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Title	A comparison of the use of whole milk and fat-filled milk powders for production of heat-stable long-life beverages
Authors	Crotty, Aisling
Publication date	2020-04
Original Citation	Crotty, A. 2020. A comparison of the use of whole milk and fat-filled milk powders for production of heat-stable long-life beverages. MRes Thesis, University College Cork.
Type of publication	Masters thesis (Research)
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Download date	2026-11-07 11:54:51
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A comparison of the use of whole milk and fat-filled milk powders for production of heat-stable long-life beverages

Thesis presented by

Aisling Crotty, B.Sc.

For the degree of

Master of Science

in

Food Science and Technology

April 2020

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Overall conclusions and suggestions for future work

Declaration

I hereby declare that the work submitted is entirely my own and has not been submitted as an exercise for a degree at this or any other university or higher education institute, or for any other academic award in this university.

Name: _____ Date: 09/04/20

Aisling Crotty

Acknowledgments

I wish to thank my supervisors Prof. Alan Kelly and Dr. Seamus O'Mahony firstly for the opportunity to pursue an MSc at University College Cork, and secondly for their supervision, guidance, and patience throughout the (ever-extending) length of my studies.

I would like to thank the Dairy Processing Technology Centre (DPTC) for funding the project, the industry partners for their powders and their perspective, and everybody who I have met through the DPTC network, for their interest and opinions.

My thanks also to the technical staff in the School of Food and Nutritional Sciences, both upstairs and downstairs. In particular, I would like to thank Dave Waldron who helped design my trials and kept the lab in ship-shape.

Thanks to all of my fellow post-graduate (and post-doctorate) students who lent a hand over the years, Ryan and Loreto in particular for deciphering the Lumisizer! For the camaraderie, I wish to extent my gratitude to all in team MiST (Patrycja, Nikki, Bozhao, Sonia, Naomi, Nishmita, Jasper, Anuya, and Megan, and all who have passed through) and to the usual occupants of the tea-room. Thank you for consuming my stress-bakes and lending an ear when the going got tough. I would not have a shred of sanity left had it not been for you all.

And finally, a massive thank you to my family. To Dad for the coffee and endless encouragement at every step, and to Mum for the cakes and constant support, regardless of the issue. And to my siblings: Rory, Eimear, and Aoife, my personal international cheering squad.

Dedication

I would like to dedicate this thesis to my parents, without whom this thesis would simply not exist. Thank you both.

Abstract

Commercial bovine milk is 3.5% fat, the level of which is affected by seasonality, stage of lactation, feed, health, breed, and even the individual teat. Milk (and other liquid dairy products) are highly perishable due to their nutritional quality, as they are the sole source of nutrition for the neonate. As a result, milk is often dehydrated into powder form, which enhances its shelf life, its storage stability, and the convenience. Another way to enhance the shelf life of milk is to subject it to heat to destroy pathogenic bacteria, enzymes, spores, and to enhance the shelf life of the product. As most dairy products are subjected to some form of heat treatment, their heat stability is integral to the overall quality of the product. In Chapter 2, two reconstituted dairy powders (fat-filled milk powder (FFMP) and whole milk powder (WMP)) were compared under two heat treatments (UHT-processing and retort sterilisation), and three protein contents (2.3, 3.3, and 5%). These variables significantly affected the apparent viscosity, the pH, the colour, the emulsion stability, and the average particle size of the samples. Chapter 3 investigated the influence of calcium-chelating salts on heat stability. These salts are an often-used ingredient in dairy products, as they enhance the heat stability of the system by binding the calcium ions, which are important for casein micelle integrity. The influence of trisodium citrate (TSC), disodium hydrogen phosphate (DSHP), and sodium hexametaphosphate (SHMP) on heat stability, colour, and apparent viscosity was examined. SHMP had the most significant effect on heat stability, colour, and apparent viscosity due to its chelating capacity and its influence on cross-linking between the casein micelles. DSHP had no

significant effect on viscosity or colour, whereas the addition of 20 mmol/L of TSC significantly affected the colour of the solution.

Chapter 1

Literature review

Optimising production and quality of dairy products containing different fat types

1.1 Milk composition

1.1.1 General composition

Milk is a biological fluid secreted by mammals. As it is the only food which the neonate will consume during the early stage of life, it is a highly complex emulsion, as well as colloidal suspension, of essential growth and energy components such as proteins (and amino acids), lipids, carbohydrates (lactose being the major sugar), minerals, and vitamins. (Bylund, 1995; Sun, 2006). The levels of each constituent in milk vary between species, and is also affected by the stages of lactation (early-, mid-, or late-lactation), seasonality, feed, health, breed, and even vary between each individual teat of an udder (Walstra, 1999). An example of this variation can be seen in Table 1.1; in addition to solids, bovine milk contains about 87% water (Bylund, 1995). The levels at which the components are present are not indicative of their importance, for example, vitamins and enzymes are both present in relatively low amounts, yet they are very important from nutritional and indeed processing perspectives.

Table 1.1 Variation of composition between species (Bylund, 1995; Martin *et al.*, 2016).

Species	Protein total (%)	Casein (%)	Whey protein (%)	Fat (%)	Carbohydrate (%)	Ash (5%)
Human	1	0.3	0.7	3.8	7.0	0.2
Cow	3.4	2.6	0.8	3.5	4.8	0.7
Buffalo	4.0	3.5	0.5	7.5	4.8	0.7
Goat	3.6	2.7	0.9	4.1	4.7	0.8
Sheep	5.8	4.9	0.9	7.9	4.5	0.8

1.1.2 Milk fat

Fat globules are the largest particle found in milk (Bylund, 1995). They are also the least dense particle, which results in creaming during storage, if milk is not homogenised. The majority of fat in milk exists as triacylglycerols (98% of total fat) (Taylor and MacGibbon, 2011b); the remainder is composed of phospholipids (originating from the milk fat globule membrane), sterols, mono- and di-acylglycerols, free fatty acids, the colour pigment β-carotene, fat-soluble vitamins, and flavour compounds.

1.1.2.1 Milk fat globule

Milk fat is present in milk in the form of milk fat globules; small spherical droplets ranging between 0.1 and 15 μm in diameter (in unhomogenised milk) which are dispersed in the milk serum (Hardham *et al.*, 2000; Wilbey, 2003; Singh, 2006). The droplets are spherical in shape due to the interfacial tension between the two immiscible phases, as a sphere has the smallest surface area-to-volume ratio of all geometrical shapes (Fox, 2011). The membrane surrounding the globule, known as the milk fat globule membrane (MFGM) is a complex bilayer of phospholipids, lipoproteins, glycerides, cerebrosides, proteins, nucleic acids, over 25 enzymes, metals and water (Figure 1.1) (Bylund, 1995). Its composition resembles the apical plasma membrane of the secretory cells (Singh, 2006). Approximately 90% of the dry weight of the MFGM is made up of proteins and lipids. Two major proteins in the MFGM are xanthine oxidase and butyrophilin (Singh, 2006). Xanthine oxidase is a loosely associated enzyme and converts hypoxanthine to xanthine to uric acid which is excreted by the kidneys (Waud *et al.*, 1975; Mather *et al.*, 1977). Butyrophilin is the

most abundant protein in the MFGM, accounting for 40% of total protein associated with the MFGM in milk from Holstein cows (Singh, 2006). It is firmly associated with the MFGM and it has been proposed to be essential in the secretion of the milk fat globule (Robenek *et al.*, 2006). An interesting side-note: the name "butyrophilin" originates from the Greek words "butyros" and "philos", which roughly translated means "having affinity to milk fat" (Franke *et al.*, 1981).

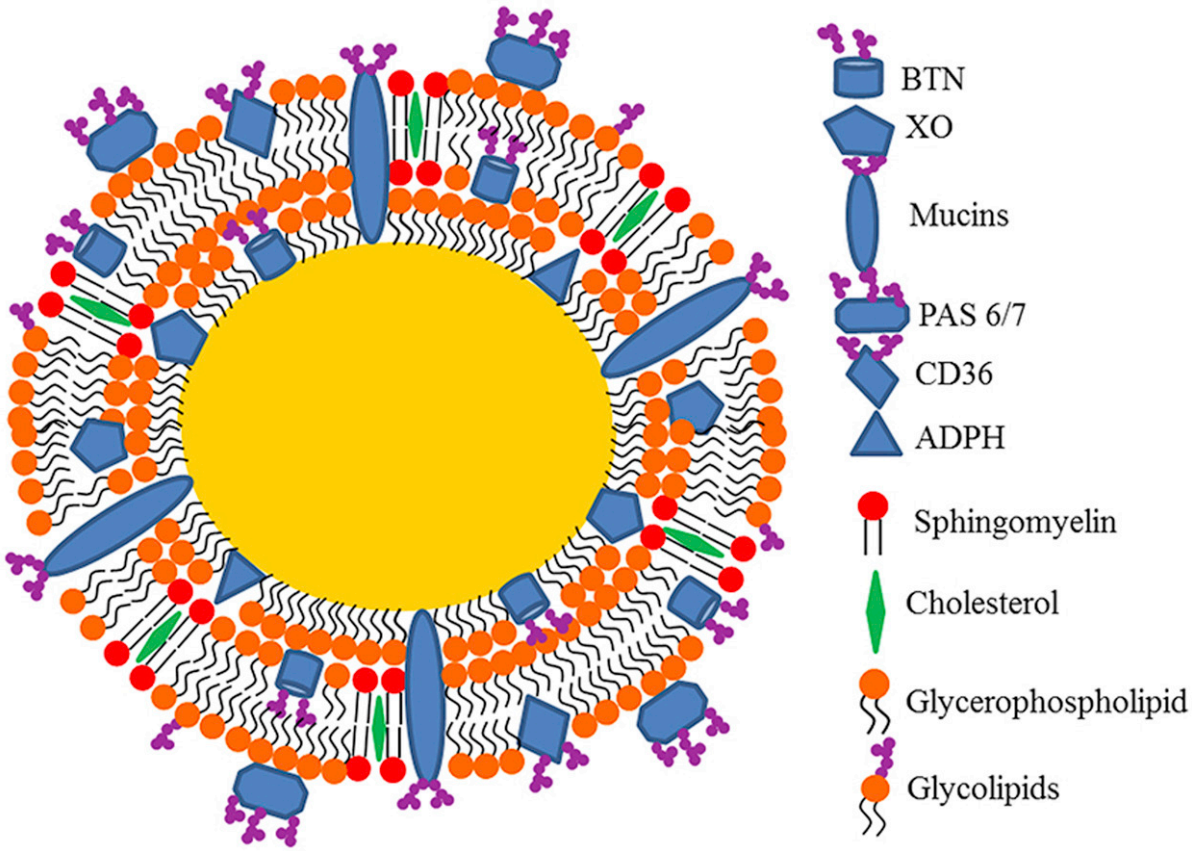


Figure 1.1: The complexity of the milk fat globule (Singh and Gallier, 2017).

