5

Improvements in the pasteurisation and sterilisation of milk

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5.1 Introduction

Liquid milk for consumption is mostly either pasteurised or sterilised. Pasteurisation is a mild process, designed to inactivate the major pathogenic and spoilage bacteria found in raw milk. It should produce minimal chemical, physical and organoleptic changes in the product. If pasteurised milk is then cooled and packaged hygienically and stored under refrigerated conditions, it should have a shelf-life of over 10 days. Further improvements in shelf-life can be obtained by careful control of post-pasteurisation contamination (PPC), by use of good quality raw milk and manipulations of the processing conditions. Eventually, however, such milks will spoil due to survival and growth of thermoduric bacteria or any post-pasteurisation contaminants. Heat treatment regulations require pasteurised milk to show a negative alkaline phosphatase activity and a positive lactoperoxidase activity.

To keep milk for longer than a few days at ambient temperature, it needs to be sterilised. The traditional process involves heating milk in a sealed container in the temperature range 114–120ºC for 20–30 min. The product would be subject to considerable changes in its nutritional value and its sensory characteristics, such as a cooked or caramelised flavour and brown colour. Originally it was heated in glass bottles and sealed with a crown cork, but now it is sterilised in plastic bottles with a foil cap.

More recently UHT processes have been introduced. These are continuous sterilisation processes and involve temperatures in excess of 135ºC for times of greater than 1s, followed by aseptic packaging. UHT milks are subjected to much less chemical change compared to sterilised milk, in terms of colour development, thiamine inactivation, lactulose formation and whey protein

denaturation. However, the resulting cooked flavour (cabbagy or boiled) is generally not liked by the UK consumer. Use of direct processes involving steam injection or infusion allow further improvement in cooked flavour and further reduce other chemical changes, due to the combination of the rapid heating and the flash cooling, which is quick and also removes volatiles. Both sterilised and UHT milks have a shelf-life of up to 6 months, but chemical reactions and physical changes will take place during storage which will alter the sensory characteristics of the milks. These usually result in a deterioration in product quality and take place more quickly at elevated temperatures.

Raw milk is extremely complex. Although water is the main component, milk also contains proteins, fat, lactose, a wide range of vitamins and minerals and many other trace elements and minor components. In addition, there are numerous active enzymes including acid and alkaline phosphatases, lactoperoxidase and lactoferrin. Raw milk from healthy animals has a very low microbial count, but it easily becomes contaminated with spoilage and perhaps some pathogenic micro-organisms. These need to be inactivated and this is readily achieved by heat treatment. From a milk processor's standpoint, raw milk composition and its microbial loading will vary from day to day. Milk is also available from other animals such as goats, sheep, buffalo and camels, to name but a few.

Milk composition can also be easily modified and milk is now available as a wide variety of products: skim, semi-skim, full cream, lactose-reduced, calcium or vitamin fortified and flavoured, as well as a range of creams with different fat contents. It is also concentrated, in the form of evaporated (31% TS) or sweetened condensed (72% TS). It is also produced as powders, which may be subsequently reconstituted to drinking milk or converted into a wide range of other milk products. Heat treatment is a critical step in the production of safe, high-quality liquid milk, cream and milk-based products. The main treatments for milk for consumption are pasteurisation and sterilisation and currently 91.5% of milk for drinking is pasteurised, 0.7% sterilised and 7.8% UHT treated in the UK (*Dairy Facts and Figures*, 2001). This balance is different in other European countries and in some, such as France and Germany, UHT milk is the main product. After both these heat treatments it is also crucial to reduce as far as possible post-processing contamination (PPC). This chapter will consider some of these issues.

5.2 Kinetic parameters in heat inactivation

One of the main purposes of heat treatment is to reduce the microbial population in raw milk. Also, when milk is heated enzymes are inactivated, chemical reactions take place and there are changes in its physical properties. An overview of the changes taking place when milk is heated is given by Walstra and Jenness (1984). Some important ones are a decrease in pH, precipitation of calcium phosphate, denaturation of whey proteins and interaction with casein,

Maillard browning and modifications to the casein micelle. The overall effect is to alter the sensory characteristics, i.e. overall appearance, colour, flavour and texture, and the nutritional value, as well as to improve the keeping quality.

The two most important kinetic parameters are the rate of reaction or inactivation at a constant temperature (e.g. D and k values), and the effect of temperature change on reaction rate (z and E values). The heat resistance of vegetative bacteria and microbial spores at a constant temperature is characterised by their decimal reduction time (D value); this is the time required to reduce the population by 90% or one decimal reduction (one log cycle). D values for vegetative organisms are quoted in the range 60–80ºC and for spores in the range 100–140ºC. Generally heat inactivation follows first-order reaction kinetics. The number of decimal reductions ($\log N_0/N$) can be evaluated from:

$$\log (N_0/N) = \frac{\text{heating time}}{D}$$

where $N_0 =$ initial population, $N =$ final population.

Two important points follow from this. Firstly, it is not possible to achieve 100% reduction; for example, four decimal reductions is equivalent to a 99.99% reduction. Secondly, for a specified heat treatment, the final population will increase as the initial population increases. Therefore sterilisation by heat is not regarded as an absolute form of sterilisation and the microbial quality of the raw material will have a big effect on the final population and hence the keeping quality of pasteurised milk and the spoilage rate for sterilised milk.

The temperature dependence of a reaction is measured by its z value, i.e. the temperature change that brings about a tenfold change in the D value. Inactivation of vegetative bacteria is very temperature sensitive ($z = 4$–8ºC), whereas most heat-resistant spores are found to have a z value in the region of 10ºC. Thus, one can also say for spores that a temperature rise of 10ºC will result in a tenfold reduction in the processing time to achieve the same lethality. Chemical reaction rates are less temperature sensitive than spore inactivation. Thus using higher temperatures for shorter times will result in less chemical damage occurring for an equivalent level of microbial inactivation. Table 5.1 gives a summary of heat resistance data for some important spores, enzymes and chemical reactions that occur when milk is heated. It should be appreciated that chemical reactions, physical changes and reactions catalysed by any residual enzyme activity will continue to take place during storage.

