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The technology of pasteurisation and its effect on the microbiological and nutritional aspects of milk

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The technology of pasteurisation and its effect on the microbiological and nutritional aspects of milk

THE TECHNOLOGY OF PASTEURISATION AND ITS EFFECT ON THE MICROBIOLOGICAL AND NUTRITIONAL ASPECTS OF MILK

ABSTRACT

Pasteurisation of milk involves heating the milk to at least 72°C for 15 s or to 63°C for 30 min. Such heat treatment is necessary to reduce pathogenic bacteria to an acceptable safe level and reduce spoilage organisms, thus extending the shelf-life of the milk and improving public health. There are few adverse effects on the nutritional quality of the milk.

In this Bulletin, the technological process of pasteurisation is outlined, the microbiological aspects of the impact of pasteurisation on public health are explained and the scientific basis demonstrating that milk pasteurisation does not significantly impact the nutritional properties of milk are described. Thus, according to the currently available knowledge, drinking pasteurised milk is still the safest way to enjoy the health benefits of drinking milk. The focus of the Bulletin is on pasteurised cow's milk for direct consumption; milk from other animal species or milk intended for further processing have not been considered in this publication. Although homogenisation is now an integral part of the pasteurisation process in many regions, it has not been considered for the purposes of this Bulletin.

This Bulletin will be of value to the dairy sector and to a broader audience, as it provides an overview of the process of pasteurisation of milk, the advantages of milk pasteurisation from a public health perspective and demonstrates that milk pasteurisation has little impact on the nutritional properties of milk.

Keywords: *Pasteurisation, heat treatment, cow's milk, safety, nutrition, technology.*

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FOREWORD

We are pleased to present this new publication on pasteurisation, a process which is crucial for the dairy industry and a major tool for public health protection.

Since the withdrawal of the IDF's previous pasteurisation Bulletin n°200 (1986), there has been a need for an overview of some key technical, microbiological and nutritional aspects of milk pasteurisation, and this new Bulletin fills that gap.

The focus of the publication is on pasteurised cow's milk for direct consumption. It showcases the advantages of milk pasteurisation from a public health perspective and the scientific basis demonstrating that milk pasteurisation does not negatively impact the nutritional properties of milk. The outputs of the publication will be valuable not only to the dairy sector but to a wider audience as well.

This Bulletin was prepared by a joint Action Team (AT) of the IDF Standing Committees on Microbiological Hygiene (SCMH), Nutrition and Health (SCNH) and Dairy Science and Technology (SCDST), under the lead of Kieran Jordan (IE).

IDF wishes to thank the AT leader, AT members below and all contributors to this work, which also includes members of the Society for Dairy Technology. Kieran Jordan (IE) – AT leader, Walter Bisig (CH), François Bourdichon (FR), Helen Dornom (AU), David Everett (US), Bita Farhang (CA), Choreh Farrokh (FR), Claus Heggum (DK), Phil Kelly (IE), Judith Narvhus (NO), Rosalind Robertson (NZ), Allen Sayler (US), Geoffrey Smithers (AU), Phillip Tong (US), Ellen Wemmenhove (DK).

Caroline Emond
Director General
International Dairy Federation

Brussels, February 2019

BACKGROUND

Pasteurisation of milk is a key unit process critical to the global dairy industry. This Bulletin provides an overview of some key technological, microbiological and nutritional aspects of milk pasteurisation. The focus is on pasteurised cow's milk for direct consumption; milk from other animal species or milk intended for further processing have not been considered. Although homogenisation is now an integral part of the pasteurisation process in many regions, it has not been considered for the purposes of this Bulletin. It is anticipated that this Bulletin will be of value to the dairy industry, as well as a broader audience as it provides an overview of the process of pasteurisation of milk, the advantages of milk pasteurisation from a public health perspective and the scientific basis demonstrating that milk pasteurisation does not impact the nutritional properties of milk.

INTRODUCTION

Pasteurisation is the heat treatment of milk which aims to (i) reduce pathogenic microorganisms associated with milk to an acceptable level and thus avoid public health hazards arising from consumption of milk contaminated by these microorganisms; and (ii) extend the shelf-life of milk through the inactivation of spoilage microorganisms and indigenous enzymes which can cause quality defects over time. Pasteurisation is a process consistent with minimal chemical, physical and organoleptic changes in the milk (FAO/WHO, 1986). It involves heating the milk at temperatures sufficient to inactivate the most heat-resistant vegetative pathogenic bacteria which could be present in the raw milk (i.e., *Mycobacterium tuberculosis* and *Coxiella burnetii*) to an acceptable level (at least 5-log reduction), and thereby make it safe for human consumption (Kelly et al., 2005). In addition, pasteurisation, in combination with good manufacturing practice (GMP), reduces non-pathogenic indigenous microflora (e.g., lactic acid bacteria [LAB]) to an acceptable level and inactivates indigenous/endogenous enzymes such as lipoprotein lipase (Deeth, 2006), which can be associated with non-microbiological spoilage of milk.

Originally introduced in the 1860s and 1870s by Louis Pasteur to control spoilage in wine and beer, pasteurisation was first used to control milk spoilage in the 1880s and later used as a means of protecting public health. Batch pasteurisation was initially used, while in-bottle pasteurisation was not widely accepted, although it is still used in some settings. Eventually, continuous flow pasteurisers were developed and became widely available for industrial application in the 1920s. Along with potable water and sewage treatment, pasteurisation has been one of the most important technological developments to combat infectious diseases transmitted from food, such as tuberculosis (Cohen, 2000). The health and hygiene of cows, the environment in which cows are housed and milked, the procedures used in cleaning and sanitising the milking and milk storage equipment and the temperature and duration of storage are all key factors in influencing the quality of raw milk, which in turn will influence the quality of the pasteurised milk.

1 TECHNOLOGICAL ASPECTS OF MILK PASTEURISATION

1. HEATING CONDITIONS AND METHODS

Two types of pasteurisation are used: (i) *batch*, sometimes called "holder" or "low-temperature, long-time" (LTLT); and (ii) *continuous*, often referred to as "high-temperature, short-time" (HTST). HTST is most commonly used in the dairy industry although batch pasteurisation is still used for small-scale operations. The minimum conditions for these processes are 63°C for 30 min and 72°C for 15 s, respectively, although some countries have 16 s as the minimum holding time at 72°C. The relationship between time and temperature is usually described using the *Arrhenius* model[1] which is based on a linear relationship between ln (time) and temperature. For time-temperature combinations above 72°C, the *Kessler equation*[2] (Kessler, 1985) should be applied. Some food safety authorities have established guidance on combinations at temperatures above 89°C; this is termed "higher-heat, shorter-time" pasteurisation (Meunier-Goddik & Sandra, 2011). The relationships and some national guidance combinations are graphically illustrated in Figure 1. Any combination of time and temperature at any point on the solid lines of the graph presented in Figure 1 will be equivalent. When sugars or other solutes are added, then the lower water activity (a_w) has a protective effect on bacteria and the LTLT or HTST treatment time-temperature combinations should be modified accordingly (see later).