The MFGM allows the non-polar fat to exist in the polar serum as an oil-in-water emulsion. The complex array of materials in the membrane originates from the milk-secretory cells and from the material acquired during the secretion of fat globules from the cell from the apical membrane and the Golgi vesicles (Burgess *et al.*, 1991; Mather,

2011). The interior of the globules is comprised mostly of triglycerides (the majority of which seem to contaminate the fat globule's core only during membrane isolation), but also diglycerides, monoglycerides, fatty acids, carotenoids, sterols and their esters, and vitamins A, D, E, and K (Walstra, 1985; Bylund, 1995; Jensen and Newburg, 1995; Singh, 2006).

Milk fat globules influence the colour, mouthfeel, and viscosity of milk (Fox, 2011). In its natural state (i.e., before homogenisation), the MFGM has been determined to be approximately 10 nm thick, but this increases to 15 nm after homogenisation due to the binding of protein fragments as part of the newly-formed milk fat globule membrane (Burgess *et al.*, 1991; Fox, 2011). The MFGM acts as a barrier for the enzyme lipoprotein lipase, so it cannot act on its substrate, i.e., lipid. Therefore, fat-filled milk powder (FFMP) might be expected to be more susceptible to lipase action than whole milk powder (WMP). The MFGM is quite delicate, so abrasive processes must be avoided when possible on dairy processing lines (Burgess *et al.*, 1991). There have been multiple proposals for the structure of the MFGM; however, neither a structure nor a secretory mechanism have been universally accepted as the membrane structure is not fully understood (Walstra, 1999; Heid and Keenan, 2005).

1.1.2.2 Creaming and instability of milk fat: Stokes' Law

The flotation of the milk fat globules in the serum can be explained by Stokes' Law, although it does not account for coalescence or cold agglutination (Bylund, 1995; Wilbey, 2003). The equation is:

$$V = \frac{2r^2(\rho_1 - \rho_2)g}{9\eta}$$

where V is the rate of creaming, r is the radius of the globule, ρ_1 is the density of the continuous phase (water in the case of milk), ρ_2 is the density of the dispersed phase (fat in milk), g is the acceleration due to gravity, and η is the viscosity of the system (Fox, 2011). The system must fulfil several conditions for the law to hold, but it is a very useful tool in anticipating potential trends (Walstra, 1999).

Phase separation of emulsions is very closely linked to Stokes' Law and can be broadly described by the following four phenomena: flocculation, clustering, clumping, and coalescence (Figure 1.2). Flocculation is the weak association of two fat globules, still retaining their individual identity; this is easily reversed through gentle agitation e.g., stirring (Burgess *et al.*, 1991). Clustering occurs when these flocculated globules begin to share their membranous material. Clustering may occur if there is not sufficient surface-active material available to stabilise the interface upon homogenisation, and clusters are more difficult to disrupt than flocs. This is overcome in practice by the second stage of homogenisation (Wilbey, 2003; Anema, 2019). Direct fat-fat interaction results in clumping of the globules; this is only possible when the fat is solid. This state is actually essential during the churning of cream, when agitated or when subjected to a shear force; however, it is undesirable when ultra-high temperature-treated (UHT) whipping cream forms a plug. The adsorption of crystallised fat at the MFGM interface is very important in this destabilisation mechanism. Coalescence is the total merging of fat droplets, resulting in the loss of their individual identities (Chiralt, 2009). Granules can also occur if there is fat crystallisation present, and generally cannot be disrupted (Walstra, 1999). Stokes' Law can also estimate the rate of sedimentation of a sample if certain parameters such as the density difference between the phases are known (Dalgleish, 1992).

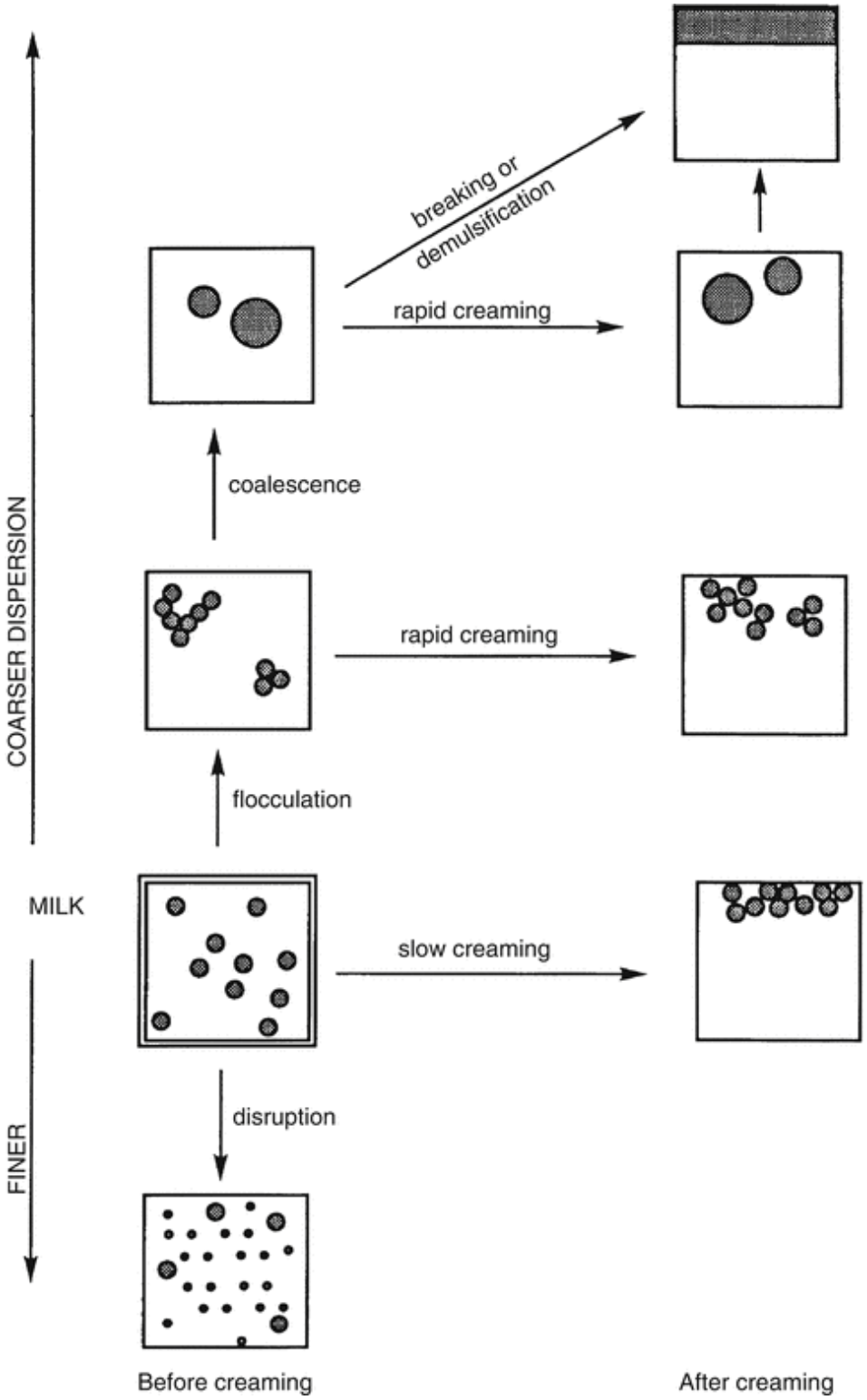


Figure 1.2: Emulsion breakdown through various destabilisation mechanisms (Fox *et al.*, 2015).

1.1.2.3 Triglycerides

A triacylglycerol (also known as a triglyceride) molecule (Figure 1.3) consists of a glycerol backbone, with three positions (*sn*-1, *sn*-2, and *sn*-3) to which non-polar fatty acids can be esterified. Because they account for 97–98% of the total fat found in milk, they greatly influence the melting profile and density of milk, depending on what fatty acids are esterified at which position, which is not a random assignment (Walstra, 1999; Parodi, 2009). For example, butyric acid and caproic acid are commonly esterified in the *sn*-3 position, while stearic acid is found in the *sn*-1 position. Triacylglycerols are not highly reactive; however, they can undergo oxidation and lipolysis, which can result in the production of off-flavours in the milk (Fuquay *et al.*, 2011).