5.3 Thermisation and tyndallisation

The mildest of the heating processes is known as thermisation. This can be used to extend the keeping quality of raw milk, when it is known that raw milk may be held for some time under chilled conditions, prior to it being further processed into other products. The aim is to reduce the growth of psychrotrophic

Table 5.1 Values of D and z for microbial inactivation, enzyme inactivation and chemical reactions (from Lewis, 1999b, with permission)

	D_{121} (s)	z (°C)
Bacillus stearothermophilus NCDO 1096, milk	181	9.43
B. stearothermophilus FS 1518, conc. milk	117	9.35
B. stearothermophilus FS 1518, milk	324	6.7
B. stearothermophilus NCDO 1096, milk	372	9.3
B. subtilis 786, milk	20	6.66
B. coagulans 604, milk	60	5.98
B. cereus, milk	3.8	35.9
Clostridium sporogenes PA 3679, conc. milk	43	11.3
C. botulinum NCTC 7272	3.2	36.1
C. botulinum (canning data)	13	10.0
Proteases inactivation	0.5–27 min at 150°C	32.5–28.5
Lipases inactivation	0.5–1.7 min at 150°C	42–25
Browning	–	28.2; 21.3
Total whey protein denaturation, 130–150°C	–	30
Available lysine	–	30.1
Thiamin (B_1) loss	–	31.4–29.4
Lactulose formation	–	27.7–21.0

bacteria, which may release heat-resistant protease and lipase enzymes into the milk. These enzymes will not be totally inactivated during pasteurisation and may give rise to off-flavours if the milk is used for cheese or milk powders. Conditions used for thermisation are 57–68°C for 15 s, followed by refrigeration. Raw milk thus treated can be stored at a maximum of 8°C for up to 3 days (IDF, 1984). The milk should also be phosphatase negative in order to distinguish it from pasteurised milk. It is usually followed later by pasteurisation or a more severe heat treatment.

Another thermal process which has been considered is tyndallisation, which involves successive heat treatments in order to inactivate spores. According to Wilbey (2002), Tyndall in 1877 suggested that if a medium was heated at 100°C for 3 min on three successive days, first the vegetative cells would be killed and the spores would germinate and then be killed on either the second or third day. In general, such double heat treatments are rarely encountered and the process is not successful in totally inactivating spores because of the unpredictability of the spore germination process.

5.4 Pasteurisation

The history of pasteurisation is documented in Cronshaw (1947) and this makes interesting reading. In fact the first stage in the history of pasteurisation between 1857 and the end of the nineteenth century might well be called the medical stage, as the main history in heat-treating milk came chiefly from the medical profession interested in infant feeding. The first positive Holder pasteurisation system was introduced in Germany in 1895 and in the USA in 1907. Thus by 1895 what was required for an effective pasteurisation process was well recognised: 'we know that this process (pasteurisation) if properly carried out will destroy all disease germs' and 'a thoroughly satisfactory product can only be secured where a definite quantity of milk is heated for a definite period of time at a definite temperature. Then too, an apparatus to be efficient must be arranged so that the milk will be uniformly heated throughout the whole mass. Only when all particles of milk are actually raised to the proper temperature for the requisite length of time is the pasteurisation process complete.'

In 1927, North and Park established a wide range of temperature–time conditions to inactivate tubercle bacillus (Cronshaw, 1947). These experiments were performed by heating milk heavily infected with tubercle bacilli under different conditions and injecting them into guinea pigs. A selection of conditions where negative results were found, i.e. where the animals survived, were 212ºF (100ºC) for 10 s, 160ºF (71.1ºC) for 20 s, 140ºF (60ºC) for 10 min, and 130ºF (54.4ºC) for 60 min.

HTST (high temperature–short time) continuous processes were developed between 1920 and 1927 and for some time the ability of the HTST process to produce safe milk was questioned. In answer to the question whether HTST pasteurisation results in as good a bottle of milk as does the Holder process, Yale in 1933 concluded that one method of pasteurisation produces as good a bottle of pasteurised milk as does the other when good methods are used and when conditions are comparable. Further developments were made in the classification of tests for evaluating the pasteurisation process. These included tests for the following:

- Raw milk quality (platform test)
- Pasteurisability (survival of thermodurics)
- Efficiency of pasteurisation (pathogens and phosphatase)
- Recontamination (thermophilic and coliform bacteria and the methylene blue test)
- General bacterial quality, including organisms surviving pasteurisation plus contaminating organisms (plate count).

The methylene blue test is now little used, but the detection of alkaline phosphatase activity is still used as a statutory test in many countries.

Pasteurisation is now defined by the International Dairy Federation (IDF, 1986) as a process applied with the aim of avoiding public health hazards arising from pathogenic micro-organisms associated with milk, by heat treatment which

is consistent with minimal chemical, physical and organoleptic changes in the product. Pasteurised products should last for up to 48 hours without refrigeration and for several days when stored refrigerated. However, UK retailers of pasteurised milk are now demanding keeping qualities of 12 days. Traditionally, milk was pasteurised batchwise in the Holder process at 63ºC for 30 min but, as discussed earlier, the high temperature–short time (HTST) process was introduced later. Typical conditions are 72ºC for 15 s but these vary from country to country. Originally pasteurisation was introduced to destroy the pathogen *Mycobacterium tuberculosis*. It was established that the conditions required to achieve this corresponded to those required for inactivation of alkaline phosphatase, and since this was easier to measure, this was adopted as one of the procedures for establishing that milk had been adequately pasteurised. More recently, it has been required that pasteurised milk should show a positive lactoperoxidase activity, to prevent the milk being overprocessed (Statutory Instruments, 1995). Milks which showed a negative lactoperoxidase activity would be designated high pasteurised. Freshly pasteurised milk should be deemed to pass the coliform test and the plate count tests if its coliform count is less than one per ml and its plate count is less than 30 000 per ml.

Pasteurisation is a mild form of heat treatment, causing minimum whey protein denaturation, little loss of heat-sensitive vitamins and, for the majority of consumers, no change in its colour, flavour and texture.

5.5 Factors affecting the effectiveness of pasteurisation

The main control points for ensuring good quality pasteurised products are:

- Raw milk quality
- Processing conditions: temperature and time
- Post-processing contamination (PPC)
- Storage temperature.

These will be discussed in turn.