All dairy processing equipment should be of a sanitary design, which ensures that all surfaces in contact with the product demonstrate the following properties: Smooth, impervious, non-adsorbent, non-porous, corrosion resistant, non-toxic, non-reactive, durable and cleanable (Hauser et al., 2004). Stainless steel is the required general-use metal for contact surfaces in pasteurisation equipment because of its corrosion resistance and durability in dairy applications. However, not all stainless steel is of the same quality. In general, the properties of the stainless-steel alloy are related to its relative composition with regard to chromium and nickel content. Corrosion resistance varies with chromium content and structural strength varies with nickel content. Stainless steel is commonly used to produce surfaces which come into contact with food during processing as it is relatively resistant to corrosion by chloride. Any other materials in contact with food should comply with sanitary standards and accepted practices (guidance is available from http://www.3-a.org/knowledge-center/resource-papers/a-primer-for-3-a-standards-practices or https://www.ehedg.org/guidelines/).

1 Ln (t) = 2.71 + 61330*(1/T – 0.002899), where t is the holding time (in seconds) and T the absolute temperature (in kelvin)
2 Log_{10} (t) = 14885/T - 41.97, where t is the holding time (in seconds) and T the absolute temperature (in kelvin)

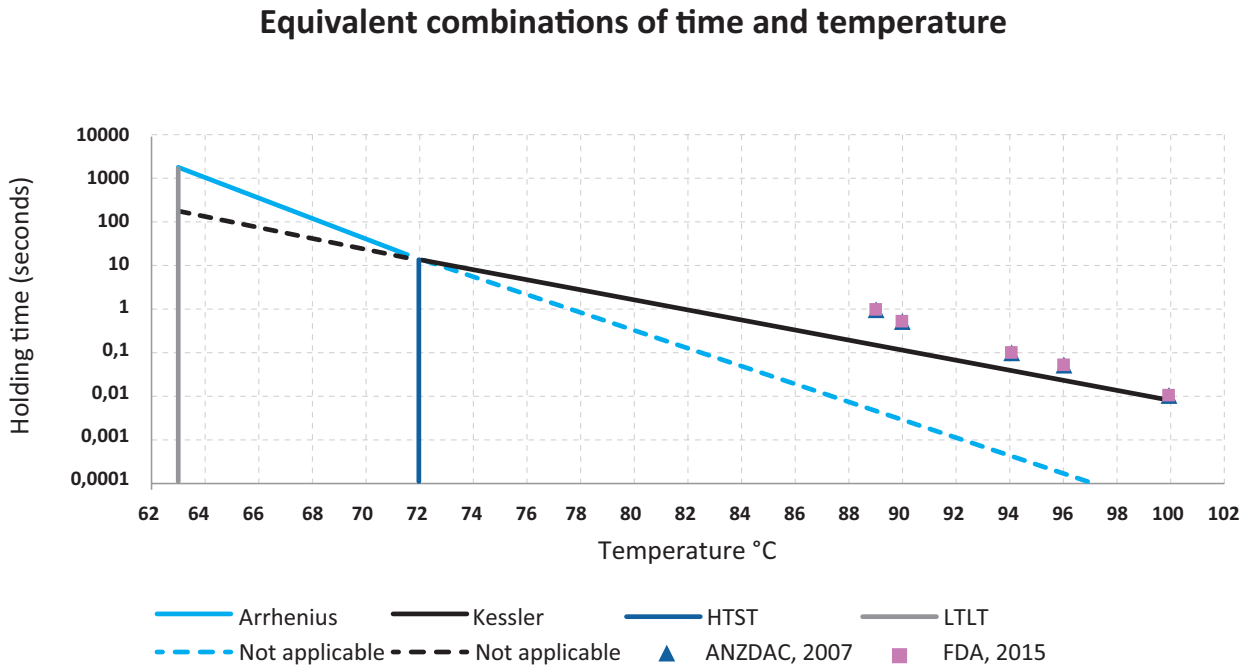


Figure 1. Minimum temperature-time combinations for pasteurisation. Batch (LTLT) pasteurisation is at 63°C for 30 min and continuous (HTST) pasteurisation is at 72°C for 15 s, assuming Z = 8°C. Various guidelines exist on equivalent/appropriate time-temperature combinations above 72°C. Some of these are shown as symbols on the graph.

2. LOW-TEMPERATURE LONG-TIME (LTLT) BATCH PASTEURISATION

Batch or LTLT pasteurisation of milk is typically carried out in double-jacketed, insulated vats (also referred to as tanks, cookers, kettles or boilers) equipped with a stirrer. The tanks are commonly constructed of stainless steel and the milk is heated indirectly by hot water or steam, either in the jacket or in heating coils fastened to the inner wall of the vessel. The milk is heated slowly under agitation until the temperature reaches the desired inactivation temperature and is held at that temperature for the required period of time (e.g., 63°C for 30 min) before cooling to ≤ 4°C. Key considerations for batch heating systems include:

1. Achieving uniform heating of the material undergoing pasteurisation;

2. Vessel volume versus surface area and associated implications for slower heating rates in larger batches;

3. Agitator design and rate of rotation to ensure rapid and uniform mixing of the entire material volume;

4. Choice of heating medium and implications regarding product burn-on and fouling when using low pressure steam;

5. Temperature of the headspace between the surface of the fluid being treated and the lid of the vat as this will influence the temperature of the fluid during the required hold period;