Lipid oxidation occurs between oxygen and unsaturated fatty acids and is catalysed by light and transition metals. Free radicals are formed, as well as hydroperoxides, both of which break down to form highly flavoured carbonyl compounds, which can impart off-flavours, even at very low concentrations. This reaction can be slowed down or inhibited by antioxidants (such as α-tocopherol) or air removal (Choe and Min, 2009; Lobo *et al.*, 2010).

Lipolysis is the hydrolysis of the ester linkages which link the fatty acids to the glycerol backbone, releasing the fatty acids as free fatty acids. Once their levels increase to above the detection threshold, they contribute to off-flavours in the milk. Lipolysis is catalysed by lipases, both indigenous and bacterial (Taylor and MacGibbon, 2011a). Pasteurisation will inactivate lipoprotein lipase, which is the native lipase present in milk, however, the lipases produced by bacteria are more heat-stable than lipoprotein lipase, so they generally remain active after pasteurisation (Muir, 2011). Milk fat, when exposed to both oxygen and lipolytic enzymes,

decomposes to fatty acids and glycerol; this is accelerated by daylight and results in the characteristic smell of butyric acid. Oxidative decomposition also results in the production of peroxides, which exhibit a rancid taste (Písecký *et al.*, 1997).

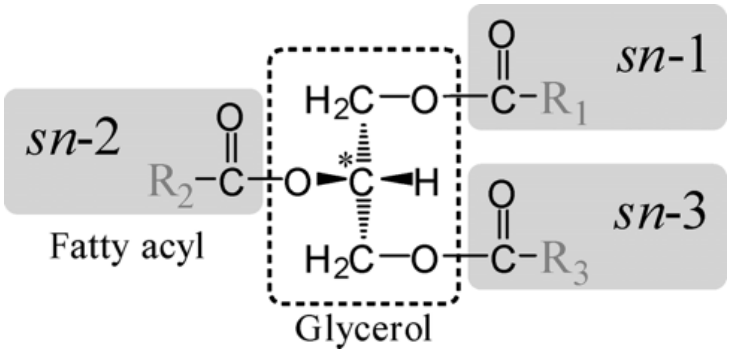


Figure 1.3 Structure of triacylglycerol (Motoyama *et al.*, 2010).

Triglycerides are very energy dense (9 kcal g^{-1}) in comparison to proteins and carbohydrates (both 4 kcal g^{-1}). This makes fat a more desirable energy store in the body but can cause problems when consumed in excess of dietary limits (O'Brien and O'Connor, 2002). Diglycerides and monoglycerides are also present in milk fat, albeit at much lower concentrations to triglycerides. Monoglycerides are quite amphiphilic and, as a result, they accumulate at the oil-water interface (Walstra, 1999).

1.1.2.4 Fatty acids

One of the reasons why milk fat is considered to be so complex is that it contains in excess of 400 fatty acids of varying chain length and degree of saturation (Parodi, 2009; Taylor and MacGibbon, 2011a). Although there is a wide variety of fatty acids in milk, the majority are present in extremely low quantities (Burgess *et al.*, 1991).

Saturated fatty acids account for 65–70% of the total fatty acids found in milk, of which palmitic acid (C16:0) is the most abundant. Of the unsaturated fatty acids present in bovine milk, oleic acid (C18:1), also known as *cis*-9-octodecanoic acid, is the most abundant, being present at levels varying between 17–25% of the total fat (Guo and Hendricks, 2007).

Diet can influence the fatty acid profile, as seen in the variation of the levels of vaccenic acid (C18:1) when a cow is fed on hay and concentrate (1.5%) or on pasture (5%). Due to the occurrence of bio-hydrogenation in the cow's rumen, polyunsaturated fatty acids (PUFAs) generally are only present at low levels. Another example of diet affecting the composition of milk was shown by Średnicka-Tober *et al.* (2016), who showed that organic milk (i.e., milk that is sourced from cow's who have been fed with organic materials) had significantly higher levels of total PUFAs such as eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexanoic acid (DHA) compared with conventionally-produced milk. The levels of minerals varied between the two milk types too.

Factors other than diet can also affect the fatty acid composition in milk, such as the stage of lactation and the time of year (seasonality) (Taylor and MacGibbon, 2011a; O'Callaghan *et al.*, 2020). According to Burgess *et al.* (1991), the fatty acid range present in milk is dependent on genetics, but the quantity of each fatty acid is a direct consequence of the diet. Fatty acids can vary in chain length and degree of unsaturation (number of double bonds in the chain). According to Taylor and MacGibbon (2011a), short-chain fatty acids (SCFAs), butyric and caproic in particular are almost exclusively esterified at position *sn*-3.

1.1.2.5 The influence of milk fat on health

The negative perception of dairy fat seems to stem from the efforts to reduce the amount of saturated fats in the diet (Parodi, 2009; Lordan *et al.*, 2018). Saturated fats in particular contribute approximately 40% of total energy in Western diets, with dairy contributing about 15-20% of daily intake of saturated fatty acids, 10% of which comes from 16:0 (palmitic acid) and 18:0 (stearic acid) (German *et al.*, 2009). In the past few decades, there has been a marked decline in the consumption of full-fat dairy products (Lordan *et al.*, 2018). Price is one of the contributing factors for this change, but some areas of medical opinion, some nutritionists, and the media have been pushing the public towards switching to a more low-fat or even plant-based diet (Burgess *et al.*, 1991; German *et al.*, 2009).

There has been debate about reducing or modifying the amount and nature of fats in the diet to benefit the overall health of the population. This is, of course, dependent on the country and the stage of development, as populations in developing countries often suffer from insufficient dietary energy intake, which could be rectified through an increase of fat in their diets. The opposite is the case for most developed countries, where many authorities and nutritionists are recommending a reduction in dietary fat to avoid obesity and other associated chronic diseases (Burgess *et al.*, 1991; Parodi, 2009; Bockisch, 2015). Studies are showing that the balance of fats (and indeed nutrients in general) in the diet is more important than omitting certain fats (Burgess *et al.*, 1991; O'Brien and O'Connor, 2002). As a result, foods and their constituents must not individually be considered healthy or unhealthy. For example, dairy products provide a wide range of vitamins, minerals (including up to 70% of the recommended daily allowance of calcium), proteins, fats, and other micro-constituents, and encouragement of consumers to reduce their milk intake means that their diet will be

deprived of several components which have potential health benefits (Heaney, 2000; Parodi, 2009; Weaver, 2014; van Ballegooijen and Beulens, 2017; Wade *et al.*, 2017).

Lipids are important in the diet and must not be entirely excluded, as they perform important functions, such as providing a store of energy and protecting vital organs. They are also an important source of fat-soluble vitamins and essential fatty acids (EFAs) (O'Brien and O'Connor, 2002). Short- and medium-chain fatty acids are not stored in the adipose tissue but are transported to the liver where they are metabolised. Milk fat contains significant amounts of short chain fatty acids, such as butyric acid (C4:0) (Bockisch, 2015).

Milk fat, although it can contain about 70% saturated fatty acids, as previously stated, also contains unsaturated fatty acids such as oleic acid, linoleic acid, linolenic acid vaccenic acid, myristoleic acid, and palmitoleic acid (Burgess *et al.*, 1991, Bockisch, 2015). However, due to the hydrogenation of polyunsaturated fatty acids in the cow's rumen, dairy products are relatively poor providers of EFAs (Burgess *et al.*, 1991). If essential fatty acids are not supplied in a young mammal's diet, this can lead to the development of metabolic disorders such as growth retardation and dermatitis. The two major EFAs required in the diet are linoleic (C18:2) and linolenic (C18:3) acids.

Another important group of unsaturated fatty acid present in bovine milk is conjugated linoleic acid (CLAs), which is produced in the rumen during biohydrogenation of PUFAs from the feed and the pasture (Parodi, 2009; Taylor and MacGibbon, 2011a). CLAs include a mix of geometric and positional isomers of linoleic acid (Bauman *et al.*, 2011). In ruminant milk fat and meat fat, the predominant isomer of CLA is *cis*-9, *trans*-11 18:2 CLA, also known as rumenic acid (Figure 1.4).

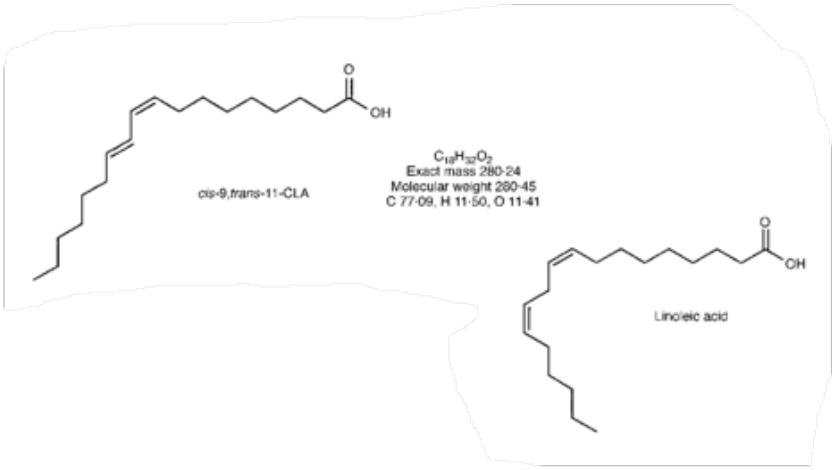


Figure 1.4: The structure of cis-9, trans-11 CLA and linoleic acid (Roche *et al.*, 2001).

