with three replications. The three products were:

1 A standard *sous vide* product at day 0.
2 A commercially produced product.
3 A modified *sous vide* product at day 0 (bolognese meat sauce minus wine).

Discrimination

The mean percentage scores of the panel for each descriptor were plotted on a 'spider plot'/radar chart (Microsoft Excel: displays changes in values relative to a centre point) to create a product profile for the different products tested. The profiles were then used to assess if the panel had detected known product differences (i.e. exclusion of wine in modified *sous vide* product compared with standard *sous vide* product).

A two-way repeated measures ANOVA applied to each panellist's replicate scores for each descriptor was used to determine panellist ability to discriminate and whether or not descriptors were being used to discriminate accurately.[73, 74] A probability value of <0.50 was used to indicate discriminatory ability within panels and of descriptors.[68, 73] ANOVA interaction tables highlighted whether products were significantly different for individual descriptors. These tables were also used to determine if descriptors were being used to discriminate.

Consistency

The product profiles for the different *sous vide* products (standard and modified) were used to indicate panel consistency in response. For example, the mean percentage score of descriptors that were not expected to be significantly affected by the modifications were compared.

A two-way repeated measures ANOVA applied to each panellist's replicate scores for each descriptor was used to determine the degree to which panellists responded consistently, through evaluation of probability values.[62, 75] A probability value of < 0.50 was used to indicate consistency of response within panels and of descriptors.[68, 73]

Panel agreement

Correlation coefficients and ANOVA interaction tables provided indications of the levels of agreement between panellists in the definition and use of descriptors.[62, 76–78]

After completion of panel and descriptor evaluation (post-QDA training), all panellists attended a further training session which concentrated on the further refinement (or in some cases removal) of descriptors which had caused unsatisfactory levels of performance (discriminatory ability, consistency and/or panel agreement). Those panellists whose scoring did not demonstrate satisfactory levels of performance attended further training sessions, as described previously.

Consistency of response and panel agreement within the panel was assessed during the storage study to complete the profile on panellist performance. Those panellists whose scores did not demonstrate satisfactory levels of performance during the storage study and whose profile reflected unsatisfactory performance in previous evaluation studies were not included in the final storage results. Panellist ability to discriminate was not measured during the storage studies, as whether or not the products tested contained detectable differences was unknown. An evaluation of descriptor performance was also not carried out on

the storage study data, as the panel had agreed the final score sheet before this phase of testing (after the previous evaluation study).

QDA: experimental design and statistical analysis

A five-product balanced block design was conducted with three replications. The five products included day 0, 10, 20, 30 and 40 products. The five products (from different batches) were tested together to allow simultaneous comparison of products in single testing sessions. Testing the products together also minimised the number of testing sessions, which was essential in maintaining panellist motivation. This production and sensory testing schedule (see Table 8.4) was supported by the demonstration of a high level of product uniformity between production runs in panel evaluation studies. To ensure

Table 8.4 Production and sensory testing schedule of *sous vide* products in storage study

Day	Production	Sensory testing
0	'Day 40^1' product	
1	'Day 40^2' product	
2	'Day 40^3' product	
10	'Day 30^1' product	
11	'Day 30^2' product	
12	'Day 30^3' product	
20	'Day 20^1' product	
21	'Day 20^2' product	
22	'Day 20^3' product	
30	'Day 10^1' product	
31	'Day 10^2' product	
32	'Day 10^3' product	
40	'Day 0^1' product	'Day 0^1' product 'Day 10^1' product 'Day 20^1' product 'Day 30^1' product 'Day 40^1' product
41	'Day 0^2' product	'Day 0^2' product 'Day 10^2' product 'Day 20^2' product 'Day 30^2' product 'Day 40^2' product
42	'Day 0^3' product	'Day 0^3' product 'Day 10^3' product 'Day 20^3' product 'Day 30^3' product 'Day 40^3' product

Note: Superscript numbers = different product production batches.

microbiological safety, all products presented for testing had been microbiologically tested.

All panellists who attended the training sessions participated in the three individual storage studies. Product scoring data from individual panellists who had demonstrated an ability to discriminate, be consistent and be in agreement with the rest of the panel periodically and in this study were included in the data analysis of the storage study.

The individual product scores reported by each panellist during the storage study were converted to a 0–100 scale, from which means were calculated. These mean scores were plotted on a 'spider plot' (radar chart) to create a product profile.[71] Two-way analysis of variance (ANOVA) determined whether or not significant differences existed among products tested for each descriptor.[75] Figure 8.2 represents 'spider plots' for day 0 and stored *sous vide* bolognese meat sauce and the level of product differences between samples. The least significant difference (LSD) test was applied when significant differences were found to identify which products were different.[79]

8.3.3 Consumer acceptance testing

Two affective quantitative sensory tests (9-point hedonic scale, paired preference test) were conducted by laboratory consumer panels to determine the effect of storage duration on consumer acceptance of *sous vide* bolognese meat sauce.

Consumer acceptance testing: experimental design and statistical analysis

Balanced verbal 9-point hedonic scales, ranging from like extremely to dislike extremely[80] were used to assess the degree of liking/acceptance of the aroma, appearance, flavour and texture of day 0 and stored products. A paired preference test[61] was used to determine consumer preference between the day 0 or stored *sous vide* product. An incomplete two-product balanced design[61] was conducted for both affective tests (9-point hedonic scale, paired preference test). The two products tested together included:

- Day 0 and day 10.
- Day 0 and day 20.
- Day 0 and day 30.
- Day 0 and day 40.

Untrained consumers (200, 40 per test) were recruited from within the university. The project was widely advertised by posters and by 'word of mouth'.

Significant product differences in the acceptance (hedonic scale data) of products tested together (by the same consumers) for each descriptor were calculated, using the Wilcoxon signed rank test.[79,81] Table 8.5 represents product differences in the acceptance of day 0 and stored *sous vide* bolognese meat sauce, tested by the same consumers. Significant product differences in the acceptance of products not tested together (by different consumers), for each

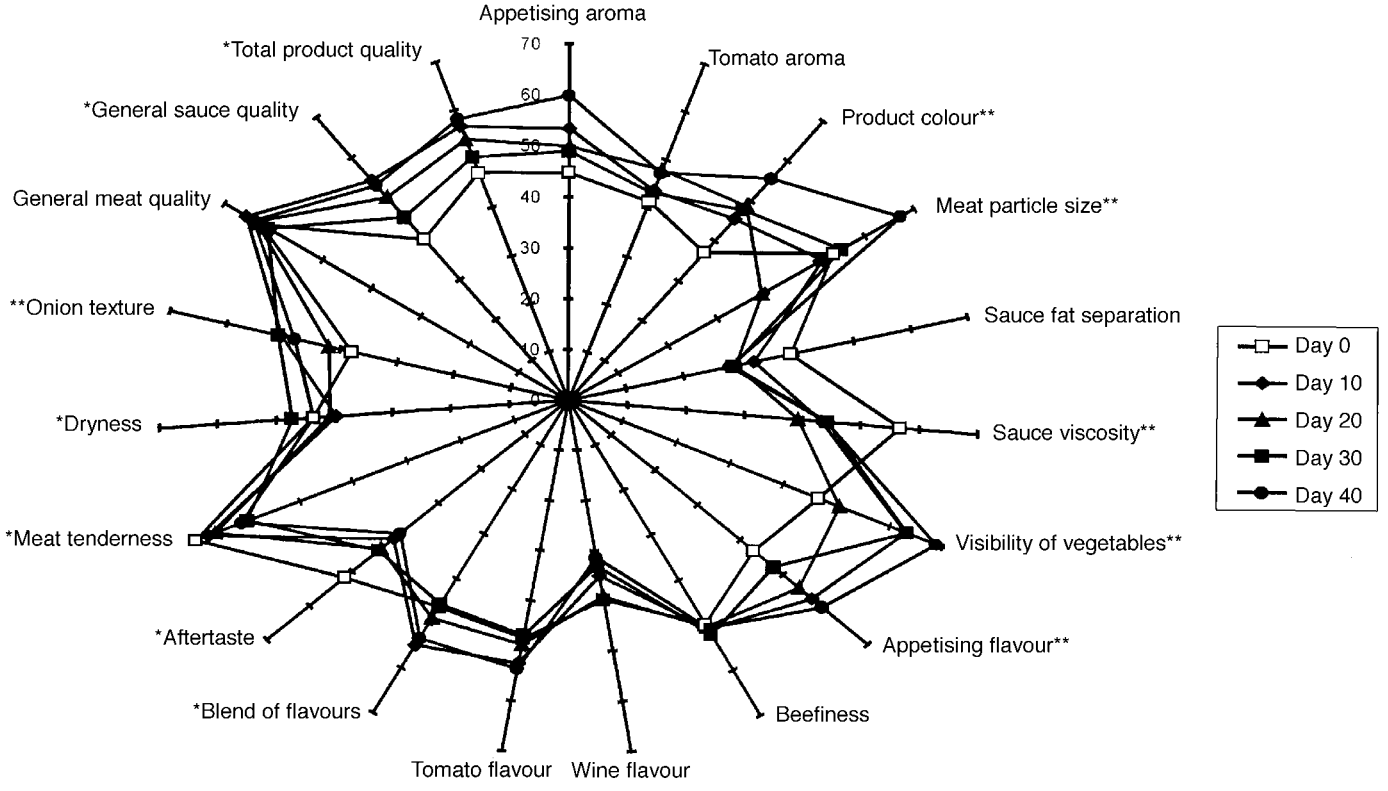

Fig. 8.2 Spider plot (mean percentage scores) of bolognese meat sauce during storage. Significance levels: ** $p < 0.01$, * $p < 0.05$ (using two-way ANOVA for all products).

Table 8.5 Product differences in the acceptance of day 0 and stored bolognese meat sauce using the Wilcoxon signed rank test

Attribute	Mean values for day 0 and stored products derived from hedonic scale data					
	Day 0	Day 30		Day 0	Day 40	
Aroma	3.52	NS	3.42	3.87	NS	4.23
Appearance	2.85	NS	3.00	3.64	*	4.36
Flavour	3.27	NS	3.37	3.56	NS	3.79
Texture	3.02	NS	3.30	3.49	NS	3.92

Hedonic scale rated from 1 = like extremely to 9 = dislike extremely.
Significance level: * significant $P < 0.05$, NS = non-significant.

descriptor were calculated using the Mann–Whitney U test.[79] Significant differences in preference between day 0 and stored products were determined by reference to binomial paired-preference tables.[79]

8.3.4 Interpretation of results

The simplest way of using the results produced by a storage study such as this is the use of the acceptance data (hedonic scale and preference test) to determine product shelf-life and the descriptive (QDA) data to determine which factors are controlling the shelf-life.[63] Using the descriptive data to identify the factors responsible for changes in acceptability can also provide valid information for the effective development of new or existing products.[82]

8.3.5 Instrumental evaluation

Although sensory evaluation is the final arbiter of product stability, instrumental evaluation can be used to provide an objective and often less costly measure.[83] Instrumental evaluation can be used in the assessment of product appearance, aroma, flavour and texture. Instruments that measure appearance are based on colour systems developed to quantify colour and can be useful in determining colour differences during storage.[84] Instrumental measures of aroma and flavour can develop a better appreciation of product freshness during storage. Analytical analysis of aroma compounds involves the extraction of volatiles from food, their concentration, and the separation and identification of individual components.[85] The acidity of a food, usually increased by product spoilage, can be effectively determined by measuring pH.[86] Instrumental texture methods can provide a useful quantitative measure of product texture, before it is placed in the mouth. Common instrumental approaches include shear force tests, compression force tests, tensile tests and extrusion.[87]

Valid instrumental methods are those that correlate highly with sensory data and therefore should be used in addition to sensory evaluation.[83] Their need in

determining storage stability can be determined by the nature of sensory evaluation being carried out. Quantitative sensory methods may require less instrumental evaluation to support findings than more qualitative sensory methods.

8.4 Extending shelf-life

In recent years the developmental aspects of product formulation, in addition to process parameters, have been reported[36, 88] to enable extension of *sous vide* product shelf-life. A review of the literature and findings of recent studies[36, 89] indicate that the quality shelf-life of *sous vide* products can be extended through effective recipe development, which concentrates primarily on retaining appearance and aroma attributes during storage. Particular considerations that appear to be important in the retention of the sensory quality and consumer acceptance of stored products involve:

- The inclusion of herbs and spices to meat products (to mask negative changes in sensory quality and to act in some capacity as antioxidants and as microbe inhibitors).
- The inclusion of sauce components, which include industrially modified starches (to avoid syneresis and to minimise undesirable changes in sauce consistency and subsequent appearance).
- The inclusion of a pre-heat treatment to vegetables before *sous vide* processing (to allow strong brassica volatile aromas/flavours, for example, to be released prior to *sous vide* processing and not to be retained within the pouch).

Formulations of products with physiochemical and microbially-based hurdles (pH, water activity, salt, organic acids, competitive microflora) in addition to physical hurdles in the *sous vide* process (thermal processing and temperature control) can also be used to extend product shelf-life.[24, 32] In combination these hurdles control growth of spoilage and pathogenic microorganisms, despite each hurdle not being sufficient on its own to achieve the same effect.[90] The hurdles, however, need to be carefully selected to minimise negative changes in sensory characteristics and to ensure the production of acceptable products. Many workers[36,91,92] have reported a need for such research as a means of increasing the safety and shelf-life of *sous vide* products in the retail market, where the chill chain is not robustly controlled.

Ultimately, the production of *sous vide* products using a HACCP approach and/or good manufacturing practice (GMP) approach can extend product shelf-life by shifting the emphasis from finished product testing to raw material and process control.[93] Tightly controlled temperature monitoring throughout the *sous vide* process and during storage in particular, is essential in the extension of product shelf-life. The application of time–temperature integrating systems[94] that change colour or become visible upon significant temperature abuse may be

one means of extending shelf-life through continuous monitoring and control of distribution and storage temperatures.

8.5 Future trends

Sous vide technology has been shown to have the capability to satisfy increasing consumer demands for high sensory quality and extended durability beyond that of any other cook–chill technology. Considering that the technology also offers convenience in consumer preparation, high nutritional content, freedom from preservatives and products that are perceived to offer 'freshness',[95,96] it has very significant market potential. However, before this opportunity can be fully utilised by the retail market and subsequent consumer, stringent control of processing parameters and of the chill chain is required to ensure microbiological and sensory quality. Until such control can be achieved in the retail market, the application of multiple hurdle technology (MHT) will be one method of developing *sous vide* technology as a viable technology in this well-established and growing market for chilled products.

In general, research into the quality of *sous vide* products needs to take on a much greater consumer focus. The challenge in *sous vide* product development will be in the design and selection of hurdles which balance the diametrically opposing demands of safety and acceptable sensory quality.[97,98] Although the microbiological quality of *sous vide* products is important, consumer acceptance as a primary determinant of food choice is beginning to gain appreciation.[99] Already, research on *sous vide* is beginning to address the need for effective product development and the production of valid and reliable information on sensory quality and consumer acceptance. However, this emphasis needs to continue, if the benefits of *sous vide* technology are to be fully utilised.

8.6 Sources of further information and advice

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9

Milk and milk products

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9.1 Introduction

There is no straightforward objective definition of the shelf-life of milk and milk products because criteria that may be appropriate for one product may be inadequate for another. For this reason, we choose to define shelf-life as the period following manufacture during which the product meets consumer expectations. This definition is somewhat elastic, not least because the expectations of individual consumers vary. Nevertheless, its utility lies in the recognition that, in a diverse range of products, the end of shelf-life may be signalled by changes in appearance, smell or flavour. The essence of the definition is that a *change* in quality of sufficient magnitude to influence consumer opinion has taken place.

Changes imply transformations and these may be physicochemical, chemical or biochemical in nature. Examples of such processes include the following:

- *Physicochemical* – creaming of fat, gelation of protein solutions, syneresis of curds and crystallisation of minerals.
- *Chemical* – non-enzymic browning and oxidation of fat.
- *Biochemical* – growth of microorganisms, enzymic degradation, ripening of cheese and fermentation.

This chapter will highlight the various transformations that tend to limit the shelf-life of milk and milk products. As a general background, brief consideration will be given to the composition and important chemical properties of milk components. The bacterial flora of milk with reference to their potential for limiting shelf-life will then be considered and the effect of temperature on growth of spoilage bacteria discussed. Finally, examples will be

given of the factors influencing the shelf-life of specific products together with comments on methods of control.

9.2 Chemical composition and principal reactions of milk

Milk was designed by nature to provide complete nourishment for the newborn and, as might be expected, is a highly complex mixture. The four main chemical classes present in milk, irrespective of species, are fat, protein, carbohydrate and mineral and each component plays a key nutritional role. In Europe, most milk is now derived from the dairy cow and the composition of typical mid-lactation milk is shown in Table 9.1. Transformation of milk protein and fat are responsible for most of the changes that govern shelf-life.

9.2.1 Milk protein

The proteins in milk are classified into two families, caseins and whey proteins. Their respective abundance are shown in Table 9.2. Casein is the most important group constituting over 80% of the protein in bovine milk in mid-lactation milk.

Casein, the major milk protein is split into five main classes, α_{s1}-, α_{s2}-, β-, γ- and κ-caseins, as shown in Table 9.3. The primary structure of every casein in bovine milk has been defined. All the caseins are modestly sized and are not thought to possess an organised structure. As a result, the caseins cannot be

Table 9.1 Average composition of milk

Constituent	Concentration ($g\,l^{-1}$)	Proportion solids (%)
Fat	37.0	28.9
Protein: casein	27.6 } 34.0	26.6
whey protein	6.4 }	
Non-protein nitrogen	1.9	1.5
Lactose	48.0	37.5
Ash	7.0	5.5
Total solids	127.0	100.0

Table 9.2 Protein distribution in skim milk

Milk protein	(%)
Casein	82.2
Whey protein	
β-lactoglobulin	9.6
α-lactalbumin	3.8
bovine serum albumin	1.4
minor 'proteins'	3.0

Table 9.3 Composition and properties of casein fraction

Fraction	Molecular weight[a]	Proportion whole casein (%)	Serine phosphate residues	Calcium sensitivity	Sugar residues
α_{s1}	23 000	38.1	7–9	++	–
α_{s2}	25 000	10.2	10–13	+++	–
β	24 000	35.7	5	+	–
γ	11 600–20 500	3.2	0 or 1	–	–
κ	1 980	12.8	1	–	+

[a] Molecular weight of monomer.

Table 9.4 Minerals in milk

	Total (mmol l^{-1})	Diffusible (mmol l^{-1})
Calcium	30.1	9.5
Magnesium	5.1	3.3
Sodium	25.5	–
Potassium	36.8	–
Chloride	30.3	–
Inorganic phosphate	20.9	11.2
Citrate	9.8	9.2
Zinc, selenium, molybdenum and iodine	trace levels	

denatured, for example by heating. The caseins are phosphoproteins (Table 9.3) and the extent of their reaction with multivalent ions such as calcium is very dependent on the number of serine phosphate groups present on the molecule. This ability to interact with other ions is an important aspect of the functionality of caseins, e.g. in cheese making or in the production of fermented products. In addition it plays an important role in determining the stability of in-can sterilised milk products (evaporated milk and cream) and is the primary cause of age gelation in UHT sterilised milks.

In raw milk, caseins are associated with calcium and phosphate into small particles – with an average size of approximately 100 nm – called micelles. The mineral content of milk is shown in Table 9.4. About two-thirds of the calcium and about half the phosphate are bound to the colloidal, i.e. micellar, phase. The partition of calcium (and phosphate) between the micellar and the serum phase may be manipulated by technological means. Calcium can be withdrawn from the micelle by addition of sequestrants, such as trisodium citrate, hexametaphosphate or polyphosphate. In the micellar structure there is a network of α_s-casein and calcium phosphate within which β-casein is held. The surface of the micelle is rich in κ-casein but this component is also located within the micellar structure. The 'hairy' micelle model best fits the known behaviour of casein micelles.

Another important property of caseins is derived from their primary structure. Within the caseins, the acidic amino groups (carboxyl and ester phosphate) are

unevenly distributed along the polypeptide chains. As a result, the proteins have highly charged polar regions and contrasting domains of a hydrophobic nature. Such heterogeneity confers very good emulsifying properties on the molecules because the polar regions can associate with the aqueous phase while the hydrophobic regions bind well to lipids. Thus the proteins stabilise fat droplets in solutions or in semi-solid matrices such as meat emulsions.

In contrast, the whey proteins are globular proteins with classical tertiary structures. The structure of the main whey protein, β-lactoglobulin, is stabilised by disulphide bridges. Such links are disrupted by heat treatment above 65 °C and, as a result, the proteins are denatured. On the other hand, undenatured whey proteins are not greatly affected by multivalent ions and do not readily precipitate.

Four types of reaction can influence the functional properties of milk protein:

1 Protein degradation can take place as a result of attack by milk plasmin or by bacterial enzymes.
2 The second important reaction of milk proteins occurs when they react with reducing sugars – the Maillard reaction. This reaction is characterised by browning of products but, in its early stages, there is a significant loss of nutritive value because lysine, an essential amino acid, reacts very readily with reducing sugars. The extent of loss of lysine depends on the severity of heat treatment, the pH of the product and the amount of reducing sugar present. By careful avoidance of such prejudicial conditions during manufacture, the nutritive value of milk proteins can be conserved. Nevertheless, the Maillard reaction can limit the shelf-life of dried milk products.
3 Acidification forms the basis of production of all fermented milks. The gels of fermented milks, such as yoghurt and quarg, are formed by acidification of milk. As the pH is reduced, the casein precipitates selectively. The first signs of aggregation occur around pH 5 and once the pH falls to 4.6 all the casein becomes insoluble.
4 Another property of casein is its ability to aggregate in the presence of calcium under specific conditions. As described above, casein micelles are stabilised by a κ-casein that behaves like a 'hairy' layer at the micellar surface. Chymosin, the principal enzyme in calf rennet, can selectively break down the surface κ-casein and reduce micellar stability. If the temperature of the rennet-treated milk is above 10 °C and calcium is present (as it always is in milk, viz. Table 9.4), aggregation takes place and a rennet gel is formed.

9.2.2 Milk fat

Milk fat consists almost entirely of triglycerides (triacylglycerols), i.e. esters of fatty acids with the molecule glycerol. Fatty acids in milk are derived from a number of sources and the pathways from feed to milk are not straightforward.

Fat consumed by the cow is first hydrolysed to free fatty acid in the rumen or first stomach. Because of the strongly reducing conditions in the rumen, unsaturated fatty acids are hydrogenated. The saturated acid then passes to the gut where it is absorbed into the circulating blood. Some fatty acid is stored in the animal's fat reserves, after reconversion to triglyceride. Another portion is broken down to provide energy for the animal, while the remainder passes to the mammary gland where it can be re-esterified into milk triglyceride. Such pre-formed fatty acids are predominantly of chain length 16 or higher, though chain lengths of 12 and 14 can be found when the cow is fed diets rich in these acids. However, the cow also has the ability to synthesise fatty acids with chain lengths from 4 to 16 in the mammary gland. These acids can account for over a third of the total triglyceride. A further complication arises from the presence of a specific enzyme in several tissues of the cow. This enzyme is capable of taking a saturated fatty acid of chain length 18 (stearic acid) and converting it to the mono-unsaturate (oleic acid). As a result of this series of transformations the fatty acid composition of milk is fairly heterogeneous, as shown in Table 9.5. The distribution of the fatty acids in the triglycerides adds another layer of complexity, because the distribution among the three potential sites for esterification is not random. The short chain acids are preferentially linked to the hydroxyl group at one end of the molecule.

As with milk protein, fat occurs naturally as a complex structure. Milk fat globules range in size from 0.1 to 12 μm in diameter (median 3 μm). The globules are spherical droplets of triglyceride coated by a double membrane rich in phospholipid. The milk fat globule membrane (MFGM) is fragile and is damaged and disrupted by physical treatment. This reaction forms the basis of butter-making. By arranging optimum conditions for disruption of globule membrane, the droplets are induced to clump. The fat surface exposed by

Table 9.5 Fatty acid composition of April milk

Fatty acid	Mole (%)
4:0	9.6
6:0	4.0
8:0	2.0
10:0	3.5
12:0	3.6
14:0	9.9
14:1	2.1
16:0	24.7
16:1	3.2
18:0	10.5
18:1	22.6
18:2	3.0
18:3	1.4
Carbon number	42.7

removal of the membrane is very hydrophobic and quickly associates with exposed fat surface on other droplets. This process is called churning. The clumps of granules are first washed to remove protein, lactose and minerals (as buttermilk) then physically worked to yield a plastic mass – butter.

Milk fat is susceptible to several important reactions:

- Raw milk has an abundance of lipoprotein lipase, an enzyme that will rapidly hydrolyse milk fat to free fatty acids.
- Bacterial lipase causes serious degradation of milk fat.
- The delicate MFGM is also susceptible to enzymatic degradation.
- Another important reaction is oxidation. Reaction is initiated by free radicals of oxygen at the unsaturated bonds (especially conjugated double bonds) in fatty acids. The reaction is catalysed by light and by heavy metals such as copper. Phospholipids in milk are more prone to attack in milk than are the triglycerides which are mostly saturated. Lipid oxidation is best controlled by exclusion of oxygen, light and potential contaminants, hence packaging plays a key role.
- Milk fat droplets in raw milk are readily susceptible to creaming. The rate at which fat globules rise depends on the density difference between the fat globule and the serum, the viscosity of the serum which is influenced by temperature, the concentration of a cold agglutinin and fat globule size. In practice, creaming is inhibited by reduction of the fat globule size by homogenisation. The milk fat globules are reduced in size by pumping at very high pressure (up to 400 bar) through a small slit or orifice. The size reduction results in an increase in specific surface area and this newly-formed fat surface is immediately coated with milk protein from the serum phase. The threshold globule size below which creaming does not occur is *ca.* 0.8 μm diameter. Control of fat emulsion size is critical in products that are prone to creaming.

9.3 Bacteria in milk and related enzyme activity

9.3.1 Psychrotrophic Gram-negative bacteria

The bacteria in freshly drawn milk from a healthy cow are largely derived from the environment within which the cow is kept – the byre and milking parlour – and from the equipment through which the milk passes and in which it is stored. The majority of milk in Western Europe is cooled and refrigerated promptly after milking. As a result, conditions favour the survival and subsequent growth of organisms adapted to a low-temperature environment. Many such bacteria have an optimum growth temperature between 20 and 30 ºC but also grow, albeit more slowly, at refrigeration temperature. They are known collectively as psychrotrophs.

Psychrotrophic bacteria from farm bulk tanks and from creamery silos have been extensively studied because of their potential commercial importance.

Table 9.6 Psychrotrophic Gram-negative bacteria in milk and associated enzyme activity

	Isolates in genus (%)		Isolates with stated activity (%)		
Bacterial genus	Creamery	Farm	Lipolytic	Proteolytic	Lipolytic + proteolytic
Pseudomonas					
fluorescing	33.5	50.5	5	2	71
non-fluorescing	44.1	31.5	32	1	11
Enterobacteriacea, Aeromonas, Pasturella or *Vibrio*	8.5	15.8	2	2	31
Acinetobacter, Moraxella or *Brucella*	6.2	0.0	5	9	36
Flavobacterium	4.0	1.3	6	6	24
Chromobacterium	2.2	0.0	25	6	41
Alcaligines	1.5	0.9	0	0	92
Number isolates	735	85			

Typical results for creamery silo milk collected in South-west Scotland and from a farm bulk tank are presented in Table 9.6. The Gram-negative bacteria, which make up over 90% of the total flora, are classified according to genus. Bacteria of the genus *Pseudomonas* were by far the most common organisms, about half being of the fluorescent type. The main species *Pseudomonas fluorescens* is characterised by the production of a diffusible fluorescent pigment during growth on an appropriate medium. Although the optimum temperature for growth lies between 25 and 30 °C, pseudomonads will also grow at temperatures just above freezing. The genera are widely distributed in water and in the soil. The second most common family of psychrotrophic bacteria in raw milk is that of the *Enterobacteriaciae*. These organisms are also small, motile, Gram-negative rods. Their optimum growth temperature tends to be higher (i.e. > 30 °C) than that of the pseudomonads but they adapt well to growth at refrigeration temperature. The usual source of coliform contamination of raw milk is from the digestive tract of the cow via faecal contamination of the bedding or udder. Some strains of *Escherichia coli* produce verotoxins and constitute a food-poisoning hazard. A number of other types of psychrotroph are also frequently found (Table 9.6), albeit at low frequency. Included in the list of common contaminants are bacteria of the genera *Flavobacterium, Chromobacterium* and *Alcaligenes*. They are all Gram-negative rods capable of low-temperature growth and, like the pseudomonads, are commonly found in soil and water.

Many of the psychrotrophic bacteria isolated from milk produce extracellular enzymes that degrade milk fat and protein (Table 9.6). Some genera have great destructive potential. For example, over 70% of isolates classified as *P. fluorescens* exhibit both proteolytic and lipolytic activity. At least 20% of all psychrotrophs isolated from raw milk can cause protein breakdown and lipolytic

rancidity. It is also worth noting that all genera examined possessed some degree of extracellular degradative activity and thus pose a significant threat to milk quality and to products manufactured from milk.

9.3.2 Heat-resistant bacteria

The psychrotrophic bacteria considered above are almost all killed by modest heat treatment (e.g. pasteurisation, 72 °C/15 seconds). However, some survivors from the natural flora, given suitable conditions, are able to promote spoilage. Bacteria typical of those isolated from milk and cream are shown in Table 9.7. In general, only *Bacillus* spp. and *Corynebacteria* are found in any number, though thermoduric micrococci and lactococci are occasionally recovered. The coryneforms, micrococci and lactococci are usually incapable of further growth in pasteurised product provided the temperature is held below 6 °C. *Bacillus* spp. are the other major thermoduric group of organisms and are of greater technical significance because of their ability to grow under refrigeration conditions. Of the *Bacillus* spp. found, *B. cereus*, *B. licheniformis* and *B. coagulans* predominate. The vegetative cells of the bacilli are readily destroyed by pasteurisation and it is the spore form of the organism which is heat stable. These residual spores may – given the correct conditions – germinate after heat treatment and subsequently grow in pasteurised products. The degradative activity associated with thermoduric bacteria isolated from pasteurised cream is shown in Table 9.7. Coryneforms are largely inactive but the *Bacillus* spp. have, in general, great potential for spoilage. Almost 40% of isolates could degrade both milk fat and protein while 80% of isolates exhibited phospholipase activity. As indicated earlier, phospholipase action can destroy the native MFGM, resulting in destabilisation of the fat emulsion in milk.

In summary, the psychrotrophic thermoduric floras of milk are able to survive pasteurisation, can subsequently grow in product and also possess the

Table 9.7 Heat-resistant bacteria recovered from milk and associated enzyme activity

	Bacillus spp.	*Coryneform*
Proportion isolates, %[a]		
Heated at 63 °C/30 min	54	46
Heated at 80 °C/10 min	61	37
Enzyme activity, %		
Lipolytic only	0	0
Proteolytic only	34.1	3.3
Lipolytic + proteolytic	37.0	10.0
Phospholipase	80.4	0
Tri-butyrin hydrolase	16.8	20.0
Inactive	12.1	66.7
No. isolates	316	30

[a] No Gram-negative organisms were found.

extracellular enzyme activity necessary to induce spoilage. Thus they constitute a significant threat to the shelf-life of pasteurised product.

9.4 Raw milk enzymes

As reported above, the bacterial floras of milk are associated with extracellular enzyme activity which can lead to spoilage of milk and milk products. However, bacterial enzymes are not the only enzymes present in raw milk. Bovine milk is a biologically active product and around 50 different enzyme activities have been reported in clean, freshly drawn milk. Fortunately, only two of these native enzymes have a substantial impact on the quality or shelf-life of milk and milk products. Therefore we will consider only native enzymes with relevant activity.

9.4.1 Lipoprotein lipase

Milk lipase is a lipoprotein lipase that catalyses the breakdown of milk triglycerides to produce free fatty acids (FFAs). Some of these FFAs have low organoleptic thresholds and produce odours and flavours that are described variously as rancid, bitter, soapy or unclean. The purified enzyme is relatively unstable and can be inactivated by heat, ultraviolet light, acid or oxidising reagents. In milk, the association of the enzyme with casein affords some protection but it is generally accepted that the enzyme is almost completely inactivated by high-temperature short-time pasteurisation (i.e. heat treatment at 72 ºC for 15 s). In milk, the enzyme is not normally active since the potential substrate – milk fat droplets – is encapsulated by MFGM.

Two distinct types of lipolysis by lipoprotein lipase are recognised. When freshly drawn milk is found to be rancid the condition is referred to as spontaneous lipolysis and is influenced by stage of lactation, season, diet and plane of nutrition. Nevertheless, spontaneous lipolysis is not a determinant of shelf-life because the fresh milk is unacceptable.

On the other hand, induced lipolysis can lead to spoilage of products which have not been heat treated. The key factor for expression of enzyme activity is damage to the MFGM. Two common types of damage occur – first, the membrane may be damaged by physical means such as foaming, agitation or homogenisation; and second, the integrity of the membrane may be prejudiced by temperature cycling. In all cases, the end result is similar: lipolysis proceeds. Thus products which contain active lipase must be treated with extreme care.

9.4.2 Plasmin

Although more than one proteinase has been identified in raw milk, the major proteinase is a serine proteinase with trypsin-like activity called milk plasmin. At acid and neutral pH, the enzyme is stable to pasteurisation but, at alkaline pH, it is rapidly inactivated. Some plasmin activity resists UHT processing (heat

treatment at 140 ºC/3 s). Nevertheless, the occurrence of plasmin is associated with physiological conditions in which the tight junctions in the basal membrane of the mammary gland are 'leaky' and allow some passage of blood components into the milk. For example, in very early lactation, very late lactation and when disease is present in the udder, abnormally high concentrations of plasmin are found in milk. Provided plasmin levels are low in milk, problems will not be manifest in short shelf-life products. However, even modest levels of proteinase activity may be deleterious in long-life products. This aspect of proteinase activity will be discussed later.

9.5 Control of the quality of short shelf-life products

Short shelf-life products are those with a normal shelf-life of three weeks or less. Such products include pasteurised milk and cream, cottage cheese and yoghurt. A range of dairy desserts is also now available. The changes that occur in fresh products after manufacture are associated with physical separation of phases and with the growth of microorganisms. Chemical changes, the action of raw milk enzymes and pathogens, have no significant effect on the shelf-life of fresh dairy products. Physical separation, i.e. creaming, may be a minor consideration and is controlled by reducing the fat globule size by homogenisation. However, the main limitation on shelf-life of fresh dairy products is spoilage by bacteria, moulds and yeasts that grow at refrigeration temperature (< 8ºC).

9.5.1 Pasteurised milk and cream

The shelf-life of pasteurised milk and cream is governed by the same factors. Historically, shelf-life was limited by the ingress of Gram-negative spoilage bacteria after the pasteurisation process. This problem is now universally recognised and is under strict control. Nevertheless, once the Gram-negative contamination is excluded, steps must still then be taken to moderate the outgrowth, albeit slow at refrigeration temperature, of psychrotrophic spore-forming bacteria.