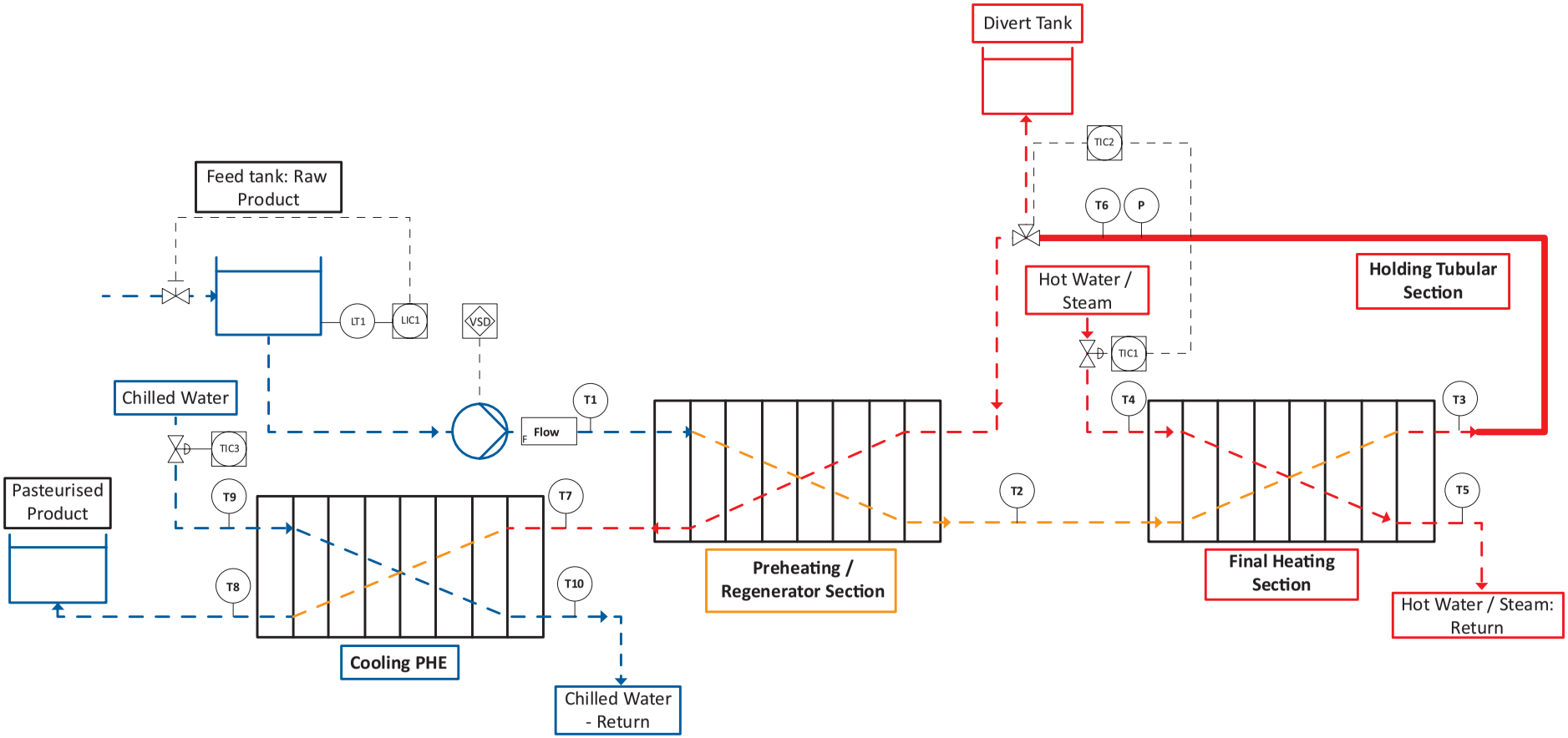
6. Location of temperature probes, normally at the vessel wall, which could overestimate product temperature when compared to the centre of the vessel, thus underestimating the length of time at which the minimum pasteurisation temperature has been achieved; and

7. Rapid cooling is crucial to avoid over-heating. A positive peroxidase value as an indicator for minimal processing (see later) can only be reached with rapid cooling using ice-water or another cold cooling medium.

The key to LTLT pasteurisation is to ensure that all material within the vat reaches the minimum time-temperature combination to ensure a microbiologically safe product is produced. It is worth noting that LTLT pasteurisation is more commonly practiced in artisanal production scenarios for small scale manufacture of specialised cheeses.

3. HIGH-TEMPERATURE SHORT-TIME (HTST) CONTINUOUS PASTEURISATION

HTST pasteurisation uses continuous flow-through heat exchangers, either a plate heat exchanger or tubular heat exchanger. Plate heat exchangers are the most commonly used for milk and cream (Figure 2). Typically, the gap between the plates is 2.5 – 5 mm. Tubular heat exchangers are favoured for viscous products and products which are prone to fouling and/or contain particulate matter. Tubes can withstand higher pumping pressure than plate heat exchangers, which are limited to about 2 MPa. Regardless of how the heat is transferred from the heating medium to the product (by plate or tubular heat exchangers), the actual guarantee of a minimum time-temperature combination is almost universally attained in a stainless-steel tubular holding section (which might be insulated) that incorporates temperature monitoring and flow diversion capabilities. Other than heat exchangers, new technologies such as continuous microwave heating could be integrated into the future design of some pasteurisers to minimise fouling.



LT1 – Level Transmitter
LIC1 – Level Control
VSD – Variable Speed Drive
T1 – Raw Feed Temperature

T2 – Final Heater Product In
T3 – Final Heater Product Out
T4 – Hot Water / Steam In
TIC1 – Temerature control for Hot Water / Steam

T5 – Hot Water / Steam Out
P – Pressure Indicator
T6 – Holding Tube Temperature
TIC2 – Temperature Control to Divert Tank

T7 – Regenerator Temperature out
T8 – Pasteurised Product Out
T9 – Chilled Water Temperature In
TIC3 – Temperature Controle for Chilled Water
T10 – Chilled Water Temperature Out

Figure 2: Schematic illustration of a continuous flow high temperature short time pasteurisation system.

The steps involved in HTST pasteurisation of milk are as follows:

1. Cold milk, which might previously have been thermised at sub-pasteurisation temperatures, is fed from the bulk storage vat or silo into the balance/feed tank of the pasteuriser.

2. Pumping of the milk in the pasteuriser is normally achieved by centrifugal pumping systems.

3. Heating of the milk is normally carried out in two stages. The first stage pre-heats the milk in the regenerative section of the pasteuriser by transferring heat (energy) from the pasteurised product (discussed below) to the incoming cold milk stream. Dependent upon system configuration and design, the regenerative section recovers most of the heat from the pasteurised milk, reducing the energy requirements in the heating section and improving the economics of the entire operation. Regeneration efficiencies of over 95% heat recovery are possible.

4. The pre-heated milk is then heated in the second stage to the required final pasteurisation temperature (at least 72°C) using hot water in the second section of the heat exchanger.

5. Product exiting from the final heating section enters a tubular holding section comprised of a tube of a defined length, which might be insulated. The outlet of the holding section might or might not (depending on legal and other issues) contain a back-pressure valve and single/double temperature probes. The temperature probes at the outlet of the holding tube control the temperature in the final heater, with product normally exiting the final heater at several degrees higher than the pasteurisation temperature to account for any temperature loss across the holding section. The holding section ensures that a minimum time-temperature condition is met to ensure legal pasteurisation is achieved. The milk is maintained at the pasteurisation temperature while it flows through a tube, the volume of which ensures that the residence time of the milk is at least 15 s (HTST pasteurisation). The design of the tube ensures that all of the milk is held for at least the minimum required holding time. Thus, the volume of the tube must be designed to meet the required holding time of the fastest moving volume of milk based upon the rheological properties of the fluid, the pipe geometry and the flow conditions. The average residence time (t) in the tube can be calculated as follows:
   t (in seconds) = length of holding tube (in metres) / maximum speed (V_{max}) of the liquid (in m/s)
   The maximum speed is dependent on the flow conditions.
   V_{max} is $V_{average}$ x 1.2 for turbulent flow, and
   V_{max} is $V_{average}$ x 2 for laminar flow
   Turbulent flow is highly recommended to achieve a more uniform heat treatment and pasteurisation effect.