The level of rumenic acid can be doubled by switching a cow's diet to pasture-fed. A significant increase can also be achieved by supplementing the cow's feed with fish oils or seed oils, which naturally contain high levels of linoleic acid (Bauman *et al.*, 2011). CLAs have been shown to inhibit tumour growth in mice, and displayed anti-diabetic, anti-obesity, and anti-atherogenic effects (Ip *et al.*, 1991; Pariza *et al.*, 2001; Nagao and Yanagita, 2005; Koba and Yanagita, 2014). Research has shown that CLAs could play a role in the reduction of breast cancer risk in women who consume higher levels of milk (O'Brien and O'Connor, 2002). However, the mechanism through which CLA could work as an anticarcinogenic factor has yet to be identified. As CLAs are fatty acids, higher levels will be naturally present in higher fat dairy products such as cheese, cream, butter and whole milk.

Milk is an important source of fat-soluble vitamins in the diet. One of the major precursors to vitamin A, β-carotene, provides about 25% of the UK's daily intake of vitamin A (Burgess *et al.*, 1991). Vitamin D is important in the absorption of calcium, both of which are found in milk, and it is imperative that children, lactating mothers

and pregnant women obtain sufficient amounts. The effect of milk fat on blood cholesterol level varies. Gurr *et al.* (1989) stated that, when milk fat was consumed alone, it raised blood cholesterol levels, but consumption of milk lowers blood cholesterol. Controlled studies of the consumption of milk fat have shown that milk fat is hypercholesterolemic, but, when milk is consumed as part of a varied diet, it does not significantly increase blood cholesterol levels (Menotti *et al.*, 1999; German *et al.*, 2009). The Masai tribe in east Africa have very low blood cholesterol levels, even though their traditional diet contains a high level of fermented milk and other animal products (O'Brien and O'Connor, 2002). Fermented milk has been shown to contain bioactive peptides which reduced both systolic and diastolic blood pressure in subjects suffering from hypertension (Seppo *et al.*, 2003).

Phospholipids (0.8% of total fat in cow's milk) are one of the major structural components of the membranous material in milk (Ferreiro *et al.*, 2015). The major phospholipids present in the MFGM include sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine (Mather, 2011). Even though they are only present in bovine milk at about 0.8% of the lipid fraction (Taylor and MacGibbon, 2011b), they are very important in the stabilisation of the milk fat globules in the milk. Phospholipids act as an emulsifier in milk, as they are composed of a hydrophilic head (stable in water), and a hydrophobic, or lipophilic, tail. Not only are they structurally important in milk, but recent research suggests that they are also beneficial to our health (Contarini and Povolo, 2013). Such benefits include reducing the development of arthritis, protection against GI infection and regulating inflammation, and they may even play a role in protecting against various forms of cancer (Contarini and Povolo, 2013; Lordan and Zabetakis, 2017; Lordan *et al.*, 2017; Verardo *et al.*, 2017).

Cardiovascular diseases (CVDs) are the leading cause of death globally (Lordan *et al.*, 2018). These are linked to the consumption of dairy, as previous research has linked their high saturated fatty acid content to increased levels of low-density lipoprotein (LDL) cholesterol, which increases the risk of CVDs (Artaud-Wild *et al.*, 1993). However, there is no clear evidence linking the consumption of dairy fat with an increased risk of developing a CVD (German *et al.*, 2009). In fact, dairy products improve some CVD risk factors, such as blood pressure, as they are rich in calcium which is linked with reduced blood pressure (Allender *et al.*, 1996, Bucher *et al.*, 1996). Coronary heart disease (CHD) has been long-associated with dietary fat intake through the 'lipid hypothesis', which theorises that consumption of saturated fat in particular elevates the cholesterol levels in the blood, which will eventually result in the formation of plaque on the inner walls of the arteries, known as atherosclerosis, and lead to a heart attack (Parodi, 2009; Lordan *et al.*, 2018). However, dairy products contain short-, medium-, and long-chain saturated fatty acids, and research has proven that saturated fatty acids increase the level of high-density lipoproteins (HDL) in the blood, decreasing the ratio of total:HDL cholesterol (Mensink *et al.*, 2003). This effect increases with decreasing chain-length of saturated fatty acid, so lauric acid (12:0) has a greater influence on HDL levels than myristic (14:0) and palmitic (16:0). However, they also adversely affect the LDL cholesterol levels (Fernandez and West, 2005). Saturated fatty acid intake has been found to be inversely correlated to the risk of stroke, and reduces total mortality (Dehghan *et al.*, 2017).

1.1.2.6 Infant formula

While breast milk is deemed to be the best source of nutrition for infants, known as the gold standard, sometimes it is necessary for infants to consume infant formula in

cases where the mother is unable to, or does not wish to, breastfeed (O'Brien and O'Connor, 2002; Martin *et al.*, 2016). Infant formulae are modelled on the nutritional composition of breast milk, but often use cow's milk as a base, or soy as a non-dairy option (Figure 1.5) (O'Callaghan *et al.*, 2011).

Bovine milk is not suitable for consumption by infants (McCarthy *et al.*, 2012). Bovine milk contains higher levels of protein, fat and minerals than breast milk. Table 1.2 shows that cow's milk contains a higher proportion of short-chain fatty acids compared to breast milk, thus, if cow's milk is used as a base for infant formula, the solution must be fortified with unsaturated fatty acids as well as adding minerals, vitamins, iron and manipulating the whey protein:casein ratio (Guo and Hendricks, 2007; Martin *et al.*, 2016).

Table 1.2: Difference in fat between breast milk and cow's milk (Guo and Hendricks, 2007; Martin *et al.*, 2016).

Component	**Breast milk (%)**	**Bovine milk (%)**
Total Fat	3.8	3.5
Saturated fatty acids (% of total)	48.2	65.6
Butyric (4:0)	*-*	*3.5*
Caproic (6:0)	*-*	*1.9*
Caprylic (8:0)	*-*	*1.3*
Capric (10:0)	*1.4*	*2.5*
Lauric (12:0)	*6.2*	*2.8*
Myristic (14:0)	*7.8*	*10.7*
Palmitic (16:0)	*22.1*	*27.8*
Stearic (18:0)	*6.7*	*12.6*
Monounsaturated fatty acids (% of total)	39.8	30.3
Palmitoleic (16:1)	*3.1*	*2.5*
Oleic (18:1)	*35.5*	*26.5*
Gadoleic (20:1)	*0.96*	*Trace*
Cetoleic (22:1)	*Trace*	*Trace*
Polyunsaturated fatty acids (% of total)	10.82	4.5
Linoleic (18:2)	*8.9*	*2.9*
Linolenic (18:3)	*1.2*	*1.6*
Parinaric (18:4)	*-*	*Trace*
Arachidonic (20:4)	*0.72*	*Trace*
Eicosapentaenoic (20:5)	*Trace*	*Trace*

Fat is the primary source of energy for the developing infant, and thus must account for 40-54% of the total energy provided in infant formula, in line with the energy provided from breast milk (Koletzko *et al.*, 2001; Thompkinson and Kharb, 2007). The fat also carries fat-soluble vitamins such as vitamins A, D, E, and K.

A common practice in the infant formula industry is to use (not hydrogenated) vegetable oils to achieve the desired fatty acid profile (Nelson *et al.*, 1998; Kelly *et al.*, 2014). The absorption of fat from bovine milk is not as high as that from human milk, hence the substitution with vegetable oil. The absorption is influenced by the length of the carbon chain and the degree of saturation (Aggett *et al.*, 1991; Thompkinson and Kharb, 2007).

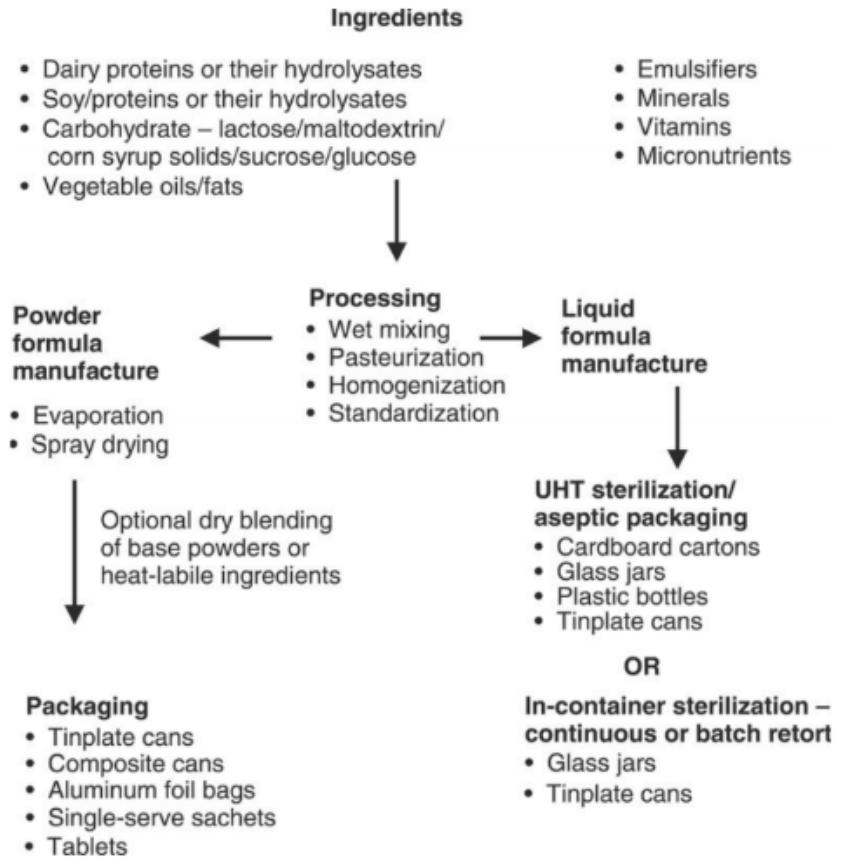


Figure 1.5: Production of infant production, including powdered formula and liquid formula (O'Callaghan *et al.*, 2011).