Post-heat treatment contamination

Gram-negative spoilage bacteria pose a risk to shelf-life. These bacteria are completely inactivated by pasteurisation but are regularly found in pasteurised products. They are post-heat treatment contaminants (PHTC). A schematic of processing sequences for pasteurised milk and cream is shown in Fig. 9.1. The most commonly used sequence relies entirely on pasteurisation to reduce the bacterial load and to inactivate enzymes with degradative potential. Provided the process downstream of the heat exchanger is aseptic, Gram-negative psychrotrophic bacteria play no part in spoilage. However, this situation is seldom realised in practice. Most problems arise in the filling line where open containers

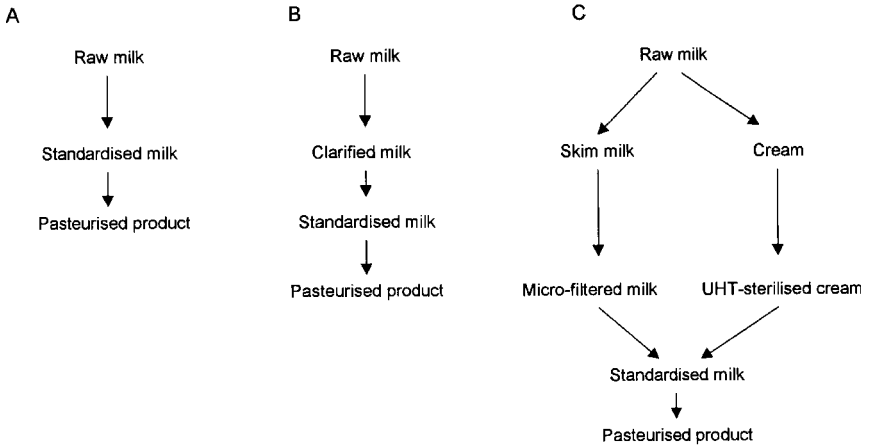

Fig. 9.1 Strategies for manufacture of pasteurised milk

permit ingress of contaminants. This can be kept to a minimum by flooding the filling line with a curtain of sterile air. Nevertheless, disruption of the high-speed packaging line by physical misalignment of containers is inevitable. When this occurs, operator intervention is inevitable and the integrity of the aseptic environment is breached. The key to limiting PHTC lies in stringent exclusion of contamination during the filling and packaging operations. In particular, it is essential to control the number of stoppages on high-speed lines.

Measurement of the extent of PHTC is not straightforward. The number of contaminating bacteria required to induce spoilage depends on the storage temperature of the product. During storage at 8 ºC, ten colony-forming units (cfu) per litre of a typical pseudomonad would reduce shelf-life by several days. Because of the difficulty of enumerating low numbers of bacteria, pre-incubation techniques have been introduced to enhance the process. A necessary prerequisite for success is that the growth of Gram-positive organisms is inhibited during the pre-incubation to allow selective growth of the Gram-negative flora. Methods developed in our laboratories use a cocktail of inhibitors (penicillin, crystal violet and nisin) to inhibit the growth of Gram-positive bacteria during pre-incubation at 21 ºC for 24/25 hours. After pre-incubation the extent of PHTC may be assessed by enumeration of bacterial numbers using ATP-photometry (rapid), visual counting (rapid), impedimetry (slow) or by plate-counting (slow). The pre-incubation step is rate-limiting and the overall measurement takes at least 25 hours. Nevertheless, routine estimation of the extent of PHTC is an essential tool for quality control.

Heat-resistant organisms

Provided PHTC is absent, the shelf-life of pasteurised milk and cream is anticipated to be at least eight to ten days at storage temperatures in the range 6–8 ºC. Outgrowth of spore-forming bacteria (mainly *Bacillus* spp.) forms the

ultimate limitation on shelf-life. Because these bacteria are not inactivated by pasteurisation and can grow, albeit slowly, at refrigeration temperature, three strategies have been explored to control their growth:

1 Destruction of spores by heat treatment.
2 Control of growth by low-temperature storage.
3 Reducing the number of spores in milk.

The simplest method of reducing the numbers of bacterial spores in milk is to increase the severity of pasteurisation. Unfortunately, spores are not effectively destroyed until temperatures in excess of 110 ºC are employed. Typically, heat treatment at 120 ºC for 30 s will destroy almost all psychrotrophic spore-forming bacteria. However, this severe treatment induces flavour changes in the product and reduces its appeal to the consumer. The effect of heating temperature on the sensory character of milk has been explored in the laboratory and flavour change is detected once the heating temperature exceeds 82 ºC (15 s hold). As a result, high-heat treatment is not often used for extending the shelf-life of liquid milk or cream.

Although many *Bacillus* spp. grow at refrigeration temperature, growth is slow. Significant extension of shelf-life can be achieved by storing product at or below 4 ºC throughout its shelf-life. This condition is readily achieved at the dairy and in the distribution chain but is likely to be ignored by retailers and customers. Despite scientific and technological advances leading to improved milk quality, the shelf-life of the product can easily be spoiled by temperature abuse.

The best strategy to control spoilage of milk by spore-forming bacteria is to reduce the number of spores in the raw milk supply. This objective can be achieved on the farm by implementing a detailed protocol for the milking operation, e.g. washing and drying of the udder before milking and the use of teat disinfectant have significant effects. Spores can be removed from milk at the processing factory by high-speed centrifugation. The separation exploits the density difference between the spore and the milk serum. However, the process is not absolute and clarifiers and bactofuges – specially designed to remove spores – achieve an efficiency of ca. 95% in a single pass. The equipment is situated upstream of the pasteuriser (e.g. sequence B in Fig. 9.1). Inclusion of a bactofuge in the processing line might extend the shelf-life of pasteurised milk by up to three days. However, there is an inevitable increase in processing cost and the waste stream from the bactofuge or clarifier may be as high as 5% of the raw material. These costs must be offset against the further extension in shelf-life by three days.

Another method of removing bacterial spores from raw milk is to employ membrane filtration. Spores (and vegetative bacterial cells) are readily removed from skim milk using cross-flow ultrafiltration with ceramic membranes with a nominal pore size of 1.4 μm – typically a five log-cycle reduction in bacterial count is achieved. Unfortunately, a proportion of the native milk fat globules is similar in size to bacteria and must be removed by centrifugal separation before

the microfiltration step. The cream portion is heat-treated independently. A typical processing sequence (c) is shown in Fig. 9.1. It is claimed that a shelf-life in excess of 21 days can be attained by application of this process. Notwithstanding this substantial increase in shelf-life, the added production cost and complexity of processing cast doubt on the viability of the method.

Although extension of the shelf-life of milk or cream is undoubtedly of benefit to the retailer, present technology has already increased the shelf-life of pasteurised products to such an extent that the consumer may safely buy fresh products on a weekly basis. A guaranteed shelf-life of two weeks blurs the concept of 'freshness' and consumer resistance may develop.

9.6 Yoghurt and fermented milk

Yoghurt and fermented milk are inherently safe. A milk base, usually fortified with protein, is severely heated to denature the whey protein and inoculated with a lactic acid starter. The starter converts lactose to lactic acid and, as a result, the pH of the mixture falls. Several concurrent changes take place – calcium phosphate is solubilised, the integrity of the casein micelles is weakened and, as the isoelectric point of the protein (pH 4.6) approaches, a gel is formed. The yoghurt is then cooled to inhibit further growth of starter. The combination of severe heat treatment, low pH and a dense population of living starter bacteria (typically 10^7–10^9 cfu ml^{-1}) inhibit growth of spoilage bacteria. Nevertheless, yeast and mould may thrive under these conditions and can spoil the product. Precautions to exclude their ingress follow the same principles as avoidance of PHTC described for milk and cream. Notwithstanding these minor problems, yoghurt may deteriorate during storage owing to fermentation continuing after the manufacturing process is complete. The product continues to develop acidity and syneresis may occur with the formation of an unsightly layer of serum. This limits shelf-life but may be avoided by prudent selection of starter bacteria that 'stop' when the product is cooled.

9.6.1 Cottage cheese

Cottage cheese is a minor dairy product but has a high added value. It is manufactured by a process in which a curd is formed, annealed and then coated with a cream dressing. The curd is made by acidification of skim milk by lactic starter bacteria (some rennet is added but this is not the primary cause of clotting). After the curd is cooked and washed, a cream dressing is added, together with fruit, herbs, or spices in some cases.

The shelf-life of the product is essentially determined by the microbiological quality of the cream dressing and microbial status of the other additives, as well as their pH. Particular attention must be paid to the quality of the water used to wash the curd. The factors which affect the shelf-life are similar to those found for other pasteurised milk products. PHTC can be enhanced if the additives –

herbs, etc. – are not properly treated before addition. The problems associated with PHTC can be ameliorated by culturing the cream dressing with lactic acid starter. The resultant drop in pH effectively inhibits growth of most commonly occurring Gram-negative rods. However, yeast and mould can grow at the acid pH values achieved and must be strictly controlled.

9.7 Factors influencing the stability of long shelf-life products

The stability of short shelf-life dairy products depends on the moderation of the growth of and subsequent degradation by spoilage microorganisms. In contrast, the shelf-life of intermediate and long-life dairy products is largely determined by enzymic degradation or by chemical deterioration. In this section, degradative enzymes in dairy products, their heat resistance, methods of detection and strategies for inactivation are considered.

9.7.1 Heat-resistant enzymes

A notable feature of the spoilage bacteria found in raw milk is their almost universal ability to produce extracellular degradative enzymes. While the bacteria – mostly Gram-negative psychrotrophs – are readily killed by pasteurisation, such heat treatment has little effect on the extracellular degradative enzymes. In this section the effect of UHT processing, a heat treatment designed for sterilisation, on proteinase, lipase and phospholipase activity will be discussed. UHT treatment represents the most severe heat treatment applied to dairy products other than those like evaporated milk and sterilised and clotted creams which are in-container sterilised.

An overwhelming proportion of the psychrotrophic floras found in milk produce heat-stable enzymes. Typical results from work conducted in our own laboratories are shown in Table 9.8 for the residual proteinase, lipase and phospholipase C activity found after treating cell-free supernatants at 140 ºC for 5 s. Of the bacterial types examined, only *Acinetobacter*, *Aeromonas* and *Bacillus* spp. had residual activities below 10%. The fluorescent pseudomonads that predominate in the flora of refrigerated milk and are enzymically active had residual enzyme activities ranging between 14 and 51%. In addition, very high

Table 9.8 Residual enzyme activity after heat treatment

	Residual enzyme activity (%)	
Type of degradation	Pasteurisation	UHT treatment
Lipolysis	59	31
Proteolysis	66	41
Hydrolysis of phospholipid	30	21

residual levels of phospholipase C survived UHT treatment. When enzymes from 46 isolates exhibiting both proteolytic and lipolytic properties were compared, there was little difference in the ability of the enzyme to withstand either pasteurisation or UHT sterilisation. These results are typical of those found throughout the world for enzymes from ex-farm milk, e.g. enzymes isolated from ex-farm milk in New Zealand were equally heat-resistant.

The effect of stage of growth cycle on the thermostability of cell-free extracts from eight cultures of psychrotrophs grown for 2 to 3 days at 30 ºC and at 30 ºC for 14 days has been studied. At the extremes of the logarithmic phase of the growth cycle, the heat stability of the enzymes after pasteurisation or UHT treatment was the same. Furthermore, there was little difference in the thermostability of extracellular protease produced by psychrotrophic cultures grown at temperatures ranging from 2 to 30 ºC. Therefore, the spoilage bacteria found in raw milk have the potential to produce extracellular degradative enzymes irrespective of the conditions of growth. Once produced, these enzymes are not destroyed by simple heat treatment. Consequently, these enzymes play a key role in the spoilage of intermediate and long shelf-life products.

9.7.2 Potential methods of reducing the effect of heat-stable enzymes

Significant inactivation of extracellular proteinase and lipase is observed above the optimum temperature for maximum activity. For example, heat treatment at 55 ºC for 1 h promoted a marked reduction in proteinase activity. The most efficacious combination was UHT treatment followed by low-temperature inactivation at 55 ºC for 1 h. Proteinase and lipase activity were reduced by this treatment to 17 and 7% respectively of their original value. Nevertheless, the logistics of holding large volumes of sterile milk for extended periods has precluded the application of these findings. The overwhelming conclusion to be reached is that, once extracellular enzyme activity is present in a product, it is almost impossible to inhibit its action. Attention must therefore be focused on detection of the degradative ability.

Methods of detection of extracellular enzyme activity

The simplest method of detecting extracellular enzyme activity is to use a diffusion assay. Agar or another suitable gel is cast with an indicator component and cell-free supernatant is inoculated into a well cut in the agar. Enzyme activity is then detected either as a zone of clearing or by a colour reaction with a suitable indicator compound. In our experience, skim milk agar is an effective indicator medium for proteolytic activity. Enzyme activity is detected as a zone of clearing or a zone of precipitation around the agar well. The concentration of proteinase present is directly proportional to the square of the true zone radius (that is, allowing for the diameter of the well) and there is also a relation between the area cleared and incubation time. A similar principle may be used for detecting lipase activity using tributyrin agar as the substrate. Furthermore, a high correlation exists between the ability to hydrolyse tributyrin and hydrolysis

of butterfat. Diffusion using egg yolk emulsion in a blood agar base is also effective for detection of phospholipase activity.

Various colorimetric assay methods have also been developed based on liberation of a dye from a substrate by the enzyme action. The use of hide powder azure for proteinase detection is an apparently robust technique for use in quality control laboratories. It is reported to be sufficiently sensitive to detect the proteinase activity of 2.5×10^6 cfu ml^{-1} of an enzymically active pseudomonad grown in refrigerated whole milk. An equally robust colorimetric assay for lipase is based on the hydrolysis of colourless β-naphthol-caprylate to yield β-naphthol which is readily complexed with an azo dye.

9.8 Control of the stability of long-life milk products

In response to consumer pressure for more sophisticated and diverse food, the number of intermediate and long-life dairy products in the market place has increased significantly. As a result, it is impractical to give comprehensive details of the factors controlling the shelf-life of every product in this class. Moreover, generalisations are dangerous because of the specificity of many shelf-life problems. To illustrate the diversity of the problem, a range of specific examples has been selected and the key factors controlling shelf-life are outlined for each type of product in turn.

9.8.1 Butter and spreads

Preservation of milk fat by conversion into butter involves separation of milk into cream and skim milk. The cream is subject to phase inversion by physical disruption of the natural MFGM. When the membrane is damaged, the fat globule surfaces lose their stability in the aqueous phase and coalesce (or churn) to form fat-rich granules. After washing with clean water to remove milk solids, the granules are physically worked into a uniform mass that is called butter. Butter should comprise at least 80% fat and contain less than 16% water in the form of very small, evenly distributed water droplets.

Control of shelf-life of butter is multifactorial. Raw material quality is especially important because the droplets of aqueous phase entrained in the fat phase have the potential to support bacterial growth. Consequently, heat treatment of raw milk must be efficient and levels of heat-stable extracellular enzyme must be low. The psychrotrophic count in the raw milk should not exceed 5×10^6 cfu ml^{-1}. After heat treatment, the total bacterial count in the cream should be $<10^3$ cfu ml^{-1} with fewer than one yeast, mould or coliform organism detected per ml. Furthermore, dispersion of the water droplets within the butter must be maintained. Coalescence of droplets to form free water offers the potential for rapid spoilage even when contamination is slight.

Even under optimum production conditions the shelf-life of butter is limited at room temperature. Butter is best stored at $-25\,^{\circ}C$ and sweet cream, salted

butter keeps satisfactorily for several years. Oxidation is an important feature of shelf-life. The problem is not as great as might be expected because of the low temperatures employed for prolonged storage. Moreover, slightly oxidised flavours are expected by many consumers and are disguised by salt addition. Nevertheless, shelf-life can be usefully prolonged by exclusion of oxygen during packaging and during storage. Various barrier types of wrapping have been employed with success.

Dairy-based spreads are manufactured by margarine-based technology and may have fat contents from 37.5 to 76.3%. Usually the amount of butterfat present is low but, in contrast to butter, high levels of milk protein may be incorporated to stabilise the product. Because of the high water content, the water in oil emulsion may have limited stability and this limits shelf-life – especially when the product is subject to temperature cycling. A further problem, associated with the large increase in water content is the potential for bacterial growth and spoilage. As a result, the shelf-life of spreads is often limited, especially at storage temperatures above 4 °C or when preservatives are not incorporated in the blend.

9.8.2 Dried milk products

Preservation of milk by drying involves heat treatment to reduce bacterial load, concentration by evaporation to about 45–52% solids before atomisation into a stream of hot air. The milk droplets are converted into a powder within a short time (5–30 s) and are separated from the air-stream by cyclones or bag filters. The essential feature of spray drying is that the moisture content of the powder is reduced to a level at which no bacterial growth occurs and there is little damage to the functionality of the milk components.

Shelf-life is determined by three factors: quality of the raw material, the drying process itself and the conditions under which the powders are stored. The heat treatment applied during processing ensures that the final bacterial load of powder is low. For all but low-heat powders the bacterial load bears little relation to raw milk quality. Nevertheless, heat-resistant, extracellular enzymes are not destroyed. The bacterial count in the raw milk should not exceed a level at which extracellular enzymes from psychrotrophic bacteria can initiate degradation – this threshold is about 2×10^6 cfu ml^{-1}.

The second factor to influence the shelf-life of dried milk is the nature of the drying process. It has been found that the extent of heat treatment applied to the milk during powder manufacture (measured by the extent of whey protein denaturation) is associated with a reduction in the solubility of dried skim milk during storage for six months at 30 °C.

The final and most important factor controlling shelf-life of dried milk is the condition in which it is stored. Although storage conditions are more critical for whole milk powder than for its fat-free analogue, the moisture content of all powders must be maintained in the critical range of 2–4% if deterioration is to be avoided. Skim-milk powder stored in barrier bags at normal ambient

temperature has a shelf-life of at least one year and the deterioration observed during storage for a further year is slight. However, if moisture penetrates the powder rapid deterioration occurs even when enzyme activity is absent. The main cause of deterioration is associated with protein/lactose interaction. Such deterioration is exacerbated by storage of powder at high temperature.

In the case of dried whole milk, autoxidation of milk fat affects shelf-life. Where addition of antioxidants is permitted, a useful extension of shelf-life can be achieved but their use is associated with marked consumer resistance. To ameliorate the problem, dried whole milk is given a very severe heat treatment during manufacture. Such heating results in the liberation of free sulphydryl groups in the proteins and these reactive groups compete with lipids for oxidants. In addition, the oxygen level of the powder may be reduced by replacing the air with an inert gas but special rigid packaging must be used, adding significantly to the cost.

In summary, control of moisture content and protection from exposure to oxygen hold the key to extending the shelf-life of powders. Because all the reactions associated with powder deterioration are temperature sensitive, where possible powder should be stored in the cold (4–8 °C) and out of direct strong light.

9.8.3 In-can sterilised cream

In contrast to butter and dried milk, the shelf-life of sterilised cream is determined by chemical reactions involving minerals and protein. Bacteriological and enzymic deterioration are unusual in products sterilised in cans because of the severity of the heat treatment. Almost all the sterilised cream (23% butterfat) manufactured at present in the UK contains the sodium salts of orthophosphate and those of carbonate and citrate. These stabilisers inhibit calcium–protein interaction with considerable success. In addition, storage at refrigeration temperature has beneficial effects. Serum separation is almost completely inhibited and viscosity is increased. There is little penalty in terms of cream texture if storage is carried out at 6 °C but severe problems can occur if sterilised cream is frozen.

9.8.4 Sterile concentrated milk

Full cream evaporated milk is an important commodity in terms of the international trade in dairy products and is usually made to contain 9% fat and 31% total solids. Control of quality must take into account: (a) cream separation during storage, (b) age-gelation and (c) deposition of calcium salts. Cream separation is avoided by manipulation of the homogenisation conditions during manufacture. Homogenisation should be as severe as possible without prejudicing heat stability. Age gelation is inhibited by application of a severe heat treatment to the milk before concentration and by addition of mineral stabiliser. Finally, mineral deposition is moderated by limiting the use of mineral stabiliser. Where very extended shelf-lives are required, the addition of

small amounts of lecithin to the concentrate can promote a useful increase in stability. While manufacture of in-can sterilised concentrated milk is well established and the control factors are known, successful manufacture of the equivalent UHT concentrate is more difficult. UHT sterilised concentrate is very susceptible to premature age-gelation and stringent conditions must be applied to the raw material to avoid contamination with bacterial proteinase.

9.8.5 Sterilised UHT processed milks and creams

UHT treatment is based on the principle that the thermal characteristics of bacterial destruction are substantially different from the rates of chemical reaction. By increasing the temperature of heat treatment and reducing exposure time (e.g. to 4 s at 142 °C), equivalent bacterial lethality can be maintained to that used in heat sterilising canned milk or cream but with a significant reduction in chemical interaction such as Maillard browning.

In UHT milk, the main cause of premature spoilage is a result of proteolytic action. Two sources of heat-stable enzyme have been implicated. Although plasmin has been implicated, its concentration in mid-lactation milk from normal, healthy cows is low and it is likely to be of secondary importance in spoilage. On the other hand, enzyme from psychrotrophic bacteria is important and the general rule is that product should not be manufactured from raw milk in which the bacterial load exceeds 10^6 cfu ml^{-1}.

The shelf-life of UHT cream is substantially shorter than that of milk even when proteolysis is absent. For UHT single cream (18% butterfat), the main customer complaint is associated with feathering when the cream is added to hot coffee. The problem has been identified as one of calcium-induced aggregation and can be ameliorated, but not overcome, by the careful use of mineral stabilisers that interact with calcium. In commercial practice, additions of sodium carbonate and tri-sodium citrate have been found to extend the period before the onset of feathering in hot coffee. Storage temperature also has a significant effect on shelf-life and, although not necessary for bacteriological stability, refrigeration promotes a marked improvement in shelf-life.

9.8.6 Cream liqueurs

Cream liqueur is a class of compound beverage containing a substantial proportion of dairy ingredients, e.g. 16% butterfat and 3% sodium caseinate. Shelf-life is determined by the onset of gelation, by creaming and fat plugging and, infrequently, by deposition of calcium citrate-rich deposits. The liqueurs are made by emulsifying cream in a solution of sodium caseinate to yield a dispersion of fat particles. Sugar, colour and flavour are then added and the mixture treated by severe homogenisation to obtain a very fine dispersion of the fat. Creaming during storage is related to the efficiency of homogenisation and it has been established that, by ensuring that all fat particles are less than 0.8 μm in diameter, creaming does not occur on extended storage.

The second problem which may limit the shelf-life of liqueurs is the onset of gelation. This defect is associated with calcium interactions with milk protein and can be avoided by the following:

- Addition of trisodium citrate.
- Reduction of the calcium content of cream.
- Use of anhydrous milk fat as a lipid source.
- Use of the citric acid ester of glycerol mono-stearate to replace some of the protein present for emulsification.

The third defect of cream liqueurs is associated with the use of trisodium citrate as an inhibitor of age-gelation. On prolonged storage, crystalline particles may form a deposit, largely of calcium citrate. This salt becomes progressively less soluble as temperature increases and its formation can be slowed by reducing processing temperatures after citrate addition or by reduction of the concentration of added salt.

9.8.7 Cheese

Cheese is a family of products ranging in shelf-life from several days to many years. It is thus difficult to generalise and, for this reason, only a single type – Cheddar – representing the most popular variety consumed in the UK will be considered here. The standard of identity limits the moisture to an upper limit of 36% and the fat in dry matter to a minimum of 48%. Nevertheless, the 'Cheddar' label spans a wide range both in terms of flavour and texture. The major classification is on the basis of maturity. 'Mild' Cheddar may have been matured for only 3 months while 'extra-mature' cheese may be 18–24 months old. The complexity of cheese lies in the fact that it is a biologically and chemically active product. Manufacture is simple in theory but complex in practice. A lactic acid starter culture is added to heat-treated milk and, after a short ripening period during which the pH drops, the milk is coagulated by addition of rennet. The active ingredient of rennet is the enzyme chymosin that cleaves the κ-casein specifically. This action results in destabilisation of the micellar casein in the presence of calcium – in large excess in acidified milk – and a protein gel forms in which milk fat globules are entrapped. The coagulum or cheese curd is then cut into small pieces, and syneresis is encouraged by scalding, stirring and piling of the curd. After further curd processing and salting, the curd is pressed.

The pressed curd is then ripened by storage in permeable packaging at between 6 and 12 °C (sometimes complex temperature profiles are used). During ripening, simultaneous reactions occur which lead to breakdown of the curd texture and development of flavour. Proteolysis is the key reaction controlling maturation rate but its origin and control is complex. Lipase action plays a secondary, but probably underrated, role in flavour development. Clear guidelines for the relation between bacterial load in raw milk and off-flavour development associated with excessive lipolysis in cheddar cheese have been

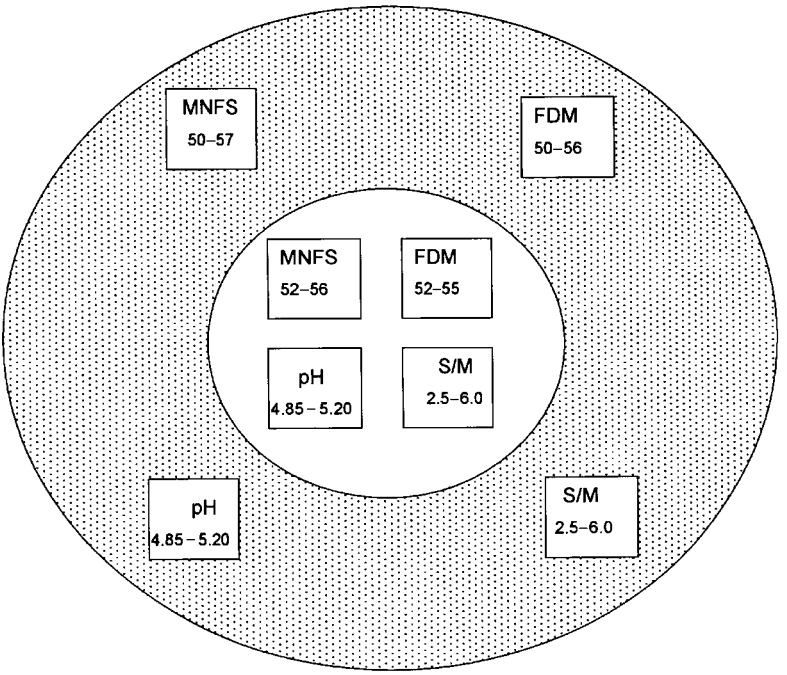

Fig. 9.2 Compositional range for optimising quality of Cheddar cheese. Adapted from Gilles and Lawrence. MNFS = moisture in non-fat solids; FDM = fat in dry matter; S/M = salt in moisture; pH = pH of cheese. Inner ring = premium grade; outer ring = first grade.

established. Rancid flavours developed in cheese after only 16 weeks' storage when the psychrotroph count of the raw milk used for manufacture reached a threshold of between 2×10^6 and 8×10^6 cfu ml^{-1}.

The maturation rate of cheese depends not only on the amount and type of enzyme present but also on the composition of the product, because composition determines the environment in which enzyme (and subsequent chemical) activity can be expressed. Guidelines proposed by the New Zealand Dairy Research Institute relate cheese composition to the ultimate quality of long-hold mature product and these have stood the test of time. The compositional ranges for first and premium grade cheese are shown schematically in Fig. 9.2. Four factors are important: salt in moisture (S/M), moisture in non-fat solids (MNFS), fat in dry matter (FDM) and pH. It has been found both in New Zealand and in the UK that by careful control of cheese composition, optimal quality and shelf-life can be attained.

Although space does not permit detailed consideration of other cheese varieties, similar principles apply, i.e. shelf-life is controlled by initial composition and by subsequent proteolysis. Flavour defects are usually associated with either residual enzyme activity derived from psychrotrophic bacteria or by a gross imbalance in initial composition.

9.9 Summary

The examples given illustrate the complexity of control of the shelf-life of intermediate- and long-life dairy products. Each type of product is associated with specific problems and the critical control points may be different for apparently similar defects. Some defects, such as those associated with enzymic degradation, are common to a range of goods. Clearly, raw material quality is paramount. The available evidence has implicated the heat-stable extracellular enzymes of the common psychrotrophic bacteria found in milk with both proteolytic and lipolytic defects. Manufacturers of long-life products would therefore be well advised to ensure that the psychrotroph count is not allowed to exceed a level of 10^6 cfu ml^{-1} if they wish to avoid potential problems.

In contrast to short shelf-life products, chemical reactions can limit the durability of long shelf-life products. High fat products are prone to oxidation and, short of excluding oxygen and controlling storage temperature, there is little scope for significant alleviation of the problem. The other major chemical reaction limiting shelf-life in several products is calcium-induced aggregation of milk protein. Unlike fat oxidation, control of this problem is often possible. Modifications to processing conditions, especially those involving heat treatment and homogenisation, are often successful and the addition of the appropriate mineral stabiliser can often be effective.

Cheese poses a particular problem for not only is composition important but the starter culture and coagulant used significantly affect the rate of ripening. It is perhaps inappropriate to define a shelf-life for cheese since many varieties are acceptable to the consumer for a large part of their maturation period – albeit with suboptimal flavour or texture.

In conclusion, no panacea can be provided for control of the shelf-life of dairy products. Each must be considered in turn and, as new products are developed, it is anticipated that further problems will emerge.

9.10 Acknowledgement

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10

Confectionery products

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10.1 Introduction

Confectionery products, in comparison with other foods, are generally stable and have relatively long shelf-lives. The high level of sugar present in confectionery products makes them less prone to microbiological spoilage. Therefore, physical and chemical changes, which lead to a deterioration of flavour, texture, colour or odour of the product, are the main causes of spoilage. However, the shelf-life of some confectionery products is shortened by the presence of ingredients that are inherently unstable, e.g. cream, making them prone to microbial spoilage. The level and type of microbial spoilage of a food product can be predicted to a large extent by its water activity (a_w). Food products with a water activity lower than 0.75 will be stable against microbial spoilage[1] and could be said to be ambient-stable products. Since most confectionery products have a very low water activity, they are able to be stored under ambient conditions.

10.2 Factors affecting shelf-life

10.2.1 Product composition

The shelf-stability of confectionery products, as in the case of all food products, is governed by their composition. Although the high level of sugar in these products imparts significant microbial stability in most cases, microbial spoilage can occur if the products contain ingredients that are prone to microbial spoilage. The presence of other ingredients, such as fats, will make the product prone to chemical and physical changes.

Since the presence of sucrose or other sugars is common to all standard confectionery products, some deterioration related to the changed state of the

sugars can be considered as a common problem occurring in most confectionery products. The stability of some confectionery products is directly related to the stability of particular ingredients in the products. An example of such ingredients is lactose, which when incorporated into confectionery, can cause the premature crystallisation and graining of products such as toffee. There are, of course, many ingredients that are added to confectionery products to increase their stability. Examples of such ingredients include antioxidants to minimise oxidation, humectants to retain moisture, and emulsifiers to reduce separation of water and oil from products.

10.2.2 Product structure

Product structure determines the textural attributes of a product. Therefore, a study of the microstructure of a product can help us to understand how the ingredients and processing parameters affect the sensory characteristics of the product and also how these influence product stability. Different textural characteristics can be obtained in a product based on the same recipe by changing the processing conditions to bring about a change in the structure of the products. A simple example of such a change is that of toffee, which changes from being a chewy product to being a product with a very short texture (fudge) through the physical beating of the mix, which causes crystallisation of the sugar. Since the toffee product has one phase and the fudge two separate phases (syrup and crystalline), the equilibrium relative humidities (ERH) of the two products are very different. The ERH is related to the concentration of sugars in the syrup phase in the case of fudge. The stability of the products under a standard set of storage conditions will therefore be different.

Another example of structural influence on shelf-stability can be found in the case of aerated confectionery. Aerated products have a lower density than non-aerated products and are often more fragile to handle. Air can become trapped in the porous structure of any aerated product, which can then accelerate oxidative changes (e.g fat rancidity and oxidation of vitamins) and thereby reduce the shelf-life of the product. In the case of very sensitive products, oxidation can be minimised by replacing the air with nitrogen or carbon dioxide during the stage of beating. However, it should also be borne in mind that these gases can also cause some tainting of the products. Nitrogen can dissolve in the fat phase, and carbon dioxide is soluble in water, which can then lead to a change in the flavour of the product.

Most of the changes in the textural attributes of products during storage, referred to as ageing, are caused by structural changes in the product. The subsequent product-specific sections cover this subject in more detail.

10.2.3 Moisture migration and equilibrium relative humidity

The driving force for moisture absorption from the environment by the product or moisture migration within a multi-component product and/or the environment

Table 10.1 Typical equilibrium relative humidity values for confectionery products

Type of confection	ERH (%)
Biscuits/wafers	Less than 30
Boiled sweets	20–30
Butterscotch	Less than 40
Caramels	45–55
Gums and pastilles	50–65
Liquorice paste goods	53–66
Fudge	60–70
Nougat (grained)	60–70
Jellies	65–75
Marshmallow	65–75
Fondant centres	75–80
Plain chocolate	70–72
Marzipan	68–84
Coconut ice	73–76

Adapted from Lees.[2]

is determined by the differences in the ERH of the individual components and the relative humidity of the environment. ERH is the humidity at which the product neither loses nor gains moisture from the environment. The greater the differences in the ERH between adjacent components, the greater the tendency for moisture migration, which leads to quality deterioration, particularly textural changes. The ERH, which is related to a_w ($a_w \times 100$ = ERH), of a range of confectionery products is given in Table 10.1.

Moisture migration can be minimised by formulating the adjacent components of products to have similar ERH values. However, this may sometimes mean an unacceptable change to the product characteristics. In such cases, where it may be difficult to change the formulation, moisture migration needs to be reduced by applying a physical barrier, to stop the movement of moisture within the product. Fats and fatty coatings such as chocolate have been used as moisture barriers. The influence of composition of special fat blends on effectiveness as moisture barriers has been studied by Talbot.[3] Fats with high solid fat contents were found to be very good moisture barriers. The addition of sugar to these fats improved their performance. Although liquid oils were shown to be ineffective as moisture barriers at ambient conditions, it is possible that they could exhibit moisture-barrier properties at freezer temperatures. The temperature at which barrier fats are applied was found to be critical to their performance. A fat applied at a temperature of 50 °C was shown to provide a better moisture barrier than when applied at 40 °C or 20 °C, which, when combined with rapid cooling, produced imperfections in the barrier, allowing moisture migration. This study also showed that, at solid fat contents of 20–60%, shorter-chain fats (C8–C14) were more effective than long-chain fats. Based on

these findings, commercial speciality fats have been developed to be used as moisture barriers in products.

Gums have also been used to coat sensitive ingredients against both moisture and fat migration. A common example is the use of gum arabic solution, to coat nuts prior to chocolate panning or adding into multicomponent confectionery bars. Edible coatings consisting of various hydrocolloids are also being developed for the same purpose.