5.5.1 Raw material quality

Raw milk may contain pathogenic micro-organisms from the farmyard environment, including vegetative bacteria such as *Staphylococcus aureus, Campylobacter jejuni, Salmonella*. spp, *Escherichia coli*, *Yersinia enterocolitica*, and spore formers such as *Bacillus cereus and Clostridium* spp. It is considered that these major vegetative pathogens can be effectively controlled by pasteurisation and that they are not a major determinant of keeping quality. Pasteurisation achieves in the order of 5–8 decimal reductions of *Campylobacter* and *Salmonella*, both of which have been reported to cause food poisoning outbreaks in milk. *Listeria* is also inactivated. Pasteurisation also reduces the population of acid-producing spoilage bacteria and coliform

bacteria, including *E. coli* O157. The main interest is in what survives pasteurisation or mild heat treatments. Thermoduric bacteria are defined as those which survive 63ºC for 30 min, whereas spore-forming bacteria are those which survive 80ºC for 10 min. *Bacillus cereus* spores are relevant here, being the main pathogen which will survive pasteurisation and grow at low temperature. It will certainly cause spoilage in heat-treated milk, for example bitty cream, and produce an intense bitter flavour, but it rarely causes food poisoning because infected products are so unacceptable.

Spore counts in raw milk have been rarely reported to exceed 10^3 per ml, although Bramley and McKinnon (1990) reported that they may reach 5000 per ml. They are higher in winter than in summer; however, the proportion of psychrotrophic spores is higher in summer. The main psychrotrophic types are *B. cereus*, *B. circulans* and *B. mycoides.* Spores are mainly derived from surfaces of teats in contact with bedding materials. Very heat-resistant spores, e.g. *B. stearothermophilus*, form only a small proportion of the total. These are also more prevalent in winter. The most common sources of *Bacillus* spores from teat surfaces are *B. licheniformis*, *B. subtilis* and *B. pumilis*; there are lower numbers of *B. cereus*, *B. firmus* and *B. circulans.* Most common in raw milk are *B. licheniformis*, *B. stearothermophilus* and *B. cereus. Clostridium* spores are commonest in winter: they are derived from feed silage and bedding material (fewer than one spore per ml for cows on grass). In general they do not grow well in heat-treated milks because of the high redox potential, but they may cause problems in some cheeses made from both raw and pasteurised milk. In a more recent survey on spore-forming bacteria, mesophilic spore formers were found to be predominant, with a mean value of 7600 spores per ml but occasional counts of over 2.4×10^5 per ml. Psychrotrophic spore counts were very low, with a maximum of 3.5 spores per ml; thermophilic spore counts were slightly higher, with a maximum of 54 spores per ml (McGuiggan *et al.*, 2002). Suggested reasons for the higher results included the improved recovery techniques used.

Enzymes in raw milk may give rise to problems in pasteurised milk. For example, indigenous lipases may give rise to soapy off-flavours, especially if raw milk is subjected to excessive agitation at temperatures of about 50ºC, e.g. when mixing flavoured milks or other similar products. However, it is unlikely that bacterial lipases and proteases, which are very heat resistant, will cause problems in pasteurised milks because of their relatively short shelf-life and refrigerated storage conditions. Excellent reviews on the heat resistance of indigenous milk enzymes between 60 and 80ºC are given by Andrews *et al.* (1987) and Griffiths (1986).

5.5.2 Processing times and temperatures

Normal HTST conditions for milk are 72ºC for 15 s. One interesting question relates to the use of higher temperatures for pasteurisation. Gomez Barroso (1997) and Barrett *et al.* (1999) both showed that milk heated at 80ºC for 15 s in general had a reduced keeping quality compared to milk heated at 72ºC for 15 s.

Although this is not a new finding and has been identified previously by Kessler and Horak (1984), Schroder and Bland (1984) and Schmidt *et al.* (1989), it is one that should often be revisited, since it would be logical to expect a more severe heating process to result in an improved keeping quality. The usual explanation for this unexpected observation is that the more severe conditions cause heat shocking of the spores and that their activity then reduces the keeping quality, but recent evidence suggests that the lactoperoxidase system (LPS) also plays a role. The LPS involves the enzyme lactoperoxidase (LP), hydrogen peroxide and thiocyanate, all of which are present in raw milk. The oxidation products, e.g. hypothiocyanite, exhibit strong anti-microbial activity by oxidising sulphydryl groups of bacterial cell walls (Reiter and Harnulv, 1982). The LPS can be further activated in raw milk by small additions of thiocyanate and hydrogen peroxide and can be used to keep raw milk longer in countries where refrigeration is not widespread (IDF, 1988). Lactoperoxidase inactivation is shown in Fig. 5.1 and, in contrast to many enzymes, is very temperature sensitive, with *z* values of about 4ºC. Heat treatment regulations now require that pasteurised milk should show a positive lactoperoxidase activity.

Marks *et al.* (2001) showed that pasteurisation conditions of 72ºC for 15 s, resulting in an active lactoperoxidase system, were found to greatly increase the