6. The pasteurised milk is then cooled by pumping back through the regeneration section of the pre-heater and then through one or more final cooling sections of the pasteuriser, using either a single or several cooling towers or chill water.

7. When marketed as drinking milk for direct consumption, the cold pasteurised milk is then transferred to finished product buffer tanks and then packaged into retail packs, which can be paperboard cartons, glass or plastic bottles or pouches. Storage and distribution of pasteurised milk is carried out at <4–6°C.

8. Recording of temperatures and maintenance of batch records can be carried out manually or electronically and in meeting this requirement local legislation has to be taken into account.

9. It should be noted that the pasteurised product side of the heat exchanger (plate or tubular) must always operate at a higher pressure than that of the heating and cooling medium side and of the incoming unpasteurised milk. This ensures that in the event of a leak, the heating and cooling medium and unpasteurised milk do not contaminate the product. This pressure differential is often maintained via a centrifugal booster pump.

The increase in heat load is limited in some countries to ensure minimal processing (Lewis & Deeth, 2009). The indicator enzyme, lactoperoxidase, is required to remain residually active in some countries (e.g., Switzerland).

The time-temperature combinations referred to above are minimum conditions for unconcentrated milk. However, many milk processors use either higher temperatures or longer times or both, to ensure that the minimum heating conditions are always achieved, or to account for the unsubstantiated claim that a higher temperature and/or a longer holding time will contribute to inactivation of *Mycobacterium avium* subsp. *paratuberculosis* (Lund et al., 2002).

Increasing the temperature of the pasteurisation of milk above the regulated minimum of 72°C, without reducing the holding time, has been shown by several authors to not increase the shelf-life of the pasteurised milk over that of milk pasteurised at 72°C. In fact, in some cases it has been shown to decrease the shelf-life (e.g., Brown et al., 1980; Schröder & Bland, 1984; Schmidt et al., 1989; Barrett et al., 1999). Explanations for this finding include activation of spores and subsequent germination and growth of the spore-formers (mostly *Bacillus*), inactivation of the antibacterial enzyme lactoperoxidase and reduced competition by the normal spoilage bacteria.

4. OTHER TIME-TEMPERATURE COMBINATIONS

A HTST pasteuriser designed for liquid milk might not be suitable for higher fat or higher solids products as these typically have a higher viscosity and lower heat transfer coefficients, thus requiring larger surface areas to achieve an equivalent thermal load.

Increases in viscosity can be critical as it is essential that turbulent flow is maintained in the holding tube to achieve the desired minimum holding time with the smallest possible average value. Under turbulent flow conditions, the minimum holding time can be as high as 83% of the mean, whereas this falls to 50% when the flow is laminar.

The time-temperature combinations shown in Figure 1 are applicable only to unsweetened liquid milk with less than 10% milkfat and/or less than 18% milk solids. In the case of liquid milk products with higher fat or solids contents, or to which sugar/salt has been added, then the lower water activity (a_w) has a protective effect and the LTLT or HTST treatment time-temperature combination should be modified accordingly. The performance criterion of 5 log reduction of *Coxiella burnetii,* as defined by Codex (FAO/WHO, 2004), applies. Several default approaches are recommended by various national food safety authorities and the Codex Alimentarius Commission (CAC). The FDA Grade "A" Pasteurised Milk Ordinance, recommends adding +3°C to the temperatures specified for whole milk (Meunier-Goddik & Sandra, 2011) to achieve pasteurisation of the following products:

- Milk with a fat content of 10% or above;
- Milk with milk solids content above 18%;
- Milk with added sugar.

CAC provides examples of recommended time-temperature conditions for pasteurisation of cream: 75°C for 15 seconds (10–20% fat), 80ºC for 15 seconds (above 20% fat) and 65ºC for 30 minutes (batch) (FAO/WHO, 2004). Table 1 presents the guidance by Food Safety Australia New Zealand (FSANZ) for the minimum pasteurisation conditions applicable to milk of different solute concentration (FSANZ, 2009).

	Milk with < 10% fat (no added sweeteners and particles)			Dairy products with ≥ 10% fat and/or added sweeteners and concentrated dairy products with > 15% total solids and particles		
Particle diameter	**< 200 µm Ø**	**200 to < 500 µm Ø**	**500 to < 1000 µm Ø**	**< 200 µm Ø**	**200 to < 500 µm Ø**	**500 to < 1000 µm Ø**
Minimum holding time	**Minimum temperature (°C)**					
5 s	75.7	76.5	79.0	78.5	79.3	81.8
15 s	72.0	72.1	72.7	74.8	74.9	75.5
1 min	69.4	69.4	69.5	72.2	72.2	72.3
10 min	65.1	65.1	65.1	67.9	67.9	67.9
15 min	64.3	64.3	64.3	67.1	67.1	67.1
20 min	63.8	64.8	64.8	66.6	66.6	66.6
30 min	63.0	63.0	63.0	65.8	65.8	65.8

[a]based on data from FSANZ (2009)

Table 1. Minimum heat treatment conditions for pasteurisation of various types of milk.

5. ENSURING PASTEURISATION CONDITIONS ARE SUCCESSFULLY ACHIEVED

Based on monitoring the critical control points, HTST pasteurisation systems employ a number of automated flow diversion scenarios to minimise the risk of failure in the pasteurisation system and these ensure that inadequately pasteurised material does not enter the food chain. At a minimum, the required parameters to monitor are:

1. Temperature, monitored through single or double-temperature probes at the outlet of the holding section to ensure that minimum temperature set points are achieved. In the event of a deviation in temperature below the legal requirement for milk, the system will automatically divert the product back to the balance tank or to an intermediate holding tank (for possible rework). Additionally, deviation outside of a pre-defined limit between the two outlet temperature probes will also initiate a diversion of the milk.
2. Minimum holding times at a given pasteurisation temperature are guaranteed based on maintaining a minimum temperature at a maximum flow rate. If the maximum flow rate is exceeded, then for a given hold-tube configuration, the holding time will become too low and the critical time-temperature combination will not be met. This flow rate is monitored by means of a flow/mass metering system within the heating plant, which triggers a flow diversion in the event of a flow deviation beyond the maximum set point.