10.2.4 Storage conditions

The ERH of a product indicates its tendency either to absorb or to lose moisture, depending on the relative humidity (RH) of the environment. The humidity of air in cool temperate climates will range between 45 and 55%, but can be as high as 80% in tropical regions. Products with a higher ERH than the RH of the environment will tend to dry out, but those with a lower ERH than the RH of the environment will absorb moisture during storage. From Table 10.1 it can be seen that products such as fondant and marzipan will have a tendency to dry out, but products of the other extreme, with low ERH values, such as high-boiled sweets (sugar glass) will be prone to moisture pick-up from the environment. Any such changes in the moisture content during the storage time will lead to major changes in the sensory quality of the product. The choice of correct packaging is vital to minimise moisture transfer between product and the environment.

10.2.5 Packaging

The use of appropriate packaging is most important in maintaining the quality of the products and achieving the required shelf-life. The role of packaging and the factors governing the choice of packaging for any particular product are covered in detail in Chapter 7. The water vapour permeability required for individual confectionery products depends on the ERH of the product. Products such as sugar glass require good barrier properties to minimise moisture pick-up by the products. However, products with high ERH values will tend to sweat if the permeability is too low. The build-up of moisture on the surface in such cases could then lead to mould growth on products.

In addition to the considerations regarding permeability to moisture and oxygen, the requirements relating to pack format and performance on the packing line, such as ability to be twist-wrapped, sealed and printed, are important factors that will influence the choice of packaging materials for products.

10.3 Chocolate and chocolate products

Chocolate is composed of cocoa mass, sugar, cocoa butter, lecithin and, in the case of milk chocolate, milk solids. In certain countries, other vegetable fats may

Table 10.2 Solid fat content of cocoa butter

Temperature (ºC)	Solid fat (%)
0	83
20	83
25	76
30	55
35	1
40	0

also be permitted. Cocoa butter (CB) is the most important fat present in chocolate and is the most expensive of all the bulk ingredients used in chocolate. Cocoa butter has a unique composition, its fatty acids comprising about 25% palmitic (C16), 36% stearic (C18) and 35% oleic (C18:1), with minor amounts of other fatty acids.[4] The triglyceride composition is simple, being about 12% POP, 43% POS and 35% SOS (where P = palmitic, O = oleic, S = stearic). The triglyceride composition is affected by the origin of the cocoa beans. The percentage of solid fat in a typical CB at different temperatures is shown in Table 10.2. The level of liquid fat present in a product is significant not only in determining the sensory (particularly textural) quality but also in influencing the shelf-life of chocolate products. The fast-melting characteristic of CB between 30 ºC and 35ºC is responsible for fast meltdown of chocolate in the mouth. A high solid fat content at body temperature would be perceived as an unpleasant waxy mouthfeel.

The limitation in shelf-life of chocolate products can be due to various deteriorative process. The most common deteriorative change is the development of fat bloom, the causes of which are discussed in section 10.3.1. Apart from bloom, many other deteriorative changes take place during the storage of chocolate products. These include major changes in the sensory attributes, causing the staling of the product. In the case of solid chocolate, these changes are likely to be induced by the changes in the polymorphic state of CB or by rancidity development. However, in the case of enrobed and shell-moulded products, the changes may be driven by migration of moisture and/or fat from the centre component into the chocolate, and vice-versa. Typical shelf-lives for chocolate products, adapted from Martin,[5] are given in Table 10.3.

10.3.1 Fat bloom

The polymorphic nature of cocoa butter affects the processing and the shelf-stability of chocolate products. It is generally accepted that cocoa butter can exist in six polymorphic forms, although many believe forms V and VI to be identical. The relationship between CB forms and classification according to X-ray patterns are shown in Table 10.4, adapted from Willie and Lutton.[6] Forms I to IV are termed as unstable as they have a tendency to convert to the higher

Table 10.3 Deteriorative changes and typical shelf-lives for chocolate products

Product	Major deteriorative changes	Typical shelf-life at temperate conditions* (months)
Plain chocolate bar	Fat bloom, sugar bloom, stale flavour	24
Milk chocolate bar	Fat bloom, sugar bloom, stale flavour	16
White chocolate bar	Fat bloom, sugar bloom, stale flavour	16
Milk chocolate-coated peanuts	Fat bloom, rancidity in peanuts, sugar bloom, stale chocolate	12
Chocolate bars with raisins	Fat bloom, stale chocolate flavour, drying of raisins	12
Chocolate-coated wafer	Staling of wafer, fat bloom, sugar bloom, stale chocolate flavour	12
Chocolate-coated fondant	Fat bloom, sugar bloom, drying out of fondant	18
Chocolate shells with soft caramel centre	Sugar bloom, change in caramel texture, fat migration causing fat bloom	12
Chocolate shells with praline centre	Fat bloom by fat migration, softening of shell, rancidity of nut paste	12

* Adapted from Martin.[5]

Table 10.4 Polymorphic forms of cocoa butter

Form (DSC)	X-ray pattern	Melting point (ºC)
I	γ	17.3
II	α	23.3
III	β'	25.5
IV	β'	27.5
V	β	33.9
VI	β	36.3

forms V and then VI. Although form V will eventually convert to the higher form, form VI, it is termed stable, as this conversion occurs over a long time (12–18 months) at 20 ºC. During the manufacture of chocolate, a tempering stage is necessary to ensure that all the CB crystallises in form V, thereby making the product stable and giving it a high level of gloss and snap.

The crystallisation of fat on the surface of the chocolate is referred to as fat bloom. During storage of a well-tempered chocolate under standard storage conditions, the polymorphic transformation continues, and form V of CB transforms to form VI. This transformation is commonly accepted as being responsible for bloom in chocolates stored under cool ambient conditions. Some believe that cold storage (lower than 18ºC) will prevent this polymorphic transformation of form V to VI, keeping the chocolate free from bloom indefinitely.[7]

In addition to this, two other common causes of bloom in chocolate are the melting and recrystallisation of the fat due to storage at high temperatures, and crystallisation of fats due to incompatibility of CB with other added fats. In the case of chocolate-coated products, particularly with a nut oil-based filling, the migration of oils from the centre into the chocolate coating during storage makes the coating prone to bloom formation. Incorrect processing, such as inadequate tempering and forced cooling during manufacture, can also cause the cocoa butter in the chocolate to crystallise in an unstable form and cause bloom formation.

The progression of bloom on products can be monitored by measuring the surface gloss of products objectively by using a gloss meter or subjectively by comparing the surface with tiles of known level of light reflectance, since bloom is often preceded by a dulling of the surface. However, in some cases, the product can remain very glossy, but show the presence of bloom crystals. Hence, low-power microscopy is useful in confirming the presence of bloom on the surface of samples.

10.3.2 Sensory changes during storage

Although fat bloom is a major problem limiting the shelf-life of solid chocolates, sensory changes occurring during storage are also known to be important in determining their shelf-life. In many cases, the sensory changes precede bloom development. Research work carried out at the author's laboratory has studied the sensory changes in a plain chocolate containing only CB as the fat and milk chocolate based on milk powder as opposed to crumb.[8] These changes were monitored both at 20 ºC/50% RH and under a set of accelerated storage conditions of 28 ºC/70% RH. Changes in the plain chocolate samples stored under the two conditions were monitored at two-weekly intervals up to 12 weeks. The samples stored at 20 ºC/50% RH continued to be monitored at two-monthly intervals up to 12 months. The times to indicate a ten-unit change in the various attributes of the plain chocolate are shown in Table 10.5.

The results of the samples stored at 28 ºC/70% RH confirmed that the sensory changes could precede the onset of bloom (which occurred after four weeks). Similarly, the deteriorative changes (flavour and texture) in milk chocolate were found to be accelerated by storing the samples at 28 ºC/70% RH. As in the case of the plain chocolate, sensory changes were noted prior to the bloom development in samples. Samples were found to decrease in smoothness, lose chocolate and caramel flavours, and develop a stale flavour before the appearance of bloom.

The shelf-life of many commercial chocolate products have also been found to be limited by sensory characteristics. Typical times noted for unacceptable levels of changes are given in Table 10.6.

Nuts incorporated into chocolate are sensitive not only to oxygen but also to moisture pick-up. As they absorb moisture, they develop a soft texture, which causes the product to be perceived as stale. Dried fruit remains fairly stable (two

Table 10.5 Number of weeks to ten-unit change in sensory attributes at different storage temperatures

Product	Sensory attribute	20 °C	24 °C	28 °C	20 °C/28 °C cycling every 12 h
Plain chocolate bar	Surface gloss	26	6	6	8
	Flavour impact	–	–	4	4
	Stale flavour	27	8	4	6
	Crumbliness	–	8	6	5
	Hardness	42	9	10	4
Chocolate-coated marzipan	Surface gloss	28	8	3	4
	Marzipan staleness	–	–	4	10
	Marzipan mouthfeel	17	8	3	4
	Marzipan breakdown rate in mouth	–	–	3	8
	Marzipan texture	16	8	2	5
Chocolate-coated wafers	Surface gloss	28	10	4	8
	Stale flavour in wafer	36	–	2	4
	Stale texture in wafer	36	18	4	8
	Breakdown rate of wafer in mouth	–	13	8	9

Table 10.6 Typical shelf-lives for chocolate products based on sensory changes

Product	Deteriorative changes	Time to change at temperate ambient conditions
Plain chocolate	Stale flavour, change in bitterness	12 months
Milk chocolate bar	Stale flavour	12 months
Milk chocolate coated peanuts	Softening of nuts, stale chocolate	12 months
Chocolate bars with dried fruit	Stale chocolate (dry texture in fruit)	12 months (24 months)

years) when coated with chocolate and does not show significant changes in texture. Loss of chocolate flavour and the development of stale notes in the chocolate component are thought to be related to changes in the crystalline state of the CB. As the CB begins to transform from form V to form VI during storage, it is thought to affect the rate of flavour release from the chocolate. Others relate the development of 'cardboardy' stale flavour to oxidative rancidity.[5] The presence of antioxidants in the cocoa liquor are said to reduce the susceptibility of plain and milk chocolate to oxidative rancidity. The lack of naturally present antioxidants in white chocolate makes it prone to rancidity and very sensitive to light, thus giving it a shorter shelf-life relative to that of milk and plain chocolates. Further research is required to improve understanding of the causes of flavour changes in chocolate during storage.

10.3.3 Sugar bloom

Sugar bloom, or the crystallisation of sugar, on the surface of chocolate products is caused by moisture absorption on the surface. Moisture can be caused by condensation of water on the products, poor storage conditions such as high humidity or, in the case of multicomponents product, moisture movement within the product. The condensation of water on products can occur during the cooling stage of the chocolate, when the cold surface of the product comes in to contact with warm ambient air at the end of the cooler. To prevent this from occurring, the cooled product should always be warmed in a final stage, before leaving the cooler. A product temperature of higher than 13 °C is said to prevent such condensation on the surface of the chocolate.

Chocolate can pick up moisture if stored at high humidity conditions. However, since the ERH of chocolate is about 70%, the humidity of the air needs to be higher than this to cause a pick-up of moisture by the products.

10.3.4 Anti-bloom agents

Many different ingredients are claimed to have an anti-bloom effect and improve shelf-life, including speciality fats, emulsifiers and other more novel ingredients, such as water-soluble fibre.[9] Butterfat (milkfat) has been traditionally incorporated into chocolate recipes to delay the onset of bloom, but has the negative effect of causing softening of the chocolate. The level of addition is kept below 4% to avoid over-softening of the product.[10, 11] High-melting fractions of butterfat are offered as an alternative to overcome the softening.[12–14] Other speciality fats are also available in the form of CBEs (cocoa butter equivalents) for adding to chocolate as anti-bloom agents. Anti-bloom filling fats are also available for delaying the bloom formation on chocolate-coated products. These fats are said to act by migrating with the liquid fat portion from the centre into the coating and stabilising the fat phase in the chocolate. The effectiveness of some of these commercial fats has been tested, and it was shown that the anti-bloom effect of commercial anti-bloom fats is significantly greater than that of butterfat.[9]

The incorporation of anti-bloom fats into chocolate does require some modifications to the processing conditions. Since butterfat slows down the rate of crystallisation of the CB, the seed temperature reached during tempering needs to be lower than that for the CB-based chocolate. Similarly, the incorporation of some of the commercial anti-bloom fats will require modifications to the tempering conditions to account for a change in the rate of crystallisation of the fat.

A wide range of emulsifiers has been studied for anti-bloom effect; however, only a small number have proved to be useful. The emulsification properties are not directly related to the anti-bloom property of the emulsifiers. The best surfactants are said to be those that are solid at room temperature, with a high melting point. Sorbitan monostearate (SMS), sorbitan tristearate (STS), ethoxylated sorbitan esters of fatty acids and lactylated mono-diglycerides have been suggested as having anti-bloom properties in chocolate.[15, 16] Blends of

SMS and ethoxylated SMS have been found to be particularly effective in delaying bloom. Lecithin is also claimed by some to delay bloom progression. However, more research is required to study the effectiveness of emulsifiers relative to that of the anti-bloom fats in delaying bloom.

The fractionation of CB separates the liquid triglycerides, called oleines, from the solid material, called the stearines. The addition of CB stearines into chocolate is also said to improve the anti-bloom property of chocolate, but only by increasing the hardness of the product.[17] Stearine can be made to be particularly rich (over 90%) in SOS, the highest-melting triglyceride, thus increasing heat resistance.

Apart from the obvious considerations of effectiveness and cost relating to the use of different anti-bloom agents, a vital factor to consider is whether the ingredient is permitted in chocolate in the country of use. A major advantage of butterfat is that it can be used without restriction. However, vegetable fats other than CB are permitted in chocolate only in certain countries.

In those countries where vegetable fats are not permitted in chocolate, possible options would be to add an anti-bloom fat into the filling of chocolate-enrobed products or to coat the inside of the chocolate shell with a barrier fat to restrict fat migration from the filling to the chocolate. Restrictions also apply to the use of emulsifiers in chocolate, which need to be considered.

Although much work has been carried out on different anti-bloom agents, there is still a great deal to be understood about the mechanisms by which they delay bloom formation. As research continues in this area, the chocolate manufacturer will have many more options available for improving the bloom-free shelf-life of chocolate products.

10.3.5 Moisture migration

Moisture pick-up is not a major problem in the case of chocolate bars, but is an issue to consider in chocolate products that contain components that are high in moisture content or which have a tendency to absorb moisture from the atmosphere (e.g. chocolate-coated wafer). The chocolate coating acts as a moisture barrier in enrobed products. Therefore, any imperfections in the coating, such as cracks and pinholes, can allow moisture migration into the centre. Even when the coating is perfect, a certain amount of moisture can move through thin layers of chocolate.

Moisture migration through the chocolate coating into the centre component during storage can change the structure of the centre and cause stresses in the chocolate layer. An example of this effect is seen in wafers, which expand to the point of cracking the chocolate coating (of thickness 0.065 cm) on absorbing enough moisture to increase the moisture content of the wafer by 1%.[18] Increasing the thickness of the chocolate coating will make the product more stable against such stresses caused by the centres. In order to minimise moisture pick-up by the wafer centres, they are conditioned in humidity-controlled storage rooms, which reduces their tendency to absorb moisture during storage.

The opposite, moisture loss through the chocolate coating, is a problem during the storage of chocolate products containing centres with high ERH values. The loss of moisture from centres (such as marshmallow) causes them to shrink away from the coating, making them susceptible to cracking. Improving the quality of coating to reduce pin-holing and increasing the coating thickness can help to improve the shelf-life of these products. Moisture migration can also occur within multicomponent products, where adjacent layers of components are widely different in ERH. Reformulation of the components to achieve similar ERH values in adjacent layers may be possible in some cases. However, in cases where this is not an option, some form of moisture barrier needs to be applied to reduce moisture movement. In many products, chocolate itself is applied to act as a moisture barrier.

10.3.6 Accelerated storage tests

Accelerated storage tests can be used to predict the shelf-life of chocolate under normal ambient storage conditions. The procedure for setting up such tests is described in Chapter 1. However, when dealing specifically with chocolate products, it important that the storage temperature is set below that of the melting point of the chocolate. In general, temperatures lower than 30 °C are recommended to prevent the fat phase from melting and giving rise to changes not usually seen under normal ambient storage conditions. However, high ambient conditions may be the norm in tropical climates, in which case the accelerated storage test temperature needs to be sufficiently high to induce the changes occurring under these conditions.

Temperature cycling is said to lead to a faster rate of bloom development than isothermal storage at elevated temperatures. The literature is full of examples where various cycling tests have been used. The tests are claimed particularly to accelerate the rate of fat migration, owing to the continuous melting and re-crystallisation of the fat. However, in the case of solid chocolate products, no advantage seems to be gained by the use of temperature cycling. In fact, plain chocolate-coated wafer and marzipan products were found to bloom faster under isothermal conditions (28 °C/70% RH) than under temperature cycling (cycling between 20 °C and 28 °C every 12 h), as can be seen from Table 10.7.

Table 10.7 Time to bloom development at various storage temperatures

Product	Time to bloom (weeks)		
	20 °C/50% RH	28 °C/70% RH	20 °C/28 °C cycling every 12 h
Plain chocolate bar	> 78	1–4	10
Plain chocolate-coated wafer	> 78	1–4	8
Plain chocolate-coated marzipan	> 78	1–4	6

The storage time of four weeks at 28 ºC/70% RH has been thought to be equivalent to 18–24 months at 20 ºC in the case of plain chocolate. However, this relationship may vary depending on the product composition. The results of accelerated tests are very useful for estimating the real shelf-life of products under normal storage conditions. However, it is important that all results are validated to confirm the relationship between the rate of ageing under accelerated conditions and the rate under normal test conditions.

10.4 Sugar glass

The sugar glass product is perhaps the simplest of all the confectionery products, containing sugars, water, acid, flavour and colouring. The range of products now varies from the traditional high-sugar products to the newer sugar-free products. The composition is important in determining the characteristics of the sugar glass.

10.4.1 Structure and influence of composition on glass transition

High-boiled sweets, often referred to as sugar glasses, are products of very low moisture (typically 1%), formed by cooking sugar solutions to high temperatures. The products have an amorphous glassy structure formed by the cooling of the melt supersaturated with sugars. This gives rise to the hard and brittle texture. The glassy structure can change to a viscous liquid state over a small temperature region close to room temperature. This change is called glass transition and the temperature at which it occurs is referred to as the glass transition temperature (T_g). Such a phase transition is critical to the shelf-life of glassy products as it is accompanied by substantial changes in the physical properties of the glass matrix, such as volume, heat capacity and viscosity,[19] which lead to the promotion of sugar crystallisation (graining).

The measurement of glass transition temperature can therefore be useful in predicting the relative stability of the sugar glass products against graining, the primary cause of deterioration of these products. Any product stored below its T_g should remain in the glassy state. The influence of moisture content, syrup composition and storage temperature on the rate of graining has been investigated.[20, 21] The studies found that graining did not occur below a specific moisture content, referred to as the 'threshold moisture content', even if initiated. A low threshold moisture content was found to give a high T_g or a higher level of stability against crystallisation.[22] However, the relationship between T_g and graining rate has been found to be complicated, and therefore this relationship is not always valid for products with a wide range of compositions. In the case of products containing a mixture of different sugars, the crystallisation behaviour was thought to be related to the type of sugar present in the highest concentration in the products. Nevertheless, moisture content has been shown to have the most dramatic effect on glass transition, as even a marginal increase in moisture can cause a significant decrease in T_g.[23–29]

Other compositional factors affecting T_g include the degree of polymerisation and average molecular weight of the ingredients.[19] The viscosity of the supercooled melts has also been found to be important. Increasing the viscosity of the melt has been found to act against graining. In contrast, the higher the level of supersaturation the greater the risk of graining.

10.4.2 Shelf-life improvement

The most common changes limiting the shelf-life of sugar glasses are stickiness and graining. The high level of hygroscopicity (ERH of 20%) of these products causes them to absorb moisture at normal ambient conditions. The increase in moisture content causes the product to become sticky and adhere to the wrapper. Surface moisture dilutes the sugar concentration and lowers the viscosity, promoting the crystallisation of sucrose and inducing graining.

Susceptibility to graining can be reduced by decreasing the level of invert sugar produced during cooking. The use of lower-DE (dextrose equivalent) glucose syrup or maltose syrup can increase the viscosity of the mix and thereby improve the stability against graining.[30] Care during the manufacture of the products, retaining a temperature and low humidity in the packing area can also help to improve the shelf-life of the products.

10.5 Toffee

There are no clear differences between the definitions of toffee and caramel. However, in Europe, the term toffee is often used to describe a hard-boiled chewy product of low moisture content (typically 7.5%), and the soft-textured and the flowable products with higher moisture and fat contents are referred to as caramels. Fudges have the basic composition of toffees but are grained to give a short texture.

10.5.1 Structure and composition

Toffees and caramels are made by blending sucrose, corn syrup, milk ingredient (typically sweetened condensed milk), fat, emulsifier and flavouring. The mix is then homogenised and cooked to a high total solids content. The structure of a toffee is that of fat droplets dispersed in a highly concentrated sugar matrix, in which the milk solids, not fat, are dispersed. Butterscotch products, which have a very low moisture content of less than 3%, have a glassy sugar matrix, but the caramels used in multicomponent bars have been found to have a more syrup-like sugar matrix. The flavour and texture characteristics of the products are determined both by the ingredients and the processing parameters used.

Heat-induced interaction between the proteins (amino acids) and reducing sugars, referred to as the Maillard reaction, is responsible for the development of the caramel flavour and colour. The rate of this reaction increases with increases

in temperature, heating time, and free amine and aldehyde groups. The reaction is promoted by alkaline conditions and, therefore, increasing the pH of the mix as far as possible will increase flavour and colour development. In the case of toffee and caramel products, a value of pH 6 would be adequate to produce a good-quality product.

The combination of corn syrup with sucrose affects the final level of sweetness, flavour profile and texture. The regular grade of 42 DE syrup is commonly used in toffee. However, other grades are used in special cases. The higher-DE syrups result in softer and darker products, which are more likely to cold flow (lose shape) during storage. The low-DE syrups (less than 42 DE) have a higher viscosity and are recommended for the manufacture of toffees for tropical climates as they reduce the tendency to cold flow.[31] High-maltose corn syrups are also recommended for use in products formulated for tropical storage conditions because they are less hygroscopic. However, these have a lower level of sweetness, which has to be compensated for in the formulations. Other syrups finding limited use are high-fructose corn syrup (HFCS) and invert sugar. These affect the colour, viscosity and stickiness of caramels.

Fat plays an important role in toffee, acting as a mouth lubricant by reducing stickiness and affecting flavour release. In most toffee systems, the fat is present as droplets of various size along with a certain small amount of free fat. The free fat is important in contributing mouthfeel and flavour. The level of emulsification of the fat has an important bearing on flavour in that too much emulsification can lead to a lack of flavour. However, homogenisation is also important in determining the level of smoothness achieved in the toffee. Butterfat was the only fat used in traditional toffee. Although butterfat still makes an important contribution to the flavour in toffee, other fats are used in the recipes to improve storage stability. The most commonly used fat is hydrogenated palm kernel oil (HPKO). A range of special fat blends with different melting points can now be obtained for use in toffee formulations. The ideal fat should melt sharply at a temperature of about 40 °C although higher-melting fats are used in toffees intended for tropical climates.

10.5.2 Microstructural changes affecting texture

The structure of a toffee is that of fat droplets dispersed in a highly concentrated sugar matrix, in which the milk solids, not fat, are dispersed. The microstructure of toffee products can vary from a glassy sugar matrix in the case of low-moisture toffees to a syrup-like matrix in the case of soft caramels.[32] The milk component is said to be the most important in toffee manufacture, as it affects not only the flavour and colour but also, most importantly, the texture. A study carried out by Dodson *et al.*[33] showed that the two major milk proteins – casein and whey – have different functions in toffee. The roles of the milk proteins are shown in the schematic diagrams in Fig. 10.1.

The study showed that, during cooking, the whey protein denatures and gradually unfolds and associates to form a membrane around the fat globules.

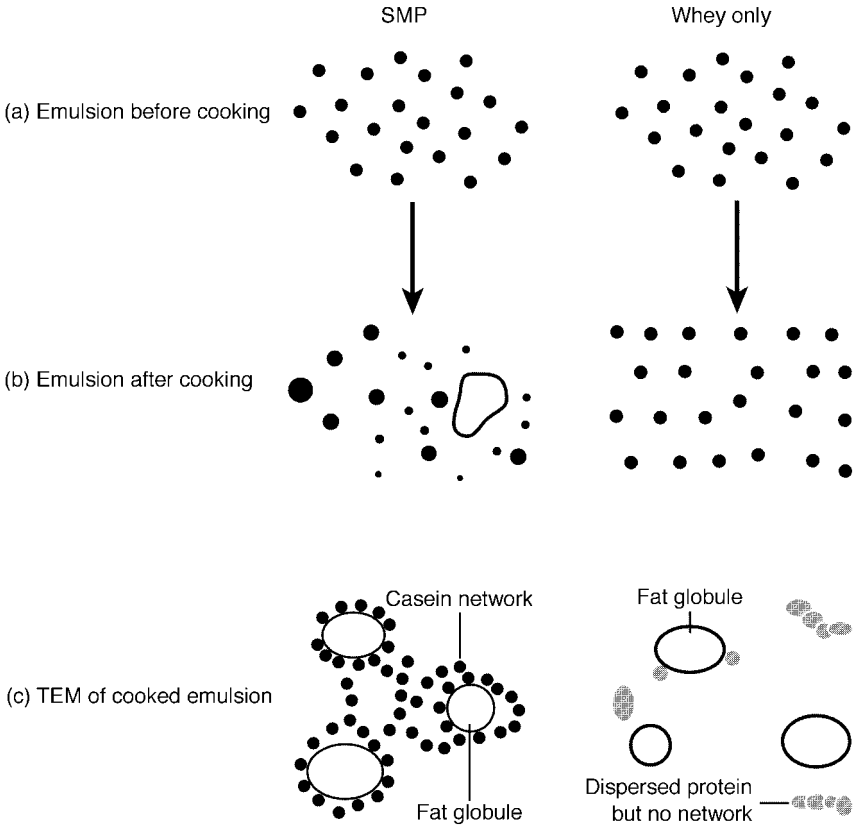


Fig. 10.1 Schematic diagram of toffee emulsions adapted from Dodson *et al.*[33] containing SMP (left), and whey only (right), showing globules (a) before cooking, (b) after cooking, and (c) structure of cooked samples as seen under transmission electron microscope (TEM).

The casein micelles gradually associate with the whey around the membrane, making the membrane more rigid. As the temperature increases, these changes become more rapid, the protein chains interacting with each other to form large-molecular-weight complexes, which produce a network to give rise to the plastic and elastic properties that give toffee its shape, body and stability against cold flow during storage. During cooking and shearing, the membrane breaks down, causing the fat to coalesce to some extent, which increases the fat globule size in the cooked toffee. The extent of the breakdown of the fat droplets is said to be related to the size of the casein micelles present. The increase in the droplet size affects the rate of flavour release from the toffee during mastication. A low calcium content in the milk has been found to give rise to small casein micelles and a finer emulsion in the cooked toffee.

The use of high levels of whey proteins without the presence of casein was found to give a darker-coloured toffee with a lower viscosity, which lacked

body. The products tended to be very unstable during storage as they had a greater tendency to cold flow. Examination of their microstructure showed that a protein (casein) network had not formed to give a firm texture.[33]

10.5.3 Shelf-life assessment

The major deteriorative changes in toffee during storage include loss of shape or distortion (cold flow), rancidity and staleness development, and changes in the texture, causing the product either to become soft and sticky or to grain (crystallise), which reduces the chewiness of the sample.[34]

Loss of shape can be the result of a high residual moisture content or the use of an unbalanced formulation lacking in milk protein, to produce a structure that will not collapse. The use of a low-DE glucose syrup has been found to improve stability against cold flow.[34] The tendency to cold flow can be predicted to some extent by the glass transition temperature of the product. The T_g is the temperature at which the product changes from a glassy state to a plastic state, where the product will deform and flow. It reduces with an increase in the moisture content. The ERH of a standard toffee with a moisture content of about 7% is approximately 52%.

When the humidity level of the storage environment increases above the ERH value, the product picks up moisture during storage, inducing graining on the surface. The surface therefore becomes soft and sticky and will adhere to the wrapper. Once graining starts, it progresses quickly to the centre of the sweet, giving a shorter texture. Graining is accelerated at high temperatures and delayed at low temperatures. However, low temperatures have the negative effect of increasing stickiness of the product. Stickiness is also promoted by the presence of high levels of invert sugars (more than 4%), but high proportions of milk solids and fat reduce stickiness and give an improved shelf-life.[34] Graining can be delayed by increasing the amount of glucose syrup in the formulation.[30]

Toffee products can also lose moisture from the surface if stored in dry conditions. Toffee samples stored unwrapped at 20 °C/50% RH have been found to show surface hardening after one week.

Shelf-life assessments on products should be carried out at typical ambient storage conditions using temperature- and humidity-controlled environments. The changes in the sensory characteristics are monitored by the use of a trained profile panel, which will assess changes in attributes such as those given in Table 10.8.

The measurement of moisture content and textural changes by an instrumental method will aid in the interpretation of the shelf-life data collected by the sensory panel. Instrumental cut tests, such as the incisor test, have been found to be useful in measuring the hardness of toffee samples using a texture analyser. This test mimics biting and involves attaching the samples to a fixed metal blade and then cutting through the sample using a similar blade moving down at a controlled speed until the two blades are 1 mm apart.

Table 10.8 Sensory attributes monitored during storage of toffee

Attribute	Definition
Colour	Brown shade of toffee
Uneven surface	Uneven samples have rough surfaces or protrusions
Hardness on first bite	Force required to break through sample as assessed on front teeth
Stickiness	The degree to which the sample adheres to the tongue and roof of the mouth
Graininess	The feeling of gritty particles in the mouth
Uneven texture	The texture of the sample is not uniform throughout
Toffee flavour	Overall toffee flavour expected in the fresh sample
Sweetness	Level of sweetness
Staleness	Flavour of old toffee variously described as musty, cardboard-like and tasting of packaging.
Meltdown	Rate at which the sample dissolves

10.6 Gums and jellies

10.6.1 Physical characteristics and microstructure

Gums and jellies can be made to contain a wide range of gelling agents, giving different textural properties to the sweets. The soft jellies tend to have higher moisture content and ERH than gums. Typical texture, moisture content and ERH found for different jelly products are shown in Table 10.9.

The sweets are coated with either sugar crystals or special glazing agents in order to protect them from the influences of humidity from the surrounding air, to stop them from sticking together, and to improve the appearance. The presence of a rigid sugar coating also reduces compression damage of products in the case of their being packed tightly in large bulk packs. A complete coating of the surface is important in achieving a high level of storage stability against moisture absorption.

Very fine caster sugar is normally used for the coating as the coarser sugar sticks badly to the surface and gives a less attractive appearance. The success of the sugar-coating operation depends on even wetting of the samples. For jelly

Table 10.9 Texture, moisture content and ERH of non-sugar coated gums and jellies containing different gelling agents

Product	Texture	Moisture content (%)	ERH (%) measured at 25 °C
Pectin jelly	Short, soft	17.0	67
Agar jelly	Short, rubbery, soft	18.0	70
Pectin/starch jelly	Slightly chewy, soft	14.5	62
Starch jelly	Chewy, soft	15.0	60
Gelatin gum	Chewy, firm	15.0	58
Gum arabic gum	Hard, chewy	12.5	58
Starch/gelatin gum	Chewy, hard	13.5	60

sweets, the wetting is done by steam, which needs to be controlled so that the surface does not become too wet. If the sweets are too wet, the moisture will transfer to the sugar and cause large lumps to form on the surface. The sweets need to be tumbled in the sugar at a controlled speed so that they do not rest on each other during tumbling, which can lead to uncoated patches on the surface. For very firm sweets, it is also possible to wet them with gum arabic solution. If the sweets are to be oiled or glazed, a similar method to sugar sanding is used but without the need to pass through the steam or wetting zone.

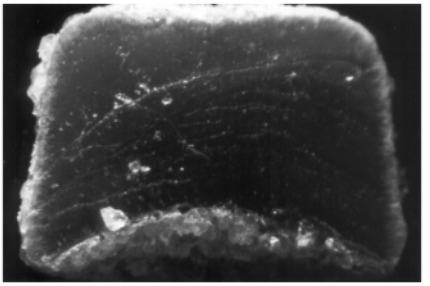
In the case of sweets that require a thicker and denser coating of sugar on the surface, another process is carried out, termed crystal coating or wet crystallisation. In this case, the sweets are submerged in a supersaturated sugar solution, to cause the crystallisation of sugar on the surface of the sweets. The supersaturated solution is prepared by boiling sugar and water. This leads to the formation of a continuous solid layer of crystals once the sugar solution has been drained off. The products that are coated by this process are more stable to humidity changes.

The textural characteristics of the gums can be related to the microstructure of the sweets, and therefore the use of a combination of sensory assessment and microscopic examination can be very useful in understanding the changes occurring during product storage. A study carried out by Lewis[35] related the microstructure of three fruit pastilles to the texture as assessed by a sensory panel. The results showed that sweets made with the same ingredients but by different processes can give rise to very different textures. In this study, all the pastilles were known to contain gelatin and starch, but the products had been made by different manufacturers. Figures 10.2–10.4, adapted from Lewis[35] show the stereo light micrograph view (a) and schematic diagrams of the structures (b) of the three pastilles. A star diagram of the texture attributes of the pastilles, adapted from Lewis,[35] are shown in Fig. 10.5. The sensory results showed that pastille 2 had a hard initial bite but softened fairly quickly on chewing; pastille 1 had a hard initial bite and continued to be tough during chewing; pastille 3 was found to have a soft initial bite and remained soft during chewing.

Examination of the microstructure found that the hardness on first bite corresponded with the level of development of the layer of crystallised sugar on the surface of the sweets. The texture on chewing could be related to the microstructure of the pastilles. Sample 2 was found to have a more substantial crystal layer than samples 1 and 3. A structure containing a protein (gelatin) continuous matrix with some dispersed starch was found to give pastille 1 the toughness experienced on chewing. The soft texture of pastille 3 was found to be the result of a starch-continuous structure containing inclusions of protein. In the case of pastille 2, the starch appeared to be dispersed in a syrupy matrix and the protein in the form of discrete pockets within the matrix, making it easier to break down during chewing.