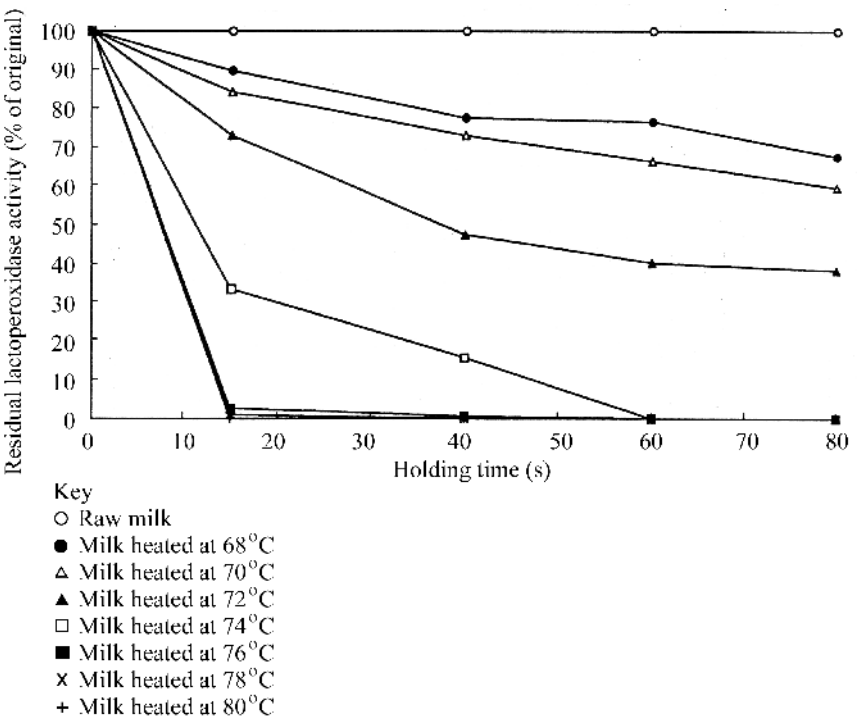

Fig. 5.1 Variation in lactoperoxidase activity with holding times and temperatures (from Barrett *et al.*, 1999, with permission).

keeping quality of milks inoculated with *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus thermophilus*, when compared to heating at 80ºC for 15 s. However, pasteurisation temperature had no effect on the keeping quality of milks challenged with *Bacillus cereus* spores. Later experiments confirmed that pasteurised milk produced from high quality raw milk could be stored for up to 20 days at 8ºC and for between 30 and 40 days at 4ºC. However, it must be emphasised that these experiments were performed with good quality raw milk, i.e. the counts immediately after pasteurisation were never above 10^3 per ml, even after the raw milk had been stored for 8 days at 4ºC prior to pasteurisation. These results also illustrate that good keeping quality can be achieved by eliminating PPC and can be further enhanced by using low storage temperatures.

In HTST pasteurisation, the holding time is controlled either by using a positive displacement pump or by a centrifugal pump linked to a flow controller, the temperature usually being controlled and recorded. A flow diversion valve diverts under processed fluid back to the feed tank. In continuous processing operations there is a distribution of residence times, and it is vital to ensure that the minimum residence time, i.e. the time for the fastest element of the fluid to pass through the holding tube, is greater than the stipulated time, to avoid under-processing. In a fully developed turbulent flow situation, the minimum residence time is about 0.83 times the average residence time.

Problems arising from a build-up of thermophilic bacteria in the heating and cooling sections associated with long operating times in continuous heat exchangers have long been recognised (Cronshaw, 1947). It may be possible to exploit some other natural anti-microbial systems in raw milk. These have been described in more detail by the IDF (1994). Double pasteurisation processes have been found to be ineffective (Brown *et al.*, 1979) and as such are rarely used.

There has been recent interest in *Mycobacterium avium* ssp. *paratuberculosis* (MAP) and whether it would survive pasteurisation. MAP levels found in raw milk appear to be low, but there is no real indication of true levels because of the decontamination procedures used to remove the other bacteria in raw milk and its extremely slow growth rate. MAP levels found in milks subjected to pasteurisation are also low but there are many inconsistencies in the experimental results (Grant *et al.*, 2001; Hammer *et al.*, 1998).

Using the Holder process (63ºC for 30 min), most investigators found some survivors after pasteurisation, but inoculum levels were much higher than would be found in raw milk. The D_{63} values quoted were 2.7–2.9 min, which would give a high level of inactivation (12.4 log reductions) and would provide a more than adequate process. Most other results suggested that the Holder process was not so efficient as this. Tails were also found in the survivor curves, which implied the presence of a more heat-resistant sub-population, though this could be an artefact. Results from HTST studies are also inconsistent and suggest great variability in the heat resistance data. One report suggested a D_{72} value of 11.7 s. According to this, normal HTST conditions would only achieve about 1.3 log

reductions, which would mean that all samples inoculated with 100 cfu/ml MAP would show surviving MAP after pasteurisation. However, results from milks inoculated with 10^7 and 10^4 cfu/ml indicated that about 20% and 40% of samples contained no viable MAP after HTST treatment, which suggested at least 7 and 4 decimal reductions (respectively) in some of these samples. This is inconsistent with a D_{72} value of 11.7 s.

Experiments also suggested that MAP inactivation is not temperature sensitive, although conclusions were based on the percentage of surviving bacteria rather than the numbers of decimal reductions achieved. There was a 55% survival at 72ºC for 15 s and experiments at 75, 78, 80, 85 and 90ºC also showed measurable survivor rates. The survival rates appeared to be higher after heat treatments at 80ºC than at 75ºC and 78ºC. At first sight this is unexpected but it could demonstrate that MAP is inhibited by an active lactoperoxidase system, which would be inactivated at 80ºC. This apparent lack of temperature dependence is unusual in a bacterium and is worthy of further investigation, as is any protective effect that may be conferred by the lactoperoxidase system (Marks *et al.*, 2001).

Results from surveys on raw milks and pasteurised milks are also inconclusive in that MAP was found in 2% of both raw and pasteurised milk samples tested. This again would suggest that pasteurisation is having no significant effect. Clearly, the heat resistance data generated to date for MAP is inconclusive and does not permit an accurate assessment of the efficacy of the pasteurisation process with regard to MAP. Information has been recently published by the IDF (1998). In the UK it has been recommended that HTST pasteurisation conditions should be increased to 72ºC for 25 s as part of a strategy for controlling MAP in cows' milk (Food Standards Agency, 2002).

Kessler (1981, 1989) has introduced a parameter (p^*) for characterising and comparing pasteurisation processes. According to him, 72ºC for 15s corresponds to $p^* = 1$. However, pasteurisation conditions vary from one country to another. In the USA a wide range of conditions are used, including 63ºC for 30 min, 77ºC for 15 s, 90ºC for 0.5 s, and 100ºC for 0.01 s. Other products pasteurised are creams and ice-cream mix. In the UK minimum temperature–time conditions for these products are 72ºC for 15 s and 79ºC for 15 s respectively, although conditions for them are more severe in some other countries.