6. MEASUREMENT OF THE EFFICACY OF PASTEURISATION

Microbiological inactivation during the pasteurisation process is quantified using D- and Z-values. The D-value refers to decimal reduction time and is the time required at a given temperature to kill 90% (or one log) of the expected microorganisms. The temperature is normally indicated as a subscript of the D-value and the value is given in units of time (usually minutes or seconds). For example, D_{60C} = 1 min indicates that at 60°C, it takes 1 minute to reduce the bacterial population by one log. The Z-value, sometimes called the value of thermal inactivation, is related to the D-value in terms of thermal death time. The Z-value of a microorganism in a particular medium is the temperature change required for the D-value to change by a factor of ten (or one log). For example, if Z = 8°C and D_{72C} = 15 s, the D-value calculated with a thermal shift of +8°C (80°C) will reduce the time by one log and will be 1.5 s. These values, related to the most heat resistant pathogenic microorganism, are used to set the pasteurisation protocol. As *C. burnetii* is the most heat-resistant non-sporulating pathogen likely to be present in milk, pasteurisation is designed to achieve at least a 5-log reduction of *C. burnetii* in whole milk (4% milkfat) (CAC/RCP 57-2004). Heat inactivation kinetics of six significant milk-borne pathogens under commercial-type pasteurisation conditions showed mean log reductions and temperatures of inactivation during a 15s treatment were *Staphylococcus aureus* >6.7 at 66.5°C, *Yersinia enterocolitica* >6.8 at 62.5°C, pathogenic *Escherichia coli* >6.8 at 65°C, *Cronobacter sakazakii* >6.7 at 67.5°C, *Listeria monocytogenes* >6.9 at 65.5 °C, and *Salmonella* Typhimurium >6.9 at 61.5°C (Pearce et al., 2012).

7. CONFIRMATION OF ADEQUATE HEAT TREATMENT

Alkaline phosphatase (ALP) is an enzyme which is naturally present in milk and is inactivated at approximately the same time-temperature combinations as *C. burnetii.* These characteristics render ALP as a useful tool to facilitate verification that milk has been adequately pasteurised. The ALP test has been used universally to help manage the likelihood that under-pasteurised milk, or pasteurised milk which has been contaminated with raw milk and which, therefore could contain pathogenic bacteria, is not released for human consumption. The ALP test can be applied to both batch and continuous pasteurisation, in addition to appropriate pasteuriser maintenance, continuous monitoring and record keeping, to demonstrate that all milk has been subjected to pasteurisation treatment conditions.

The ALP test was developed by Kay & Graham (1935) and applies to milk immediately post-pasteurisation. It is based on the conversion of disodium phenyl phosphate to phenol, which is measured colorimetrically after reaction with the Folin-Ciocalteau phenol reagent. Using this method, Kay & Graham (1935) could detect 0.25% raw milk in properly pasteurised milk. Indeed, it has also been reported that about 0.1% of raw milk could be detected by an improved version of this method, the Scharer Rapid Phosphatase test (Nelson-Jameson) (Cornell University, 2007). This latter method was adopted as ISO 3356|IDF 63:2009 (IDF, ISO, 2009). Subsequently, more sensitive methods such as the Fluorophos® ALP Test System (Advanced Instruments) based on fluorometic detection and the Charm®ALP/PasLite (Charm Sciences, Inc.) based on chemiluminescence have been introduced (Table 2). The fluorometric method was adopted by IDF as Standard 155 in 1992, now available in two parts: ISO 11816-1|IDF 155-1: Milk and milk products – Determination of alkaline phosphatase activity – Part 1: Fluorometric method for milk and milk-based drinks (IDF, ISO, 2013) and ISO 11816-2 | IDF 155-2:2016 – Milk and milk products – Determination of alkaline phosphatase activity – Part 2: Fluorometric method for cheese (IDF, ISO, 2016). IDF is currently working on a standard for the determination of alkaline phosphatase activity in milk and milk products by fluorometric detection based on a freely available method.

Added raw milk (%)	Whole milk	Skim milk	Chocolate milk (0.4% fat)	Half and half cream (11% fat)
0	12	12	10	8
0.05	256	262	262	156
0.10	494	508	521	327
0.20	960	995	1020	610

[1] Results are means of results from eight collaborating laboratories rounded to nearest whole number.

Table 2. Alkaline phosphatase (mU/L) in pasteurised milk products1 with three levels of added raw milk measured by the Fluorophos® method (from Rocco, 1990).

The limits of ALP activity for properly pasteurised milk are <1 µg phenol/mL for the rapid Scharer test, <350 mU/L for the fluorometric and chemiluminescence methods (or < 500 mU/L for other products) (Shakeel-ur-Rehman et al., 2003). However, these latter methods can detect much lower levels of ALP (~10 mU/L) which correspond to about 0.003% raw milk. Residual ALP activity in adequately pasteurised cow's milk is 20–50 mU/L. However, the levels common in pasteurised milk are often less than 20 mU/L, due to the higher-than-required pasteurisation time and temperature combinations commonly used; hence, levels higher than 50 mU/L might be unusual and require investigation (NZFSA, 2003).

Lactoperoxidase, another enzyme present in raw milk, is inactivated by heat treatments in excess of about 80°C for 15 s. Thus, the presence of lactoperoxidase can be used to detect milk which has not been over-heated. In some countries (e.g., Switzerland), it is compulsory that lactoperoxidase is active in pasteurised milk.

8. LIGHT-INDUCED OFF-FLAVOURS

Light-induced off-flavours can develop in pasteurised or unpasteurised milk during storage, particularly if it is packaged in transparent bottles. There are ten photosensitisers in milk (Liu et al., 2016) and all wavelengths tested caused oxidation of milk (Intawiwat et al., 2010). This light-induced defect can readily occur if the milk is stored under fluorescent lights. These lights have a wavelength range of 300–750 nm with a peak at 440 nm, which is close to the wavelength for maximum absorbance by riboflavin, an initiator of light-induced off-flavours in milk. High density polyethylene (HDPE) has about 60% transmittance of light between 300 and 700 nm, while polyethylene terephthalate (PET) has about 80% transmittance at these wavelengths. In three reports, milk in clear plastic bottles stored under fluorescent light showed development of off-flavours in less than 2 h (Dimick, 1973), 12 h (Chapman et al., 2002), and 24 h (Johnson et al., 2015). The development of such off-flavours in pasteurised milk can be overcome by using opaque or coloured light blockers or overwrap films which absorb or block a high proportion of light at the critical wavelengths for milk (Cladman et al., 1998; Webster et al., 2009). Paperboard cartons and opaque bottles exclude 75–99% of light (Tu & Apt, 2013) and improved packaging has now been developed.

2

MICROBIOLOGICAL ASPECTS OF MILK PASTEURISATION

1. THE MICROFLORA OF UNPASTEURISED MILK

Milk is a nutritious medium, ideally suited to the growth of many microorganisms. Traditionally, it is believed that bacteria found in milk result from the teat surface, the milking equipment or the external environment. Such contamination can be increased if there is an udder infection, e.g., sub-clinical or clinical mastitis or when good hygienic practices are not sufficiently adhered to.