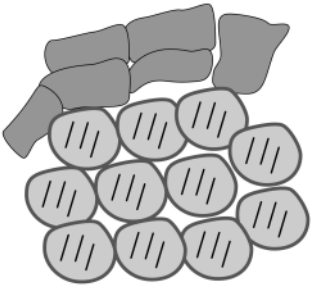
The texture of gum products changes during storage, either becoming hard as a surface crust develops on sweets owing to the loss of moisture, or softening as

Texture
High initial bite; chewy; rubbery/elastic; firm



(a) Stereo Light micrograph view

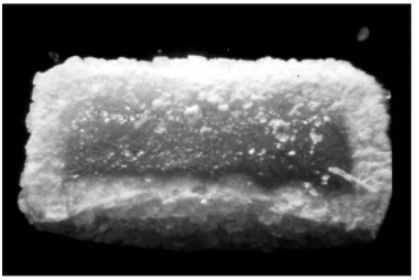
Continuous phase = protein
Dispersed phase = starch/syrup



(b) Schematic diagram of structure

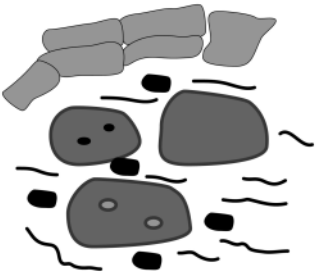
Fig. 10.2 Pastille 1 – (a) appearance under light microscope and (b) schematic diagram of corresponding structure.

Texture
High initial bite; soft; moist; breaks down



(a) Stereo Light micrograph view

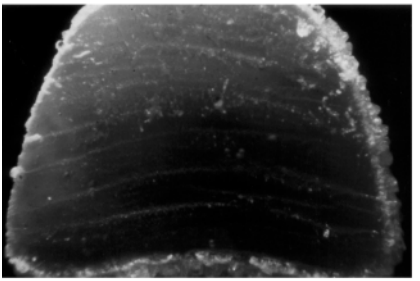
Continuous phase = syrup
Dispersed phase = protein/starch



(b) Schematic diagram of structure

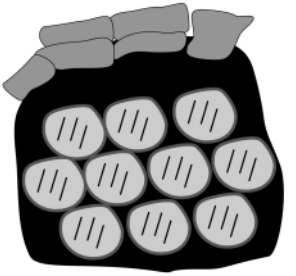
Fig. 10.3 Pastille 2 – (a) appearance under light microscope and (b) schematic diagram of corresponding structure.

Texture
Low initial bite; soft; jelly-like; moist



(a) Stereo Light micrograph view

Continuous phase = starch
Dispersed phase = protein/syrup



(b) Schematic diagram of structure

Fig. 10.4 Pastille 3 – (a) appearance under light microscope and (b) schematic diagram of corresponding structure.

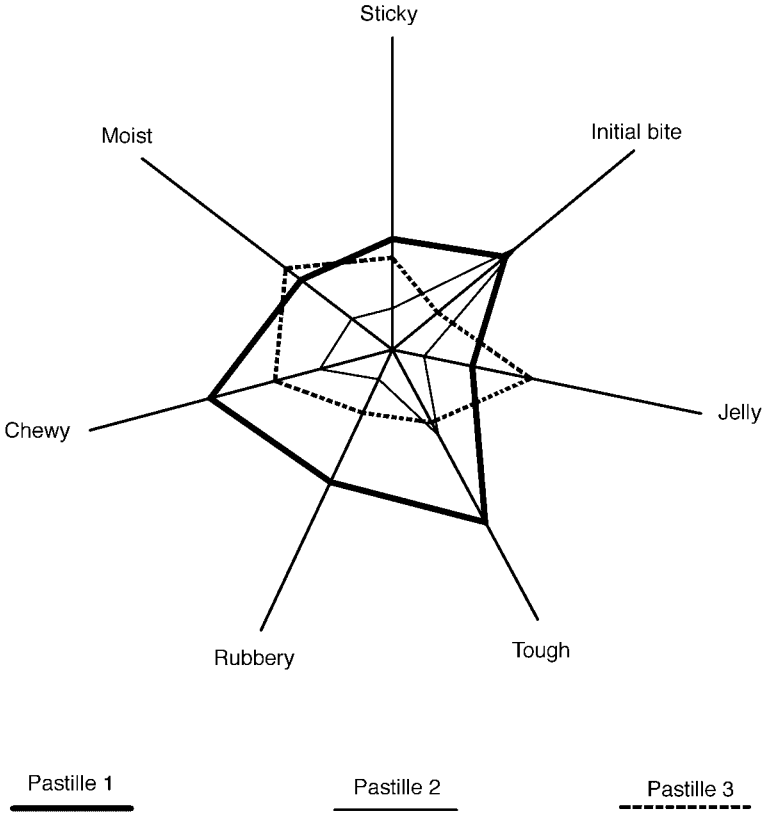


Fig. 10.5 Star diagram of texture attributes of pastilles 1, 2 and 3, adapted from Lewis.[35]

a result of the absorption of moisture under high ambient humidities. Microscopy is a useful tool in understanding such changes and can be used to develop products that have improved storage stability.

10.6.2 Shelf-life measurement

The shelf-life of gums and jellies can be assessed by storing the products under controlled storage conditions simulating ambient storage and then monitoring the changes in moisture content, ERH and sensory characteristics. Instrumental texture analysis such as that described for toffees has been used successfully to measure changes in the texture. A trained sensory profile panel can be used to characterise the changes relating to product deterioration. Table 10.10 shows some useful attributes that can be monitored during storage. Microscopy has been found to be a useful tool in understanding the changes occurring in gum and jelly products during storage.

Table 10.10 Sensory attributes assessed during the storage of fruit gums

Attribute	Definition
Gloss	Amount of shine on surface
Hardness on first bite	Resistance to bite as assessed on front teeth
Stickiness on first bite	The degree to which the sample adheres to front teeth
Chewy	Effort required to break down sample
Gelatinous	Texture of raw jelly
Stickiness	The degree to which the sample adheres to the teeth and mouth surfaces during chewing on molars
Cohesive	Degree to which sample holds together as a mass
Breakdown rate	Speed at which sample breaks down prior to expectorating
Sweetness	Sweet taste of sucrose
Fruit flavour	Level of fruit flavour and type of flavour
Staleness	Old fruit flavours
Others	Flavours not associated with fruit gums variously described as cardboard, scented, etc.

10.7 Aerated confectionery

10.6.1 Composition and structure

Aerated confectionery products have air dispersed as small bubbles throughout the matrix, which reduces the density of the products. Density measurement is often used as a means of characterising the products. Confectionery products such as gums, jellies and boiled sweets have a dense structure and density ranging from 1.3 to 1.5 g/cm^3. The density of aerated products can vary greatly. The more delicate of the aerated products, such as marshmallows, have low densities of about 0.2 g/cm^3, and firmer-textured products such as nougat will be denser at 1.1 g/cm^3.

The basic ingredients used in the manufacture of aerated confectionery are the same as those present in other standard products with the exception of the presence of air or some other gas and, in some cases, also a whipping agent. Although classed in the same category, the aerated structures of different confectionery products are created by different methods. Table 10.11 shows categories of confectionery products according to their methods of manufacture.

Table 10.11 Aerated confectionery classified according to method of aeration

Method of aeration	Product
Beating or whipping of air	Marshmallow, nougat
Expansion of small gas bubbles under pressure or vacuum	Chocolate, honeycomb
Pulling of mass followed by folding	High-boiled sugar, toffee, chews
Chemical aeration, e.g. production of gas (CO_2) through the decomposition of carbonate	High-boiled sugar

The structure and therefore the physical stability vary depending on the method used to incorporate air or gas into the products. Confectionery foams formed through beating or whipping, such as marshmallow, can be considered as colloidal systems, where gas (air bubbles) is the dispersed phase and the sugar syrup acts as the continuous phase. In these products, a whipping agent (e.g. gelatin or egg albumen) is required to change the properties of the interphase between the air bubble and the liquid (such as surface tension) in order to allow air to be incorporated. The interphase needs to be stable after aeration if the products are to remain stable, without the collapse of the air bubbles that have been created. The presence of fats causes destablilisation of foams by lowering the surface tension of the interphase.[36] Therefore, in the case of products such as nougat, where fat is an ingredient, it needs to be blended slowly at the final stage of processing after aeration.

In the case of aerated products formed by pulling, the air becomes trapped between layers of the sugar matrix, giving a denser structure than that formed through whipping. Aerated confectionery formed through pulling and through beating can be grained by the addition of icing sugar or fondant to give shorter-textured products. The graining process, which occurs during storage, needs to be controlled to achieve the desired texture in the final products, without the formation of large sugar crystals, which reduce acceptability and shelf-life.

10.7.2 Deteriorative changes during storage

Aeration allows a means of creating novel and interesting textures. Air is a cheap ingredient, but can be used effectively to increase product volume and thereby give the perception of increased value in products. However, the presence of air in the products can also affect their storage stability. Typical shelf-lives at normal ambient conditions are shown in Table 10.12.

The incorporation of air can make the product more susceptible to physical damage during handling and storage. The presence of oxygen, together with the increased surface area during aeration, also reduce the shelf-life by promoting oxidative changes that affect the flavour of products. This is a particular problem in the case of confectionery products that contain ingredients sensitive to oxygen, such as fats and nuts. In the case of sensitive products, the replacement of air with either nitrogen or carbon dioxide during processing can help to reduce the rate of flavour deterioration and extend shelf-life.

Table 10.12 Typical shelf-lives of aerated products

Product	Typical shelf-life at temperate conditions (months)
Marshmallow	9
Nougat	10
Pulled sugar	6
Aerated chews	9

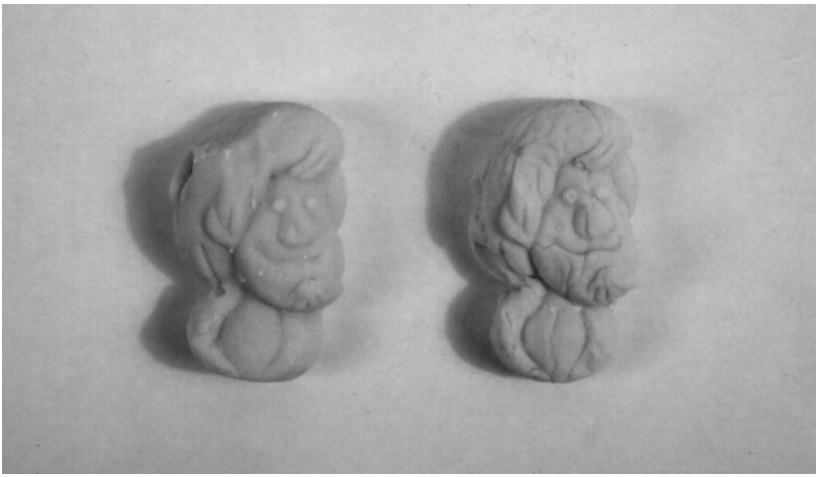


Fig. 10.6 Aerated jelly product soon after production (left), and after storage for a few weeks (right), showing premature shrinkage.

Common faults limiting the shelf-life of confectionery foams include the collapse of air bubbles, drainage of the syrup and shrinkage of the product during storage. Products such as marshmallow have a relatively high moisture content and ERH. The loss of moisture from the foams during storage can cause the air cells to collapse, causing product deterioration. Product shrinkage can occur prematurely, limiting the shelf-life, if the aerated structure is not stable. Figure 10.6 shows an aerated jelly product where the presence of starch in the product formulation was found to interfere with the air cell interphase, causing the cells to collapse and cause the product to shrink prematurely.

Three particular foam destabilisation mechanisms (often influenced by each other) have been identified depending on type of product and processing conditions used.[36] The first is disproportionation (Ostwald ripening), which involves the growth of large bubbles at the expense or loss of small bubbles. This effect can be reduced by tightly controlling the size of the bubbles, making them as large as possible (without affecting mouthfeel characteristics), narrowing the size distribution, using nitrogen gas during whipping and forming a strong hydrocolloid network around the bubbles to stop them from deforming. The second problem is weeping or the drainage of the liquid syrup, due to the difference in the density of the liquid and gaseous phases. This problem can be reduced by increasing the viscosity of the syrup phase, increasing the level of aeration and decreasing the size of the bubbles. The third physical process, coalescence of the bubbles caused by the rupture of the film between the bubbles is said to be as important as the first two processes. Stabilisation against coalescence can be achieved by changing the properties of the interphase, eliminating overbeating of the mix and limiting ingredients such as fats, that destabilise the interphase.

10.8 Sources of further information and advice

Trade/professional bodies

Biscuit, Cake, Chocolate and Confectionery Alliance (BCCCA)
37–41 Bedford Row
London WC1R 4JH
UK

The main functions of the BCCCA are to provide a meeting place for discussion of problems common to manufacturers of Alliance products; to provide a channel for representations to government on proposed legislation and other matters of concern and to keep members informed of developments in the industry.

Confectionery Manufacturers of Australasia (CMA)
PO Box 1307
689 Burke Road
Camberwell
VIC 3124
Australia

The CMA provides information and helps members with their technical queries. It represents the Asia Pacific Region on the IOCCC.

Leatherhead Food Research Association (LFRA)
Randalls Road
Leatherhead
Surrey KT22 7RY
UK

Leatherhead Food RA is a membership-based organisation offering a combination of information and applied research. It also undertakes both co-operative and confidential projects for companies. The Confectionery Products Panel of the organisation is responsible for carrying out research work in the areas of chocolate and sugar confectionery, the results of which are published as Research Reports.

International Office of Cocoa, Chocolate and Sugar Confectionery (IOCCC)
1 Rue Defacqz
B-1000 Bruxelles
Belgium

The IOCCC is a multinational organisation acting as a reference point for national associations of cocoa, chocolate and sugar confectionery manufacturers, co-ordinating and representing their interests on non-competitive issues. It also participates in and monitors worldwide guidelines, standards and scientific methods.

National Confectioners Association of the US (NCA)
7900 Westpark Drive
Ste.A-320
McLean
VA 22102
USA

This is a membership-based organisation conducting research, providing technical and governmental services, and information to the public.

Pennsylvania Manufacturing Confectioners Association (PMCA)
PO Box 176
Center Valley
PA 18034-0176
USA

The membership of this organisation includes manufacturers and suppliers of confectionery and chocolate products. It conducts research programmes and holds the annual Production Conference, the Proceedings of which are published.

Zentralfachschule der Deutschen Süsswarenwirtschaft (ZDS)
De-Leuw-Strasse 3/9
D-42653 Solingen-Gräfrath
Germany

This is the Central College of the German Confectionery Trade, offering training and education in all areas of the confectionery industry.

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11

Fruits and vegetables

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11.1 Introduction

Fruits and vegetables are unique among the food products considered in this book, in that they remain as living tissues up until the moment they are consumed, cooked or otherwise processed. All living tissues respire and the consequences of this are quite profound for the shelf-life and storage stability of these products. Slowing respiration can slow senescence and thus prolong shelf-life; however, some respiration must continue or the products will rapidly senesce and die. Cooling the produce can slow many undesirable changes in fruits and vegetables. Most plant tissues, however, will not survive freezing and many commodities are also intolerant of low temperatures well above freezing. Thus understanding the physiology of fresh produce is fundamental to understanding their stability and likely shelf-life. In section 11.2.2, the key qualities to consumer acceptability are identified as appropriate appearance, texture and flavour. In living products, all these factors can change rapidly during storage. Intrinsic and extrinsic factors, which accelerate unwanted quality changes and thus limit shelf-life, are also explored in this section.

Another factor, which differentiates fresh produce from many other food products, is the fact that each individual fruit or vegetable is unique. Its behaviour is determined by both genetic make-up (species, cultivar, clone, etc.), its stage of development (maturation, stage of ripening, etc.) and the pre- and post-harvest conditions it has experienced. This makes shelf-life prediction of fresh produce particularly difficult compared to products with a more uniform composition and stability. In section 11.3 the commercial application of shelf-life testing for fruits and vegetables and its rationale is discussed. The methods in common use for measuring fresh produce quality are reviewed. These

measurements allow an estimation of shelf-life, which is vital to successful management of the fresh produce supply chain.

The demand for all-year-round supplies at ever-higher quality standards by the retail sector is driving the development of new technical and managerial strategies. Although refrigeration throughout the cool-chain is likely to remain the most important technology for maintaining product quality, a broader range of approaches are increasingly in use such as modified atmospheres during transport, storage and in individual produce packages. In section 11.4, the broad range of post-harvest technologies used to extend storage and shelf-life of fresh fruits and vegetables are briefly reviewed. Then in section 11.5 some technologies that are likely to become available or of increasing importance to the fresh produce industry in the near future are suggested. One clear trend is that more fresh produce will be consumed partially or fully prepared for consumption. The shelf-life of these products is often much reduced compared with that of the intact product. Non-destructive, on-line quality testing, the expansion of non-chemical control of fresh produce diseases and disorders and the availability of shelf-life enhanced, genetically modified crops are predicted to have the most influence on shelf-life management on the coming years.

11.2 What determines the shelf-life of fruits and vegetables?

11.2.1 Introduction

Table 11.1 provides some examples of the variation in commercial storage conditions and expected shelf-life of some representative fruits and vegetables. The prevalence of physical damage or the presence of pathogens can, however, confound shelf-life predictions. Shelf-life of an individual product is also affected by its specific pre-harvest 'experience'. So, for example, the position of a fruit on the tree will determine its nutrient and water status and its exposure to environmental factors such as sunlight or pests and diseases. All these factors may ultimately influence post-harvest shelf-life.[1, 2] Experience may enable those who regularly handle certain produce types to predict variations in shelf-life of produce from different sources, for example, based on soil type or weather factors before and during harvest.

Fresh fruits and vegetables are not considered to be high-risk products with respect to food safety as they normally become completely undesirable for consumption long before any hazardous microorganisms or toxins might develop. There is, however, evidence that sealing fresh vegetables in modified-atmosphere packaging, may extend shelf-life, while still allowing the growth of pathogenic bacteria, in particular *Listeria* spp. and *Escherichia coli* O157.[3] For most fresh produce, shelf-life is best defined as the period within which the product retains acceptable quality for sale to the consumer. It is necessary, therefore, to identify what 'acceptable quality' means before it can be decided at what point the product no longer satisfies those expectations.

Table 11.1 Range of storage periods for selected fruits and vegetables under typical storage conditions of temperature and relative humidity

Commodity	Temperature (°C)	Humidity (%)	Storage period
Apples	−1–4	90–95	1–8 months
Aubergines (egg plants)	8–12	90–95	1–2 weeks
Avocadoes (unripe)	4.5–13	85–90	2–5 weeks
(ripe)	2–5	85–90	1–2 weeks
Bananas (green)	13–15	85–90	10–30 days
(ripe)	13–16	85–90	5–10 days
Beans (French)	7–8	95–100	1–2 weeks
Broccoli	0–1	95–100	1–2 weeks
Cabbage (green)	0–1	95–100	3 months
(white)	0–1	95–100	6–7 months
Carrots (immature)	0–1	95–100	4–6 weeks
(mature)	0–1	95–100	4–8 months
Cauliflower	0–1	95–100	2–4 weeks
Celery	0–1	95–100	1–3 months
Citrus (easy peel)	4–8	90	3–8 weeks
Courgettes (zucchini)	8–10	90–95	1–2 weeks
Cucumbers	8–11	90–95	1–2 weeks
Garlic	0	70	6–8 months
Grapefruits	10–15	90	4–16 weeks
Grapes	−1–0	90–95	1–6 months
Kiwifruits	−0.5–0	90–95	2–3 months
Leeks	0–1	95–100	1–3 months
Lemons	10–14	90	2–6 months
Lettuce	0–1	95–100	1–4 weeks
Mangoes	5.5–14	90	2–7 weeks
Melons	4–15	85–90	1–3 weeks
Mushrooms	0	90–95	5–7 days
Onions	−1–0	70–80	6–8 months
Oranges	2–7	90	1–4 months
Pears	−1–0	90–95	1–6 months
Peas	0–1	95–100	1–3 weeks
Potatoes (immature)	4–5	90–95	3–8 weeks
(mature)	4–5	90–95	4–9 months
Soft fruits	−1–0	90–95	2 days–3 weeks
Spinach	0–1	95–100	1–2 weeks
Stone fruits	−1–1	90–95	1–7 weeks
Sweet peppers (capsicum)	7–10	90–95	1–3 weeks
Tomatoes (green)	12–15	90	1–2 weeks
(ripe)	8–10	90	1 week

Note: storage conditions and storage life may differ from cultivar to cultivar. The data were adapted from the more comprehensive tables provided by Snowdon and Ahmed.[49]

11.2.2 Quality criteria in fresh produce

Different quality criteria will be important depending on the specific type of commodity and whether it is to be sold fresh or processed in some form. For the fresh produce market, specific minimum quality standards exist in many countries; however, owing to the international nature of the fresh produce

market, there is a trend towards international standardisation of quality grades. The European Commission was one of the first organisations to develop international standards for fresh fruits and vegetables.[4–6] Many of these standards have been adopted by the Organization for Economic Co-operation and Development (OECD). Usually, standards required for multiple retail outlets are considerably more stringent than these minimum standards and will be defined for the supplier by the retailer. Providing the quality standards have been met, the factors, which limit storage and shelf-life, fall into the following categories: appearance, texture and flavour/aroma.

Appearance

Appearance is the key factor for consumers in making purchases of fresh produce. As the multiple retail sector has come to dominate food retailing in many countries, consumers have come to expect fresh produce to have near-perfect visual appearance. Displays of fruits and vegetables are characterised by uniformity of size, shape and colour. Vital components of visual quality include colour and colour uniformity, glossiness, and absence of defects in shape or skin finish and freedom from disease.

Many fruits and vegetables undergo colour changes as part of the ripening process. Unripe fruits are green (the so-called 'ground colour') and in many types of fruit, the green colour becomes lighter during ripening and maturation owing to breakdown of chlorophyll, e.g. apples, grapes, papaya. This may reveal underlying yellow or red pigments.[7] In some cases, fruit colour is a strong indicator of storage and shelf-life, for example, tomatoes and bananas. For many other fruits, colour is an unreliable method of determining shelf-life. Many pre-harvest factors can affect fruit colour independently of other ripeness characteristics. So, for example, oranges grown in tropical regions may remain green despite having attained acceptable eating quality. For these fruits, factors other than colour will limit shelf-life. Yellowing of green vegetables such as broccoli and spinach may limit their shelf-life as may browning of cut tissues, e.g. butt-ends of Brussels sprouts. Other aspects of appearance that affect shelf-life include the loss of freshness, for example, wilting of leafy crops, loss of surface gloss or skin wrinkling and the development of skin blemishes caused either by natural senescence or the growth of disease organisms.

Texture

Eating quality includes a complex of textural properties, which are not readily defined or measured. Crisp, firm tissues are generally desired in vegetable crops; however, the development of tough fibres during storage in stem crops such as asparagus is not at all acceptable. Some aspects of texture can be judged visually as described above, for example, where produce has begun to wilt or shrivel. Although some degree of softening is required for optimal quality in fruit, over-softening is undesirable and is a sign of senescence or internal decay. In some fruits and vegetables (e.g. apples and tomatoes), the breakdown of intercellular adhesion between cells, leads to a condition known as mealiness.[8]

Flavour and aroma

Flavour can rarely be assessed by the consumer prior to purchase but it is critical in the repeat purchase of a particular product or product cultivar. Key taste components in fresh produce are sweetness, acidity, astringency and bitterness. Sweetness of some fruits may increase dramatically during ripening owing to starch to sugar conversions, e.g. in apples, bananas, mangoes and pears. At the same time, astringent factors (tannins) will disappear.[7] Sugar levels of fruits are often measured to determine whether produce has reached the required ripeness for marketing. Once these levels have been reached or exceeded, sweetness by itself is not a factor that directly affects shelf-life. On the other hand, sugar-acid levels can be important in the storage life of certain fruits. Acid levels are critical to the flavour balance of certain fruits such as citrus species and grapes. Acid levels generally decrease during storage. If the acid/sugar ratio falls too low, the product can become bland and lose acceptable eating quality. Bitter components can develop in various fruits and vegetables under certain storage conditions (see physiological disorders in section 11.2.3).

Aroma can be determined to some extent before purchase by the consumer but it tends to be important as a positive factor only in highly aromatic products such as certain cultivars of melons or mangoes. Since the emphasis on visual quality dominates retailing, it has been claimed that flavour and aroma has been lost from many fresh products as breeding has concentrated on cultivars that will survive the rigours of post-harvest handling without loss of visual and textural quality. Refrigeration also tends to limit the development of aroma volatiles in ripening fruits. The aroma profile can change dramatically during the post-harvest life of fresh produce, particularly in climacteric fruits in which the dominant volatile may be quite different in the unripe fruit, the ripe fruit and the over-ripe or senescing fruit.[9] Unpleasant aromas may develop due to a number of causes described in the next section. An unexpected or unpleasant aroma may make a product unmarketable even if all other quality factors are quite acceptable. Therefore aroma can be an important factor in the storage and shelf-life of fresh produce.

11.2.3 Causes of quality deterioration in harvested fruits and vegetables

Many factors can lead to loss of quality in fresh produce, hence the common description of these products as ‘perishable’. Some of these factors are part of the natural behaviour of living produce, i.e. over-ripening of fruits or sprouting in root and bulb crops. Others are a consequence of the act of harvesting. Once severed from the mother plant, the plant organ is deprived of its source of water, nutrients and anti-senescent hormones. As a consequence, normal factors such as transpiration and respiration lead ultimately to weight loss and senescence of the product. The growth of pathogens or physical damage will cause direct loss of product quality through their visual impact but both also stimulate senescence. Furthermore, the storage environment will play a highly significant role in determining the speed of all quality changes.

Respiration, ethylene and senescence

Fruits and vegetables are living commodities and their rate of respiration is of key importance to shelf-life. It has been commonly observed that the greater the respiration rate of a product, the shorter the shelf-life. Immature products such as peas and beans tend to have much higher respiration rates and short shelf-lives whereas the opposite is true for mature storage organs such as potatoes and onions.

Respiration is the metabolic process by which cells convert energy from one type of chemical structure into another form more useful to the cell for driving metabolic reactions. Under normal circumstances, fresh produce undergoes aerobic respiration, during which oxygen and glucose is consumed while carbon dioxide, water and heat are produced.[10] In non-storage tissues such as leafy crops, for example lettuce or spinach, or immature flower crops, for example broccoli, there are few energy reserves. Excessive respiration will, therefore, eventually lead to metabolic collapse. Cell membranes will break down and allow the contents to leak out. Saprophytic bacteria may grow in these tissues and give rise to off-odours. Visible symptoms of tissue collapse and yellowing due to senescence breakdown of chlorophyll in the chloroplasts may appear. Without adequate cooling, respiratory heat will further stimulate respiration leading to even more rapid deterioration.

Certain types of fruits (known as climacteric) can be harvested unripe and ripened artificially at a later stage (e.g. avocados, bananas, mangoes, tomatoes). During ripening, the respiration of these fruits increases dramatically over a short period of time.[11] Without careful temperature control, the fruit will rapidly over-ripen and senesce, leading to internal tissue breakdown and the production of volatiles characteristic of the over-ripe fruit. Failure to control respiratory heat can also increase water loss from the produce. Furthermore, the increased warmth and moisture levels which can develop in storage, are highly conducive to the development of bacterial and fungal infections.

Ethylene is a plant hormone that plays a key role in the ripening and senescence of fruits and vegetables.[12] All plant cells produce low levels of ethylene; however, anything that causes stress to the plant tissues will stimulate ethylene synthesis. Stressors may include excessive water loss, physical damage or pathogenic attack. Climacteric fruits produce high levels of ethylene during initiation of ripening and the hormone is believed to stimulate and co-ordinate the physiological and biochemical changes which occur during ripening. Exposure to exogenous ethylene can lead to an acceleration of maturation and senescence, for example, green vegetables lose their chlorophyll more rapidly, thickened fibres can develop in asparagus, premature ripening can occur in unripe fruits and cabbages and cauliflowers can lose their leaves through accelerated leaf abscission.

Breaking of dormancy

Root, tuber and bulb crops have a natural dormancy period that can be considerably extended under suitable storage conditions. Storage and shelf-life

is often limited by the breaking of dormancy. Most commonly this is seen as the growth of sprouts, for example, in onions or potatoes. Under high moisture conditions, the development of roots may also occur. Neither sprouts nor roots are acceptable in marketed produce.[13]

Water loss

Plant organs are covered with specialised tissues, which serve to protect the plant from insect and pathogen attack, physical injury and excessive water loss. The primary protective layer is the epidermis but if the plant organ undergoes secondary growth, a multilayered periderm may develop, for example on apples or potatoes. The epidermis is covered with a waxy cuticle while the cell walls of periderm tissues generally become impregnated with suberin. Both cutin and suberin can reduce water losses from plant surfaces; however, some water loss is inevitable. Water vapour can permeate the cuticle and is also lost through lenticels, which are gaps in the periderm which form to enable gas exchange for respiration. If the epidermis or periderm is damaged, water loss can be massively exacerbated.

Mature plant organs such as stems, roots and some fruits develop strengthening tissues such as collenchyma or lignified schlerenchyma to maintain their structure. The presence of tough fibrous components is not, however, desirable in fresh produce, so many vegetable crops are harvested immature. Structure and thus textural properties of fresh produce is almost entirely dependent on the maintenance of adequate cell turgor pressure, i.e. the force generated when the solute-filled vacuole presses against the relatively inelastic cell wall. If too much water is lost from the tissues, turgor pressure will fall, leading to wilting or shrivelling of the product.

The speed of post-harvest water loss is dependent primarily on the external vapour pressure deficit; however, other factors will influence the situation. Products with a large surface to volume ratio such as leaf crops will lose a greater percentage of their water far quicker than large, spherical fruits. The specific structure of the cuticle and the extent of suberisation in the periderm appears to be more important than thickness in improving resistance to the movement of water vapour. Produce varies in the percentage of water that can be lost before quality is markedly reduced. Fruits with thick peels can lose a considerable amount of moisture from the skin without compromising edible quality, e.g. citrus species, bananas. The appearance of the fruit will, however, deteriorate steadily with increasing water loss. Other thin-skinned fruits are more susceptible to water loss, for example, table grapes.[14]

Fungal and bacterial pathogens

The most important microorganisms causing post-harvest wastage of fresh produce are fungi. This is particularly true for fruits, where the relatively acid conditions tend to suppress bacterial growth. Vegetables with a higher pH can, however, suffer high losses from bacterial infections. The most important

pathogens of fruits and vegetables are described by a number of authors.[15–19] The majority of pathogens rely on damaged tissues to obtain entry into fresh produce (wounds or sites of physiological injury). For example, the *Penicillium* species which cause blue and green mould infections of citrus and other fruit crops are classic wound pathogens, incapable of invading an undamaged fruit. An intact, fresh commodity is resistant to the majority of potential pathogens. The physical barrier of the skin and the presence of antimicrobial compounds in the skin and flesh provide sufficient protection.

Some pathogens can gain entry through natural openings such as stomata and lenticels. Bacteria may use this penetration route. The most common group of bacteria causing significant reductions in shelf-life is the soft rotting species of the genus *Erwinia*. Under suitable conditions of warmth and the presence of free water, the bacteria can readily colonise produce such as potatoes through the lenticels. They produce large quantities of extracellular enzymes, which rapidly macerate the tissues. Sometimes, soft rots are accompanied by the growth of saprophytic bacteria which give rise to highly unpleasant off-odours.[20]

Only a small number of fungal pathogens are capable of direct penetration of the undamaged skin of the produce. On the whole, these latter pathogens are particularly problematic owing to the fact that they may infect produce before harvest but remain quiescent in the tissues until conditions become favourable for growth. This phenomenon is largely seen in fruits, where initial pathogen development and subsequent quiescence occurs in the unripe fruit. As the fruit ripens, quiescence is broken and the pathogen colonises the fruit tissues.[21] *Colletotrichum gloeosporioides* is a common pathogen showing this behaviour on a number of tropical fruits such as mango and papaya. Typical symptoms on ripe fruits are sunken, lens-shaped lesions, which may develop salmon-coloured sporing structures. *Colletotrichum musae* causes similar symptoms on bananas. *Botrytis cinerea* may also show quiescent behaviour on certain fruits, for example, in strawberries, fungal spores contaminate the flowers, germinate and the hyphae grow into the developing fruit where they remain symptomless until the fruit is fully ripe. The subsequent disease development can be extremely rapid and the whole fruit is completely colonised and covered with a grey, sporulating mycelium within a few days at 20 °C.

Skin diseases may remain superficial but cause large market losses owing to the blemished appearance of the produce. The potato industry has a major problem with a number of skin diseases, such as black scurf (*Rhizoctonia solani*), black dot (*Colletotrichum coccodes*), silver scurf (*Helminthosporium solani*) and common scab (*Streptomyces scabies*) which can spread rapidly on the tubers after the temperature rises in retail outlets.[19]

On the whole, fungal and bacterial infections are stimulated under high humidity conditions and in particular in the presence of free water. Pathogens of fruits and vegetables are very variable with respect to their ability to grow and reproduce at different temperatures; however, most will grow between 6 and

35 ºC. Some will survive and even grow slowly at temperatures as low as 1 ºC, for example, *B. cinerea*. The incidence of particular pathogen species is thus affected by both pre-harvest and post-harvest conditions. So, for example, *B. cinerea* is particularly important on produce grown in cool temperate climates, whereas infections caused by *Botryodiplodia theobromae* or *Aspergillus niger* tend to cause serious losses in warm regions.

Physiological disorders

Physiological disorders are adverse quality changes that occur in fresh produce owing to metabolic disturbances. These disturbances can be caused by internal factors such as mineral imbalances or may be due to non-optimal environmental factors such as inappropriate storage temperatures or atmosphere composition. The symptoms may be unique to a particular condition on a specific produce type; however, in many cases the symptoms are similar in a range of conditions with differing underlying causes. Mild symptoms are often confined to superficial tissues which may not be too significant if the produce is to be processed but can strongly decrease marketability of the fresh product because of visual disfigurement. Furthermore, physiological disorders can increase the susceptibility of the commodity to invasion by pathogens. The onset of disorders may be determined by pre-harvest conditions, the cultivar, maturity and stage of ripeness.

Poor nutrition will generally give rise to poor field growth and field symptoms. There are, however, a number of nutritional imbalances that have no obvious pre-harvest significance but which give rise to symptoms during post-harvest storage. One of the most important nutrients in this respect is calcium which plays an important role in maintaining cell wall stability. A classic example is bitter pit of apples in which hard, sunken brown pits develop both on the skin and internally. Affected tissues have a slightly bitter taste.

There is a wide range of disorders related to exposure of produce to temperatures which are too high or too low. High temperatures due to excessive exposure to the sun, for example, or inappropriate post-harvest heat treatments, may cause skin damage and uneven fruit ripening. A few commodities, for example, parsnips and onions, can survive mild freezing; however, the majority of fruits and vegetables cannot tolerate any freezing at all. Ice crystals form inside the cells leading to membrane rupture and the tissue collapses upon defrosting. The freezing point of a particular product is dependent upon the solute concentrations present in the cells (predominantly sugars), for example, lettuce may freeze at -0.2 ºC whereas sweet black cherries may not freeze until the temperature is reduced to -3 ºC.

Chilling injury is quite distinct from freezing injury and may occur at temperatures well above freezing point.[22] Tropical and subtropical commodities are particularly susceptible although there may be considerable differences in chilling sensitivity between cultivars and between immature and mature or unripe and ripe produce. Symptoms include water-soaking, surface pitting, internal discoloration, failure to ripen, accelerated senescence and increased

susceptibility to decay. Symptoms may not become obvious until the produce temperature has been raised to non-chilling levels.