5.5.3 Post-pasteurisation contamination (PPC)

PPC is now considered to be a very important determinant of keeping quality, and Muir (1996a, 1996b) describes how this was recognised both for milk and for cream in the early 1980s. PPC encompasses the recontamination of the product anywhere downstream of the end of the holding tube. It can occur in the regeneration or cooling sections, in storage tanks and in the final packaging of the product, due to poor hygienic practices. It can be greatly reduced by ensuring that all internal plant surfaces in contact with the product are heated at 95ºC for 30 min. It can only be completely eliminated by employing aseptic techniques down-

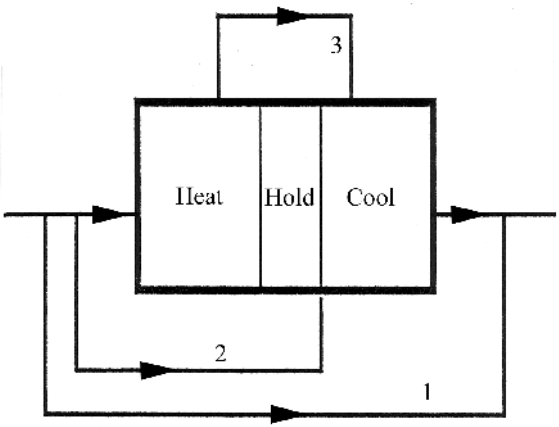

Fig. 5.2 Bypass routes in a commercial pasteuriser: 1, via cleaning routes; 2, via flow diversion route; 3, via regeneration section (e.g. pinhole leak in plate) (from Lewis, 1999a, with permission).

stream of the holding tube. One of the main safety concerns is recontamination of the product with pathogens from raw milk, which could occur due to bypassing of the holding tube by a number of possible routes (see Fig. 5.2), including pinhole leaks in plates. In terms of reducing keeping quality, recontamination with Gram-negative psychrotrophic bacteria is likely to be very important.

The presence in a pasteurised product of high counts of micro-organisms (e.g. coliform bacteria) which should be inactivated by pasteurisation is indicative of PPC, and the IDF (1993) have catalogued a large number of tests which can be used to determine the extent of the problem. In practical situations where the keeping quality of milk starts to deteriorate or is below expectations, the most likely explanation would be an increase in PPC and this should be the first factor to be investigated.

5.5.4 Storage temperature

In general the lower the storage temperature, the better is the keeping quality, bearing in mind the costs and practical problems of ensuring low temperatures throughout the cold chain and in domestic refrigerators. Before domestic refrigeration was commonplace, Cronshaw (1947) reported that the keeping time of pasteurised milk was about 24 h. Domestic refrigeration helped to improve this considerably; in the UK 10% of households had a refrigerator by 1957, increasing to 30% by 1962 and up to 90% by 1979. Raw milk is typically stored at 4°C; temperatures in the cold chain are slightly higher and they are likely to be higher still in domestic refrigerators. Some results on the effects of temperature on keeping quality were given earlier in the chapter.

HTST pasteurisation permits the use of continuous processing and regeneration of energy. Most HTST pasteurisers are of the plate type and these should be periodically tested for leaks. Consideration should be given to

ensuring that if leaks do occur, they do so in a safe fashion, i.e. pasteurised milk is not contaminated with cooling water or raw milk in the regeneration section. The control instrumentation, diversion valves and other valves should be checked regularly. Pasteurisation is crucial to many processes, for example cheesemaking, ice-cream manufacture and powdered milk production, to ensure that these are free of pathogenic micro-organisms.

5.6 Extended shelf-life milks

There is a requirement to further increase the shelf-life of pasteurised products, both for the convenience of the consumer and to provide additional protection against temperature abuse. However, it is important to avoid the onset of cooked flavour, which would result from more severe pasteurisation temperatures. It is the author's experience that this occurs at a temperature of about 85–90ºC for 15 seconds. Therefore one approach is to use temperatures above 100ºC for very short times. Wirjantoro and Lewis (1996) showed that milk heated to 115ºC for 2 s had a much better keeping quality than milks heated at both 72ºC for 15 s and 90ºC for 15 s. There is no doubt that temperatures in the range 115–120ºC for 1–5 s are more effective than temperatures below 100 ºC for extending the shelf-life of refrigerated products.

A second approach is to use small amounts of a bacteriocin. The addition of small amounts of nisin (40 IU/ml) was also effective in reducing microbial growth following heat treatment, at 72ºC for 15 s and more so at 90ºC for 15 s. It was particularly effective at inhibiting *Lactobacillus* at both temperatures. Results for milk heat treated at 117ºC for 2 s with 150 IU/ml nisin were even more spectacular. Such milks have been successfully stored for over 150 days at 30ºC with only very low levels of spoilage (Wirjantoro *et al.*, 2001). Local regulations would need to be checked to establish where nisin is a permitted additive in milk and milk-based beverages.

In general UK consumers do not like the taste associated with UHT milk, so it is important to reduce cooked flavour intensity. Direct processes (injection or infusion) offer one solution to this problem and milks processed by this method at 138ºC for 2–4 s are known in the USA as ultrapasteurised. A further strategy is to store pasteurised products at 2ºC, rather than 5–7ºC. This would further increase keeping quality but may not be practicable.

5.7 Sterilisation

Sterilisation of milk became a commercial proposition in 1894. Milk can be sterilised either in bottles or other sealed containers or by using ultra-high temperature (UHT) processing, which involves continuous sterilisation followed by aseptic packaging.

5.7.1 Safety and spoilage considerations

From a safety standpoint, the primary objective is the production of commercially sterile products with an extended shelf-life. The main concern is inactivation of the most heat-resistant pathogenic spore, namely *Clostridium botulinum*. Since milk is a low-acid food (pH > 4.5), the main aim is to achieve 12 decimal reductions for *C. botulinum*. This involves heating the product at 121ºC for 3 min, at its slowest heating point (IFST, 1991). The microbial severity of a process is traditionally expressed in terms of its F_0 value. This takes into account the contributions of the heating, holding and cooling periods to the total lethality and is expressed in terms of minutes at 121ºC. It provides a useful means of comparing processes. The minimum F_0 value for any low-acid food should be 3. The minimum *botulinum* cook will produce a product which is safe but not necessarily commercially sterile. Thus although *C. botulinum* is rarely found in raw milk, there are more heat-resistant spores which may cause spoilage but are not pathogenic, such as *B. stearothermophilus* and more recently *Bacillus sporothermodurans* (Hammer *et al.*, 1996). For foods which may contain such spores, a heat treatment achieving two or more decimal reductions is recommended, corresponding to an F_0 value of 8. Target spoilage rates should be less than one in every 10 000 containers.