Aseptically drawn milk has very few bacteria which can be cultured by traditional agar-based methods. Recent metagenomic studies indicate that milk from clinically healthy quarters can harbour genetic markers of diverse bacterial groups, many of which have not been associated with mastitis. This suggests that the microflora of milk could be more diverse than originally thought (Derakhshani et al., 2018), and that the presence of bacteria in milk is not merely the result of external colonization, but there is a possibility of an endogenous route for bacteria to enter milk. These findings do not provide unequivocal evidence for viability or functionality of the detected bacterial groups. Further data using methods such as metatranscriptomics, metaproteomics and metametabolomics are required for this.

The health and hygiene of the cow, the environment in which the cow is housed and milked, the procedures used in cleaning and sanitising the milking and milk storage equipment, and the temperature and time of storage are all key factors that influence the level of microbial contamination of raw milk. In milk, the number of bacteria increases with storage time, the rate of increase depends on the initial contamination level and time-temperature conditions of storage. An elevated number of bacteria could potentially lead to increased spoilage and public health issues.

The dominant microflora of raw milk generally includes (i) species of lactic acid bacteria (LAB; *Lactococcus* and/or *Lactobacillus* spp.), (ii) *Pseudomonas* spp., (iii) the group *Micrococcaceae* (*Micrococcus* and *Staphylococcus* spp.), and (iv) yeasts. Other microbial groups which could be present in raw milk belong to the LAB (including *Leuconostoc*, *Enterococcus*, and *Streptococcus* spp.), *Bacillus, Clostridium, Listeria* spp. and *Enterobacteriaceae*; there are also many other Gram-negative (including, *Acinetobacter*, *Alcaligenes, Flavobacterium,* and *Aeromonas*) and Gram-positive (including, *Arthrobacter*, *Corynebacterium, Brevibacterium,* and *Propionibacterium*) species (Quigley et al., 2013).

The microflora of unpasteurised milk might also contain other pathogenic bacteria. For example, pathogenic *Escherichia coli*, *Salmonella enterica*, *Listeria monocytogenes* or *Campylobacter* spp. are essentially ubiquitous pathogens and can be detected in milk-producing animals and their milk, as indicated by prevalence data from raw milk testing (EFSA, 2015; FSAI, 2015; Giacometti et al., 2012; Hill et al., 2012; Jayarao et al., 2006; Marshall et al., 2016; Park et al., 2007; van Kessel et al., 2011). *Brucella melitensis* and *Mycobacterium bovis* have been associated with disease outbreaks involving raw milk, but these are less common and more geographically restricted than the other pathogens, as control programmes have generally been successful in reducing the number of infected animals and therefore, human diseases (FAO, 2003; Cousins, 2001; CDC, 2012).

Recent studies using culture-independent methods such as metagenomics have shown that the microbial flora of unpasteurised milk is likely to be more complex than is apparent from culture-dependent methods, implying that much of the microbial flora of milk might not be culturable (Quigley et al., 2013). However, using metagenomics, there is limited definitive confirmation that the bacteria are viable and careful interpretation of the results is required (Ceuppens et al., 2014).

2. THE MICROFLORA OF PASTEURISED MILK

The microflora of pasteurised milk consists of thermoduric bacteria which survived pasteurisation and, possibly, microorganisms from post-pasteurisation contamination which could arise from pipework and storage equipment, or from personnel or from packaging. Hygiene at milking and maintaining the cold-chain pre-pasteurisation reduces the number of bacteria in pre-pasteurised milk, thus resulting in a lower number of bacteria in pasteurised milk.

The risk of food poisoning arising from pathogenic bacteria in pasteurised milk is relatively low, although isolated cases have been reported (CDC, 2008, 2011; RRMF, 2011). Since pasteurisation inactivates pathogens to acceptable levels, such contamination results from inadequate pasteurisation or post-pasteurisation contamination.

3. INACTIVATION OF MICROORGANISMS AND EXTENSION OF SHELF-LIFE

Originally, the minimum heat treatment of pasteurisation was intended to inactivate *Mycobacterium tuberculosis* (and *Coxiella burnetii*), the two most heat resistant vegetative organisms associated with milk. *M. tuberculosis* was the organism of most concern to public health at that time. Using these two organisms as reference organisms for adequate pasteurisation had the advantage that other pathogenic and spoilage organisms were also inactivated, improving the safety of milk and extending the shelf-life. Pathogens which caused concern recently, such as Shiga-toxin producing *Escherichia coli* (e.g. *E. coli* O157:H7) and *Listeria monocytogenes,* are also inactivated by pasteurisation.

Recently, there has been concern that *Mycobacterium avium* subspecies *paratuberculosis* (MAP) can partly survive pasteurisation. This organism is the agent of Johne's disease in cattle and is suspected, but not confirmed, to be a cofactor of Crohn's disease in humans. There are three main issues with regard to MAP in milk (Robertson et al., 2017):

1. A definitive causal link between MAP and Crohn's disease has not been established. MAP is suspected, but not confirmed, to be a cofactor of Crohn's disease in humans, along with *Campylobacter concisus*, measles virus, *Yersinia, Escherichia coli* O157:H7, yeasts and others

2. Although several reports (Chiodini & Hermon-Taylor, 1993; Grant et al., 1996; Gao et al., 2002) have shown survival of MAP at pasteurisation temperatures in static conditions in laboratory experiments, all experiments undertaken in pilot-scale turbulent flow systems (Pearce et al., 2001; Lynch et al., 2007; McDonald et al., 2005; Rademaker et al., 2007; Stabel & Lambertz, 2004) have shown complete inactivation of the organism during pasteurisation under commercial conditions. Thus, the probability of the presence of viable MAP in pasteurised milk is very low considering the initial concentration in raw milk (reported as 1.2–2.8 cfu/ml [Serraino et al., 2014] and 0.54–7.03 cfu/ml [Okura et al., 2013]) and the effect of pasteurisation under commercial conditions which shows at least a four log reduction. Analytical methods which have not been validated or are not unanimously accepted by the scientific community can result in false or misleading positive results. Indeed, published results on the prevalence of MAP in pasteurised milk are challenged by several lines of argument including the possibility of faulty pasteurisation or post-pasteurisation contamination (Cerf et al., 2007).

3. The methodology for demonstrating the presence of infective MAP cells in food requires validation and standardisation: It is clear that validated methods have to take into account several factors such as: (i) accuracy and precision in the relevant operating range (expected concentration of MAP), (ii) suitability of the assay for the sample matrix, (iii) ability to differentiate between viable and inactivated organisms, (iv) understanding of the limitations of the analytical and diagnostic sensitivity and specificity, (v) repeatability of the test results when conducted on the same specimen, and (vi) reproducibility of results when the test is conducted by different laboratories.