If produce is stored in an atmosphere with insufficient oxygen or excessive carbon dioxide, for example, in poorly ventilated stores, respiratory disorders can develop. At higher temperatures, the produce respires more quickly so that an unsuitable atmosphere can develop more rapidly. Symptoms depend on the product in question, so for example, potatoes may develop a black centre whereas lettuces may have pale midribs. Some apple cultivars suffer external injury and others develop internal browning due to excessive carbon dioxide (CO_2) in the tissues. Very low oxygen (O_2) levels can lead to alcoholic fermentation with accompanying off-odours. Tolerance levels are variable, for example, some apple cultivars tolerate levels less than 1% O_2, whereas sweet potatoes are highly sensitive and fermentation may set in if O_2 levels fall below 8%. Anaerobic conditions will also encourage the growth of soft-rotting bacteria in potatoes.

A range of specific symptoms in stored fruits and vegetables have been attributed to exposure to ethylene.[23] Some examples include russet spotting of lettuce (at concentrations > 0.1 ppm) which is associated with increased activity of phenylalanine ammonium lyase (PAL) and phenolic content, formation of the toxin pisatin in peas, production of phenolics in sweet potatoes and in carrots. In carrots, the phenolic, isocoumarin, gives a bitter flavour and bitter flavours have also been noted in beetroot.

There are also a number of well-defined miscellaneous disorders of certain fresh produce which are beyond the scope of this book. Further information can be found, for example, in books by Snowdon.[18, 19]

Physical injury

Physical injury is possibly the most important cause of loss in fresh produce. This is not due to the direct losses, although these can be significant in some crops but rather to the indirect effect of creating a wound in the surface of the produce. This wound is an ideal entry point for many post-harvest pathogens as described above. Injury also allows water loss which compromises the quality of the produce. Furthermore, physical injury stimulates ethylene production in plant tissues, which can lead to premature yellowing or ripening of commodities.

Physical injury can arise at any stage of the life of the crop, in the field post-harvest due to insect damage or due to poor handling. Many fungi invade through the stem-end where the produce was severed from the mother plant. Poor packaging can create problems from cuts due to sharp edges or hard parts of adjacent produce, e.g. pineapple crowns to grazes due to lack of padding or under-filling of cartons allowing movement of produce within the pack during transport and handling. Bruising can occur because of drops. Compression bruising can occur if produce is stacked too high or packs are overfilled. Significant levels of wastage occur in the potato industry due to internal bruising of potato tubers during storage and handling.[24] The shelf-life of many fresh

products is considerably reduced by physical damage caused by rough handling at the retail level, particularly where the produce is loose and can be 'picked over' by the potential customer.

11.3 How the shelf-life of fruits and vegetables is measured

11.3.1 Introduction

Knowledge of the likely shelf-life of such variable products is absolutely essential to those who manage the fresh produce chain from the grower through to the retailer. Some types of produce may need rapid transport, e.g. out of season, highly perishable produce may need to be air-freighted rather than carried by ship from overseas. Other products with a longer shelf-life can be stored and released as the market requires.

The commercial measurement of shelf-life of fresh produce is usually carried out by the quality control staff of retail supply companies (importers and distribution centres). It is considered to be part of the due diligence procedure expected by the customer. Samples of product are removed from the packing line and placed in shelf-life rooms at ambient temperature which roughly reflects the likely retail conditions. Where produce is retailed in chilled cabinets, shelf-life tests may be carried out in refrigerators at the appropriate temperature, e.g. 7 ºC for table grapes. In other cases, accelerated shelf-life tests may be carried out at higher temperatures to reveal the likely development of pathogenic rots. The produce will be assessed for quality changes over a period of time which covers the shelf-life period expected by the retailer for a particular product plus a couple of extra days. Commodity-specific evaluation sheets will be filled in and archived. Because of different quality and shelf-life requirements by individual retailers, samples will be assessed from each separate product line. Shelf-life tests are used to forewarn of potential quality problems and will enable action to be taken promptly to identify and limit the problem. They provide some comeback to retailers if there is a problem, which may have occurred since the produce left the supplier. For larger organisations, providing particular products all year round, shelf-life testing may reveal temporal patterns in quality, which can be used in decisions such as when to change the supply source.

At the present time, accurate prediction of shelf-life is not really feasible for fresh produce. Efforts to try to develop predictive models for produce shelf-life based on both internal quality factors and environmental factors experienced by the produce have been described in the scientific literature;[25] however, success in this area remains elusive. The difficulty is primarily due to the inherent variability in all the quality factors of fruits and vegetables that might be used to determine shelf-life. Even if the measurement of certain qualities were able to predict shelf-life accurately, individual differences in produce means that, ideally, each individual item would need to be assessed and tests would need to be extremely rapid. Currently, many of the tests in use cause damage to the produce and therefore can only be used on a small sample of the produce.

11.3.2 Measurement of visual qualities

Colour

Measurement of colour in horticultural crops is reviewed by Francis.[26] The fresh produce industry uses produce-specific colour matching charts to assist in the grading and shelf-life assessment of many fruits. These charts are cheap and easy to use for training personnel. In larger pack-houses, photoelectric techniques may be installed to sort strongly coloured products into at least three grades. For research purposes, colour is generally measured using a surface colour-difference meter (e.g. those manufactured by Minolta or Hunter). This type of instrument measures the characteristics of light reflected from the product surface. The output is processed to give a standard data based on a tri-stimulus system, for example, numbers for hue, chroma and lightness which together accurately describe the colour of the object.[27] The main limitation to this kind of spot colour measurement is the lack of uniformity in the produce itself, for example an apple or mango may be a completely different colour on one side than on the other.

External and internal defects

The assessment of visual defects such as skin blemishes or greening in root crops is largely carried out by manual operators. Produce may be removed if it has greater than a certain percentage of its surface covered with the blemish in accordance with set quality standards. Some commercial applications of video imaging techniques (machine vision) exist: for example, some factories use machine vision-based sorting to pick out green, black or unpeeled tubers from potatoes due for processing.[28] Currently, the only method in use commercially for determining the presence of internal defects is to cut open samples of produce from each consignment of produce or removed at regular intervals from the pack line, and score the incidence of any discoloration, cavitation or other defects.

11.3.3 Measurement of textural properties

Firmness

The firmness of produce is, in many instances, a fairly good indicator of textural properties and is relatively easy to measure mechanically. Firmness can be assessed visually to some degree, e.g. whether a product appears shrivelled or flaccid. Resistance to light manual pressure is still a common means of evaluating firmness, although clearly this is highly subjective, with considerable experience required for accurate assessment. The most common method of assessing firmness is with a penetrometer such as the Magness–Taylor firmness tester or the Effegi penetrometer. These measure the total force required to puncture through a given portion of the fruit or vegetable to a standard depth using a standard diameter probe. The test may be carried out through the peel or a portion of the peel may be removed and the flesh firmness only determined.

Non-destructive compression testers are also available on the market and can be created simply from penetrometer devices.[29] Shear instruments are used to measure the tenderness of peas and broadbeans destined for processing; for example, the 'Tenderometer', which uses two sets of hinged grids which simulate the action of chewing jaws.[30]

Firmness can also be assessed using vibration tests. If produce is tapped sharply, sound waves are propagated through its tissues and can be picked up with a microphone or piezoelectric sensor. The characteristics of these sound waves vary depending on the stiffness of the tissues (among other factors) and have shown good correlations with fruit firmness. Although the underlying physical principles of these tests have long been understood, it is only relatively recently that the tests have been applied commercially. An Israeli company (Eshet Eilon) is producing a non-destructive bench top firmness tester 'Firmalon' based on acoustic resonance for use with various fruits, e.g. apples and pears. An on-line acoustic resonance firmness tester 'AvoScan' has been developed by a UK-based machinery company (Sinclair International, Norwich) based on research by Peleg *et al.*[31] This is being used commercially to categorise fruits such as avocados into separate retail categories (for example 'ready to eat' with an expected short shelf-life).

Other textural factors

In the laboratory, universal testing machines (e.g. those made by Instron) are in common use for evaluating various components of the strength of plant tissues, which change with storage. For example, mealiness is a textural defect common in some apple and potato varieties as they age. The development of artificial jaws attached to force gauges can simulate bite action and better evaluate textural qualities such as mealiness which limit shelf-life with respect to eating quality. These kinds of measurements are only used for research as suitable commercial applications have not yet been developed.

11.3.4 Measurement of flavour factors

Taste components

Sweetness is an important component of fresh fruit quality and will give a good indication of the state of fruit ripeness and hence potential shelf-life. In the fresh produce sector, sweetness is normally measured in terms of total soluble solids (TSS) content in ^{o}Brix. In most fruits and vegetables, sugar makes up the main component of TSS which is thus a reasonable indicator of % sugar levels. TSS is measured using a refractometer or a hydrometer. The former instrument operates on the basis of the refraction of light by juice samples and the latter on the basis of the density of the juice. Light reflectance in the near infrared has been correlated successfully with TSS in a number of commodities. This property is being developed as a non-destructive method of measuring sugar levels in crops such as melons.

Acidity is generally measured by titration with a suitable alkaline solution such as sodium hydroxide. Maturity standards for citrus species are based on Brix-to-acid ratios and both TSS and acidity are important measures of table grape quality. There is no rapid objective method for measuring bitterness or other undesirable flavours in fruits and vegetables. Sensory evaluation is the only commercial test used in the fresh produce sector. In the laboratory, bitter or astringent components (generally caused by phenolic compounds) can be extracted and measured by various analytical procedures, for example, high-performance liquid chromatography.

Aroma components

The measurement of aroma is currently assessed by the industry on an informal basis, relying on off-odours in shelf-life samples being noted by produce quality managers. Laboratory measurements have traditionally been conducted by headspace analysis using gas chromatography.[32] Separated components can be identified chemically or objectively using 'odourmeters'.

11.3.5 Sensory evaluation

There are relatively few instrumental tests that give results that correlate well with consumer assessment of quality in fresh produce. Colour measurement is one of the few exceptions. The most comprehensive way of assessing overall quality is to use panels to conduct sensory evaluation of the products. People on the panel may be trained to assess certain quality components in a statistically quantitative fashion.[33] Alternatively a consumer panel may be used. In this case the assessment is hedonic, that is, made in terms of personal preferences. In the fresh produce sector, the use of sensory tests may simply involve the quality controller acting as a single 'expert' taster. Alternatively, informal taste panels may be run, say, once a month, using up to 15 members of staff, who may or may not be regular members of the panel. Recent initiatives by retailers, particularly in the UK, are encouraging the industry to standardise the use of trained sensory panels for the measurement of quality attributes.

11.4 Extending the shelf-life of fruits and vegetables

11.4.1 Introduction

The main factors causing deterioration in fresh produce were described in section 11.2. Extending shelf-life thus requires taking action to limit these factors. In some cases these are preventative measures, for example, providing suitable packaging to prevent physical injury. However, a wide range of pro-active technologies must be applied to maximise the shelf-life of perishable commodities. Of primary importance are methods to reduce produce respiration, water loss and the growth of pathogens. Of these, refrigeration dominates as the most fundamental of all post-harvest technologies.

11.4.2 Pre-cooling

Pre-cooling to remove field heat as quickly as possible after harvest is essential for slowing down the rate of deterioration of highly perishable products. The method chosen is largely determined by the type of product in question and the cost to benefit ratio.[34, 35]

Room and forced air cooling

In room pre-cooling, harvested produce is placed in a refrigerated area. Typically refrigerated air is blown horizontally just below the ceiling, sweeping over and down through the containers of produce below. Upon reaching the floor, it moves horizontally to the return vent to be recycled. More rapid cooling is effected with forced air or pressure pre-cooling. In this case, refrigerated air is forced along a pressure gradient through each package. This is achieved by lining up stacks of containers (pallet loads or individual cartons) on either side of an exhaust fan to give an air plenum chamber. Air is prevented from moving down between pallet loads or the sides of cartons by sealing these gaps with flexible baffles. The cold air from the room thus has to pass through the holes in the packaging and around the produce inside. This greatly speeds up the cooling time from one quarter to one-tenth of that of conventional room cooling.

Hydrocooling

Water is better than air at transmitting heat. Many types of produce can be cooled by bringing them into contact with flowing cold water (hydrocooling). Packaging restricts water movement and greatly reduces cooling efficiency. Produce is therefore usually hydrocooled in bulk bins and is rarely used after packaging. This method is commonly used for stem vegetables, many leafy vegetables and some fruits, e.g. tomatoes and melons. Some crops cannot be cooled this way, e.g. strawberries, because free water on the surface greatly increases the risk of disease. Proper chlorination of the water is required to prevent the build up of bacteria in the water and subsequent contamination of the produce.

Icing

Application of crushed ice may be appropriate for a few crops. This is generally used for temporary cooling during transport from the field, e.g. leafy greens, for package icing during shipment to retail outlets and in displays of produce at the retail level, e.g. root and stem vegetables, Brussels sprouts and some flower-type vegetables, e.g. broccoli. The primary disadvantage is the additional weight for transport.

Vacuum cooling

One of the most rapid and uniform methods of cooling is vacuum cooling. It involves decreasing the pressure around the produce to a point at which the boiling point of water is reduced. The consequent evaporation of the water absorbs heat. This is most efficient with produce that has a large surface area to

volume ratio, e.g. leafy crops such as lettuce, spinach and cabbage. Adequate cooling can normally be achieved with no more than about 3% water loss and this can be reduced by spraying the produce surface with water prior to cooling.

11.4.3 Pre-storage treatments

Surface coatings and wraps

Many fruits and vegetables benefit from a surface coating which can slow down the loss of water.[36] This is particularly true for crops that are washed because hot water or the inclusion of detergents can remove natural waxes from the fruit surface. Coatings can also reduce the movement of O_2 and CO_2 in and out of the fruit respectively. This internal atmosphere modification can slow down respiration; however, the layer must not be too thick or O_2 levels may fall too low and lead to fermentation problems. Many of the coatings applied are derived from plant extracts, e.g. carnuba, sugar cane waxes or polymers of sugar esters; however, petroleum-based products such as paraffin wax may be added to improve water loss control. An alternative approach to controlling water loss in fresh produce is to shrink wrap the product individually in plastic films. High-density polyethylene is highly suitable for this as it can be applied in a very thin layer, which is a good water vapour barrier but does not affect the movement of respiratory gases and the danger of off-flavours developing.[14]

Curing of roots and tubers

Some root and tuber crops, for example, sweet potato and Irish potato, retain an ability to heal minor wounds after harvest provided conditions are correct.[37, 38] This involves the development of a new periderm layer at the wound site. As these crops are highly susceptible to physical injury during harvesting and handling, it is generally beneficial to encourage wound healing before storage. This process is known as curing and requires the produce to be held at elevated temperatures and high relative humidity (RH) for a period of time. The actual conditions used depend on the likelihood of disease development. At higher temperatures, curing will be faster but bacterial infection becomes more likely. Irish potato tubers are typically cured at 15–25 ºC, RH 85–98% for 7–15 days. There is evidence, however, that curing at lower humidities may reduce the incidence of superficial infections.[39] Sweet potato roots are typically cured at 29–32 ºC, RH 85–98% for 4–8 days.

Dehydration ('curing') of bulb crops

Bulb crops, i.e. onions and garlic, are unusual among fruits and vegetables in that some water loss is highly desirable in preparation for storage. This dehydration process is known as curing but is a quite different process from curing of roots and tubers. For bulb crops, the aim of curing is to lose water from the outer scales and stalk remnant. In temperate climates, artificial curing is often carried out (although field curing may still be carried out in some

countries). Onions are topped and placed in store. Hot air is blasted over them. Temperatures are initially 30 ºC until the outer scales are dried. The temperature is then dropped to 27 ºC for about four weeks before storing the bulbs at low temperatures.[40]

Chemical control of fungal and bacterial pathogens

In many instances, the fresh produce is washed prior to grading, processing and packing. The quality of the water is extremely important, particularly if it is recycled. Bacteria and fungal spores can build up in the water and become an excellent source of inoculum unless they are controlled. The most common control method is the addition of chlorine at an active level of between 50 and 200 ppm. Ozone is also being used in some parts of the industry.[41]

As described in section 11.2.3, a number of pathogens that cause significant post-harvest losses in fresh produce are pre-harvest in origin. There are many ways of limiting the extent of pre-harvest infection that are beyond the scope of this book. The use, however, of resistant cultivars, good crop sanitation, any measures that maintain crop vigour and hence their natural resistance to infection and the application of fungicides will all go a long way to minimising post-harvest disease problems. The use of antibiotics for bacterial control in crops is not accepted in many countries, owing to fears concerning the possibility that any antibiotic resistance arising from field applications might be transferred to human pathogens.[20]

After harvest many crops which are to be stored are treated with one or more fungicides. There are about 20 types of fungicide with approval for use on fresh produce,[42] although approval varies from country to country. Fungal resistance to the benzimidazole-based fungicides, such as benomyl, thiabendozole and thiophthanate methyl, is extremely widespread and has led to an increasing use of the ergesterol synthesis inhibitors such as imazalil, etaconizole and bitertanol. Application methods are highly dependent on the fungicide type and the crop type. Fruits such as apples, pears, mangoes, citrus and various root crops are often either sprayed or dipped in fungicide baths. Some fungicides may be incorporated into waxes for surface application on, for example, citrus. Where it is undesirable for the product to be wetted fumigants may be used, for example, potatoes may be fumigated with 2-aminobutane to control gangrene and skin spot and sulphur dioxide is applied to control grey mould on table grapes.[43] Many crops are not treated with any post-harvest chemical despite high perishability due to pathogens, e.g. strawberries.

Sprouting suppressants for root, tuber and bulb crops

Control of sprouting in root and bulb crops can be carried out by pre-harvest applications of maleic hydrazide. The compound must be applied to the foliage three to six weeks before harvest. Root crops can also be treated post-harvest with various sprout suppressants,[38] for example, propham/chlorpropham (IPC/CIPC) which is normally applied as a mixture at about 10 g/tonne. These compounds must be applied after curing as they suppress wound healing.

Tecnazene (TCNB) is a commonly used alternative, which has some advantages over IPC/CIPC in that it has little effect on wound healing and also has some fungicidal properties. Application rate of active ingredient is about 135 mg kg^{-1}. There are a wide range of alternative chemicals which have sprout-suppressant properties but they all have limitations compared with the conventional compounds described above.[44]

Post-harvest chemical treatments to reduce disorders

Superficial scald is a skin disorder of certain apple cultivars which develops during storage and is due to the oxidation of a natural compound in the skin called α-farnesene. Commercially, the antioxidant compounds diphenylamine and ethoxyquin can be applied as a post-harvest dip to control this disorder (at 0.1–0.25% and 0.2–0.5% respectively). Diphenylamine may also be applied in wax formulations or in impregnated wraps.[19]

Another important post-harvest treatment of apples is the use of calcium, either as a pre-harvest spray or as a post-harvest dip, to control the storage disorder, bitter pit.[45] Although calcium treatment can improve storage quality of many other fruits, it has not been developed because of problems with getting sufficient calcium into the tissue by infiltration without causing fruit damage.

Irradiation

Many benefits of applying ionising radiation (X-rays, γ-rays or high-energy electrons) to fresh produce have been shown, including sprout inhibition in root, tuber and bulb crops, control of some fungal diseases and increased storage potential through delays to the ripening processes of fruits.[46] A range of treatments have been approved in many countries, including the UK; however, consumers have shown considerable reluctance to accept irradiated food.[47] In practice, very little fresh produce is actually irradiated owing to both these consumer concerns and legislative restrictions.

11.4.4 Refrigerated storage

As discussed in section 11.2, the storage/shelf-life of fresh produce is considerably extended if respiration can be slowed down using refrigeration. Lists of recommended storage conditions for a wide range of fruits and vegetables are given in a number of publications.[48–50] Following pre-cooling, it is important that the cold-chain is maintained throughout the life of the product. This means that refrigeration should take place throughout transportation[51] and storage and preferably be maintained during retailing and in the home of the consumer. Typically, road and sea containers are refrigerated, as are the storage units at exporters, importers and retail distribution centres. Airfreight is rarely cooled and relies on adequate pre-cooling, good pack insulation and the speed of transport to maintain adequate quality.[52] The cool-chain tends to be broken in the retail store where fruits and vegetables are rarely displayed in chilled cabinets.

Control of humidity

Most cool stores or refrigerated containers are refrigerated by a direct expansion system.[53] Fans are usually necessary to circulate the storage air over the evaporator coils and then through the produce in the cooling space. Heat is removed from the cooling space, when the refrigerant gas is allowed to expand in the evaporator coils. The temperature gradient between the coil and the produce is accompanied by a vapour pressure deficit, which increases water loss from the produce. To reduce water losses during longer-term storage it is important to have as small a difference between coil temperature and produce storage temperature as possible. For produce particularly susceptible to water loss, e.g. leafy vegetables, an indirect cooling system may be used. Storage air is cooled to about 1–2 °C and humidified to an RH of over 98% by passing it through a shower of cold water that has been cooled by mechanical refrigeration.

Control of ethylene

The presence of ethylene can stimulate senescence and give rise to a number of disorders as described in section 11.2.3. Good store management is needed to ensure that ripening fruit is not stored with unripe fruit or other produce that is sensitive to ethylene.[54] Exhaust gases from vehicles contain ethylene and must be kept well apart from produce stores. For fruits and vegetables, which produce only low levels of ethylene, adequate ventilation from a clean air source is usually sufficient to keep ethylene at safe levels. Where ventilation is not sufficient to manage ethylene levels, ethylene can be destroyed by oxidation. Store air can be passed over an oxidising compound, e.g. potassium permanganate held on an inert substrate. Alternatively, ultraviolet (UV) light is in use commercially to destroy ethylene. The UV generates ozone production. It is believed that the ethylene is destroyed by active intermediates produced during the formation of the ozone.[12] Ethylene can also be destroyed using catalytic converters by heating the air to over 200 °C in the presence of a suitable catalyst such as platinum.[55]

Control of chilling injury

Chilling injury in tropical and sub-tropical crops may limit the use of refrigeration to temperatures well above freezing. Chilling injury is dependent not only on the temperature but also on the length of exposure at that temperature. The early stages of chilling injury are believed to be reversible and some produce can tolerate chilling temperatures for short periods of time without development of symptoms. A range of methods are available to limit chilling injury.[56] These include stepwise reduction in storage temperature or intermittent warming during storage may reduce chilling injury (e.g. nectarines and peaches). Some fruits may become less susceptible to chilling when held under appropriate modified atmospheres, e.g. mango, avocado.

11.4.5 Controlled atmosphere storage

Respiration can also be controlled in many crops by reducing the levels of O_2 in store and/or by raising levels of CO_2. This is known as controlled atmosphere

storage (CA) and its use with fruits and vegetables is reviewed by Thompson.[57] Lists of recommended CA conditions for a wide range of crops are provided in a number of other publications.[50, 58] CA has long been in use as a means of extending the storage life of apples well beyond that achieved just by refrigeration. Up to ten months storage can be achieved for some cultivars such as Granny Smith.[59] CA can also be useful for chilling sensitive crops, where refrigeration alone may not give adequate storage life. Transport of bananas is increasingly being carried out under CA (typically O_2–3% and CO_2–5%), giving reduced levels of premature ripening and controlling crown rot disease. CA storage of onions can give substantial extension of storage owing to its inhibitory effect on sprouting. The technology is, however, quite expensive to install and needs well-trained technical staff to be operated effectively.

High levels of CO_2 can also have a direct inhibitory effect on certain pathogens. The upper limit for carbon dioxide levels depends on the sensitivity of the crop. Many berry crops have a high tolerance for CO_2; for example, blackcurrants destined for processing into juice are often held under 40% CO_2. Levels above 15% will significantly reduce incidence of grey mould on strawberries, raspberries, cherries and grapes[58] and small-scale CA storage structures are in increasing use with these crops.

11.4.6 Packaging

Conventional packs

It is essential to minimise physical damage to fresh produce if it is to have optimal shelf-life. The use of suitable packaging is vital in this respect.[34, 50] The most common form of packaging in this sector is the use of the fibreboard carton; however, for most produce, additional internal packaging, e.g. tissue paper wraps, trays, cups or pads, is required to reduce damage from abrasion. For very delicate fruits, smaller packs with relatively few layers of fruits are used to reduce compression damage. Moulded trays may be used that physically separate the individual pieces of produce. Individual fruits may also be wrapped in tissue or waxed paper. This improves the physical protection and also reduces the spread of disease organisms within a pack.[60]

Modified-atmosphere packaging (MAP)

Polymeric films have been used to package fresh produce for over 35 years, with a number of benefits, including control of water loss, protection from skin abrasion and reduced contamination of the produce during handling. They also provide a barrier to the spread of decay from one unit to another.[61] These films will also affect the movement of respiratory gases depending on the relative permeability of the film. This can lead to the development of lowered O_2 and raised CO_2 levels within the package and, as with CA storage, this can reduce the respiration of the produce and potentially extend shelf-life. Bananas are commonly transported in sealed polyethylene bags. It has been shown that if a

stable gas content of 2% O_2 and 5% CO_2 can be achieved, the shelf-life of bananas can be extended five-fold.[62]

A modified atmosphere can be created within the pack in two ways. Active modification involves the pulling of a slight vacuum within the pack and then replacing the atmosphere with the desired gas mixture. Absorbers of CO_2, O_2 or ethylene may be included within the pack to control the concentration of these gases. In passive modification systems, the atmosphere is attained through the respiration of the commodity within the pack. The final equilibrium atmosphere will depend on the characteristics of the commodity and the packaging film.[61] Temperature control is extremely important with MAP, as this will influence the gas permeability properties of the film as well as the respiration rate of the product. One of the main drawbacks to MAP is the potential for O_2 levels to fall too low and cause the production of undesirable off-odours due to fermentation of the tissues.

11.5 Future trends

11.5.1 Minimally processed products and MAP

One of the fastest growing trends in food retailing is that in ready-prepared foods. In the fresh produce sector, this is observed in growing sales of so-called fresh cut or minimally processed salads. New developments are having to be made in MAP to prevent the rapid deterioration that occurs once fresh produce has been cut open.[63, 64] Up to now, the development of new MAP solutions has remained something of an art, with selection based on trial and error. Attempts to put MAP design on a more theoretical basis have led to a number of models being developed; however, their general applicability has been limited by the complexity of the systems involved.[61] With the continued expansion in computing power available, eventually models that can be used successfully to predict suitable MAP solutions will be developed.

These developments in MAP will be accelerated by the commercial availability of films for so-called ‘active packaging’; for example, polymer films that become more permeable to respiratory gases at higher temperatures.[65] Packaging may include components that remove aroma or off-flavours, scavenge O_2, ethylene or water vapour or emit CO_2 or other preservative vapours.[66] Novel gas combinations such as high levels of O_2, argon or neon may have useful applications in this field.[63]

11.5.2 On-line technologies for non-destructive grading and shelf-life evaluation

Another market of growing importance is the ‘ready-to-eat’ market where the consumer is led by the product label to expect a fully ripe fruit for immediate consumption. To really guarantee good eating quality while minimising post-harvest losses, the development of robust non-destructive quality testing

equipment for use on packing lines is required. This type of equipment will also be used for the detection of external and internal defects, thus reducing staff costs in the pack-house.

The physical science behind many non-destructive techniques for evaluating internal quality of fresh produce such as the use of near infrared, X-ray scattering, acoustic resonance, etc., is well understood.[67] The goal of turning the science into technologies, which can be applied commercially within the fresh produce sector, has proved somewhat elusive. Flavour factors such as sugar content may eventually be routinely measured using near infrared.[68] Aroma profiles of fruits may be assessed using electronic nose technology based on polymer arrays which are sensitive to volatile compounds.[69] Currently, the response time of this equipment is too slow to be of practical use, i.e. in the order of minutes per sample rather than seconds. Some of this additional information could be incorporated on to labels applied on-line, perhaps indicating the expected shelf-life and percentage sugar content of each individual product.

Machine vision applications for the detection of external blemishes are rapidly making progress towards commercialisation.[70, 71] Among the novel techniques being developed for the non-destructive detection of internal defects are computer-aided X-ray tomography and nuclear magnetic resonance (NMR) imaging. These are based on the measurement of differences in tissue density or proton mobility respectively and can be used, for example, to detect cavities or tissue disruption caused by insects, disease development or developmental disorders.[63]

11.5.3 Replacements for post-harvest chemicals

In many countries there is a strong trend towards reducing the use of chemicals in horticulture, including post-harvest fungicides, sprout suppressants and antioxidants for scald control. Increasingly, consumers are prepared to pay for organic products and the retail sector is encouraging the trend.[72] Another and perhaps more significant factor in the trend to reduce usage of post-harvest chemicals is the escalating costs to the agrochemicals industry of the registration of new pesticides or re-registration of currently used pesticides.[73] Post-harvest use of pesticides on fruits and vegetables is an extremely small market compared with pre-harvest applications on major world crops such as cereals and oilseed crops. Many chemicals are now being voluntarily de-registered by their producers for post-harvest use. Others have been de-registered by regulatory bodies on the basis of new health and safety data. In 1994 the EU began the process of harmonising maximum residue levels (MRLs) for each crop/pesticide active ingredient combination in use across EU countries. Where the chemicals have been found to be out of patent and where no chemical company is willing to pay the cost of the new data requirements, the active ingredient is being or has been banned. The implications of this pesticide ‘harmony’ in Europe are potentially serious for the European horticulture industry as well as international growers exporting to Europe.[74]

It is clear that the fresh produce sector urgently needs alternatives to post-harvest chemicals, and developments of these technologies will grow in the future. Among the technologies already in use or in development are controlled and modified atmosphere storage, for example, to manage scald in apples[75] and physical treatments such as heat,[76] the use of biocontrol agents,[77] 'natural' chemicals such as plant extracts and methods to stimulate natural disease resistance in crops such as UV applications.[78]

One new chemical, which may gain future approval for use on fresh produce, is the gaseous inhibitor of ethylene action, 1-methylcyclopropene (1-MCP). 1-MCP inhibits ripening in climacteric fruit and ethylene stimulated senescence and is active at very low concentrations (ppb).[79]

11.5.4 Genetically modified (GM) fruits and vegetables

Despite consumer concerns about the desirability of genetically engineered crops, it is likely that new GM products will become available on the market in the near future. The first GM fresh product to be marketed was the FlavrSavr tomato which was engineered using antisense RNA technology to have reduced levels of polygalacturonase.[80] This increased the shelf-life of the tomato by preventing the excessive softening, which accompanies over-ripening. Other fruits such as tomatoes and melons have been manipulated to reduce ethylene synthesis. Such fruits can have extremely extended shelf-lives. Susceptibility to post-harvest damage and disorders has been manipulated in a number of crops, for example, polyphenol oxidase activity has been reduced in potatoes, decreasing their sensitivity to bruising.[81] Researchers are also trying to reduce PPO activity in other crops, including pineapples, apples, lettuces and grapes, with the aim of preventing the browning reactions which accompanies physical and physiological injury.[82] There are other ways in which the shelf-life of fresh produce could be extended genetically, for example, by enhancing the synthesis of anti-microbial compounds in their tissues.

11.6 Conclusions

The fresh produce sector is a growth market driven by improvements in quality, variety and all-year-round availability. The industry has to satisfy ever-higher quality requirements combined with high labour costs, an emphasis on reductions in chemical inputs, both pre- and post-harvest, and market demand for ready-prepared products. For growth to continue, the industry has to be prepared to adopt a wide range of technologies to enable extended shelf-life while maintaining product quality. Continued research and development is therefore needed worldwide to find improved ways of increasing the stability and shelf-life of fruits and vegetables. Providing consumer confidence can be gained, genetic engineering may hold the key to dramatic changes in the management of fresh produce shelf-life in the future.

It can be concluded that those who wish to improve the prediction and control of fresh produce shelf-life need a broad knowledge base, including aspects of horticulture, physiology, biochemistry and plant pathology. They also need to be familiar with a wide range of technologies, ranging from refrigeration to molecular biology. The management of fresh produce in the future promises to be a challenging but exciting activity.

11.7 Sources of further information and advice

Most countries have one or more research organisations who carry out postharvest studies on fruit and vegetable crops. The following lists are by no means comprehensive; they are limited to those European institutions with whom the author has had professional connections in relation to research on the storage and shelf-life of fruit and vegetables.

11.7.1 UK-based research organisations

Campden & Chorleywood Food Research Association, Chipping Campden, Gloucestershire GL55 6LD. This government- and industry-sponsored research organisation has research and training programmes in aspects of MAP and HACCP for fresh produce.

Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA. A research organisation supported by grants from the Biotechnology and Biological Sciences Research Council. Carries out basic and strategic research on food safety, quality, nutrition and chemistry.

Horticulture Research International (Headquarters), Wellesbourne. A multi-site government research organisation with a number of groups carrying out research to extend the storage potential of UK grown fruits and vegetables.

Leatherhead Food Research Association (Fruit and Vegetable Panel), Randalls Road, Leatherhead, Surrey KT22 7RY. Industry-sponsored research organisation with a product panel on fruits and vegetables and some training programmes relating to fresh produce processing.

Shipowners Refrigerated Cargo Research Association, 140 Newmarket Road, Cambridge CB5 8HE. Industry-sponsored organisation that carries out research on shipping of cargo, including fresh produce.

Silsoe Research Institute, Wrest Park, Silsoe, Bedford MK45 4HS. Government-funded with relevant research being conducted on physical properties of fresh produce, non-destructive testing techniques and machine vision technology for harvesting and grading horticultural products.

The following university sector organisations are known by the author to conduct research and/or provide training on aspects of shelf-life extension of fresh produce:

Cranfield University at Silsoe (Postharvest Technology Laboratory), Silsoe, Bedford MK45 4DT; Natural Resources Institute (Postharvest Horticulture Group), University of Greenwich, Chatham, Kent ME4 4TB; Nottingham University (Plant Sciences Division), Sutton Bonnington Campus, Loughborough LE12 5RD; Reading University (Department of Agricultural Botany), Reading RG6 6AS; Scottish Agricultural College (Food Systems Division), Craibstone Estate, Buckburn, Aberdeen AB21 9YA; Writtle College, University of Essex, Chelmsford, Essex CM1 3RR; Wye College, University of London (Department of Agriculture and Horticulture), Ashford TN25 5AH.

11.7.2 Other European research organisations

ATO-DLO Agrotechnological Research Institute, Centrum De Born, Gebouwnummer 118, Bornsesteeg 59, Postbus 17, NL-700 AA Wageningen, The Netherlands.

CEBAS-CSIS, Apdo Correos 4195, 30080, Murcia, Spain.

VBT Research Centre, Tiensevest 136, 3000, Leuven, Belgium.

11.7.3 Written and electronic sources

The following books should be referred to for an overview of fresh produce biology and relevant postharvest technologies for fruits and vegetables: refs 7, 10, 48, 50, 65, 83. The journal *Postharvest Biology and Technology* publishes scientific papers relating to horticultural produce. Review articles and abstracts of relevant papers can be found in the CAB International publication, *Postharvest News and Information*. The following two websites provide detailed procedure fact sheets, including recommended conditions for the storage of fruits and vegetables:

http://postharvest.ucdavis.edu/ is produced by the Postharvest Technology Research and Information Centre, Department of Pomology, University of California, Davis, CA, USA.

http://www.postharvest.com.au/ is provided by the Sydney Postharvest Laboratory, Sydney, Australia.