5.7.2 In-container methods

Foods have been sterilised in sealed containers, such as cans, for over 200 years. Milk was originally sterilised in glass bottles sealed with a crown cork but more recently plastic bottles are used. The main aim is to inactivate heat-resistant spores, thereby producing a product which is ‘commercially sterile’, with an extended shelf-life. Practical drawbacks of in-container sterilisation processes are that the product heats and cools relatively slowly and that temperatures are limited by the internal pressure generated. However, many dairy products are still produced this way worldwide, including sterilised milk, evaporated milk, custards, and canned puddings and desserts.

Sterilised milk is still produced in many countries and in essence the manufacturing procedure is not too far removed from that used over 50 years ago. However, the legal requirement then was that it would be expected to remain fit for human consumption for at least 7 days (Davis, 1955), though as a general rule it would keep sweet for several weeks at ordinary temperatures and there were examples of it being in usable condition after 15 years’ storage. Milk sterilisation really developed after 1930 with the advent of the crown cork, which helped with the mechanisation of the bottle filling process and the reuse of bottles. In general the basic principles have remained the same. For more details of the process refer to Ashton and Romney (1981).

Milk is clarified using a centrifuge or by bactofugation, with claimed spore removal. It is heated using similar equipment to that used for pasteurisation. It is then homogenised at 63–82ºC, for example at a single-stage pressure of 206 bar or at double-stage pressures of 34 and 172 bar. Glass bottles are then filled at

74−80ºC in conditions which give minimal frothing and sealed using a crown cork. Plastic bottles are sealed at a lower temperature, 54−55ºC. Care should be taken to avoid conditions in balance tanks which may be conducive to growth of thermophiles. Ashton and Romney (1981) cite sterilisation processing conditions of 110–116ºC for 20–30 min, depending upon the extent of cooked flavour required. Batch or continuous retorting processes may be used (Davis, 1955). Other processing details are outlined by Ashton and Romney; these include more detail on continuous retorts such as hydrostatic or rotary valve sealed sterilisers which are capable of higher temperatures and shorter times (132–140ºC for 12 min) and the use of steam for glass bottles or steam/air mixtures for plastic bottles.

The test for ensuring adequate sterilisation is the turbidity test, developed by Aschaffenburg in 1950. This test measures whey protein denaturation and is an indirect test (similar to phosphatase), as complete denaturation would indicate that the milk was adequately sterilised. Milk (20 ml) is mixed with 4 g of ammonium sulphate, which causes casein and any associated denatured whey protein to precipitate. The mixture is filtered, producing a clear filtrate which contains any undenatured whey protein present in the milk sample. The filtrate is then boiled, which causes any undenatured whey protein to be denatured, thereby producing a turbid solution, the amount of turbidity being proportional to the amount of undenatured whey protein in the milk. Thus properly sterilised milk should produce a negative turbidity result. It is interesting that the phosphatase test is not suitable, as heat-induced compounds increase the intensity of the blue colour used in the conventional test and hence erroneously indicate under-processing.

Some developments in the process have included the introduction of retortable plastic bottles and a combined process that involves the production of milk under UHT conditions (e.g. 137ºC for 4 s), which is filled into bottles that are then sealed and passed through a conventional retorting process, although the retorting is much reduced, generally just sufficient to ensure that a negative turbidity is produced. In terms of determining the sterilisation effect, if this is to be treated as a single process, the critical point is to ensure that the milk does not become recontaminated in the intermediate filling process, especially with bacterial spores. This process was found to reduce the incidence of spoilage due to spore survivors (Ashton and Romney, 1981). There is also plenty of opportunity to promote spore production due to high temperatures being maintained for some considerable time.

Sterilised milk has a rich creamy appearance, perhaps helped by Maillard browning components, and a distinct cooked flavour (rich, nutty, caramelised) which once acquired makes other heat-treated products taste insipid. It is considerably browner than raw milk, the extent of browning depending upon the severity of the heat treatment. Davis (1955) recognised the need for ensuring that raw milk to be used for sterilisation was not heavily contaminated with spore-forming bacteria. Today this remains an important control variable. Sweet curdling was the chief bacterial fault, due to highly resistant spores of *B. subtilis*

and *B. cereus*. Bacterial growth was found to produce other taints such as carbolic, bad egg, oxidised or cardboard taints. Ashton and Romney (1981) reported that the failure level of well-produced sterilised milks is of the order of 1 in 1000 units, although it may be as high as 5–10% in situations where there are large numbers of thermotolerant spores in the raw material or other contamination arising in the process. There is some loss of nutrients (Kessler, 1981, 1989). Sterilised milk cannot be coagulated with rennet unless calcium chloride is added. It should now comply with the colony count for UHT milk (see next section).

5.8 Ultra-high temperature (UHT) sterilisation

More recently, continuous processes have been introduced. Although aseptically canned milk was produced in 1921 and a steam injection system was developed in 1927 in the United States, regulations permitting the use of indirect UHT treatment came into force not until 1965 in the UK and direct processes were permitted in 1972. Ultra-high temperature (UHT) or aseptic processing involves the production of a commercially sterile product by pumping the product through a heat exchanger. To ensure a long shelf-life the sterile product is packed into pre-sterilised containers in a sterile environment and an airtight seal is formed. It has also been known for a long time that the use of higher temperatures for shorter times will result in less chemical damage to important nutrients and functional ingredients within foods, thereby leading to an improvement in product quality. This has been illustrated for reactions such as whey protein denaturation, thiamine loss, Maillard browning and HMF formation by Kessler (1981, 1989). Thus UHT sterilisation of milk is achieved by rapid heating to temperatures of about 140ºC, holding for several seconds followed by rapid cooling. Ideally, heating and cooling should be as quick as possible. Both indirect and direct heating methods are available.

At a temperature of 141ºC, a time of 1.8 seconds would be required to inactivate *C. botulinum*, but longer times would be required to achieve two decimal reductions (2D) for *Bacillus stearothermophilus*. For UHT products, an approximate value of F_0 can be obtained from the holding temperature (T, ºC) and minimum residence time (t, s):

$$F_0 = 10^{(T-121.1)/10} \times t/60$$

In practice the real value will be higher than this estimated value because of the lethality contributions from the end of the heating period and the beginning of the cooling period as well as some additional lethality from the distribution of residence times.