Recent consumer demand for less heat-processed but equally safe food, has led to intense exploration of potential non-thermal/less-thermal alternatives to heat-based pasteurisation (Morris et al., 2007). Such alternative technologies include pulsed electric field (Mosqueda-Melgar et al., 2008), ultraviolet light (Koutchma, 2009), microfiltration (Pouliot, 2008), and high hydrostatic pressure (an in-pack batch process) (Mújica-Paz et al., 2011). While these technologies appear to be equally effective to thermal pasteurisation in terms of microbial reduction and shelf-life extension, and all appear to avoid the denaturation of heat-sensitive proteins, currently none of these technologies

have been approved in the EU, the US or Canada as stand-alone alternatives to thermal pasteurisation of milk. However, in Australia high pressure processed raw milk (so-called 'cold-pressed raw milk') has recently been approved for commercial sale (Schuh, 2016).

4. THERMODURIC BACTERIA

Bacteria which survive pasteurisation, termed thermoduric, are mostly non-pathogenic organisms, with the exception of some spore-forming bacteria, some of which might be pathogenic. Depending on their number, and especially their heat resistance, they are classified into three categories:

- Moderately thermoduric bacteria, such as *Micrococcus*, *Streptococcus*, *Enterococcus*, *Lactobacillus*
- Strongly thermoduric bacteria, resistant to a treatment of 75°C for 12 min, like the genus *Microbacterium (M. liquefaciens)*
- Highly thermoduric bacterial spores, resistant to temperatures in excess of 80°C for 10 min. Such spore-forming bacteria include members of the genus *Clostridium* (for example, *C. butyricum, C. tyrobutyricum*) and *Bacillus* (*for example, B. subtilis, B. cereus, B. licheniformis*).

The presence of spores of *Bacillus cereus* group or *Paenibacillus* species are a limiting factor for the potential shelf-life of pasteurised drinking milk (te Giffel et al., 1997; Ranieri et al., 2011), and could be a potential food poisoning agent. *B. cereus* is commonly found in soil and could be frequently found in milk during the grazing season when the risk of teat contamination with soil is greatest (Slaghuis et al., 1997; Christiansson et al., 1999; Saleh-Lakha et al., 2017).

Hygienic practices at milking are the best way to control these organisms: (http://www.fao.org/ag/againfo/resources/documents/MPGuide/mpguide1.htm http://onlinelibrary.wiley.com/doi/10.1002/9781444301649.ch1/summary).

5. THE SHELF-LIFE OF PASTEURISED MILK

In general, the lower the storage temperature, the longer the shelf-life of pasteurised milk. However, psychrotrophic bacteria such as *Pseudomonas* spp. can grow below 4°C, and subsequently can produce extracellular enzymes which lead to the formation of off-flavours. Of particular relevance in this case are psychrotrophic thermoduric bacteria (for example *Bacillus* spp.) as these can survive pasteurisation and grow at 4°C.

Pasteurised drinking milk can have a shelf-life in the range of five to 20 days, depending on the quality of the initial milk, the degree of post-pasteurisation contamination and the

time-temperature profile of pasteurised milk storage. Apart from the bacteria metabolising lactose to lactic acid, adventitious contaminant bacteria can also produce a range of enzymes: it is through these enzymes acting on the milk components that off-flavours are formed and the physical structure of the milk is changed. The best known enzymes are proteases producing peptides, some of which are bitter (Richardson & Newstead, 1979), and lipases producing free fatty acids (Lawrence, 1967). Along with these primary enzymes is an array of other endogenous enzymes which cause a range of defects seen in spoiled milk.

3

NUTRITIONAL ASPECTS OF MILK PASTEURISATION

The purpose of this section is to review the impact of pasteurisation on the nutritional value of milk and will focus on nutrients that are found in nutritionally significant quantities as well as some antimicrobial factors which are present in milk. Milk is widely regarded as a highly nutritious food which contributes significantly to nutrient and micronutrient intakes in many populations and pasteurisation has been shown to have minimal impact on these nutrients.

1. MILKFAT

Milkfat is a source of energy and particular fatty acids including essential fatty acids. Unpasteurised milk typically contains 4% fat, but during processing could be standardised to fat levels of about 3–3.5%.

De Souza et al. (2003) and Nunes & Torres (2010) reported that pasteurisation had little effect on the fatty acid profile of milk, confirming earlier work (Badings & Neeter, 1980; Henderson et al., 1980; Renner & Baier, 1971), especially that on polyunsaturated and essential fatty acids of milkfat. In studies by Herzallah et al. (2005) and Costa et al. (2011) there was no difference between pasteurised and raw milk in the short-chain fatty acid profiles of butyric acid (4:0), caproic acid (6:0) and caprylic acid (8:0). Herzallah et al. (2005) also demonstrated that milk pasteurisation did not have a significant effect on the amount of conjugated linoleic acid (CLA), and there was no significant difference in the content of the trans isomer.

It can thus be presumed that heating has a minor effect on the nutritional value of milk fat as such (Pestana et al., 2015). Moreover, the fatty acid content of milk is known to vary significantly by genetics (e.g., breed) and environmental factors (e.g., diet and management) (Caroli et al., 2009; MacGibbon & Taylor, 2006).

Therefore, pasteurisation does not substantially change the proximate composition and fatty acid profile in raw bovine milk, regarding its potential nutritive properties and consequent benefits for human health.

2. MILK PROTEINS

Pasteurisation does not cause a significant change in protein quality, although minor levels of denaturation of whey proteins have been reported due to pasteurisation (Lucey, 2015). The direct nutritional value of milk proteins depends on their digestibility and their contribution to the intake of essential amino acids (Claeys et al., 2013). A key essential amino acid in milk is lysine. Only small losses (1–4%) of the available amount of lysine were observed after pasteurisation of milk and the effect of heating on the other amino acids appeared to be negligible (Claeys et al., 2013; Andersson & Oste, 1995; Schaafsma, 1989). Some studies have shown that HTST pasteurisation treatments mainly modified the functional properties of milk proteins (e.g., emulsifying and water binding properties, solubility), but had little effect on their digestibility or nutritional properties (Claeys et al., 2013; Douglas et al., 1981; Lacroix et al., 2006). A study in 2010 (Inglingstad et al., 2010) showed that the protein digestion patterns of both raw and high temperature heated (at 95 °C for 1 min) bovine, caprine, equine and human milk were different among the species. However, heat treatment of the milk (at 95 °C for 1 min) did not seem to affect the protein digestion pattern, except for increased degradation of α-lactalbumin in milk from all species. It has also been reported that proteins in both pasteurised and high heat-treated goat and cow milk were more resistant to hydrolysis compared to the raw milk (Almaas et al., 2006; Tunick et al., 2016). Qi et al. (2015) demonstrated that HTST pasteurisation caused neither appreciable chemical changes, nor remarkable secondary structural reduction in the major proteins in pasteurised milk.