The composition of infant formula is strictly regulated, with all major components having minimum and maximum levels of addition (Koletzko *et al.*, 2005; Martin *et al.*, 2016). Long-chain polyunsaturated fatty acids (LC-PUFAs) have been shown to aid immunity, visual, and neurological development during growth, so it is encouraged to include them in infant formula (Koletzko *et al.*, 2003; Kuratko *et al.*, 2013). Blends of vegetable oil provide sufficient levels of PUFAs (Thompkinson and Kharb, 2007). Arachidonic acid and docosahexaenoic acid are present at high levels in membrane lipids in the nervous system and the brain (O'Brien and O'Connor, 2002). Even the positioning of fatty acids in the triacylglycerol differs between bovine and human milk which is an important factor in the absorption of the fat (Thompkinson and Kharb, 2007; O'Callaghan *et al.*, 2011). For example, palmitic acid is located at positions *sn*-1 and *sn*-3 positions in vegetable oil but is most commonly located at the *sn*-2 position in breast milk (Christie *et al.*, 1991). Transesterification is carried out to change the position of the vegetable-based palmitic acid which will, in its new position, mimic the digestion of human milk (Bar-Yoseph *et al.*, 2013). Fractionated bovine milk fat globule membrane is also used to a limited extent in the infant formula industry as a source of gangliosides, which are important for infant growth and development (O'Callaghan *et al.*, 2011).

1.2 Milk processing

The vast majority of commercially available milk has been processed at some point before being sold. The aim of milk processing is to enhance the physical stability and the shelf life of the product (Chevallier *et al.*, 2016). Homogenisation is a process which occurs before heat treatment and is always used when processing milk and cream. It reduces the size of the milk fat globules, which increases the physical stability of milk by reducing the fat globules propensity to cream (Hardham *et al.*, 2000). This is based on Stokes' Law, which states that the velocity of movement of the fat globule will increase with an increase in fat globule diameter and an increase in the density difference between the serum and the fat. The rate of creaming will decrease as the viscosity of the milk serum increases (Wilbey, 2003). Homogenisation must be followed immediately by heat treatment in order to minimise lipolysis (Deeth and Fitzgerald, 2009).

Heat treatment of milk was first attempted in 1810 by Nicolas Appert and has been common in Sweden and Denmark since the mid-1880s (Westhoff, 1978; O'Connell and Fox, 2002). Today, any milk on the market is subject to at least one form of heat treatment and it is used not only to reduce or eliminate spoilage and pathogenic bacteria, and inactivate enzymes, but it also takes advantage of protein denaturation as an integral step in such processes as yoghurt production (Lewis and Deeth, 2009; Sharma and Rajput, 2014; Mahomud *et al.*, 2017). The heat treatment of milk can include thermisation, low-temperature, long-time (LTLT) pasteurisation, high-temperature, short-time (HTST) pasteurisation, ultra-high temperature (UHT)

processing, and sterilisation (Bylund, 1995). Each heat treatment has its own advantages and disadvantages that must be considered.

1.2.1 Homogenisation

The main reasons why creaming occurs is due to the density difference between the fat phase and the water phase (0.9 and 1.036 g cm^{-3}, respectively (Fox, 2011)), and differences in polarity (fat being non-polar, and water being polar). Due to the density difference between the milk serum and the fat globules, the fat tends to rise and for a cream layer on top of the milk (Wilbey, 2003). Homogenisation is a process which operates on the principle of disrupting the milk fat globule under sufficiently severe conditions, followed by the maintenance of the new fat globules in dispersion (Wilbey, 2003). During homogenisation, milk is forced through a very small passage at a high pressure (about 13–20 MPa) at temperatures of at least 40°C, to ensure all of the fat in the milk is liquid, which increases the mobility of the fat to form new fat globules (Fox, 2011). The two determinants of fat globule size in homogenisation are: the temperature at homogenisation and the pressure at which homogenisation occurs (Fuquay *et al.*, 2011). Nieuwenhuijse (2016) reported that an increased temperature at homogenisation (55-60°C versus 45-50°C) is preferable to increase the heat stability of the product. Homogenisation reduces the average fat globule diameter to below 1 μm, which results in better fat distribution in the milk, and a reduced creaming rate (Hardham *et al.*, 2000). The reduction in fat globule size results in an increase in surface area of the fat globules, which cannot be fully covered by the existing MFGM, and as a result, casein forms the majority of the new fat globule membrane, although whey proteins have been found as part of the new membrane also (McCarthy *et al.*,

2012; Zamora *et al.*, 2012). Strong homogenisation also increases the negativity of the zeta potential of the fat globules (from approximately -13.5 mV to -20 mV), increasing the colloidal repulsion between the fat globules which should reduce the propensity of fat to cream (Michalski *et al.*, 2002).

Cryoglobulins are immunoglobulins present in milk which affect the creaming rate as they cause the fat globules to aggregate. This immunoglobulin is denatured when heated above time-temperature combinations equivalent to 75°C for at least 15 s, which are the conditions of high-temperature short-time (HTST) pasteurisation (Bylund, 1995; Wilbey, 2003).

Homogenisation can occur before heat treatment (upstream) or after heat treatment (downstream), the latter of which has the added advantage of minimising shear damage by breaking down any protein-protein aggregation or fat globule-protein aggregation that may have occurred during heat treatment (Hinrichs and Atamer, 2011). Upstream homogenisation avoids any microbial contamination which may occur due to seal problems in the homogeniser valve (Wilbey, 2003). Downstream homogenisation is possible in UHT treatment or continuous sterilisation processes (as long as the homogeniser is built for aseptic use) but cannot be done for in-container-sterilised products.

Homogenisers are usually two-stage in operation, using two separate valves, with the second stage operating at a lower pressure (generally about 10% of the total homogenising pressure) to disperse the clumps of fat globules (due to the sharing of casein micelles in the new recombined membrane) that may form after the first stage (Wilbey, 2003; Anema, 2019). Clumping also occurs due to the incomplete coating of

the smaller fat globules with protein (casein mostly, but also whey proteins) (Fox, 2011). The effect of two-stage homogenisation can be seen in Figure 1.6.

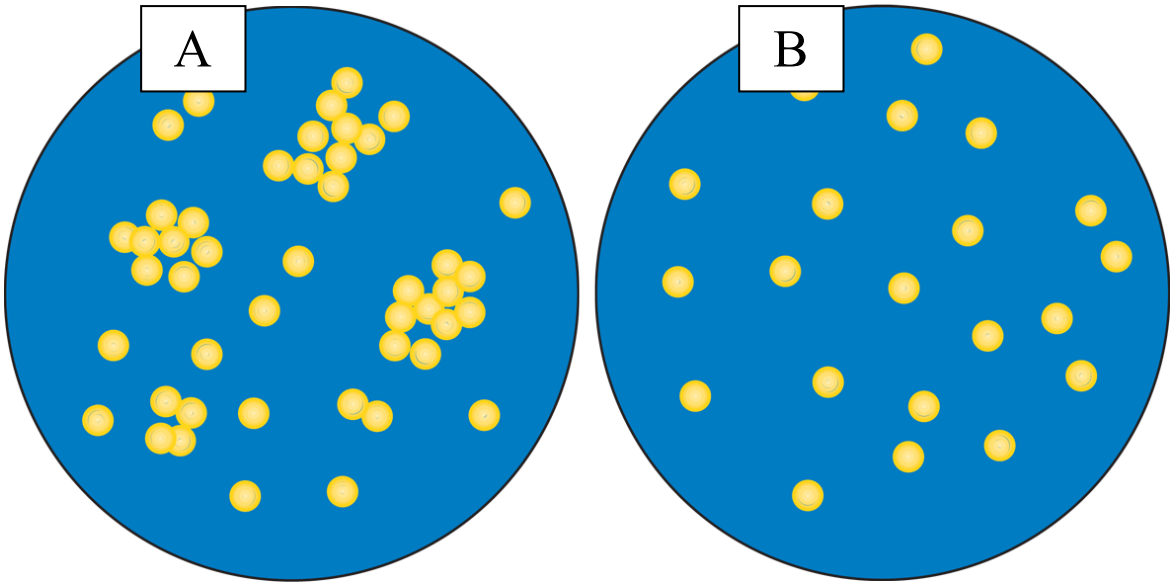


Figure 1.6: Effect of two-stage homogenisation on fat globule (yellow) disruption. **A**: Fat globules after single-stage homogenisation; **B**: Fat globules after two-stage homogenisation (Bylund, 1995).

The process of homogenisation dramatically increases the level of protein in the membrane compared with the natural membrane before homogenisation (2.3 g protein per 100 g fat, up from 0.5–0.8 g protein per 100 g fat). It also increases the fat surface area by approximately three- to six-fold (Burgess *et al.*, 1991; Walstra, 1995).

The changes that occur in milk due to homogenisation include: reduced creaming due to reduced surface tension and smaller globule size, increased susceptibility to hydrolytic rancidity (unless pasteurised immediately, due to lipoprotein lipase which is associated with the casein micelle being incorporated into the MFGM), increased susceptibility to sunlight oxidised flavours, reduced susceptibility to metal-catalysed

lipid oxidation, a whiter colour, a reduction in heat stability (more so after single-stage homogenisation), reduced curd strength upon renneting and reduced renneting time, increased viscosity, improved foaming characteristics, improved mouthfeel and flavour, and improved physical stability of cultured milk products (Bylund, 1995; Tamime and Robinson, 2007; Fox, 2011). The reduction in fat globule size also aids in digestibility and reduces free fat levels in milk powders produced from homogenised milk (Písecký *et al.*, 1997).