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12

Fats and oils

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12.1 Introduction

Edible fats and oils are not highly perishable foods because of the absence of water. They generally have a long shelf-life during which only minor changes of their sensory characteristics occur, provided that they have been correctly manufactured and storage conditions are adequately maintained. The shelf-life that is generally applied for such products ranges between three months for table spreads and twelve months for pure oils.

For many decades, the quality changes that occur in fats and oils during storage have been researched in great detail, and a wealth of information is available from a variety of sources. In this chapter this vast amount of information is condensed to a brief and informative explanation of the most important changes that have an influence on the shelf-life of fats and oils. The chapter is restricted to the consideration of the storage stability of final products of the edible oils and fats industry. The stability of composite foods that contain fats and oils as a major ingredient is dealt with in the other chapters of this book. Raw materials or intermediate products are mentioned only where this is necessary to understand the quality of the final product. Throughout the text it is the intention to make reference to a selection of the most comprehensive and up-to-date literature sources, where more detailed information on particular aspects can be found. Finally, an explanation of the terminology – edible fats and oils are essentially of the same chemical nature. Therefore, most quality changes that occur during storage are relevant to both liquid oils and solid fats. For this reason, the term ‘oil’ is used to include the two physical states throughout the text and the term ‘fat’ is used when the context of a section is relevant to the solid state only.

At the beginning of the chapter the chemical nature and composition of oils are considered because they determine the stability of appearance, texture, flavour and mouthfeel throughout the shelf-life. The use of the term 'stability' is somewhat misleading because chemical reactions and physical changes occur continuously during the storage of oils. For this reason, the stability of an oil must be regarded as the ability to maintain the original sensory and texture characteristics that are present immediately after manufacture for as long a time as possible despite the ongoing changes in its molecular structure. Following on, the basics of the major chemical reactions, i.e. rancidity development, and physical changes, i.e. crystal type transition, that occur in oils and fats during storage, are explained. Although of lesser importance in fats than in other foods, the microbial stability of margarine products is also considered.

In section 12.3 the methods that are currently available for the measurement of oil quality and for the prediction of the shelf-life of fats and oils are described. These methods include sensory, chemical and physical analyses. There is also a section on the set-up of storage trials. Section 12.4 considers the implications of raw material quality and processing techniques for the stability of fats and oils. The various options during production of oils and fats to extend the shelf-life of the final products are described. At the end of the chapter some current developments of raw material manipulation and measuring techniques are briefly reviewed for their impact on the shelf-life of oils.

12.2 What determines the shelf-life of fats and oils?

For understanding the chemical reactions and physical changes that occur during the storage of oils, and that eventually lead to significant changes in their sensory characteristics and texture, it is necessary to consider the composition of oils and the molecular structure of their main constituents – the triglycerides. Refined and deodorised oils consist of about 98% triglycerides.

In Fig. 12.1 the molecular structure of a model triglyceride is shown. Three fatty acids are esterified with a glycerol backbone. Because the fatty acid in the middle position of the glycerol backbone points in the opposite direction to the fatty acids in the outer positions, the triglyceride molecule resembles the shape of a chair. A wide range of different fatty acids are present in edible oils. They differ in the number of carbon atoms (chain length) and the number, position and type of double bonds within the chain of carbon atoms. The figure shows different ways in which fatty acids can be symbolised. For reasons of clarity, it is common either to draw only the chain of carbon atoms, or to symbolise the chain by a zigzag line where each kink stands for a carbon atom. This way it is more obvious where double bonds are located, and of which type they are.

The fatty acids can be cleaved from a triglyceride by either chemical or enzymic hydrolysis, which means that moisture must be present. Once liberated, the fatty acids are responsible for a variety of off-flavours in an oil. The exception are extra virgin olive oils which obtain their distinctive flavour notes

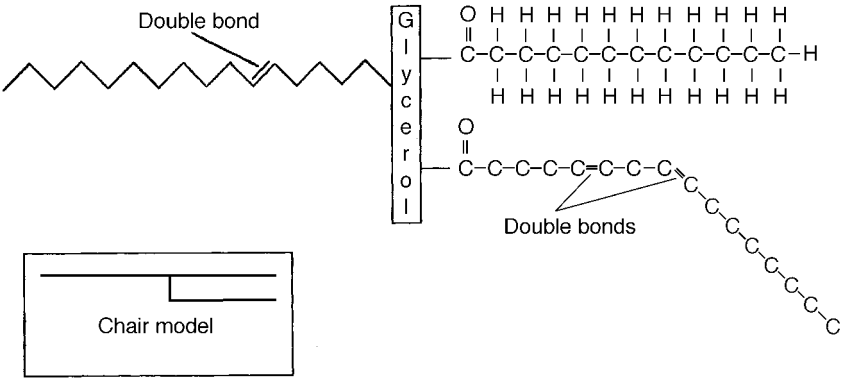

Fig. 12.1 Model triglyceride molecule.

in part from the type and level of naturally present free fatty acids. The fatty acids with short and medium carbon chain lengths (C4:0–C12:0) have particularly noticeable and unpleasant off-flavours.[1] The development of such off-flavours by the liberation of free fatty acids from triglycerides during storage is called hydrolytic rancidity development. Its occurrence makes an oil unpalatable and, therefore, shortens the shelf-life. A high level of free fatty acids causes low smoke-, flash- and fire-points of an oil, which are important when considering the safety of cooking oils.

Another type of chemical reaction occurs with the unsaturated fatty acids that contain one (mono-unsaturated) or more (poly-unsaturated) double bonds in their carbon chain. The unsaturated fatty acids are very important for the stability of oils because of these reactions occurring at the double bonds. A double bond between two carbon atoms is characterised by a shared electron pair which is highly reactive. In the presence of other reactive molecules, e.g. reactive oxygen species, the electron pair can react with such molecules, which eventually leads to the formation of completely new compounds. Such reactions occur with free unsaturated fatty acids as well as with those that are still esterified in a triglyceride molecule. The newly formed compounds have characteristic flavours, which are usually unpleasant. The formation of such off-flavours by oxidative degradation of unsaturated fatty acids is called oxidative rancidity development.

While the types of fatty acids that are present in an oil, and in particular their number of double bonds, determine the type and extent of chemical reactions that occur during the storage period, it is the molecular size and structure of the triglycerides themselves that determine the crystallisation properties of a fat and therefore the type of physical changes that may happen. There are several crystal types in which natural fats and oils can crystallise. The type of crystal that will be formed by an oil or oil blend on cooling depends on the structure of all triglyceride molecules that are present. The texture of a fat depends on the types of crystal formed. Since one crystal type can transform into another during the storage period of a fat, its texture can change dramatically.

Even after refining, natural oils contain a range of minor components such as sterols (e.g. cholesterol), tocopherols (vitamin E), colour compounds (e.g. chlorophyll), trace metals (e.g. iron, copper) and others. Of particular interest with regard to stability of oils during storage are the tocopherols because they are the natural defence to oxidation processes and, on the other hand, iron and copper ions, which are powerful oxidation catalysts.

In addition to this natural composition of pure oils, margarine contains an artificial water phase by definition of margarine as an alternative for dairy butter. This aqueous phase contains a variety of water-soluble food ingredients and additives such as milk protein fractions, thickeners, emulsifiers, salt, flavours, food colours and preservatives. The components of this water phase must be stable on their own and in combination with each other in order to ensure that they do not unacceptably limit the shelf-life of a margarine. The water phase in margarine may also contain microorganisms, which makes margarine liable to microbial spoilage. However, the risk of a shelf-life reduction of margarine as a result of microbial spoilage is low compared with rancidity development or texture changes.

In the following sections the major causes of spoilage of edible fats and oils are explained in more detail.

12.2.1 Chemical stability

As mentioned before, the use of the term 'stability' is somewhat misleading because chemical reactions occur continuously during the storage of oils. These complex reactions are called hydrolytic and oxidative rancidity development.

Hydrolytic rancidity development

The initial chemical reaction that leads to the development of rancid off-flavours is the cleavage of fatty acids from the triglyceride molecule in the presence of moisture as shown below.

$$\begin{array}{l} CH_2OCOR' \\ | \\ CHOCOR'' + 3\,H_2O \\ | \\ CH_2OCOR''' \end{array} \xrightarrow[\text{heat}]{\text{lipase}} \begin{array}{l} CH_2OH \\ | \\ CHOH + R'CO_2H + R''CO_2H + R'''CO_2H \\ | \\ CH_2OH \end{array} \quad [12.1]$$

The hydrolysis of triglyceride molecules is either an enzymic or a spontaneous chemical reaction. In both cases, water must be present for the reaction to take place. This is certainly the case in margarine products. However, it has been shown that the small residual moisture content of deodorised oils is sufficient to trigger the reaction, when an oil is stored partially crystallised at a temperature below its melting point.

Andersen and Roslund have confirmed previous Japanese observations that spontaneous hydrolysis of triglycerides can occur during room temperature storage of deodorised palm kernel stearin.[2] This is because during crystal-

Table 12.1 Flavour characteristics and threshold levels of some fatty acids and oxidative decomposition products[3]

Compound	Threshold in paraffin oil (mg/kg)	Flavour characteristic
Butyric acid	0.6[a]	Rancid butter, pungent
Lauric acid	700.0[a]	Soapy
cis-3-Hexenal	0.09	Green, beans
cis-4-Heptenal	0.000 5	Creamy, buttery
trans-6-Nonenal	0.000 35	Hydrogenation flavour
trans-2-*trans*-6-Nonadienal	0.02	Cucumber, tallow

[a] Threshold in vegetable oil.

lisation, the moisture is concentrated in the liquid oil phase which surrounds the fat crystals. The possibility of spontaneous hydrolytic rancidity development at low temperatures is an important aspect for the design of appropriate stability tests of fats and margarine which contain milk fat or oils rich in lauric acid.

Hydrolytic rancidity development that is catalysed by lipases is also referred to as lipolytic rancidity. Lipases are enzymes that occur in living animal and plant cells, and which catalyse the hydrolysis of specific fatty acids from triglyceride molecules as shown above. Many of the fatty acids that are liberated from triglycerides by lipolysis have unpleasant off-flavours. Fatty acids of short and medium carbon chain lengths have, in addition, low flavour thresholds (see Table 12.1). For example, butyric acid with four carbon atoms has a pungent, off-putting smell, which is associated with the odour of rancid butter. Another example is lauric acid, which has 12 carbon atoms, and which causes unpleasant soapy flavour defects in foods containing oils rich in lauric acid such as coconut and palm kernel oils.

A special type of lipolytic rancidity development is the so-called ketonic rancidity. It has been shown that moulds of the genera *Penicillium, Aspergillus* and *Citromyces* can release enzymes called desmolases, which catalyse the production of methyl ketones and alcohols from the liberated fatty acids.[4] The methyl ketones formed by such enzymes have very characteristic sweet and fruity odours which resemble that of perfume.

Because enzymes are usually inactivated at temperatures above 60 ºC, lipolytic and ketonic rancidity development can only occur in fats or high-fat foods that have not been processed at temperatures higher than 60 ºC, or that have been insufficiently pasteurised. In the edible oils and fats industry this applies to milk fat and other ingredients which may be used in the formulation of some types of table spreads. However, lipolytic rancidity development can also occur during the storage–use cycle of table spreads, when the products can easily be contaminated with lipase-producing microorganisms.

It is well established that spontaneous hydrolysis of triglycerides can be triggered by heat in the presence of moisture. The compounds formed under such conditions are methyl ketones and lactones, which have different off-

flavours compared with those of the free fatty acids.[5] This type of reaction is of no practical relevance to the stability during shelf-life of edible fats and oil products because these are usually stored at or below ambient temperature.

Oxidative rancidity development

This is by far the most important complex of chemical reactions that limits the shelf-life of oils. As the name of the reactions implies, their basic principle is a reaction between unsaturated fatty acids, regardless of whether they are in their free state or esterified with a triglyceride molecule, with oxygen. In order to enable such reactions to take place, either the fatty acids or the oxygen must be excited to a reactive state. Although the exact mechanism of such excitations are not yet fully understood, three models have been established that explain the generation of the first intermediate products – the hydroperoxides of fatty acids.[5]

The first model is centred around the formation of free radicals of fatty acids ($R^{\bullet}$), i.e. fatty acids that contain an unpaired, reactive electron adjacent to a double bond within the carbon chain, from the initially unsaturated fatty acids (RH). For this to happen external energy from heat, light, or radiation and catalysts such as metal ions must be present. This fatty acid radical formation is called initiation.

$$RH \xrightarrow[\text{Catalysts}]{\text{Heat, light, radiation}} R^{\bullet} + H^{\bullet} \qquad [12.2]$$

Once fatty acid radicals have been formed they can react with oxygen to produce fatty acid peroxy radicals ($RO_2^{\bullet}$). These peroxy radicals can then react with another unsaturated fatty acid to form a hydroperoxide (RO_2H) and a new fatty acid radical.

$$R^{\bullet} + O_2 \longrightarrow RO_2^{\bullet} + RH \longrightarrow RO_2H + R^{\bullet} \qquad [12.3]$$

This reaction step is called propagation because new radicals are continuously produced, leading to an acceleration of hydroperoxide formation. The production of new radicals is finally terminated when two radicals react with each other to form non-reactive molecules.

$$\begin{aligned} R^{\bullet} \quad &+ \quad R^{\bullet} \longrightarrow R{-}R \\ RO_2^{\bullet} &+ R^{\bullet} \longrightarrow RO_2R \end{aligned} \qquad [12.4]$$

The second model is called photo-oxidation, and it is based on the generation of highly reactive singlet oxygen from the dissolved atmospheric oxygen, which is normally in the triplet state. The difference between singlet and triplet oxygen is their electron configuration.[6] The electron configuration of singlet oxygen has been found to enable rapid reaction of this species with the electrons that surround the double bond of unsaturated fatty acids.[7] For singlet oxygen to be formed, energy from a light source and sensitiser molecules must be present. Pigments such as chlorophyll and riboflavine, and heavy metal ions, which are all naturally occurring minor components in animal and vegetable fats and oils,

have been shown to function as sensitisers.[5] Synthetic dyes such as eosin and erythrosin can also act as sensitisers.[6] The singlet oxygen reacts directly with unsaturated fatty acids to form hydroperoxides.

$$^3O_2 \longrightarrow \xrightarrow[\text{Sensitiser}]{\text{Light}} {}^1O_2 + RH \longrightarrow RO_2H \qquad [12.5]$$

In this reaction, the position of the double bond in the fatty acid carbon chain is shifted, which means that the hydroperoxides formed as a result of photo-oxidation are different from those formed from fatty acid radicals.

The third model of hydroperoxide formation involves the reaction between oxygen and unsaturated fatty acids through the catalytic action of enzymes, which are called lipoxygenases. Like other enzymes, lipoxygenases are highly specific with regard to the reactions that they catalyse. Hence the hydroperoxides formed via this route are of a different nature from those resulting from the radical or photo-oxidation reactions. This model is mentioned here only for completeness. As in the case of lipolytic rancidity development, it is very unlikely that lipoxygenases are present in an active state in the final products of the edible oils and fats industry.

The hydroperoxides of unsaturated fatty acids are only intermediate products in the process of oxidative rancidity development. They are odourless and very unstable. They decompose in a cascade of various chemical reactions to form aldehydes, alcohols and hydrocarbons. The main characteristic of all of these subsequent reactions is that they result in the formation of molecules with much shorter carbon chain lengths than those of the original fatty acids. Therefore, some of the final products of fatty acid hydroperoxide decomposition are much more volatile and are responsible for the development of the rancid off-flavours. Bearing in mind the great variety of fatty acids that are present in edible oils, and the number of chemical reactions that can occur it is no surprise that a wide range of oxidative decomposition products has been identified. Based on the decomposition of hydroperoxides, which can be formed from the three most abundant unsaturated fatty acids in edible oils (oleic acid C18:1; linoleic acid C18:2; linolenic acid C18:3), Przybylski and Eskin have collated comprehensive lists of final oxidation products.[8] The flavour characteristics of a selection of these compounds can be found in Table 12.1.

Oxidative rancidity development in oils is also referred to as autoxidation. This is because the activation energy of the first two reaction steps is very low, 16–21 and 25–58 kJ mol^{-1} respectively.[5] Therefore, autoxidation in oils can neither be prevented by maintaining cool storage conditions nor by the exclusion of light. Another important aspect of autoxidation are the different rates of oxidation reactions, which depend on the number of double bonds in the carbon chain of unsaturated fatty acids. Sonntag has collated experimental results from various authors on relative oxidation rates of unsaturated fatty acids.[9] In particular, he mentions the relative rates measured by Gunstone and Hilditch at 20 °C for methyl oleate, methyl linoleate, and methyl linolenate to be 1:12:25. This means that at room temperature oils that contain large amounts of poly-

unsaturated linolenic acid C18:3 (e.g. linseed oil) will be spoiled by oxidation reactions in a much shorter time than oils that are rich in mono-unsaturated oleic acid C18:1 (e.g. olive oil). Therefore, knowledge of the fatty acid composition, and in particular of the amounts of unsaturated fatty acids, in edible oils is vital information for the assessment of their stability towards oxidation during storage.

12.2.2 Physical stability

The products of the edible fats and oils industry are either liquid oils or solid fats. There are various types of solid fat products such as pure fats, e.g. cocoa butter, tallow and lard, baking shortenings, which are mostly blends of various refined and modified vegetable oils and, optionally, animal fats, and baking margarine and table spreads, which are solidified water-in-oil emulsions. In all cases the crystal structure of the solid fats determines their texture, which is an important functional property for the success in many food applications. For example, the texture of baking fats determines their ability to stabilise highly aerated creams with sugar, which finally affects the crumb structure and volume of baked products. Another example for the relationship between the texture of solid fats and their functionality are table spreads. Here, the texture determines not only the spreadability on bread but also the eating quality during direct consumption. Therefore, the stability of the physical state of fat products is a very important aspect of their shelf-life. The following sections outline the physical changes that occur during the storage of fats and the resulting quality defects, which limit the shelf-life.

Polymorphism

The crystallisation of oils takes place when the temperature is decreased below the melting point of the triglycerides. The melting point of a triglyceride depends on the type of fatty acids present and its structure. Because each natural oil consists of triglycerides with many types of fatty acids, the triglycerides differ in their melting points. Hence, each oil has a wide temperature range in which melting or solidification occurs in contrast to the sharp melting point of a pure substance, such as water or sucrose. In other words, at any temperature within the melting range of an oil there is a balance between liquid oil and solid fat crystals.

All natural oils are able to crystallise in various crystal types. This ability of a substance to exist in more than one crystal type is called ‘polymorphism’ – a Greek term which means ‘many shapes’. There are three main types of fat crystals which have been denoted with the Greek letters alpha (α), beta prime (β'), and beta (β). The crystal types differ in the way in which individual triglyceride molecules can be geometrically arranged within the layers of a crystal. This is shown in Fig. 12.2. The main geometric features of a triglyceride layer are the space between neighbouring molecules (a), the angle of tilt (c), and the distance between the terminal points of the alternating triglycerides (b).

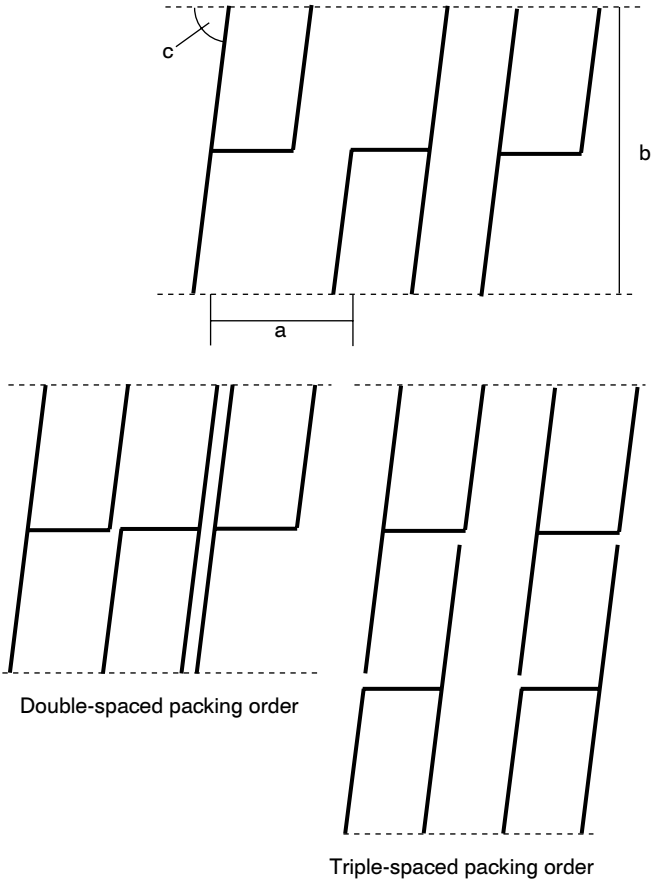

Fig. 12.2 Geometric features of triglyceride crystal layers.

Using the model of each triglyceride molecule resembling the shape of a chair it is possible to visualise two different packing options. Either the alternating triglyceride molecules of a crystal layer overlap in a way that the central glycerol backbones are all in one plane, or they overlap in a way that the glycerol backbones are located in two planes. In the first case, the distance between the terminal points of alternating triglycerides equals the lengths of two fatty acid chains, and, therefore, this arrangement is called 'double-spaced' packing order. The latter arrangement is called 'triple-spaced' packing order because the distance between the terminal points of the triglycerides is equal to three lengths of fatty acids.

The three main types of fat crystals can be distinguished by their geometric features. The least ordered arrangements with the largest spaces between triglyceride molecules are found in α-crystals, whereas the most ordered arrangements with the tightest packing of triglycerides are found in β-crystals. The tightness of triglyceride packing in β'-crystals is in between that of α- and

β-crystals. The result of these differences in triglyceride packing arrangements is that the crystal types have different melting points. The least orderly α-crystals have the lowest melting points and are, therefore, very unstable. The most orderly β-crystals have the highest melting points and are the most stable crystal type. For example, the melting points of the main crystal types of tristearin are as follows: α-crystal = 54 ºC, β'-crystal = 64 ºC, β-crystal = 73 ºC.

The crystal types of a fat differ not only in the geometric features of the triglyceride packing arrangements, but also in the size and shape of the crystals. The α-crystals are of a very small size with irregular shapes. The β'-crystals are also small and have an elongated, needle-like shape. They can arrange themselves in a loose network which contains liquid oil and, in the case of margarine, aqueous phase in the spaces between the crystals. The texture of such a network is that of a smooth cream. In contrast, β-crystals are very large and bulky. When fully established after slow growth they are large enough to be detected by the human tongue as individual particles and to be visible to the naked eye.

Exactly which crystal type is formed during cooling of an oil or oil blend depends on the type of oil and on the process conditions during cooling. Animal fats and vegetable oils can be divided into two groups according to their 'natural' tendency to form either β'- or β-crystals. This is shown in Table 12.2. The formation of α-crystals is of no practical relevance here because of their instability. With regard to process conditions, it has been established that rapid cooling with continuous forceful shearing action of an oil results in the formation of β'-crystals, whereas β-crystals are formed by slow growth of a stationary oil. The technologies for the production of margarine and high-fat foods such as chocolate have been developed to ensure the formation of the crystal type, which is desired for the texture of the finished product. For margarine and spreads the development of a network of delicate β'-crystals is desired because such a network can incorporate large quantities of liquid oil and

Table 12.2 Crystal type preference of some oils and fats

β-crystal type	β'-crystal type
Canola (low-erucic acid rape)	Coconut oil
Cocoa butter	Cottonseed
Groundnut	Fish
Lard	Milk fat (butter oil)
Maize	Palm (including fully hydrogenated palm)
Olive	Palm kernel
Safflower	Rapeseed (high-erucic acid)
Sesame	Tallow
Soyabean	Whale
Sunflower	
Fully hydrogenated and many partially hydrogenated vegetable oils (except palm)	

aqueous phase, which results in the smooth, creamy texture of such products. In chocolate, the solidification of the cocoa butter in the most stable β-crystals is desired to ensure the maintenance of the solid state of the chocolate product itself, especially its gloss.

Once established after production, the final crystal structure of a fat is not stable. Ultimately, all triglycerides have the tendency to arrange themselves into the most stable β-crystal packing arrangement where this exists. The transition of one crystal type into another can occur at any time during storage of a fat. Such transitions are promoted when the temperature of fats during storage and/or transportation fluctuates, which results in a partial melting and recrystallisation of the fat crystals. The recrystallisation generates almost exclusively large β-crystals, which can result in the loss of the original texture and appearance of the fat or food product.

Quality defects

The polymorphic transition from one crystal type to another during the storage of fats can lead to two major physical quality defects – oil migration and sandiness of margarine and spreads. Oil migration can occur when, as a result of short-term temperature increases during storage or the storage–use cycle, the fat crystals partially melt and the liquid oil can migrate from the spaces within the crystal network to form large lakes. Because such short-term temperature increases affect mainly the sides and surface of a fat block within a package, the liquid oil accumulates on the contact surface between the packing material and the fat, where it is instantly visible. The liquid oil will crystallise again once the temperature sinks below the melting point of the fat. If it is pure fat, the crystals will have a translucent and white appearance, which in the case of cocoa butter is known as chocolate bloom. However, in the case of many margarine products and spreads, the oil phase contains food colours, and uneven solidification of liquid oil on the surface of the bulk fat results in a marbling effect or mottled surface. Care must be taken not to confuse this uneven colour effect on the surface of margarine with the uniform thin layer of intense yellow colour, which can be found on the surface of some table spreads. This effect is caused by moisture loss through evaporation from the top layer of a table spread and is called the ‘primrose’ effect.

Sandiness is a quality defect that is relevant mainly for its occurrence in table spreads. The development of sandiness in a spread is caused by storage temperatures that are continuously too high. Under such conditions, a large proportion of the fat is in the liquid state and the most stable β-crystals can grow slowly throughout the bulk fat. Because the β-crystals can grow to a size large enough to be detected as individual particles on the tongue, the texture of the spread appears sandy as opposed to the desired quality of a smooth cream. Of course the development of sandiness can only occur in spreads which contain oils with β-crystal tendency.

Other quality defects that can affect the texture of a margarine, i.e. firmness, plasticity, emulsion stability, spreadability and mouthfeel, are not really a result

of the physical changes that occur during storage. These defects are caused by an inappropriate composition of the product and/or by inadequate manufacturing techniques, which means that such products have not been produced to meet the desired quality specification in the first place.

A secondary quality defect that is related to the crystallisation of fats, and also to the subsequent transition of crystal types is the increased risk of hydrolytic and oxidative rancidity development occuring. This is because the liquid phase that surrounds the fat crystals is enriched with moisture, and thus spontaneous hydrolysis can occur more easily at low temperatures. Moreover, the liquid oil phase contains a high proportion of unsaturated fatty acids in addition to the concentrated dissolved oxygen which promotes oxidation reactions. This fractionation of individual oil components during crystallisation explains how rancidity development can occur even when oils are stored frozen.

12.2.3 Microbial stability

Microbial spoilage can affect the quality of an oil in two ways: either it causes food poisoning by the presence of pathogenic bacteria, or it causes flavour deterioration as a result of lipolytic or ketonic rancidity development. There is no risk of microbial spoilage for the majority of edible liquid oils, pure fats and baking shortenings, because of the absence of water, which is vital for the existence of microorganisms. Only margarine, i.e. baking margarine and table spreads, contains a water phase which provides a living space for microorganisms, such as bacteria, moulds and yeasts. During the production process microorganisms can get into margarine either by the use of contaminated ingredients for the aqueous phase or because of unhygienic processing conditions. The chances of a contamination of margarine with microorganisms during production are small in a modern, well-managed factory. It is more likely that margarine is contaminated during the storage–use cycle in the caterers' or consumers' kitchen. Every time the package is opened, the surface of the margarine is exposed to contamination by airborne microorganisms, by the use of unclean cutlery and by skin contact with the food handler.

Even if microorganisms are present in a margarine, they still need living conditions that enable them to multiply. This depends on the composition of the aqueous phase – its water activity and pH value must be correct in order to promote microbial growth. In addition, because most margarine products are water-in-oil emulsions the size of the water droplets is of importance. According to Delamarre and Batt, and Stang and Schubert, water droplets must be large enough to provide sufficient space for the growth of colonies of microorganisms.[10, 11] In other words, sufficiently small water droplets suppress microbial growth. Last, but not least, the temperature dependency of microbial growth needs to be taken into account. Continued storage at temperatures below 8 °C drastically limits the multiplication of microorganisms.

In his review in the microbiological quality assurance of table spreads, Charteris has collated a list of coliforms, pathogens, yeasts and moulds that can

be used for challenge testing of edible spreads.[12] Although he suggested the use of *Staphylococcus aureus* and *Salmonella* species for challenge tests he did not find reports of food poisoning outbreaks caused by direct consumption of table spreads which were spoiled by either of the two pathogenic bacteria.

There is one published case of food poisoning from a liquid oil product, which occurred in Canada in 1985.[13] More than 30 people became sick after having consumed garlic-flavoured oils in restaurants. The cause of the illness was contamination of the flavoured oils with the soil bacterium *Clostridium botulinum*. The oils had been prepared in a homemade fashion by the restaurant owners rather than by an industrial process. Chopped garlic cloves were stored in non-refrigerated soyabean oil for several weeks, and provided excellent growing conditions for the bacteria. Although this food poisoning outbreak was not a result of a microbial spoilage of the actual oil, this incident is a good example of how easily an oil or fat can become contaminated during household use, not only with microorganisms themselves, but also with suitable living spaces in the form of water-containing food such as fresh herbs, spices and vegetable pieces. The problem of microbial contamination during the production of flavoured oils can be overcome by the use of sterilised flavour extracts.

12.3 How shelf-life of fats and oils is measured

12.3.1 Quality assessment of fats and oils

Sensory evaluation

Following on from the definition of oil stability as the ability to maintain the original flavour and texture characteristics for as long a time as possible, it is a logical conclusion that sensory analysis is the ultimate method for the quality assessment of fats and oils. The general rules for the set-up of sensory evaluation facilities and on how sensory evaluation should be carried out are considered in Chapter 4. Specific details on the adaptation of these general rules for the sensory evaluation of fats and oils have been comprehensively reported in a number of books.[14–16]

For the purpose of quality control, the sensory evaluation of oils begins with the visual assessment of appearance. At room temperature, liquid oils should be clear and free from foreign bodies. The colour of edible oils ranges from very pale yellow such as in rapeseed, soyabean and sunflower oils to the dark brown colours of cottonseed and sesameseed oils. Extra virgin olive oils are generally of a green-yellow colour and, because this particular type of oil is non-refined, fine particles which originate from the olive fruits may accumulate at the bottom of the container. In recent years, a special type of palm oil has been introduced in which the naturally occurring β-carotene has been preserved during refining which gives the oil a bright orange to red colour. At refrigerator temperatures, some liquid oils may be partially solidified and contain solid fat crystals at the bottom of the container. The colour of oils can be measured with special colour

reading instruments, which contain standardised intensity scales for yellow, red and blue colour components.

The surface of solid fat products such as shortenings, margarine and table spreads can be assessed for integrity, evenness, signs of liquid oil separation and for the visual absence of colonies of microorganisms. Most fats for industrial and catering applications such as baking margarine and solid frying oils are of a white colour, whereas table spreads generally contain food colours as an additive. Thus the colour of these fats ranges from pale to very intense yellow, sometimes with an orange hue. Because the colour of edible fats is unique for each individual product, there is no universal technique for its measurement in the solid state. However, after separation of the melted oil phase its colour can be measured in the same way as described for liquid oils.

On opening of the original package there is also the opportunity to assess the odour of an oil. With the exception of cold-pressed oils and products which contain flavours as additives, edible oils should be free of any odour. Cold-pressed oils, including extra virgin olive oil, have not been subjected to the usual oil-refining process and, therefore, contain higher levels of free fatty acids and other odour compounds, which form the characteristic head-space odour of these oils. Because the chemical reactions of rancidity development generate volatile decomposition products with a wide range of usually unpleasant odour characteristics, their detection in oils by odour assessment is a clear indicator that the product is approaching the end of its shelf-life. It depends on the perception of the individual consumer to make a judgement on what level of rancid odour is still acceptable.

The eating quality of oils is tested by flavour assessment which is usually carried out on the pure oils. Refined and deodorised oils should have an almost bland flavour, whereas cold-pressed oils and products that contain food flavours as additives should have a flavour that is characteristic for their type. Rancidity development in oils during storage means the development of rancid off-flavours which are caused by the emerging decomposition products of triglycerides and fatty acids. For quality control purposes, the presence of rancid off-flavours is measured using hedonic intensity scales. Various intensity scales for oil flavour scoring are being used within the food industry which differ in the number of graduations, flavour definition of individual grades and cut-off levels for flavour acceptability. It is generally accepted that trained flavour assessors are able to discriminate oil flavours on a 10-point intensity scale, an example of which is shown in Table 12.3. The two end-points of such a scale are almost of theoretical value only, and the cut-off point for ‘pass’ or ‘fail’ of an individual sample can be set as a matter of consensus.

As a result of the continuously occurring chemical reactions of rancidity development during the storage of oils, the flavour characteristics change in accordance with the generated decomposition products. In products in which hydrolytic rancidity development prevails, the typical pungent or soapy off-flavours of butyric or lauric acids respectively, emerge during storage. For the most common oxidative rancidity development, the indicative flavour changes are very complex because of the great variety of compounds, which are formed in these

Table 12.3 Intensity scale for flavour assessment of liquid edible oils[16]

Score	Intensity level	Quality level	Characteristic
10	Bland	Excellent	No flavour detected
9	Trace	Very good	Detectable, but too weak to identify
8	Faint	Good	Typical of most freshly deodorised oils
7	Slight		Typical of most commercial oils on shelf
6	Mild	Fair	Typical of oils with peroxide value (PV) <5
5	Moderate		
4	Definite	Poor	Typical of oils with PV >5
3	Strong		
2	Very strong	Bad	Typical of oils with PV >10
1	Extreme		

Note: Cut-off point for 'pass' or 'fail' usually between 6 and 7.

chemical reactions. In the initial stages of oil oxidation the sparkling clean flavour of a fresh deodorised oil becomes stale. As oxidation progresses, the flavour changes to a typical cardboardy note and further oxidation leads to the development of ever-more unpleasant off-flavours that can be described as grassy, fishy or painty, until very disagreeable rancid off-flavours occur. Most oils have their individual range of typical flavours and off-flavours and an extensive vocabulary for the description of these flavour notes has been established.[15, 16]

The assessment of the eating quality of table spreads also includes the evaluation of mouthfeel, texture and melting properties. Whereas mouthfeel and melting properties are related to the composition and processing conditions of an individual spread, the texture can change from smooth and creamy to sandy as a result of crystal type transition during storage.