In conclusion, the scientific evidence indicates that pasteurisation of milk might modify the structure of milk proteins slightly, but that the changes in the proteins are related to their functional properties, such as solubility and emulsifying, and have no significant effect on their digestibility or nutritional properties (Efigênia et al., 1997; Claeys et al., 2013).

3. ENZYMES IN MILK THAT HAVE ANTIMICROBIAL PROPERTIES

Several components of milk with potential antibacterial properties such as lactoferrin, lysozyme and lactoperoxidase, are either unaffected or minimally affected by HTST pasteurisation (Cifelli et al., 2010). Lactoferrin, which binds free iron and thus limits its availability to pathogens for growth, is not affected by standard pasteurisation (Cifelli et al., 2010; Paulsson et al., 1993; Steijns et al., 2000). Lysozyme, an antibacterial protein, is not affected by pasteurisation (Cifelli et al., 2010; Fox & Kelly, 2006). Lactoperoxidase, another antimicrobial enzyme, retains 70% of its activity after HTST pasteurisation at 72°C for 15 s, while oligosaccharides, which are known to prevent the adhesion of potential pathogenic bacteria to the intestinal epithelium, are also heat-stable (Cifelli et al., 2010; Marks et al., 2001).

4. VITAMINS AND MINERALS

Milk is a good source of calcium and phosphorus and pasteurisation has little or no impact on the concentration of these components. Heat treatment appears to have no significant effect on the bioavailability of calcium: Both the total amount of calcium and the bioavailability of the calcium in milk remain unchanged after pasteurisation (Claeys et al., 2013; Cifelli et al., 2010).

The iodine content of milk varies greatly depending on milking practices, geographical location, year, season and the cow's diet (Trøan et al., 2015). Inclusion of potassium iodide (KI) in the diet of the dairy cows increases the milk iodine levels.

While the vitamin content of milk can vary by geography and seasonality, it is widely regarded to contain significant amounts of vitamins A, B_2 and B_{12}, along with smaller, but still relevant amounts of vitamins D, B_1, B_6, B_3 (niacin) and dietary folate (ANSES-CIQUAL, 2017; Sivakumaran et al., 2017). Biotin might also be present in significant amounts, however, a lack of data in food composition tables makes this difficult to quantify. Dairy is of particular significance to dietary intakes of vitamin B_{12} because humans obtain most of their vitamin B_{12} from animal sources; while there are some plant-based sources of vitamin B_{12}, such as certain algae and plants exposed to bacterial action or contaminated by soils or insects, these sources are minor. A national survey by CSIRO in Australia, carried out in 1995/6, indicated that adults obtained around 30% of their vitamin B_{12} intake from milk and dairy products, while children obtained around 50% (Cobiac et al., 1999; Vogiatzoglou et al., 2009). This is not surprising considering that a 250 mL serving of milk can provide between 0.75 and 0.82 µg vitamin B_{12} (ANSES-CIQUAL, 2017; Sivakumaran et al., 2017) which represents 31–34% of an adults' daily requirements (Codex, 2017).

Pasteurisation might cause minor losses of water soluble vitamins, but does not affect the concentration of fat soluble vitamins like vitamin A, D, E and K (Claeys et al., 2013; Cifelli et al., 2010; MacDonald et al., 2011). Pasteurisation can cause 7–10% losses of vitamin B_{12}, but even with these losses, pasteurised milk is still a good source of B_{12}. There are varying reports on the stability of vitamin B_2 (riboflavin) (Lucey, 2015; MacDonald et al., 2011). However, food composition tables indicate milk can contain between 0.44 and 0.7mg/250mL (ANSES-CIQUAL, 2017, Sivakumaran et al., 2017) per serving and internationally accepted nutrient reference values indicate 1.2mg/day to be sufficient (Codex, 2017). Vitamin C (ascorbic acid), for which milk is not a rich source at ~ 1 mg/100 mL, was reduced by pasteurisation (Burton, 1988) and is also sensitive to oxidation by dissolved air in the milk. It should also be recognised that milk is not considered to be a primary source of vitamins E or K (Cifelli et al., 2010). Raw milk contains a variable level of vitamin D dependent on herd management; in some countries, pasteurised milk is fortified with vitamin D, which promotes calcium absorption and plays a key role in bone health (Cifelli et al., 2010).

5. SUMMARY

Milk pasteurisation can be achieved by heating milk, for example, to at least 72°C for 15 s or to 63°C for 30 min. Appropriate reduction of active ALP, as tested immediately after pasteurisation, provides a useful indicator, in addition to pasteurisation management records, to help verify that adequate heating was achieved, while the presence of lactoperoxidase indicates that the heat treatment was not too severe.

Such heat treatment will reduce pathogenic bacteria to an acceptable safe level and reduce spoilage organisms, thus providing safety and extending the shelf-life of the milk. There are few adverse effects on the nutritional quality of the milk. Thus, according to the currently available knowledge, drinking pasteurised milk is still the safest way to enjoy the health benefits of drinking milk. Maintenance of a healthy herd, hygienic housing, where appropriate and hygiene at milking and during storage, in addition to rapid cooling and storage of milk at 2–6°C prior to pasteurisation, limit the growth of the microflora of milk pre-pasteurisation, while post-pasteurisation hygiene, avoidance of re-contamination and a reliable cold-chain improves the shelf-life of pasteurised drinking milk.

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Example: 1 Singh, H. & Creamer, L.K. Aggregation & dissociation of milk protein complexes in heated reconstituted skim milks. J. Food Sci. 56:238-246 (1991).

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"	Usually double quotes and not single quotes
? !	Half-space before and after question marks, and exclamation marks
±	Half-space before and after
microorganisms	Without a hyphen
Infra-red	With a hyphen
et al.	Not underlined nor italic
e.g., i.e.,...	Spelled out in English - for example, that is
litre	Not liter unless the author is American
ml, mg,...	Space between number and ml, mg,...
skimmilk	One word if adjective, two words if substantive
sulfuric, sulfite, sulfate	Not sulphuric, sulphite, sulphate (as agreed by IUPAC)
AOAC INTERNATIONAL	Not AOACI
programme	Not program unless a) author is American or b) computer program
milk and milk product	rather than "milk and dairy product" - Normally some latitude can be allowed in non scientific texts
-ize, -ization	Not -ise, -isation with a few exceptions
Decimal comma	in Standards (only) in both languages (as agreed by ISO)

No space between figure and % - i.e. 6%, etc.

Milkfat	One word
USA, UK, GB	No stops
Figure	To be written out in full
1000-9000	No comma
10 000, etc.	No comma, but space
hours	Ø h
second	Ø s
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