High pressure homogenisation (HPH) is emerging as an alternative to conventional pressure two-stage homogenisation (Hayes and Kelly, 2003). High pressure homogenisers operate under the same design, but at a much higher pressure than the conventional homogenisers (Datta *et al.*, 2005). High pressure homogenisers work in the range 100–400 MPa, the higher end of this range (above 300 MPa) being referred to as ultra-high pressure homogenisation (UHPH) (Dumay *et al.*, 2013; Georget *et al.*, 2014). In addition to its conventional use to reduce the fat globule size distribution, high pressure homogenisers can also be used in food preservation to inactivate endospores and microorganisms in various food systems (Popper and Knorr, 1990; Diels and Michiels, 2006; Georget *et al.*, 2014). HPH can thus be used as a hurdle to reduce total bacterial counts in order to minimise the undesirable effects of more severe heat treatments. Gram-positive bacteria are less susceptible to inactivation by HPH than gram-negative bacteria, due to the protection they receive from their cell wall (Fuquay *et al.*, 2011). The susceptibility of the bacteria to HPH is dependent on the viscosity of the solution being treated. Plasmin and lipoprotein lipase activity are stable to homogenisation, but their activity can be reduced at higher homogenisation pressures (>200 MPa) combined with higher inlet temperatures (Fuquay *et al.*, 2011). It is possible to achieve sterilisation of whole milk using UHPH at 300 MPa at a much

lower temperature of between 75–85°C, compared with typical UHT treatment temperatures of 135–141°C (Amador-Espejo *et al.*, 2014). High-pressure homogenisation prolongs shelf-life while maintaining the "fresh-like" quality of products (Suárez-Jacobo *et al.*, 2009; Chawla *et al.*, 2011; Patrignani and Lanciotti, 2016). In milk, high pressure homogenisation has been shown to enhance rennet coagulation properties, decrease total bacterial counts, increase total product stability, reduce fat globule size distribution (Figure 1.7), increase the retention of hexanal (a secondary product of lipid oxidation which is also used as a biomarker for oxidative stress during storage), and prevent coalescence of fat globules post-processing (Hayes and Kelly, 2003; Chawla *et al.*, 2011; Donsì *et al.*, 2012; Asaduzzaman *et al.*, 2017; Martínez-Monteagudo *et al.*, 2017). The reduction in fat globule size can increase the occurrence of lipid oxidation in the system, due to the inadequate coverage of the increased surface area of the fat globules, which would negatively impact the sensory profile (Fuquay *et al.*, 2011).

HPH also affects the protein in milk, although to a lesser degree to its influence on fat globules (Fuquay *et al.*, 2011). HPH denatures whey proteins, with β-lactoglobulin being more susceptible than α-lactalbumin (approximately 45% denaturation versus approximately 35% denaturation) and also affects the turbidity of milk by disrupting the casein micelles on the order of approximately 10-15% (Needs *et al.*, 2000; Scollard *et al.*, 2000; Huppertz *et al.*, 2004; Datta *et al.*, 2005).

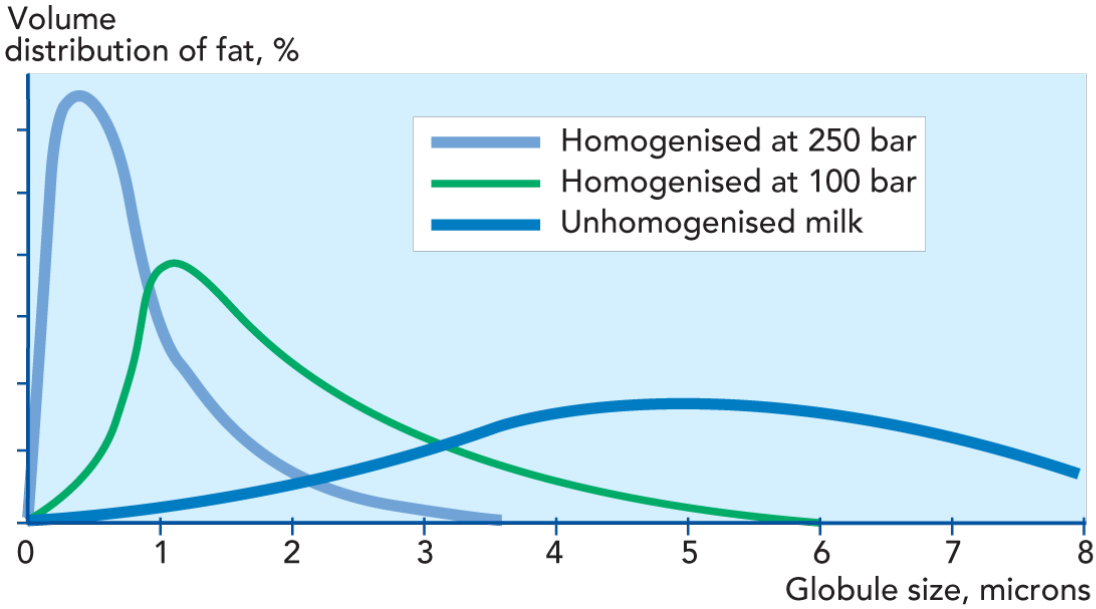


Figure 1.7: Influence of high-pressure homogenisation on fat globule size distribution, in comparison with unhomogenised milk and conventional milk homogenisation (Bylund, 1995).

Alternatives to high-pressure valve homogenisers include, but are not limited to: high speed mixers; ultrasonics; colloid mills; membrane emulsification; and microfluidisers (Joscelyne and Trägårdh, 2000; Villamiel and de Jong, 2000; Olson *et al.*, 2004; Vladisavljević *et al.*, 2004; Perrier-Cornet *et al.*, 2005; Leong *et al.*, 2009; Ashokkumar *et al.*, 2010; Fuquay *et al.*, 2011; Lee and Norton, 2013; O'Sullivan *et al.*, 2014).

Heat treatment generally follows immediately after homogenisation, to reduce action of lipoprotein lipase on the new fat globules which have an increased surface area and may not yet be fully enclosed by a new membrane. Lipase is heat-labile and so is largely inactivated by pasteurisation (Wilbey, 2003).

1.2.2 Heat treatment of milk

The most important reasons why milk undergoes heat treatment are to ensure the safety of the product for general consumption by destroying pathogenic bacteria, to increase the keeping quality by inactivation of spoilage bacteria, and to inactivate indigenous or endogenous enzymes, as well as establishing specific or desired product properties such as denaturing the whey proteins to achieve a satisfactory consistency for yoghurt (Morand *et al.*, 2011; Chevallier *et al.*, 2016; Mahomud *et al.*, 2017; Singh *et al.*, 2019). Milk is an incredibly rich source of essential food constituents, as previously mentioned; as a result, however, it is a good growth medium for many bacteria, which can be either spoilage and/or pathogenic in nature (Sun, 2006; Chavan *et al.*, 2011).

Table 1.3: Summary of variety of typical heat treatments of milk (Ashton and Romney, 1981; Huijs *et al.*, 2004; Lewis and Deeth, 2009; Melini *et al.*, 2017).

Heat treatment	Aim of heat treatment	Typical time-temperature combination	Storage	Shelf life
Thermisation	Extend keeping quality of raw milk before further heat treatment	57–68°C x 15-20 s	Refrigerated	3 days
Pasteurisation (HTST)	Reduce number of any harmful microorganisms to a level which they do not constitute a significant health hazard, with minimal chemical, physical, and sensory changes	72°C x 15 s (continuous flow) 63°C x 30 min (batch pasteurisation)	Refrigerated	3–21 days based on raw milk quality
Sterilisation	Achieve 12 decimal reductions of *Cl. Botulinum* to achieve 'commercial sterility'	110–116°C x 20–30 min (batch sterilisation)	Room temperature	8–12 months
		132–140°C x 12 min (continuous retort sterilisation)		
		135-150°C x 1-4 s (ultra-high temperature)		3–12 months
		150-200°C x 0.1 s (innovative steam injection)		

Many chemical and physical changes occur in milk when heat treated, such as a mutarotation of lactose, decrease in pH (both of which are reversible), loss of gases,

protein denaturation, enzyme inactivation, colour change (Maillard reaction), viscosity increase, vitamin degradation, and many more. These changes become more pronounced at increased heat loads (Figure 1.8). Above 80°C, free sulfhydryl groups are created on the milk fat globule surface, which protect the fat from oxidation, essentially acting as antioxidants (Písecký *et al.*, 1997). It is important during milk processing to avoid undesirable changes of sensory and physicochemical properties, as well as preserving the nutritional quality (Popov-Raljić *et al.*, 2008).