To complete the subject, sensory quality assessment of fat products also includes the manual or instrumental texture evaluation of baking fats with regard to firmness, smoothness, plasticity and stickiness. However, these quality attributes are functional properties of such fats, which depend on product formulation and correct processing conditions, and which are unlikely to change dramatically as a result of the molecular changes that control the stability of the previously mentioned sensory characteristics.

Chemical analysis

Several chemical test methods have been developed for the quality assessment of oils. They have been comprehensively described, reviewed and evaluated in a number of publications.[15, 17–20] The methods can be broadly classified into two groups – in one group are methods that determine the quantity of triglyceride and fatty acid decomposition products within the oil, and in the other group are methods that measure volatile decomposition compounds in the headspace of closed oil containers. Each individual test method can only be used for the determination of a single group of chemical compounds. Because of the complexity of decomposition products that are formed during rancidity

development there is, as yet, no single chemical test method that provides conclusive information on the quality of an oil. At least two chemical tests have to be carried out to characterise the degree of freshness of oils. In this respect chemical analysis may be more time consuming and deliver inconclusive results compared with sensory evaluation of oils.

The most frequently used chemical tests for the quality assessment of oils are the determination of free fatty acid content, peroxide value, anisidine value (AnV), total oxidation (Totox) value, thiobarbituric acid (TBA) test, and extinction at 230 and 270 nm. The determination of the free fatty acid (FFA) content during storage of oils measures the liberation of fatty acids as a result of hydrolytic rancidity development. The peroxide value (PV) determines the quantity of hydroperoxides, which are formed during the early stages of oxidative rancidity development. However, as hydroperoxides are decomposed in subsequent oxidation reactions, a low PV of an oil does not necessarily mean that it is fresh. For this reason, the determination of the AnV provides additional information because it measures the quantities of secondary oxidation products, which emerge as hydroperoxides decompose. In the edible oils industry PV and AnV are often combined for the calculation of the Totox value via the simple formula

$$\text{Totox value} = 2 \times \text{PV} + \text{AnV} \qquad [12.6]$$

Similar to the AnV, the TBA test can be used for the quantification of secondary oxidation products, in particular of malonaldehydes. Finally, the extinction measured at 230 and 270 nm determines the presence of conjugated dienes and trienes of linoleic and linolenic acids, which are formed during the initial stages of hydroperoxide formation. For all of these tests, standard methods of analysis have been established by several organisations such as the American Oil Chemists' Society (AOCS), the International Union of Pure and Applied Chemistry (IUPAC) and the members of the International Standardization Organization (ISO).

Methods for the determination of volatile oil degradation compounds in the headspace of closed oil containers, e.g. gas chromatography, require specialist equipment and are more suited for research purposes than for the routine quality assessment of oils. While the established chromatographic methods determine relative quantities of individual compounds, a new type of instrument called an 'electronic nose' is able to determine the odour intensity of mixtures of a variety of volatile oil degradation compounds.[21] This instrument has the potential to become an alternative method to sensory evaluation for odour characterisation of oils. Whether it can be established as a routine method for the quality assessment of oils depends on the consistent reproducibility of results and on the balance between benefit and costs of the instruments.

Microbiological analysis

The methods that can be used for the determination of the microbial quality and stability of fat products have been reviewed by Charteris.[12] This paper provides detailed information not only on methods of aerobic plate counts for various

types of microorganisms but also on sampling plans, microbiological challenge tests and preservative efficacy tests, which have been established for use in the development of new table spreads. Besides these direct tests for the presence of microorganisms, there are also indirect tests for the determination of microbial activity in fat products during storage. These tests determine enzyme activity, in particular lipase activity, which is important for estimating the potential for lipolytic rancidity development in fats.

12.3.2 Stability assessment of fats and oils

The commercial necessity for quick provision of information on the stability of oils and, ideally, to predict their likely shelf-life has led to the development of accelerated test methods which deliver results in a much shorter time than traditional storage trials. As oxidative rancidity development has been recognised as the predominant cause of oil deterioration during storage, all the established accelerated stability tests are based on the principle of challenging oil samples under conditions that promote oxidation reactions, i.e. elevated temperatures and increased oxygen supply. The most simple set-ups are accelerated storage trials. In a drive to reduce the time until results on oil stability are available, automated stability tests were developed, which require the use of special instruments. These tests are not just accelerated storage trials because the oil samples are actively treated with artificial oxygen supply to force oxidation reactions to occur. The tests can be grouped into either active oxygen methods or oxygen bomb methods in accordance with the principle of oxygen supply. The accelerated stability tests have been reviewed and evaluated in several publications.[4,15,17–19] As in the case of chemical tests for oil quality assessment, standard methods for the conduct of many of the oil stability tests have been established.

Accelerated storage trials

The best known and described accelerated storage test for the determination of oil stability is the Schaal oven test. In this test, the oil samples are placed in open containers, which are stored in an oven at a constant temperature. The temperature is in the range between 60 and 70 °C, depending on the method used.[4, 17] At specified intervals of time, usually daily, a single oil sample is removed from the oven and the oil quality is assessed by odour and taste evaluation, and by determination of the PV. The plot of the measured PV over time creates a curve for each type of oil tested, on which the point of time can be marked at which the odour and taste of the oil became unacceptable. For the final interpretation of the result it has been suggested to multiply the oxidative stability by the factor of 16 to estimate the shelf-life of the respective oil at room temperature.[4] However, this method has not always proved reliable for the estimation of oil shelf-life because of inconsistent correlation between the oxidative stability measured and the onset of odour and taste degradation during ambient storage.

Active oxygen methods

Several oil stability tests have been developed which can be grouped under the term active oxygen methods. The principle of these tests is to force oxidation by continuously purging the heated oil samples with a defined air flow. In the initially developed Swift test, individual samples were sequentially removed and their PV was measured. A plot of PV over time enabled the determination of oxidative stability as a measure of time, which is called the induction period. The induction period has been defined as the length of time until progressive oxidation exponentially accelerates the generation of oil degradation compounds.

Later, special instruments were developed for the automation of the Swift test, namely the Rancimat apparatus and the OSI (Oil Stability Index) apparatus. The difference between the Swift test and the automated active oxygen test is the way in which the induction period is measured. In the case of the Rancimat and OSI apparatus, the volatile compounds, which are formed during the oxidation of the oil samples, are swept from the oil with the air stream, which is then fed into a wash bottle containing distilled water. Many of the volatile compounds dissolve in the water and change its conductivity. The conductivity is continuously measured, and an induction period determined at the point of time at which the conductivity exponentially increases.

The temperature at which the active oxygen methods are usually carried out ranges between 80 and 120 °C. Therefore, results in the form of induction periods can be obtained much faster than in the case of the accelerated storage trials. Moreover, modern models of the instruments allow tests to be carried out over an even wider temperature range.

Oxygen bomb methods

In the oxygen bomb methods, the oil samples are placed in closed containers with an air or pure oxygen headspace. Oxidation is promoted by continuously heating and optional stirring of the oil samples. As the oil oxidises, the oxygen content of the headspace gas decreases which results in a drop of pressure within the closed container. This pressure drop is measured via a manometer. Induction periods can be measured, as previously described, by plotting the pressure over a time scale and determination of the point in time at which the pressure drop accelerates exponentially.

The original method which is based on the principle of an oxygen bomb is called the Sylvester test. This test was automated, and the equipment sold under the name FIRA-Astell apparatus, which is no longer available. It has been replaced by a modern instrument called the Oxidograph. A further development of the Oxidograph is the Oxipress, which enables oil stability tests to be carried out in composite food samples such as potato crisps (American = potato chips), biscuits, meat products, and others. With the Oxidograph and Oxipress apparatus the test temperature can be chosen between 60 and 135 °C.

Applicability of oil stability tests

The tests described in this section have been developed for the determination of the stability of oils towards oxidation, and for the evaluation of measures that would improve oil stability, in particular the application of antioxidants. The various tests have been compared with each other in great detail with regard to the usefulness of results.[15, 17] For the modern instruments, a good correlation between the various types of induction periods has been found. However, parallel tests between traditional storage trials and the oil stability tests have shown that induction periods can only be used for the evaluation of the likely stability of the same type of oil. There is no straightforward correlation between induction periods of various types of oils that allows the comparison of their ambient stability. For this reason, the results of oil stability tests should be used only as an indication for the possible effectiveness of measures which could improve oil stability. In this respect, it is worth mentioning that Dijkstra *et al.* have established a formula that enables the prediction of induction periods based on fatty acid composition and antioxidant properties.[22]

The results obtained from oil stability tests have been used for the prediction of their shelf-life, i.e. the stability of their sensory characteristics. Most recently, a comparative investigation was carried out which correlated the changes of sensory characteristics of rapeseed, soyabean and sunflower oils during ambient storage in the dark with Rancimat induction periods, PV and tocopherol content.[23] No correlation was found between Rancimat induction periods and off-flavour development in the oil samples. This is not surprising taking into account the big differences between reaction conditions during the oil stability test and ambient storage. The drastically elevated temperatures of the stability test not only change the rates of individual oxidation reactions in a non-linear way but also the types of reaction favoured at various temperatures. In addition, the activities of oxidation catalysts and of antioxidants are different at the high test temperature compared with those at ambient storage. Further, the artificial supply of oxygen during stability tests affects the types of oxidation reaction that take place. As a result, the degradation products that are formed during oil stability tests are different from those that cause the flavour changes in oils during ambient storage. Except the Schaal oven test, the currently available oil stability tests are unsuitable for the reliable prediction of the shelf-life of oils.

12.3.3 Set-up of storage trials

Because of the limitations of the existing accelerated methods for the stability assessment of oils, storage trials must inevitably be used for the accurate determination of shelf-life. The objectives of storage trials may vary from the evaluation of new production methods or packing materials for their effect on product stability to the shelf-life testing of entirely new products. Depending on the objective of an individual storage trial, aspects such as storage conditions, sample type, sample size, sampling schedule and procedure and test selection require careful consideration.

The storage conditions should always be chosen to mimic those the product is likely to experience during storage, distribution and use as closely as possible. Care should be taken to ensure that all samples are exposed to exactly the same conditions. This is particularly important when light exposure is part of the storage conditions. In trials where liquid oils were stored in transparent containers, it was seen that the shadow cast from neighbouring containers significantly influenced the rate of oxidation in individual containers. If it is not possible to ensure that all samples of a set can be stored under the same light conditions, the locations of individual samples in relation to the light source can be swapped in regular time intervals throughout the trial to equalise possible differences.

When choosing the sample type, there may be constraints with regard to available storage space and the timing of storage trial commencement within a complex product development project. Where possible, production trial samples in the packing materials that are intended to be used for distribution are the more appropriate sample type compared with pilot-plant trial samples. This is because, in many cases, there are still differences among these sample types, not so much in product composition but with regard to the actual exposure of the bulk of the product to treatments during the manufacturing process. For example, refined and deodorised oils from pilot-plant trials are very likely to have undergone batch processing conditions, whereas production-scale oils are more likely to come from continuous processes. For solid fat products, there are also likely to be significant differences between pilot-scale and production-scale samples. One example is the 'aeration' of many solid fat products with nitrogen gas where the way in which the gas has been incorporated determines the actual gas distribution throughout the bulk of the fat.

The choice of appropriate sample size regarding the volume of individual storage containers again depends on the available storage space and also on the intended sampling procedure. Depending on the objective of the storage trial the storage containers should be of the same quality and size as the original containers for product distribution. For the organisation of the sampling schedule and procedure, decisions have to be made on how frequently a sample will be tested and on whether subsequent samples can be drawn from a big container, or whether each sample has to come from an individual, previously unopened, container. It can be concluded from the importance of oxidative rancidity development for oil stability that each opening of a storage container and removal of a small sample introduces fresh oxygen not only into the headspace of the container but also in the oil itself. This will accelerate the oxidation reactions in the sample, which may lead to a shorter shelf-life being determined for such a sample compared with one that has not been opened between packing and final test.

While the frequent withdrawal of small test portions from a bulk sample of oil may give a misleading result for the actual storage stability during distribution, this type of sampling procedure may be the most suitable approach for the determination of product stability during the final storage–use cycle in the caterers' or consumers' household. In catering and household applications a

single package is frequently opened and closed over a longer period of time, while only small portions of the product are withdrawn. This leads to ever-increasing headspace volumes in the package. In addition, table spreads can easily become contaminated with other food particles from cutlery. The concept of challenge testing, which models the storage–use pattern of many products will provide a much more reliable answer about the stability of an oil or fat product towards the end of its shelf-life than a rigid storage trial.

The selection of appropriate tests for the quality assessment of oils during a storage trial depends on the facilities that are available. Sensory evaluation is the most appropriate test; however, it is also the most difficult to set up to ensure that valid results are obtained. Firstly, sensory evaluation of oils during a storage trial needs to be carried out by trained assessors who are able to detect the subtle flavour changes that occur during the initial storage period. The training of the assessors involves regular, e.g. weekly, tasting sessions with known reference samples to maintain consistency of results. Secondly, the availability of standard reference samples, which in most cases should represent the sensory characteristics of the test samples at the beginning of the storage trial, is very problematical. It has been recognised that storage of original oil samples in domestic freezers, i.e. at $-18\,^{\circ}C$, does not prevent flavour changes from occurring during the course of ambient storage trials. Ideally, facilities should be available for the preparation of fresh reference samples, which need to be identical in composition to the original test samples, at any time during a storage trial. For these reasons, it is recommended to include chemical tests such as PV determination for the quality assessment of the test samples.

The big disadvantage of storage trials under realistic conditions is the long time required until results on shelf-life can be obtained. For most product development work there is a big commercial pressure to launch new products as fast as possible, and no time is available to wait until a storage trial has been completed. In such circumstances it is necessary to hazard an informed guess about the likely shelf-life of a new product. This is easier the more details about product type and composition, new ingredients and additives, manufacturing conditions and likely conditions during storage, distribution and use are taken into account. It is easier to estimate the shelf-life of a new product within a well-established product category than of a completely new product type where no comparison is possible. In any case, shelf-life testing by storage trial should commence as soon as proper test samples are available for each newly developed product, not only as a matter of due diligence but also for obtaining information that may be useful for future development work.

12.4 Measures for ensuring storage stability and extending shelf-life of fats and oils

The foundation for ensuring the stability of the sensory characteristics of edible fats and oils during storage is a good manufacturing process that delivers final

products of a previously specified quality. The factors that influence the storage stability of oils have already been taken into account for the design of the oil-refining process and a complex list of oil processing 'do's and don'ts' is well established. Proper handling procedures of oils during the refining process, including bulk oil shipment and storage conditions have been reviewed by Berger.[24] Provided that the quality of the final products is as specified, the shelf-life depends on the suppression of oxidative rancidity development and the prevention of polymorphic crystal transitions.

12.4.1 Retardation of oxidative rancidity development

The big issue in preserving the sensory characteristics of any fat or oil product during storage is the delay of the development of rancid off-flavours. The mechanism of oil oxidation has been reviewed at the beginning of this chapter, and the major factors that influence the potential for and the extent of rancidity development are the degree of unsaturation, i.e. the fatty acid composition of an oil, the oxygen content, the presence of oxidation catalysts, the presence of antioxidants and the storage conditions.

Fatty acid composition

The fatty acid composition, i.e. the relative amounts of saturated, mono- and poly-unsaturated fatty acids, is an inherent feature of the individual oil types. In general terms animal fats such as butter oil, lard and tallow contain more saturated fatty acids than vegetable oils, e.g. maize, rapeseed, soyabean and sunflower oils, which are known for their high contents of poly-unsaturated fatty acids. Since the poly-unsaturated fatty acids are more prone to oxidation the oils which contain high amounts of these fatty acids are likely to develop rancid off-flavours earlier during storage compared with the more saturated oils. Partial hydrogenation of such oils with the target to eliminate highly unsaturated fatty acids is widely used to increase oxidative stability, in particular in frying oils.

Disregarding all other measures that retard oxidative rancidity development, the important aspect is to select the most suitable oil for a specific application. This means that highly unsaturated oils should not be used in applications where the food product is to be stored for a long period of time. For example, maize, soyabean and sunflower oils are best used in foods with a short shelf-life such as salad dressings or prepared foods for immediate consumption.

The oil manufacturer should select appropriate package sizes that take the vulnerability of oil products into account. This is particularly important for retail packages bearing in mind the potentially long storage–use cycle in domestic households. It would be unwise to offer a highly unsaturated oil such as walnut oil in a 1-litre container, which the consumer might want to use and store for several months.

Oxygen content

For oxidation reactions of unsaturated fatty acids to occur, oxygen must be present in the oil. Atmospheric oxygen readily dissolves in oil wherever a

contact surface between air and oil exists. The solubility of oxygen in oils at room temperature is roughly between 30 and 50 mg/kg.[4, 24, 25] Experiments by Pardun, and Becker and Niederstebruch have shown that a reduction of dissolved oxygen in sunflower oils and margarine respectively resulted in improved flavour scores after storage.[4, 26] However, even minute residues of dissolved oxygen of well below 1 mg/kg were sufficient to cause flavour deterioration through the development of rancid off-flavours.

Berger has reviewed various studies on the oxygen contents of oils during the stages of crude oil shipment and oil refining.[24] He concluded that any pumping operation introduces new oxygen into the oil, which is particularly important to be borne in mind for the pumping operations during the packing of oils. The best method of reducing contents of dissolved oxygen is by purging the oil with an inert gas such as oxygen-free nitrogen. Although it has, as yet, not been practicable to remove all dissolved oxygen from oils prior to storage, any measure that reduces the oxygen content improves the flavour stability of oils.

It is obvious that during the storage–use cycle of an individual oil container in a domestic household, fresh oxygen will be introduced into the oil every time the container is opened. The best option for minimising the effect of oxygen supply to the stored oil in such situations is to keep package sizes small to ensure that a frequent exchange with a fresh product is regarded as reasonable by the consumer.

Oxidation catalysts

Metal ions, in particular copper and iron, enzymes and pigments have been identified as catalysts for oxidation reactions of unsaturated fatty acids. A proper bleaching operation during the refining process reduces the contents of metal ions and natural pigments well below levels that have been identified as critical for oxidative rancidity development, and the heat treatment during refining also inactivates any enzymes which might be present in a crude oil.

Therefore, the aim is not to re-introduce oxidation catalysts into the refined oils. This is particularly important for the production of margarine and table spreads, where a water phase is added, which contains several ingredients and food additives. The water itself should have a low trace metal content, food ingredients should be properly pasteurised to avoid contamination with enzymes, and additives such as food colours should be carefully chosen and dosed, bearing in mind their potential pro-oxidative properties. In addition, any packaging material should be checked for the possibility of contamination of the fat or oil product during storage.

Antioxidants

Antioxidants are compounds that are able to inhibit oxidation reactions. Based on the previously explained free radical model of autoxidation in oils, primary antioxidants (AH) are able to abstract the unpaired electrons of fatty acid radicals ($R^{\bullet}$) or fatty acid peroxy radicals ($RO_2^{\bullet}$) by donation of hydrogen, which interrupts the radical chain reaction (see Equation 12.3)

$$\begin{aligned} AH + R^{\bullet} &\longrightarrow A^{\bullet} + R{-}R \\ AH + RO_2^{\bullet} &\longrightarrow A^{\bullet} + RO_2R \end{aligned} \qquad [12.7]$$

The specific molecular structure of antioxidants enables the unpaired electron to be stabilised within the molecule in such a way that the antioxidant radical cannot generate new radicals of fatty acids. Another group of compounds, which are called secondary antioxidants, inactivate oxidation catalysts such as metal ions by chelation, e.g. citric acid.

All edible fats and oils contain natural antioxidants of which the tocopherols and tocotrienols are the most important group. A particular tocopherol, α-tocopherol, is commonly known as vitamin E. In Table 12.4 the indicative ranges of total tocopherol contents are shown for animal fats and vegetable oils. The total tocopherol content is very variable for individual types of oil. However, the main feature is that vegetable oils contain more than ten times the amounts of tocopherols that are present in animal fats. This is the reason why animal fats are more vulnerable to oxidative rancidity development compared with vegetable oils despite the fact that they contain smaller quantities of unsaturated fatty acids. The tocopherols are not stable during oil refining. The

Table 12.4 Ranges of total tocopherol contents in animal fats and vegetable oils[19,27]

Type of oil	Total tocopherol content (mg/kg)
Beef tallow[19]	10
Butter oil	10–46
Lard[19]	27
Almond oil	277–593
Avocado oil	112–201
Cocoa butter	275–290
Coconut oil	31–80
Cottonseed oil	259–940
Grapeseed oil	242–676
Groundnut oil	238–489
Hazelnut oil	356–400
Linseed oil	440–493
Maize oil	941–1748
Olive oil	43–215
Palm oil	272–1176
Pine kernel oil	672–680
Poppy seed oil	241–252
Pumpkin seed oil	385–499
Rapeseed oil	598–655
Safflower oil	379–629
Sesameseed oil	475–550
Soyabean oil	666–1259
Sunflower oil	482–926
Walnut oil	410–455
Wheatgerm oil	916–4073

major losses occur during deodorisation of oils where the tocopherols are removed with the stripping steam. Syväoja *et al.* have reported refining losses of 10–33% of α-tocopherol, 20–33% of other tocopherols and 43–48% of tocotrienols.[28] This means that a careful refining operation is necessary to preserve the natural antioxidant contents in edible oils.

The use of antioxidants as food additives for the retardation of oxidative rancidity development during storage is a long-established practice in the food industry. Despite the fact that many natural and synthetic compounds have been shown to be active antioxidants in foods, only a few synthetic antioxidants, namely butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), gallate esters and *tert*-butylhydroquinone (TBHQ), are permitted food additives as antioxidants in most countries including the EU member states (not TBHQ) and the USA. The maximum permitted dosage levels differ among individual countries, but are usually in the range between 100 and 200 mg/kg, depending on the particular type of antioxidant and food. It is important to note that antioxidants are only effective when added to fresh oils of good quality. They are less effective in delaying oxidative rancidity development when oxidation has already started. More information on the application of antioxidants in oils can be found in Coppen.[29]

Storage conditions

As for all chemical reactions, the rate of oxidation reactions is roughly doubled per 10 K temperature increase. Therefore, cool storage of oils below room temperature reduces oxidation rates and thus retards the development of rancid off-flavours. However, as has been explained earlier, frozen storage of oils increases their vulnerability to off-flavour development. In industrial applications the storage temperatures of some oils, such as palm oil and some shortenings, are kept above room temperature to prevent their solidification. The effect of the higher storage temperature on the oxidation rates needs to be taken into account for the specification of the shelf-life of industrial bulk oils. However, in industrial applications fresh oils are frequently supplied, but it has to be ensured that the user has an appropriate stock rotation schedule in place.

Apart from the storage temperature, exposure to light is the other important storage condition that affects the rate of oxidation reactions in oils. As shown earlier in this chapter, oxidation of unsaturated fatty acids is triggered and accelerated by light via the action of sensitisers. In particular, short wavelength light triggers photo-oxidation. The effect of photo-oxidation on the flavour deterioration of oils is dramatic. Pardun has reported that the shelf-life of sunflower oil stored in transparent glass bottles at 20 °C, was shortened to only four days when exposed to light of 1000 lux intensity, compared with a nine months shelf-life stored in the dark.[4] Oil and fat products distributed through the retail sector are very likely to be exposed to intense light when stored on the shelves of a supermarket or shop. For this reason, the packaging of such products should be designed to reduce the intensity of the incoming light as much as possible.

12.4.2 Prevention of polymorphic crystal transitions

The solid phase of the majority of fat products such as margarine, shortenings, and table spreads consists of a network of fine β'-crystals. It is of vital importance for the maintenance of the texture characteristics of these products to keep this crystal network intact during storage and to prevent its destruction by the transition of the fine β'-crystals to discrete, large β-crystals. Modern margarine production technology, which combines rapid crystallisation in specialised machinery with slow crystal growth under controlled tempering conditions, enables the establishment of β'-crystal networks from a great variety of oil blends regardless of whether individual oils have either β'-crystal or β-crystal tendency. Once a network of β'-crystals has been established, the polymorphic transition to form coarse β-crystals is promoted by higher storage temperatures. Depending on the product, storage at room temperature is usually sufficient to enable such transition of crystal types to occur within the predetermined shelf-life leading to changes of texture characteristics and, ultimately, to the loss of product integrity.

For this reason, fat products should be stored in temperature-controlled warehouses below ambient, and temperature increases should be minimised during transportation. This is particularly important for small-volume retail packages such as tubs of table spreads, which are usually bought and transported in individual units. It is almost impossible to avoid continuous temperature fluctuations during the storage–use cycle of table spreads and margarine in a domestic or catering environment. A suitable package size that brings the average length of the storage–use cycle into safe limits, combined with clear storage instructions on product labels, is useful for ensuring the maintenance of product quality until the end of the shelf-life.

12.4.3 Prevention of microbial spoilage

Margarine and table spreads can be spoiled by microbial growth because of their water phase. However, margarine and spreads with fat contents above 60%, which have been produced under hygienic manufacturing conditions, are unlikely to provide suitable microbial growth conditions because of the small size of the water droplets in the water-in-oil emulsion and the preservative effect of salt, which is usually added to such products. In contrast, reduced- and low-fat table spreads, and also low-salt table spreads, require the addition of preservatives such as benzoic, citric, lactic or sorbic acids to the water phase in order to maintain a pH value between 4 and 6, depending on the salt level of the product. Studies on the effectiveness of various combinations of these acids with salt have been reviewed by Chrysam.[30]

12.5 Future trends

The big issue with regard to the stability and shelf-life of oils is the retardation, if not prevention, of oxidative rancidity development. The two major

compositional factors of oils that determine their susceptibility towards oxidation are the fatty acid composition and inherent antioxidant activity. Traditional plant breeding techniques and modern biotechnology, i.e. genetic modification, have been applied successfully to develop new varieties of traditional oilseed crops that provide oils with altered fatty acid compositions. From the point of view of oil stability, oils with greatly reduced amounts of poly-unsaturated fatty acids in favour of increased amounts of mono-unsaturated oleic acid, such as high-oleic rapeseed and sunflower oils, are already on the market. In addition, new oil types with high oleic acid contents from hitherto not exploited agricultural crops are likely to extend the range of edible oils. It is obvious, however, that the limitations for the manipulation of edible oil fatty acid composition are set by the nutritional requirements of the consumers for an adequate supply of essential poly-unsaturated fatty acids. For this reason, the development of new fat and oil products with altered fatty acid compositions will always involve finding a balance between these nutritional requirements and the desired oxidative stability.

A variety of developments are also under way that aim to improve and preserve the inherent antioxidant activity of oils. The target of many breeding and genetic modification projects is to increase tocopherol contents in traditional oilseed crops, and mild oil-refining processes are being developed that make it possible to retain the majority of inherent tocopherols within the refined oils. Considerable research work has been carried out in the food and food ingredient industries to investigate the antioxidant potential of a great number of natural compounds that can be obtained from plants or microorganisms. Provided that government approval is given for such novel antioxidant preparations, their use in fat and oil products will greatly enhance their stability towards oxidation.

Within the edible fats and oils industry there is still the need to develop a reliable method for the prediction of product shelf-life without having to conduct long-term storage trials. Prompted by medical research into the function of antioxidants in human health, a new type of simple and quick analytical methods has been applied for the determination of antioxidant activities in a variety of foods including oils. The principle of these new analytical methods has been reviewed in a paper by Zieliński and Kozłowska.[31] The potential advantage of these new methods is that they determine the activity, rather than the amount, of antioxidants under the test conditions. This also means that all antioxidants present in a food matrix are considered in their entirety in contrast to the determination of individual compounds. The question is whether the test conditions can be suitably adapted for modelling antioxidant reactions in foods during storage.

Combining the fatty acid composition of an oil with the antioxidant activity determination would enable the complete assessment of an oil's stability towards oxidation. However, in order to predict the shelf-life of fats and oils, their content of dissolved oxygen also needs to be taken into account. Until recently, the methods for the determination of dissolved oxygen in oils have been impractical for routine analysis. Modern sensor technology has enabled the development of oxygen-sensitive electrodes that are already being used for on-

line process control in the fermentation industry, i.e. for aqueous food matrices. It seems to be only a small step to adapt such sensors for the application in the non-aqueous matrix of oils. Based on the general model of oxidative rancidity development in oils, the easy determination of dissolved oxygen appears to complete the set of analytical techniques required to assess the shelf-life stability of edible fat and oil products.

12.6 Sources of further information and advice

In addition to the books and journals that have been referred to in this chapter, further detailed information on many aspects related to the properties, production and applications of edible fats and oils can be found in the books by Bockisch,[32] Hui,[33] Karleskind,[34] and O'Brien.[35] The original French version of the book by Karleskind is more comprehensible than the English translation.[36] Details on the crystallisation properties of fats are explained in a book by Garti and Sato.[37] A comprehensive review on oil oxidation can be found in a book by Frankel.[38] Information on many aspects related to the edible oils and fats industry can also be found in patents and on the Internet.

Professional institutions that are dedicated to dealing with technical and commercial aspects of the edible fats and oils industry exist in many countries. The contact details of a selection of such institutions are given below.

American Oil Chemists' Society (AOCS), PO Box 3489, Champaign, IL 61826-3489, USA.
Tel: ++1-217-359-2344
Fax: ++1-217-351-8091
E-mail: general@aocs.org
Website: www.aocs.org

Deutsche Gesellschaft für Fettwissenschaft eV, Postfach 90 04 40, Frankfurt/Main, D-60444, Germany
Tel: ++49-69-7917-529
Fax: ++49-69-7917-564
E-mail: F.Amoneit@gdch.de
Website: http://www.gdch.de/dgf

Institut Des Corps Gras, Rue Monge, Parc Industriel, Pessac, F-33600, France
Tel: ++33-5-56 36 00 44
Fax: ++33-5-56 36 57 60
E-mail: iterg@wanadoo.fr

Society of Chemical Industry, Oils and Fats Group, 14/15 Belgrave Square, London, SW1X 8PS, UK.
Tel: ++44-20-7598-1500
Fax: ++44-20-7823-1698
Website: http://sci.mond.org

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13

Sauces and dressings

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13.1 Introduction

The words sauces and dressings refer to emulsions such as ice-cream, milk, margarine, mayonnaise, salad dressings and condiment sauces, such as barbecue sauce, ketchup and spaghetti sauce. The natural emulsion, milk, has been an important part of the human diet for many years (Graf and Bauer, 1976). Studies into other natural emulsions by food scientists led to the discovery and production of man-made emulsions, including mayonnaise. Compared with mayonnaise, which is believed to have existed for many centuries (Robinson, 1924), the other emulsions such as cake batter (Shepard and Yoell, 1976), ice-cream (Berger, 1976), margarine (Brown, 1949; Weiss, 1970) and sausage (Schut, 1976) are relatively new products.

All these food products are liquid or solid droplets (dispersed phase) suspended in another (continuous) phase. An emulsion is a common type of dispersion. The products discussed in this chapter are either oil-in-water emulsions such as milk and mayonnaise, or water-in-oil emulsions such as margarine and butter. Products in this category vary in their fat content considerably, allowing them to go from one emulsion type to another (Table 13.1). It is not only the fat content that varies in these products; ingredients that make up the product vary extensively also. Product definitions vary from continent to continent; however, there are common grounds to be found in their definitions.

13.1.1 Mayonnaise

In Europe the term mayonnaise is defined as a condiment sauce that is obtained by emulsifying edible vegetable oil(s) in water. The resulting water-in-oil

Table 13.1 Typical fat content of various sauces and dressings

Sauce/dressing	Fat (%)
Mayonnaise	70–84
Salad dressing	30–60
French dressing	36–40
Thousand Island	30–45
Barbecue sauce	1.0–2.0
Ketchup	0.1–0.2

Adapted from Ford *et al.* (1997).

product will contain vinegar and chicken egg's yolk as the stabiliser. There are other ingredients that may also be included in the emulsion defined as mayonnaise. These ingredients may include salt, sugar, egg white, egg products, fruits and vegetables (and/or their juice), herbs, spices, mustard and dairy products and other condiments. Mayonnaise may be acidified with the aid of organic acids (or their salts) such as acetic, citric, lactic, malic and/or tartaric acid. Benzoic acid, sorbic acid (including their salts) and nisin are often used as preservatives in mayonnaise. Other ingredients are also used to boost the sensory quality of such products by including colouring, antioxidants, flavourings and flavour enhancers (e.g. monosodium glutamate).

The Codex Alimentarius Regional European Standard includes the above definition and states that the total fat content of mayonnaise must be at least 78.5% and not less than 6% pure egg yolk. The Association of the Mayonnaise and Condiment Sauce Industry of the EEC adopted a minimum total fat content (70%) and minimum egg yolk content (5%) policy (CIMSCEE, 1992). Typically the pH of mayonnaise in Europe ranges between 3.0 and 4.5 with acetic acid as the predominant acid which is typically between 0.8 and 3.0% in the aqueous phase. The level of salt and sugar in the aqueous phase are not part of any European legal requirement and most often fall between 1 and 12%, with the occasional products exceeding these levels.

US standards are slightly different from those in Europe, with the vegetable oil content set at a minimum of 65%. Also included in the US standards are set ranges for pH, salt and sugar. The pH of mayonnaise must be in the range 3.6 to 4.0 with the predominant acid being acetic acid (representing between 0.29 and 0.50% of the total product). The salt and sugar levels may not fall outside the range 9 to 12% and 7 to 10% in the aqueous phase respectively (US Department of Health, Education and Welfare, 1975a).

13.1.2 Dressings

In comparison with mayonnaise, dressings have a lower fat content, but have a starch phase, which helps to give the necessary consistency. Dressings often range in pH between 3.0 and 4.2 with acetic acid being the predominant organic

acid acidulant that may range in the aqueous phase between 0.5 to 1.5%. The salt and sugar level in the aqueous phase may vary between 1–4% and 1–30% respectively. The Food and Drug Administration (FDA) in the US defines dressings as emulsified semi-solid foods prepared from vegetable oils, vinegar, lemon juice and/or lime juice, egg yolk-containing ingredients and a cooked or partially cooked starchy paste (US Department of Health, Education and Welfare, 1975b). The definition includes minimum levels for edible vegetable oil (30%), pH range (3.2–3.9), acetic acid level (0.9–1.2% of total product), salt in aqueous phase (3–4%) and sugar in the aqueous phase (20–30%).

Becher (1957) compiled definitions of emulsions and also added his own definition to the list. He stated that an emulsion is a 'two phase system of immiscible liquids' (Lynch and Griffin, 1974) that 'posses(es) a minimal stability'.

Sauces and dressings are emulsion systems that rely on their organoleptic properties as well as their microbiological safety to sell. The definitions stated above illustrate the tip of the iceberg where formulations are concerned. With varying formulations, the most popular (sensory) emulsions may be produced. However, in most markets this is not enough. To increase sales it is necessary to be able to keep the product for very long periods of time without compromising the quality or safety of the product. Emulsions are by nature thermodynamically unstable and hence it is only a matter of time before the structure breaks down. The loss of emulsion structure integrity would in effect bring about changes in the quality of the product. These changes may involve flavour release, change in aroma, colour and appearance. The end of a product shelf-life is reached when the sensory attributes no longer satisfy the quality standards set by the manufacturer. The loss in stability arises as the emulsion structure breaks down. The breakdown of the emulsion structure may be due to emulsion properties or microbial activity, both of which influence emulsion shelf-life directly.