In the UK, there are statutory heat-treatment regulations for some UHT products:

- Milk: 135ºC for 1 s

- Cream: 140ºC for 2 s
- Milk-based products: 140ºC for 2 s
- Ice-cream mix: 148.9ºC for 2 s.

In some cases lower temperatures and longer times can be used, provided it can be demonstrated that the process renders the product free from viable micro-organisms and their spores.

5.8.1 Process characterisation

To some extent, requirements for safety and quality conflict, as a certain amount of chemical change will occur during adequate sterilisation of the food. Therefore it is important to ask what is meant by quality and what is the scope for improving the quality. One very important aspect is minimising chemical damage and reducing nutrient loss. In this aspect, UHT processing offers some distinct advantages over in-container sterilisation. Chemical reactions are less temperature sensitive so the use of higher temperatures, combined with more rapid heating and cooling rates, helps to reduce the amount of chemical reaction. There is also a choice of indirect heat exchangers for milk, such as plate or tubular types, as well as direct steam injection or infusion plants, all of which heat products at different rates and shear conditions.

For a better understanding of the UHT process, it is required to know the temperature–time profile for the product. Some examples of such profiles are shown for a number of different UHT process plants in Fig. 5.3. Considerable differences arise in the heating and cooling rates for indirect processes and

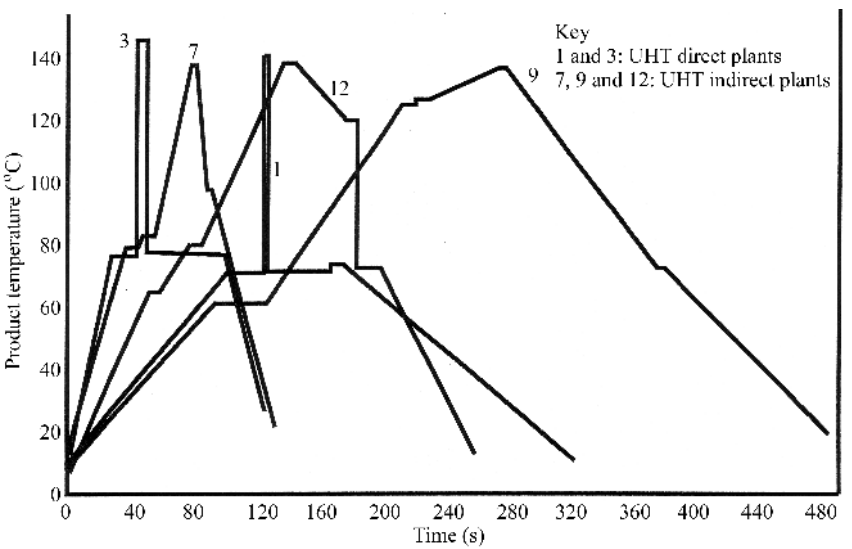

Fig. 5.3 Temperature–time profile for different UHT plants (from Lewis and Heppell, 2000, with permission).

between the direct and indirect processes due to steam injection and flash cooling. Because of these differences, similar products processed on different plants may well be different in quality. A more detailed discussion is given by Burton (1988) and Lewis and Heppell (2000).

Two other parameters introduced for UHT processing of dairy products are the B^* and C^* values (Kessler, 1981). The reference temperature used (135ºC) is much closer to UHT processing temperatures than that used for F_0 (121ºC) or cooking value (100ºC) value estimations. B^* is a microbial parameter used to measure the total integrated lethal effect of a process. A process given a B^* value of 1 would be sufficient to produce nine decimal reductions of mesophilic spores and would be equivalent to 10.1 s at 135ºC. C^* is a parameter to measure the amount of chemical damage taking place during the process. A process given a C^* value of 1 would cause 3% destruction of thiamine and would be equivalent to 30.5 s at 135ºC.

Again, the criteria in most cases are to obtain a high B^* and a low C^* value. Some effects of increasing heating and cooling periods on F_0, B^* and C^* have recently been shown by Browning *et al.* (2001) (Table 5.2). These results are based on heating the product from 80ºC to 140ºC, holding it for 2 s and cooling it to 80ºC. Heating and cooling times from 1 s (almost instantaneous) through to 120 s are shown. Increasing these periods increases both the chemical and the microbial parameters, with the ratio of C^*/B^* increasing with increasing heating time. At a heating time of about 8 s, the amount of chemical damage done during the heating and cooling periods exceeds that in the holding tube. It is this considerable increase in chemical reaction that is more noticeable in terms of decreasing the quality of the product. Lactulose is one useful indicator to assess the amount of chemical change taking place during sterilisation, as it is not found in raw milk (Andrews, 1986). Tentative proposals now being discussed include lactulose in UHT milk, being greater than 10 mg per 100 ml and for sterilised milk being greater than 60 mg per 100 ml.

High C^* values may be beneficial in those circumstances where a greater extent of chemical damage may be required, i.e. for inactivating heat-resistant proteases or lipases or for inactivation of natural toxic components, e.g. trypsin

Table 5.2 Comparison of heating and cooling rates (1 to 120 s) for UHT milk heated at 140ºC for 2 s (from Browning *et al.*, 2001, with permission)

Time (s)	F_0	B^*	C^*	Browning[a]
1	2.81	0.64	0.12	12.8
10	4.62	1.07	0.31	31.3
30	8.63	2.04	0.75	72.4
60	14.66	3.48	1.39	134.0
90	20.68	4.92	2.05	196.0
120	26.70	6.36	2.70	257.0

[a] Denotes equivalent time (s) at 121ºC ($z = 26.3$ºC) (Browning *et al.*, 2001)

inhibitor in soy milk. Chemical damage could be further reduced by using temperatures in excess of 145ºC and very short times. The best solution would be the direct process, with its accompanying rapid heating and cooling. Steam is mixed with the product, pre-heated to about 75ºC, by injection or infusion. The steam condenses and becomes an ingredient in the product. Steam utilisation is about 10–15% (mass/mass). There are special requirements for the removal from the steam of impurities such as water droplets, oil and rust. Heating is almost instantaneous. The condensed steam is removed by flash cooling, which is also very quick. This process also removes volatiles and dissolved oxygen. Advantages of the process are reduced chemical damage and a less intense cooked flavour for many products. There are claims that products produced by direct UHT are indistinguishable from pasteurised products. One problem would be the very short holding times required, and their control. In theory it should be possible to obtain products with very high B^* and low C^* values at holding times of about 1 s. However, direct processes are more expensive in terms of both capital and running costs than indirect processes.

