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Factors affecting the shelf-life of 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 is 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 abundances 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

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	

thought to possess an organised structure. As a result, the caseins cannot be 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 ºC 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 ºC 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

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 ºC/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 ºC. *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 ºC/30 min	54	46
Heated at 80 ºC/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 °C/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

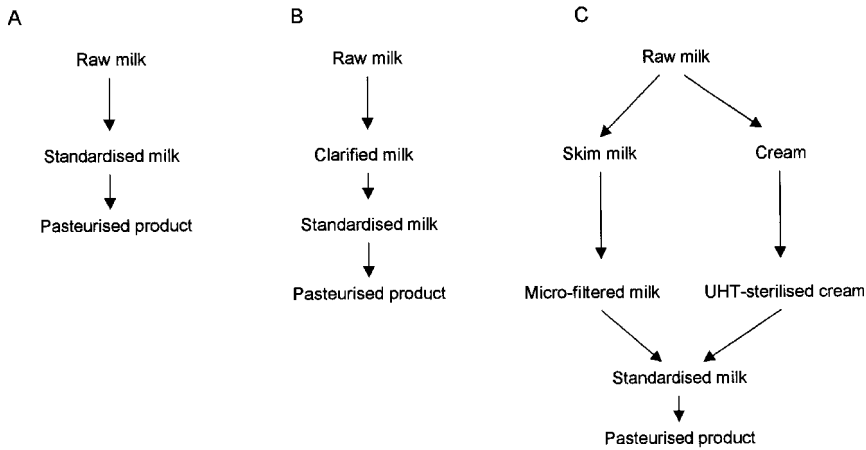

Fig. 9.1 Strategies for manufacture of pasteurised milk

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 affecting 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 ºC) 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 ºC 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 39%, 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

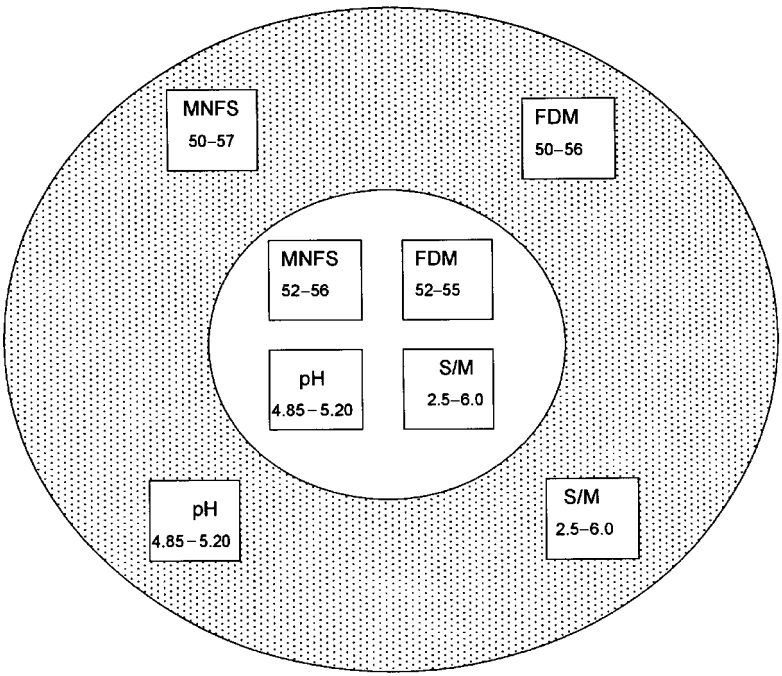

Fig. 9.2 Compositional range for optimising quality of Cheddar cheese. Adapted from Gilles and Lawrence. MNFS = moisture in non-fat solids; FDM = fat in dry matter; S/M = salt in moisture; pH = pH of cheese. Inner ring = premium grade; outer ring = first grade.

development associated with excessive lipolysis in cheddar cheese have been 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.

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