13.2 What determines the shelf-life of sauces and dressings?

The shelf-life of sauces and dressings is based on the stability and safety of the product. Stability can be defined in terms of emulsion properties or microbial activity.

13.2.1 Emulsion property

Emulsion properties may be chemical and/or physical. The characteristics of the emulsion properties are generally considered to be dependent on the properties of the continuous phase and/or the ratio of the continuous to dispersed phase (Lynch and Griffin, 1974). These properties have been categorised into eight groups by Lynch and Griffin (1974) and Bennett (1947).

Appearance

The appearance of the emulsion is affected by the ingredients used, the colour of

the ingredients, particle size of the dispersal phase and the refractive index. The continuous phase colour defines the emulsion's final colour. A dispersal phase particle size ranging between 0.5 and 5.0 μm would give an opaque emulsion.

Conductivity
One simple means of distinguishing water-in-oil from oil-in-water emulsions is by measuring the conductivity. Water-in-oil emulsions are weak electrical conductors, whereas oil-in-water emulsions are strong electrical conductors.

Dispersability and emulsion type
Oil-in-water emulsions can be dispersed in and diluted by water. Water-in-oil emulsion can be dispersed in and diluted by oils.

pH
Variation in pH may lead to ingredients coming out of solution, changing charge or altering polymer/protein structure and as a result leading to destabilisation of emulsions.

Viscosity
The viscosity of an emulsion is mainly dependent on the ratio of the dispersal to continuous phase. At low dispersal levels, the viscosity of the emulsion is very similar to that of the continuous phase. The viscosity will increase as the level of dispersal phase increases in relation to the continuous phase. Theoretically, the level of dispersal phase may not be more than 74% of the emulsion and still maintain a spherical shape. Increasing the level beyond this value would cause distortion of the particle size and allow the emulsion to increase its plasticity. Under these conditions, particle charge and size would have a greater effect on the viscosity of the emulsion.

Particle charge
All dispersal phase particles are charged and this plays a major role in the stability of fine emulsions. The charge is not as important in coarse emulsions.

Particle size
The diameter of spherical particles is taken as the particle size. Emulsions with small particle size are considered as fine emulsions, whereas those with larger particle size are coarse emulsions. High stability is often associated with fine emulsions with uniform sizes. The particle size is directly affected by the method of emulsion production. This would include the order of ingredient addition as well as the amount of work done to form the emulsion.

Stability
Emulsions are not thermodynamically stable and this instability may be revealed by observable changes of the emulsion. Since stability is a relative concept a reference material is necessary for comparison for the degree of change. The

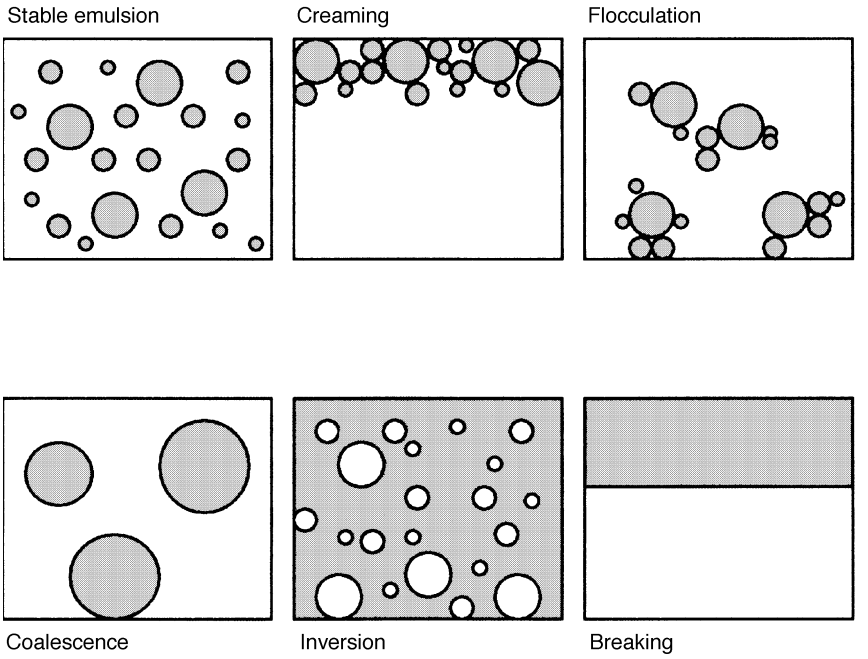

Fig. 13.1 Emulsion instability.

changes may be as a result of coalescence, breaking, inversion, flocculation or creaming (Fig. 13.1).

In an emulsion system, oil or water particles, as the dispersed phase, move within the water or oil continuous phase, respectively. The collision of two particles leads to coalescence and the formation of a larger particle. The resulting particle will have a lower surface area and a lower interfacial energy. Hence, this particle will coalesce more readily than a smaller particle. Continuation of this process would lead to complete separation of the phases and bring about inversion or breaking. In order to prevent coalescence, emulsifiers are used that prevent the dispersed phase particle from close contact. Emulsifiers achieve this by forming a potential energy barrier between the particle surfaces. The stronger the emulsifier, the less chance there is of dispersed particles colliding and hence the higher the stability of the emulsion. A potential energy barrier may be achieved by using emulsifiers that operate by varying mechanisms. The mechanism of action of emulsifiers may be categorised into three groups, electrostatic, steric and particle absorption.

Electrostatic repulsion by emulsifiers such as lecithin and proteins are seen through charged hydrophilic groups. These emulsifiers are absorbed into the interface of oil and water. Since they are charged, they repel other emulsifier absorbed particles. With this in place, the emulsion will remain kinetically stable as long as the maximum combined potential of the attractive van der Waals forces and the repulsive electrostatic forces is greater than the thermal energy of the droplet. Loss of stability will be observed only when the emulsion is subjected to external forces, such as centrifugal field or heat.

Not all emulsifiers are charged; Tween and monoglycerides are examples of non-ionic emulsifiers. The mechanism of action of these emulsifiers is known as steric stabilisation. The molecule would be absorbed into the oil droplet at the interface of an oil-in-water emulsion. The exposed tail is highly hydrophilic and as a result will attract water molecules around it and hence a layer of water will surround the oil droplet. The water barrier formed would not allow two oil droplets to come into contact and hence the emulsion will remain stable, all other conditions being constant.

The other mechanism of emulsifier action is particle absorption. Hydrophilic mustard particles or crystals of triglyceride (high melting point) are examples of this type of emulsifier (Fillery-Travis *et al.*, 1990). The solid particles sit in the interface and are believed to act as a physical barrier between the contact of the oil droplets and hence the risk of coalescence.

Continued coalescence would lead to breaking or inversion of the emulsion. However, before coalescence, flocculation and creaming may occur. This state of an emulsion is brought about when there is a minimum potential energy/distance relation. The minimum potential represents a metastable state where the droplets are kept at a fixed distance from one another, yet maintain their individual identity. This state can be reached via different mechanisms in electrostatic or steric stabilised emulsions. In the former, the set distance between droplets can be defined by the charge on the surface of the droplet, causing flocculation. In steric stabilised systems, long polymers may interact with other droplets and hold them in close proximity, bridging between two particles and once again bring about flocculation. Alternatively, when much higher concentrations of non-adsorbing large polymers are incorporated in the continuous phase, the droplets are forced together by osmotic pressure.

In an emulsion system where the droplets and continuous phase have significantly differing densities, gravitational or centrifugal forces cause creaming or sedimentation to occur. This is a phenomenon commonly observed with the natural emulsion, milk, where a layer of cream would form on the top. This is also observed in other condiments such as salad dressing (French).

Emulsion stability is not solely dependent on oil, water and emulsifier. Other ingredients included in the formulation of a real sauce or dressing also have a role. Such ingredients may include surface-active polymers, as well as chemicals that would affect the pH and ionic strength. A clear understanding of emulsifier mechanisms in a complex system is necessary for determination of emulsion stability.

13.2.2 Microbial activity

Microorganisms require favourable conditions to grow. During growth in a product, some microorganisms produce gas, some produce acids and others produce toxins (or a combination of these). Food spoilage occurs when the action of microorganisms in food causes the product to be rejected from a quality (e.g. gas production) point of view. The food may also be rejected as its consumption may lead to food poisoning. This would be due to the presence of infectious pathogens or the presence of pre-formed toxin in the food consumed. Manufacturers of food products use formulations in order to kill, stop or slow growth of the microorganisms that may be present in the finished product. This process dictates the microbiological shelf-life of a product. The tools available to manufacturers may not all be used, as this may lead to the final product quality being unacceptable. For example, it would not be possible to sterilise mayonnaise at 121 °C for 3 min. The product would be ambient stable indefinitely, but it would not be mayonnaise as we know it. On the assumption that all microorganisms are present in the product, the intrinsic and extrinsic parameters are used to achieve the desired kill, stop or slowing of microbial growth.

Intrinsic parameters would include the following:

- *pH* – it has been long established that most microorganisms grow best in the pH range 6.5–7.5. Very few bacteria are capable of growth below pH 4.0, *Lactobacilli* being one. Yeasts and moulds, however, are capable of growth well below pH 4.0 and some yeast species may grow at levels as low as 2.0 (moulds, even lower). Different microorganisms have different pH optimum for growth. As the pH is taken below this value, growth is inhibited and hence shelf-life is increased.
- *Moisture content* – this parameter has been one of the oldest methods for inhibiting microbial growth and hence increasing product shelf-life. It is often referred to in terms of water activity. Water activity (a_w) may be related to equilibrium relative humidity (ERH) by the equation: ERH = $a_w \times 100$. Most microorganisms grow best at a_w around 0.99. As the a_w drops in value, water availability is reduced and hence microorganism growth is inhibited. Most spoilage bacteria will not grow at a_w below 0.91, whereas yeasts and moulds are inhibited at much lower values (0.62 for yeasts and 0.61 for moulds) (Anon., 1996).
- *Oxidation/reduction potential* – O/R potential is zero when the level of oxidants equals the level of reductants. Microorganisms are sensitive to the O/R potential of their own growth medium. Anaerobic bacteria require negative O/R potential whereas aerobic bacteria require positive O/R potential. Alterations to the potential will inevitably inhibit bacterial growth.
- *Nutrient content* – microorganisms, like all other living organisms, require nutrients to survive and grow. Therefore, supplementation with water, energy source (e.g. sugar), nitrogen source, vitamins and growth factors as well as minerals are necessary for growth and survival.
- *Antimicrobial compounds* – some foods have shown to have antimicrobial agents such as allyl isothiocyanate in mustard (Shelef, 1983). Compounds

such as these are natural chemicals used as ingredients to inhibit or kill bacteria.

- *Preservatives* – natural chemicals exist that are capable of growth inhibition. Sorbic acid, benzoic acid and nisin are only a few that may be used in many food products to inhibit microbial growth.
- *Weak organic acids* – other natural chemicals that are used as preservatives but are also used as acidulants may be used to increase shelf-life by inhibiting microbial growth. Acetic acid from vinegar, citric acid from lemon juice and lactic acid are a few of the most commonly used acids in sauces and dressings.

Extrinsic parameters include the following:

- *Storage temperature* – microorganisms grow within a very wide band of temperature, often ranging between 0 ºC and 100 ºC. Those that may grow below 7 ºC but not above 30 ºC are referred to as psychrotrophs, those that cannot grow below 7 ºC or above 40 ºC are termed mesophiles, and those that can grow above 45 ºC are termed thermophiles. Inhibition, halting or death of different microorganisms may be achieved by selecting the correct temperature.
- *Equilibrium relative humidity* – ERH of the environment is key to surface-growing microorganisms.
- *Presence and concentration of various gases in the immediate environment* – nutrients are necessary for microorganism proliferation including gases. However, some gases may also act as inhibitors and these are mostly concentration-specific.
- *Presence of other microorganisms* – various species of microorganisms grow in the presence of others without any side effects. However, other microbes may produce chemicals during their natural growth cycle that may be toxic to others. Hence, the presence of one bacterium would effectively remove another.
- *Process* – thermal, radiation or even ultrasound techniques are used to decontaminate products. The level of process determines the level of microorganisms remaining in the product. From this the time taken for microbial levels to reach unacceptable levels can be calculated, and hence the shelf-life.
- *Emulsion structure* – in an emulsion system, the microorganisms may reside in one of three locations: oil, water or at the interphase. Different microbes can grow in different locations: Aerobic bacteria can only grow in the water phase or in the interphase, yet anaerobic bacteria only grow in the oil phase. Depending on what microorganisms are present, a different time will be required for the bacteria to grow to unacceptable levels and hence the product will have a different shelf-life.

These are intrinsic and extrinsic parameters that determine growth rates, death rates or complete inhibition of microorganisms. However, by the nature of sauces and dressings, not all microorganisms will be present or able to grow. It is

necessary to know what microorganisms are key to this product category and hence this knowledge becomes another factor that needs to be used to determine shelf-life.

The microbiological problem with sauces and dressings can be split into spoilage and food poisoning. Depending on the ingredients of the product, many species may be present. However, many would not be considered as they would not be able to grow in the product and hence would not be a significant risk to food safety or spoilage.

Microbial stability – spoilage

Yeasts and *Lactobacilli* are the main causes of spoilage to sauces and dressings. There have been reports of spoilage by moulds; however these are rare because of their lower tolerance to acetic acid, which is the base acidulant of most sauces and dressings (Smittle and Flowers, 1982). Yeasts that are capable of and have been reported to be the cause of spoilage in these products have to be resistant and capable of growth in 3% acetic acid (in aqueous phase). Problematic species include *Zygosaccharomyces bailii* and *Pichia membranaefaciens* (Thomas and Davenport, 1985). The latter yeast often grows on surfaces and it has been observed that in practice the organism requires oxygen to grow (Smittle and Flowers, 1982). Although these two yeasts have been regarded as the most common causes of sauces' and dressings' spoilage, other species have also been reported as spoilers, such as *Z. rouxii*, *Saccharomyces cerevisiae* and *Candida magnolia*. Spoilage caused by yeasts is commonly recognised by gas formation or growth of light brown colonies on the surface of mayonnaise. These sometimes appear as small oil droplets, depending on oxygen availability.

Spoilage by bacteria is mostly observed through the growth of *Lactobacillus plantarum* and *Lac. buchneri*, with *Lac. fructivorans* isolated from spoiled products less often (Smittle and Flowers, 1982). Spoilage is often observed due to gas production and a change in product pH. Occasionally the bacteria may grow to high numbers yet have no impact on the product.

Generally, moulds are not regarded as a risk to sauces and dressings, owing to high acetic acid levels present in the product. However, acid-resistant moulds do exist such as *Penicillium glucum, P. roqueforti, Moniliella acetoabutans* and *Monascus ruber* and may be spoilers of sauces and dressings (Tuynenburg Muys, 1971). Another species, *Geotrichum* species, has also been reported to be found on the surface of mayonnaise in jars with faulty seals (ICMSF, 1980).

Pathogens – food safety

There are four pathogens that may be of concern in sauces and dressings, *Salmonella, Staphylococcus aureus, Listeria monocytogenes* and *Escherichia coli* O157. Other pathogens are unable to grow in sauces and dressings as described in this chapter.

Salmonella has received much attention in recent years owing to food poisoning outbreaks. This organism is an infectious pathogen and very low numbers are required to cause food poisoning. There is no need for the pathogen

to grow; its presence in the product is enough. Therefore, it has to be eliminated from the product. Hence, from a product shelf-life point of view it is very clear what action is needed. *Salmonella* must not be present in the product. If present, then the shelf-life is zero. The product formulation (e.g. mayonnaise) pH below 4.5 and use of acetic acid as acidulant would inactivate *Salmonella* and satisfy the requirement for food safety. Similarly for other products, formulations must ensure elimination of the pathogen from the product.

Staphylococcus aureus does not cause food poisoning by infection but by intoxication. Toxins are produced by the bacteria during growth (only after reaching 10^5 per gram) and ingestion of the toxin, not the bacteria, causes food poisoning. Therefore, if *S. aureus* is present in the product, the conditions that allow it to grow will determine its growth rate and time taken to reach a toxin-producing level. This information is used to set the product shelf-life with respect to this pathogen. In most sauces and dressings the formulation is such that *S. aureus* does not grow and hence is not a significant risk.

Listeria monocytogenes carries with it the same problems as *Salmonella* and so is dealt with in a similar fashion.

Escherichia coli O157 is also an infectious pathogen and its presence in food is unacceptable. Similarly to *Salmonella* and *L. monocytogenes* this pathogen must not be present in the final product. Unfortunately, this strain is much more acid tolerant than the other pathogens (ICMSF, 1996).

Emulsion properties, ingredients and original microflora are the data that may be gathered if shelf-life measurement is desirable. Therefore, the above information may be used in combination to assist in assigning product shelf-life.

13.3 How shelf-life of sauces and dressings is measured

The most accurate method for measuring shelf-life is by carrying out product storage trials. Although time consuming and product-specific, it is the most reliable method for shelf-life measurement. Accelerated shelf-life testing can also be conducted; however, this method is only truly valid for sensory attributes and not microbiological shelf-life determination. When carrying out storage trials for sensory attributes, reference material is necessary for comparison. Emulsion stability measurement is a direct method for measuring shelf-life. For microbiological shelf-life determination, challenge tests may also be conducted, as well as the use of mathematical predictive models.

13.3.1 Storage trials – sensory

It is relatively simple to conduct storage trials but not so simple to interpret them. Product samples are aliquoted into separate containers and stored under the required conditions (set temperature, humidity, atmosphere, etc.). At predetermined times (e.g. at 1, 2, 4, 6, 10, 14, 20 weeks), duplicate or triplicate samples are removed and examined for:

- Colour.
- Odour.
- Taste.
- Texture.
- Coalescence.
- Breaking.
- Inversion.
- Flocculation.
- Creaming.

Alongside each test trial there will also be a reference sample, in order to determine at what sample time the sensory attributes changed enough to assign the end of shelf-life.

Accelerated shelf-life testing is carried out only for a product's sensory attributes. This is achieved by creating a calibration curve for the behaviour of the product, with reference to changes in the above-mentioned attributes, at varying temperatures. Having created a calibration curve of changes in product characteristics in time at varying temperature, the product is then incubated as previously described but at a designated elevated temperature. The resulting changes in the sensory characteristics will be recorded and the time at which the product is considered to be unacceptable. With the aid of the calibration curve previously created, the end of life is identified and hence shelf-life is measured.

13.3.2 Storage trials – microbiology

The underlying method is as carried out for sensory storage trials. Product samples are aliquoted into separate containers and stored under the required conditions (set temperature, humidity, atmosphere, etc.). At predetermined times (e.g. at 1, 2, 4, 6, 10, 14, 20 days), duplicate or triplicate samples are removed and microbiological examination carried out. Sauces and dressings have specific formulations such as pH. Therefore, during the timed sample examination, pH measurement is also conducted. When examining the product, end of life is determined by the specifications set for the product:

- $pH \leq 4.5$.
- No gas production.
- Plate count $< 10^5$ per gram.
- *Bacillus* spp < 200 per gram.
- Clostridia < 200 per gram.
- Coliforms/*E. coli* < 20 per gram.
- *Salmonella* spp. not detected in 25 grams.
- *S. aureus* < 20 per gram.
- Yeast and moulds $< 10^3$ per gram.

At each time point, tests for the above are carried out. At the time point that the results fall outside of the specification, that time point marks the end of product life. Hence, shelf-life can be assigned to the product.

13.3.3 Challenge test

Challenge tests are carried out in exactly the same way as storage trials, with one difference. In this case the appropriate microorganisms are inoculated into the product at a predetermined level (pathogens at a level of 10^6 per gram, and spoilage organisms at 10^4 per gram). Pathogen level is higher than the specification at the beginning, but the objective of the test is to see if the product will reduce the pathogen level to acceptable levels or inhibit growth completely. If not then the product is not safe and requires reformulation. If the numbers are reduced to the acceptance level, then it is microbiologically a safe product. If complete inhibition is achieved, then product safety may be assigned if the final product satisfies the microbial specification listed above. The level of fungi inoculated needs to be observed for any decrease also. A decrease in numbers would indicate an indefinite microbiological shelf-life (from a fungi point), all other parameters remaining unchanged. However, if the fungi numbers did not change, the tests are continued until the levels either fall or rise. If they fall, the same shelf-life as above is assigned; however, if the level begins to rise, the time at which the rise is observed would be considered the end of product life and hence shelf-life would be assigned accordingly.

13.3.4 Predictive modelling

Predictive microbiology has been receiving a great deal of attention since the early 1980s. Developing methodologies that predict growth, death and survival of food poisoning and spoilage microorganisms is hence a relatively new area. The ability to predict microbial characteristics under various conditions would help design products with extended shelf-lives and safer products. However, in order for the models or equations to be useful, they must be able:

- To predict accurately the fate of microorganisms in foods.
- To be applicable to a wide range of foods.
- To take into account intrinsic and extrinsic factors that affect microorganisms.
- To be user friendly.

Presently several mathematical models exist, each with their strengths and weaknesses. However, the information required from all for predicting shelf-life is the same. Taking Food MicroModel as an example, it is possible to insert information regarding the formulation of a dressing and the microorganism of concern. The program will calculate and present the lag times, growth rates or death rates under the specified conditions. Therefore, all the information gained from storage trials and challenge tests can be gained in minutes rather than weeks. The information is used in the same fashion as before and shelf-life is assigned.

In 1992 a revised version of the code for the production of microbiologically safe and stable emulsified and non-emulsified sauce containing acetic acid was distributed by the Comité de Industries des Mayonnaises et Sauces Condimetaires de la Communauté Economique Européenne (CIMSCEE). The code

presents two formulae, one for intrinsic safety and one for intrinsic stability of such products. The code works very well for simple sauces and dressings that contain salt, sugar and acetic acid in an emulsion. The code is unable to predict the shelf-life as days or weeks, but it can assign intrinsic safety and stability indefinitely, if the emulsion properties are maintained. However, as soon as other inorganic acids, preservatives and other antimicrobials are used, the code becomes inaccurate.

13.4 Implications of measurement for formulation and preservation

Shelf-life of sauces and salad dressings are determined by intrinsic and extrinsic factors, as described in earlier sections of this chapter. The influencing factors may affect the organoleptic and/or microbiological shelf-life. These factors, in the majority of cases, are measurable, as they are product ingredients or storage conditions:

- Oil level.
- Oil/water droplet size.
- Emulsifier concentration.
- pH.
- Preservative.
- Water activity.
- Humidity.
- Temperature.

Control of the level of each of the above, as well as other factors/ingredients, is carried out by dosing, specific processing and/or environmental condition controlling equipment. The final confirmation of the desired product specification, whether ingredient level or storage temperature, also requires the use of specialised equipment. Since these constituents are the key factors involved in product shelf-life, their control and measurement is essential. In most formulations, especially in more recent years, key shelf-life influencing factors are at their critical level. For example, a sauce with a pH of 3.9 is stable while one with a pH of 4.1 is not. It is therefore clear that there can be no room for error or inaccurate measurement.

Conducting repeated tests on presumably identical samples under presumably identical conditions, will often yield different results. There are many factors that may influence this outcome, including:

- The equipment.
- The operator.
- Calibration.
- The environment – such as temperature and humidity.

Each of the above can be overcome in order to achieve a result that is accurate and precise time and time again. It is important to define briefly what is meant by accuracy and precision.

- Accuracy – the closeness of the observed result to the true value.
- Precision – variability between repeated tests. However, this term may be expanded into two different definitions to clarify the position:

  (a) Repeatability – this refers to the closeness of agreement between results obtained from tests carried out under exact same conditions, or as close as possible (such as the use of the same equipment, same operator, same laboratory, etc.).
  (b) Reproducibility – this refers to the closeness of agreement between results obtained from tests carried out on identical samples but under as varied conditions as possible (such as the use of different equipment, different operators, different laboratories, etc.).

  In essence, repeatability and reproducibility are the two extremes of precision, the former is the minimum and the latter is the maximum variability when measurements are made.

It is possible to minimise the problems with accuracy and precision by using the same equipment (suitable for the task), use a trained operator, ensure equipment is calibrated and be sure that the environmental conditions are as similar to each other as possible. Data can be collected, based on the above, and as a result, it would be possible to quote standard deviation and assign confidence intervals at the appropriate probability level. Such a procedure may allow the product developer to know with 99% confidence that the pH of the sauce measured is in fact 3.9 ± 0.1.

Achieving an accurate and precise measurement of the factors that influence shelf-life is without doubt very important. The data are often used in product simulation (simple systems that mimic real products) studies with regard to microbial growth/death and emulsion property. The data are used to form predictive models for the different characteristics of microorganisms in environments that are accurately and precisely controlled (similarly for emulsion characteristics). These data are then used in turn to predict shelf-life of various products. Food MicroModel and the USDA Pathogen Model are two mathematical predictive models that can calculate the rate of growth or death, based on the media criteria incorporated into the equations, as discussed earlier. Although the measurable levels may be accurate and precise with the correct confidence limits, there are other 'inaccuracies' that need to be considered.

The data generated by predictive models are often based on liquid media that try to mimic the product conditions. However, there are certain product ingredients that are not represented or accounted for, such as spices. There is not yet a full understanding of the action of spices in products on microorganisms or emulsion properties. Spices are not the only ingredients that are often omitted from broth media that is used for initial modelling work. Other ingredients

include: oil, emulsifiers, natural antimicrobials and a range of chemicals that have not been characterised.

Another source of inaccuracy generated between broth and real product characteristics, is interaction between ingredients. Some chemicals when combined have an additive effect on inhibiting bacterial growth, such as use of glucose in combination with sucrose. Similar systems exist for emulsion properties. There are, on the other hand, those that do not share this characteristic. Some will be synergistic and others will have a negative effect, as the chemicals interact with one another and may eliminate the original individual characteristics.

The measurements are key to assigning shelf-life for sauces and dressings. Inaccuracies in measurements will inevitably lead to incorrect shelf-life determination and the possibility of loss in quality before time and/or causing food poisoning. In the market-place today this is not an option and hence the inaccuracies have to be minimised. Validation of broth data in a wide range of real products generates a level of confidence in the broth data. However, since not all products are included, as this would be impractical, a certain level of inaccuracy will exist. In order to overcome the inaccuracy that is generated as a result of the above, tests may need to be carried out in real products. This again indicates that challenge tests and shelf-life tests are the best methods for shelf-life analysis, as discussed earlier. Predictive models are a guideline for assisting in determining an approximate shelf-life of products. Through accurate and precise measurements, it is possible to reduce the gap between real and model systems, although one must not lose sight of the variation in measurement made on presumably identical samples under presumably identical conditions.

13.5 Extending shelf-life

Shelf-life extension is a concept that needs to be explored. Present technologies can be used to achieve this goal. Most of the methods today have not been explored to their full capabilities. The current techniques are listed below and expanded to illustrate the routes for extending the shelf-life of sauces and dressings.

- *Variation in ingredients.* Historically, sauces and dressings have been formulated using vinegar (acetic acid) as the sole acidulant. Although acetic acid is the most effective weak acid preservative, other weak acids should not be ruled out. Some manufacturers have been experimenting with the use of citric acid from lemon juice. Others have started using lactic acid in combination with citric and acetic acid. Both manufacturers have noted the increase in shelf-life. In order to use the combinations correctly, it is imperative that the mechanism of action of these weak acids is understood, since each acid has a slightly different mechanism for bacteristatic and/or bactericidal activity from the other. Also a good understanding of the pH

ranges within which they are most active is needed. The use of different sugars, monosaccharide to polysaccharide, would also have an impact on the extension of product shelf-life. This point can be easily demonstrated through the CIMSCEE code. Some microorganisms are more sensitive to one type of sugar (monosaccharide or disaccharide) than to another. Emulsion systems use vegetable oil as the base, yet the use of olive oil, which has antimicrobial activity, has not been considered widely.

- *Preservatives*. Sorbic acid, benzoic acid, nisin, parabens, spices and their oils and many other naturally occurring preservatives can be used to help extend shelf-life. The acids should be used in appropriate products, i.e. in those where the pH allows full activity. In this way lower concentrations would be needed. As in weak acids, it is important to understand the nature of action of preservatives, hence allowing them to be used with more direction towards achieving an extended shelf-life. Nisin is a useful preservative, however since it is degraded over time, the concentration eventually drops below critical level and the microorganisms will grow and spoil the product. There are a great number of spices that are used in other food sectors that have not been looked at in sauces and dressings. Spices have naturally occurring antimicrobial agents as do onions, garlic and olives. There are a range of fruits and vegetables as well as herbs and spices that contain natural preservatives with bacteristatic or bactericidal activity. The use of purified active ingredients or extracts from plants may help in achieving shelf-life extension.
- *Processing*. Microbiological safety and stability of sauces and dressings is achieved by formulations that would kill some pathogens and spoilage microorganisms and inhibit others from growing. By formulating for this objective, quality is often compromised. Elimination of microbiological problems through processing and not reducing quality is a difficult task. High-pressure sterilisation may be a way forward to achieving this goal. Product pasteurisation would be an alternative thermal process for decontamination. Sterile ingredients and production under sterile conditions (in-line) would also ensure microbiological shelf-life extension. Emulsion processing equipment for achieving a much smaller particle size would inevitably be an improvement, specifically with a suitable emulsifier.
- *Modified-atmosphere packaging*. Microorganisms associated with sauces and dressings have been identified. Their characteristics with respect to their sensitivity to various gases can be used to formulate a cocktail of gases that would inhibit the microflora. Care must be taken in the choice of gases, since selection for another microorganism may occur when elimination of another is achieved. Another point to consider is the effect of the gases on the emulsion system. As an example, ozone has been shown to be effective against a variety of microorganisms (Burleson *et al.*, 1975); however, because of its strong oxidising activity, it increases rancidity in high-lipid content foods.
- *Storage temperature*. Microorganisms associated with sauces and dressings as discussed earlier, would be four pathogens, yeasts, lactobacilli and

possibly moulds. The infectious pathogens should be eliminated and *S. aureus* has to be inhibited. Spoilage organisms (yeasts, moulds and *Lactobacilli*) also need to be inhibited. Decreasing the storage temperature would achieve this and shelf-life of these products may be extended. Temperatures below 6.0 ºC would inherently inhibit *S. aureus*, *Lactobacilli* and to an extent yeasts and moulds (Anon., 1996).

- *Mixed emulsifiers.* As with all other ingredients, it is important to gain a detailed knowledge base for the behaviour of emulsifiers. Although some emulsifiers are very good, just like hurdle technology, mixed emulsifiers have a positive effect on emulsion stability and hence the potential for increased product shelf-life. As an example, the combination of Tweens and Spans increases interaction between adsorbed molecules in the interfacial layer thereby making it stronger and more condensed.

Each of these factors has the potential to inhibit microbial growth and if necessary, by a selected factor, reduce the population to an acceptable level. Today it is common practice to use a combination of parameters to achieve the required effect. This combination technology was referred to as 'hurdle technology' by Listner in Germany, in the mid-1980s. However, the technology has been used to preserve foods for centuries. To illustrate the potential of using hurdle technology, consider *S. aureus* and its requirements for growth. *S. aureus* growth parameters are:

- Temperature: 7–46 ºC.
- a_w: 1.00–0.86.
- pH: 4.0–9.3.

Inhibition can occur in many different ways (Table 13.2). Even with all parameters in the range permitting growth, there is growth inhibition. The combination stress effects sum up to more than the bacteria can fight against.

Using this technology, the above methods may be employed to achieve an extended product shelf-life. Product formulation may be altered to include other weak acids that may act synergistically, inhibiting microbial growth further. To increase shelf-life further the suggested change can be combined with MAP packaging. Production under good hygienic conditions would ensure lower levels of contamination, hence a longer period before microbial levels reach

Table 13.2

Temperature (ºC)	a_w	pH	Growth	Inhibitor
25	0.99	6.5	Yes	None
25	0.99	3.5	No	pH
25	0.83	6.5	No	a_w
5	0.99	6.5	No	Temperature
10	0.92	4.5	No	Hurdle technology

unacceptable levels. The use of mixed emulsifiers and processing techniques to achieve smaller droplets in the dispersed phase may achieve a much more stable emulsion.

In effect, primarily there has to be an understanding of the activity of all ingredients, tools available for processing and the microflora associated with the product. Secondly, this knowledge must be used to select the correct parameters in combination, and thus the combination would serve to achieve the desired shelf-life extension.

13.6 Future trends

Emulsion structure affects sauce and dressing stability both microbiologically and organoleptically. Looking at the microbiological issues, there is a concern with the fate of microorganisms in the emulsion. Pathogens such as *Salmonella* and *E. coli* O157 must not be present in the product as described earlier. In order to achieve this, product formulation is designed specifically through pH and acetic acid levels, traditionally, that would ensure reduction of pathogen numbers to acceptable levels. However, pH and acid levels are based on the levels and values in the aqueous phase. Pathogens that may reside in the oil phase do not see this acid and pH effect and hence the levels are not reduced. This is of concern when dealing with infectious pathogens that do not need to grow in the food to cause food poisoning on consumption. It might be thought that aerobic pathogens that cause food poisoning by intoxication may not be of concern if located in the oil phase, as they will not grow. However, there may be a possibility that through the life of the product, oil residing pathogens come into contact with the aqueous phase and begin to grow. A similar situation may occur for spoilage organisms. This is an area where concern is building and plans for research are on the way at Leatherhead Food Research Association (LFRA) Food Safety Section.

Following on from the above, in emulsion systems, the location of microorganisms may be of concern when the emulsion is to be thermally processed. Wet heat (in the aqueous) is much more effective than dry heat (in the oil phase), and hence it may be necessary to reset thermal process parameters to account for microorganisms in different phases. This is another research area where LFRA has interests and has plans to build a knowledge base.

Droplet size and fat content variation has a significant effect on stability of emulsion, as discussed earlier. However, there are microbiological issues also in relation to this subject area. Preliminary work carried out at LFRA has shown that oil droplet size and oil content have specific influences on microbiological stability of a product. This apparent link between emulsion structure and microbiological spoilage requires further study. In addition to these observations, variation in emulsifiers may also be of importance.

Investigation into plant extract is a topic area where some researchers are showing great interest. Fruits, vegetables, herbs and spices and their extracts

contain natural antimicrobials and we are only at the beginning of the road to understanding their potential in food shelf-life extension. Some have been used for flavouring (mustard) and the microbial inhibitory effect has been realised by chance.

New and upcoming processing regimes such as high pressure, ultrasound and microwave are yet to be fully developed. Investigation into the combination of thermal process and product formulation has also begun. These techniques may prove to be the ones that can increase shelf-life and quality of sauces and dressings.

There is a move, and has been for a few years, away from the traditional high-fat sauces. The low calorie sauces and dressings involve fat reduction, which brings with it many problems. Apart from texture and appearance of the product, the most challenging task has been and will be to deliver the correct flavour. Therefore, product development teams are struggling to deliver a low fat sauce with low calorie but one that appears, feels and tastes the same as its predecessor. A difficult task it may be, but the solution may be just round the corner.

13.7 Sources of further information and advice

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