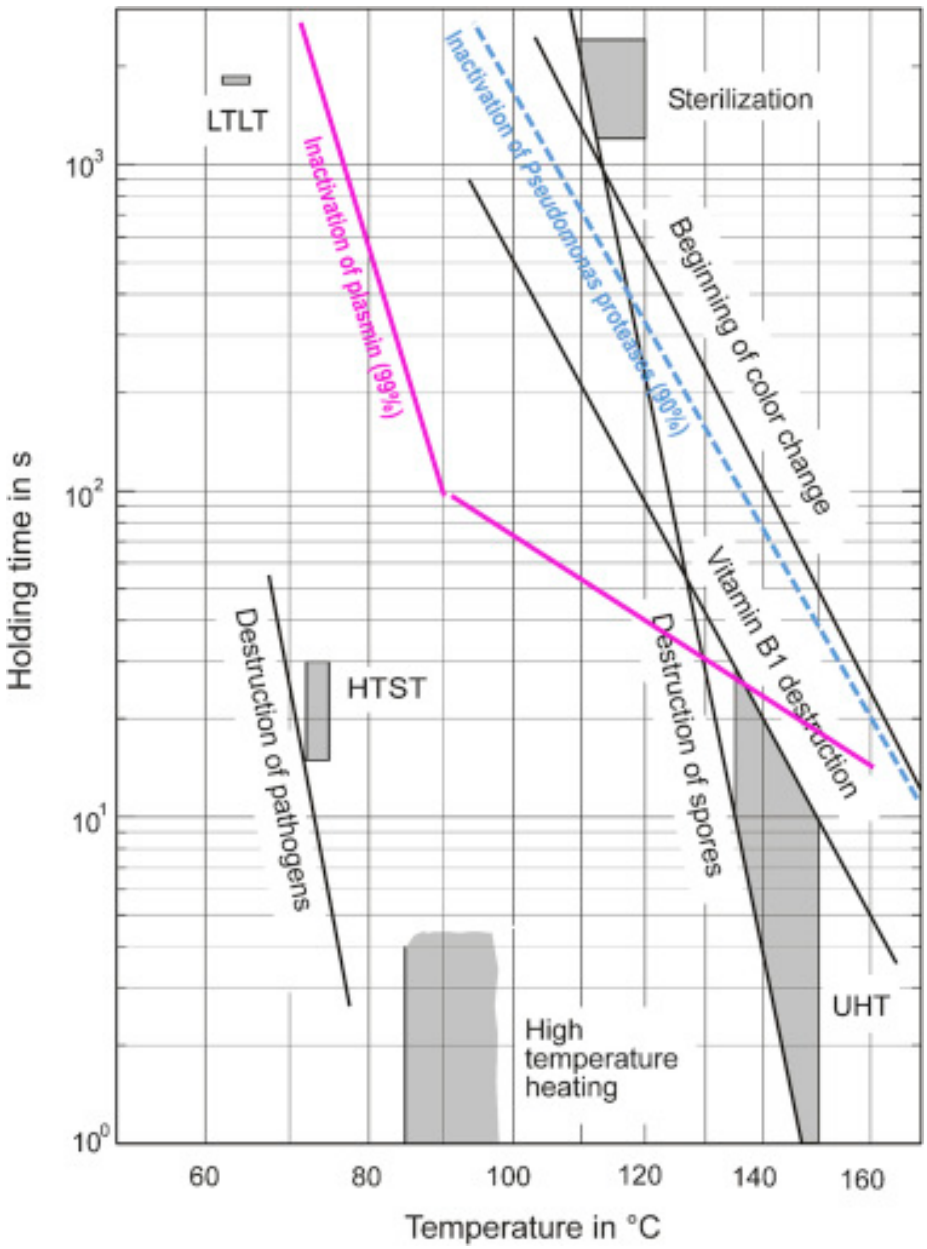


Figure 1.8: Comparison of different heat treatments (Stoeckel *et al.*, 2016b).

The aim of sterilisation is to not only destroy the thermo-sensitive bacteria present in the raw material, but also destroy the thermo-resistant spores which are produced by the bacteria (Bylund, 1995). According to Burton and Pien (1969), sterilised milk can be defined as milk that must "keep without deterioration… be free of micro-organisms harmful to consumer health" and "be free of any microorganisms whatsoever liable to proliferate". Commercial sterilisation of milk cannot guarantee absolute sterility, as the milk is not actually sterile in the microbiological sense after processing (Hinrichs and Atamer, 2011).

The sterilising effect is the concept used in commercial sterilisation processes to determine the efficiency of the system by stating the number of decimal reductions of bacterial spores achieved by the process (Bylund, 1995). The sterilising effect depends on the time-temperature combination used in the process, the product that is being processed, and the heat resistance of the spores (Datta *et al.*, 2002). Heat transfer is important here, as direct and indirect heat systems use different heat transfer mechanisms: the heating medium in direct systems comes into direct contact with the milk, so the heat is transferred almost instantaneously through convection. In indirect systems, the heat is transferred more slowly by conduction through the impermeable barrier, and then convection through the milk (Hsu, 1970; Schroyer, 1997; Lewis and Heppell, 2000; Kelleher *et al.*, 2019).

To determine the sterilising effect of a UHT process, endospores of *Bacillus subtilis* and *Bacillus stearothermophilus* are used as markers due to their significant heat resistance, whereas *Clostridium botulinum* is used in retort sterilisation (Datta *et al.*, 2002). *Bacillus* and *Clostridium* genera are the most common spore-forming spoilage organisms found in milk (Ravishankar and Maks, 2007). Lactulose is used as an index of heat treatment as it is stable during storage and is only affected by heat treatment

(Elliott *et al.*, 2003). Both UHT processing and retort sterilisation produce a product which can be stored at ambient temperature. During commercial sterilisation, enzymes present in the milk are more or less completely inactivated (Hinrichs and Atamer, 2011).

Sterilisation uses reference values to compare processes and temperatures loads. Such reference values include:

- the decimal reduction time, or the *D* value, which is the time required at a specific temperature to reduce the bacterial population in the sample by 1 log cycle (90% reduction) (Tewari and Juneja, 2008; Hinrichs and Atamer, 2011);
- *F* value, based on the *D* value, is defined as the time (in minutes) required to reduce the number of specific microorganisms by a multiple of the *D* value. The *F* value is used as a measurement of the effectiveness of sterilisation and can be used to compare heat sterilisation processes (Hinrichs and Atamer, 2011). Commercially sterile milk requires an F_0 of at least 5 or 6 (Chavan *et al.*, 2011). F_0 can be calculated using the following formula:

$$F_0 = 10^{\frac{T-121.1°C}{z}}.t$$

  Where T is the sterilisation temperature (°C) and t is the sterilisation time (minutes) (Datta *et al.*, 2002; Chavan *et al.*, 2011), and the *z*-value is explained below. Most processes base their calculations on the reference value of F_0 of *Cl. botulinum.* For example, to achieve an F_0 of 1, the product will need to be heated at 121.1°C for 1 min;

- *z*-value, this is the change in temperature which corresponds to a ten-fold increase or decrease in the D value (Datta *et al.*, 2002).

However, the *D* and *z* values have their limits, as microbial destruction does not follow first-order kinetics, i.e., it has a non-linear relationship with temperature (Hinrichs and Atamer, 2011).

The bacteriological (B*) and chemical (C*) effects can also be used to describe the effective working range of UHT processing (Chavan *et al.*, 2011). A process with a B* of 1 means that the process sufficiently reduces the thermophilic spores by 9-log cycles, which is equivalent to processing the milk at 135°C for 10.1 seconds, using a corresponding z value of 10.5°C. (Datta *et al.*, 2002). The C* value relates to the destruction of thiamine (vitamin B_1); a C* value of 1 indicates a 3% loss of thiamine. Thus, a UHT processing plant which sufficiently reduces the spore count with minimal chemical (and thus nutritional and organoleptic) damage has a B* of at least 1 and a C* of no greater than 1 (Bylund, 1995; Datta *et al.*, 2002).

1.2.3 UHT processing

Ultra-high temperature (UHT) treatment occurs between 135-150°C with a corresponding time of 2–20 s followed by cooling to a suitable filling temperature and aseptic packaging (Figure 1.9) (Chavan *et al.*, 2011; Hinrichs and Atamer, 2011). This process by design ensures microbial sterility without the overproduction of excessive "cooked flavour" usually associated with a product which has undergone UHT treatment (Newstead *et al.*, 2006). UHT-treated products are regarded as being "commercially sterile", which means that the number of surviving microorganisms post-heat treatment or any post-processing contaminants is negligible so that the product poses no threat to the health of the consumer (Fox and McSweeney, 2003). If

UHT-treated milk is packed under aseptic conditions, this should in theory ensure that microbial spoilage will not occur for a long time (Anema, 2019).

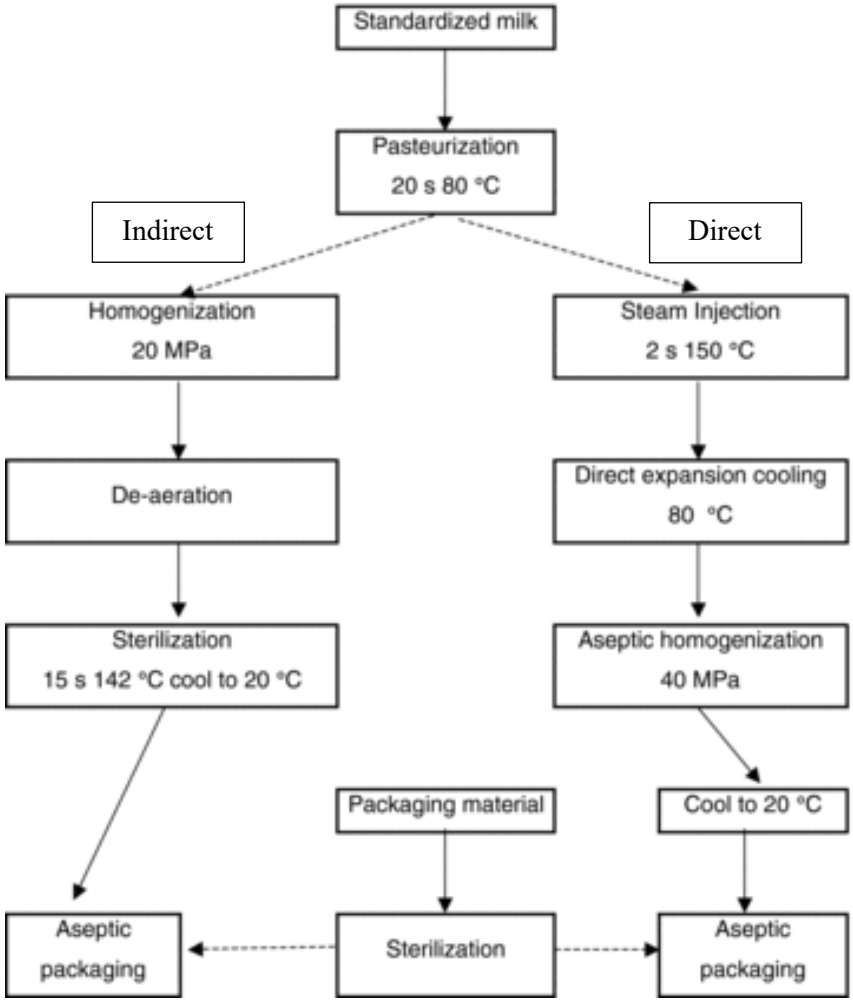


Figure 1.9: Typical steps in UHT processing of milk, both indirect (no contact between heating medium and milk) and direct (heating medium is introduced directly into the milk) (Chavan *et al.*, 2011).