5.8.2 Controlling the process

It is recognised that UHT processing is more complex than conventional thermal processing (IFST, 1991). The philosophy of UHT processing should be based upon preventing and reducing microbial spoilage by understanding and controlling the process. One way of achieving this is by using the principles of hazard analysis critical control points (HACCP) (ICMSF, 1988). The hazards of the process are identified and procedures adopted to control them. An acceptable initial target spoilage rate of less than 1 in 10^4 should be aimed for. Such low spoilage rates require very large numbers of samples to be taken to verify that the process is being performed and controlled at the desired level. Initially a new process should be verified by 100% sampling. Once it is established that the process is under control, sampling frequency can be reduced and sampling plans can be designed to detect any spasmodic failures. More success will result from targeting high-risk occurrences, such as start-up, shut-down and product changes. Thus holding time and temperature are perhaps the two most critical parameters. Recording thermometers should be checked and calibrated regularly, and accurate flow control is crucial (as for pasteurisation). For Newtonian fluids the minimum residence time will be half the average residence time. Turbulent flow will result in a narrower distribution of residence times, with a minimum residence time of 0.83 times the average residence time. In both cases, the minimum residence time should be greater than the stipulated residence time, to avoid under-processing. Residence time distributions and their implications for UHT processing are discussed in more detail by Burton (1988).

Microbial counts in sound UHT milk should not exceed 100 per ml after 15 days' storage at 30ºC. The main concern has been *Bacillus stearothermophilus*, which is very heat resistant and thermophilic and causes flat-sour spoilage.

More recently another heat-resistant spore (HRS) has been identified in UHT products in continental Europe (Hammer *et al.*, 1996). This has recently been classified as *Bacillus sporothermodurans* (Pettersson *et al.*, 1996). It is a mesophilic bacteria which has been found in some European countries and is causing UHT milk to fail the microbiological test which has been specified. However, it is a puzzle since it is not easy to grow in culture and counts in milk very rarely exceed 10^5 per ml. Furthermore it does not cause any changes in the sensory characteristics of the product, neither is it reported to be pathogenic. The question arises as to whether milks containing this micro-organism are acceptable and safe to drink.

5.8.3 Raw material quality and other processing conditions

In terms of controlling the process, the following areas will also merit some attention. All aspects of raw material are important, from an understanding of the physical properties described earlier, through to spore loadings and chemical composition. Of particular concern would be high levels of heat-resistant spores and enzymes in the raw materials, as these could lead to increased spoilage and stability problems during storage; dried products such as milk, other dairy powders, cocoa, other functional powders, and spices (if used) are ones to be particularly cautious about. Quality assurance programmes must ensure that such poor quality raw materials are avoided. The product formulation is also important, including the nature of the principal ingredients, the levels of sugar, starch and salt, and the pH of the product, particularly if there are appreciable amounts of protein. Some thought should be given to water quality, particularly the mineral content. Reproducibility in metering and weighing ingredients is also important, as is ensuring that powdered materials are properly dissolved or dispersed and that there are no clumps, which may protect heat-resistant spores. Homogenisation conditions may be important; is it necessary to homogenise and if so at what pressures? Should the homogeniser be positioned upstream or downstream of the holding tube? Will two-stage homogenisation offer any advantages? Homogenisation upstream offers the advantage of breaking down any particulate matter to facilitate heat transfer, as well as avoiding the need to keep the homogeniser sterile during processing. All of these aspects will influence both the safety and the quality of the products. For indirect processes, the use of higher temperatures may be limited by fouling considerations and it is important to ensure that the heat stability of the formulation is optimised. Heat stability and susceptibility are not easy to assess or measure quickly in a commercial situation and it may be worth developing simple tests to assess heat stability. The alcohol stability test has proved useful for milk products. Generally direct systems give longer processing runs than indirect processes.

5.9 Aseptic packaging and storage

Clean packaging systems are used for pasteurised products, where the main aim is to reduce the levels of PPC. For extended shelf-life and UHT products, aseptic packaging systems should be used of which a number are available. They all involve putting a sterile product into a sterile container in an aseptic environment. Pack sizes range from individual portions (14 ml), retail packs (125 ml to 1 litre), through to bag-in-the box systems up to 1000 litres. The sterilising agent is usually hydrogen peroxide (35% at about 75–80ºC); the contact time is short and the residual hydrogen peroxide is decomposed using hot air. The aim is to achieve four decimal reductions (4D) process for spores. Superheated steam has been used for sterilisation of cans in the Dole process. Irradiation may be used for plastic bags.

Since aseptic packaging systems are complex, there is considerable scope for packaging faults to occur, which will lead to spoiled products. Where faults occur, the spoilage micro-organisms would be more random and would include those micro-organisms which would be expected to be inactivated by UHT processing; these often result in blown packages.

Packages should be inspected regularly to ensure that they are airtight, again focusing upon those more critical parts of the process, such as start-up, shut-down, product changeovers and, for carton systems, reel splices and paper splices. Sterilisation procedures should be verified. The seal integrity of the package should be monitored as well as the overall microbial quality of packaging material itself. Care should be taken to minimise contamination during subsequent handling All these could result in an increase in spoilage rate. Rinsing, cleaning and disinfecting procedures are also very important, especially the removal of fouling deposits, which may provide a breeding ground for the growth of micro-organisms, especially thermophiles.

UHT products are commonly stored at room (ambient) temperature and good quality products should be microbiologically stable. Nevertheless, chemical reactions and physical changes will take place which will change the quality of the product. These include oxidation reactions, Maillard browning and chemical and physical changes which may give rise to age-thickening and gelation. These have been discussed in more detail by Burton (1988) and Lewis and Heppell (2000).

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