In comparison to products treated with more traditional sterilisation processes such as retort sterilisation, UHT-treated products have a reduced loss of nutritionally significant compounds such as vitamin B1 (thiamine), and lysine. This is due to the reduced exposure of the product to high temperatures in UHT treatment (Hinrichs and Atamer, 2011). Also, it is overall a much faster process (it is continuous, whereas retort

sterilisation is most commonly a batch process) which requires less space and less labour than retort sterilisation (Bylund, 1995).

It is possible to increase the efficiency of the UHT treatment plant by using regenerative heating for the initial heating and final cooling of the milk. While this is quite economical, this design causes the most chemical change, due to the higher residence times at the increased temperatures (Burton and Perkin, 1970).

Products subjected to UHT treatment can either come directly in contact with the heating element (steam), or be indirectly heated, which differ in heat transfer mechanism (Kelleher *et al.*, 2019). Indirect systems use heat exchangers, and direct UHT processing systems use steam injection or infusion, with vacuum-flash cooling after the short holding time (Newstead *et al.*, 2006). Originally, UHT processing used direct steam injection, which was followed by indirect heating about a decade later (Bylund, 1995). Direct systems most commonly use a steam injection system (steam into milk) or steam infusion (milk into steam). Steam injection systems can be further broken down into lenient steam injection, or supersonic steam injection (Murphy *et al.*, 2011; Dickow *et al.*, 2012). As the steam comes into direct contact with the milk, it must be of the highest quality and not impart any off-flavours (Chavan *et al.*, 2011).

Indirect systems use heat exchangers to achieve commercial sterility. Such heat exchangers include but are not limited to: plate heat exchangers for lower viscosity products (which are also found in HTST pasteurisation), tubular heat exchangers for mid-range viscosity products, scraped-surface heat exchangers for higher viscosity products, indirect spiral tubes, and friction heaters (Bylund, 1995; Burton, 1988). As the name suggests, there is no contact between the heating medium and the product in indirect systems, as a physical barrier such as stainless steel is present (Burton, 1988).

In direct UHT systems, steam injection results in a significantly faster heat-up (latent heats of condensation and vaporisation) and cooling rate in comparison to the indirect methods, resulting in a large reduction in thermally-induced changes compared with indirect heating methods, such as the amount of denatured β-lactoglobulin produced, which is partly responsible for the 'cooked' off-flavour often associated with UHT-treated products (Zabbia *et al.*, 2012; Jo *et al.*, 2018; Kelleher *et al.*, 2018). Direct UHT processing systems require more energy in comparison to indirect heating, but direct UHT processing results in reduced flavour changes (Elliott *et al.*, 2003; Chavan *et al.*, 2011). All UHT treatment plants process the product under pressure to allow it to reach the above-boiling point temperatures required while remaining as a liquid.

1.2.3.1 Whey protein-casein interactions

Whey proteins (including but not limited to: β-lactoglobulin, α-lactalbumin, immunoglobulins, and bovine serum albumin (BSA)) account for approximately 20% of the total protein in milk, have a high biological value (Fox, 2003; Singh *et al.*, 2019) and, according to Haraguchi *et al.* (2006) and Smithers (2008), are a rich source of essential amino acids (i.e., amino acids which humans do not naturally produce in the body), including branched amino acids and sulphur amino acids.

When milk is UHT-treated, whey protein denaturation occurs due to their low heat stability; they are denatured at temperatures exceeding 70°C, which exposes the free thiol group (-SH) of β-lactoglobulin (β-Lg) (Ashokkumar *et al.*, 2009; Mahomud *et al.*, 2017; Chevallier *et al.*, 2018). This denaturation can result in fouling of the plant, reduced run-times, and decreased heat-transfer, as well as influencing the properties and the stability of heated milk (Fickak *et al.*, 2011; Gaspard *et al.*, 2017; Singh *et al.*,

2019). The denatured whey proteins interact with themselves, with fat globules, and with κ-casein molecules, which are located on the surface of the casein micelle, through disulphide bonds and hydrophobic interactions (Figure 1.10) (Guyomarc'h *et al.*, 2009; Chavan *et al.*, 2011; Morand *et al.*, 2011; Gaspard *et al.*, 2017; Mahomud *et al.*, 2017; Anema, 2018). Chevallier *et al.* (2016) reported that when denatured whey protein-casein aggregates interacted with the fat globule surface, they caused rapid flocculation and coalescence of the fat globules which resulted in oiling off on the surface of the sample.

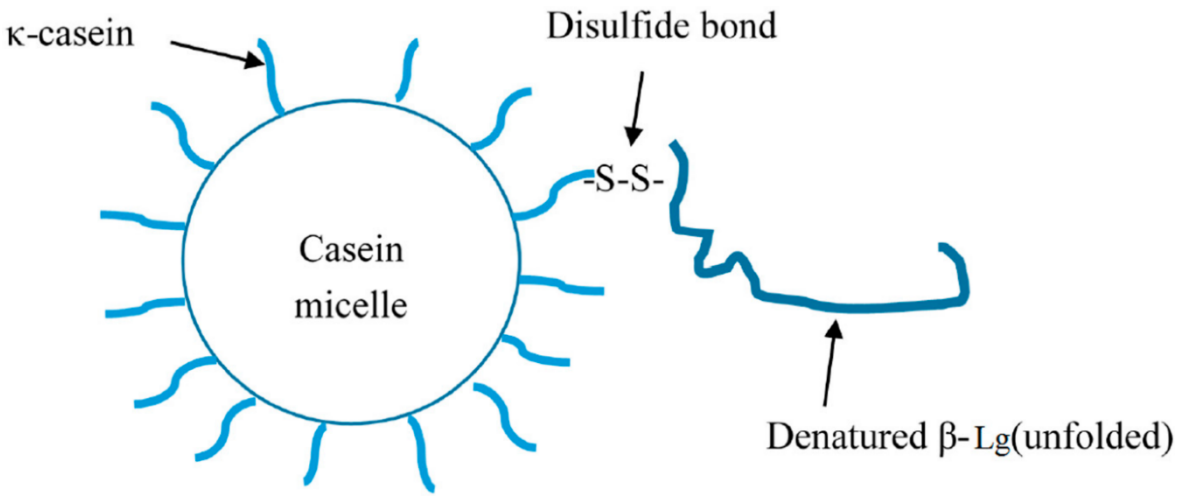


Figure 1.10: interaction of denatured β-lactoglobulin with κ-casein on the surface of the micelle (Guo and Wang, 2016).

The distribution (between the soluble phase and the micelle) and composition of this heat-induced complex depends on a variety of factors: temperature, milk pH, milk composition (total solids content and protein concentration in particular), the presence of salts, and concentration of accessible thiol groups (Mahomud *et al.*, 2017). One of the most prominent factors is the pH of the milk (Pesic *et al.*, 2014; Anema, 2018). At

acidic pH values, β-Lg forms a disulphide bond with the κ-casein (κ-CN) to complex at the surface of the micelle. In more alkaline conditions, however, these complexes are concentrated in the soluble phase, as the κ-CN dissociates from the micelle (Anema and Li, 2003; Anema *et al.*, 2014; Mahomud *et al.*, 2017).

The formation of whey protein-casein complexes can be beneficial, such as in the manufacture of yoghurt, as the formation of an acid-induced gel is improved by these complexes (Guyomarc'h *et al.*, 2009; Anema, 2018). Morand *et al.* (2011) stated that the whey protein-casein complexes improve acid skim milk gels by immobilising the water in the system, enhancing connectivity between casein clusters, and aiding the interactions required for gel formation such as hydrophobic interactions, covalent bonds, and electrostatic interactions.

Whey protein-casein complex formation is also beneficial as it enhances the heat stability of the system, compared with whey protein aggregates, which is due to the chaperone activity (Guyomarc'h *et al.*, 2009; Gaspard *et al.*, 2017). Chaperone molecules stabilise proteins and prevent them from precipitation, aggregation and unfolding (Morgan *et al.*, 2005). The mechanism behind this chaperone activity is still not yet understood, and research is ongoing (Liyanaarachchi *et al.*, 2015; Gaspard *et al.*, 2017). κ-casein, α_{s1}-, and β-casein can reduce the propensity of the whey proteins to form larger aggregates when denatured (Zhang *et al.*, 2005; O'Kennedy and Mounsey, 2006). According to Guyomarc'h *et al.* (2009), the ability of κ-casein to inhibit the increase in size of the whey-casein complexes is somewhat similar to its role in the stabilisation of the casein micelle. In model systems, casein has been observed to protect whey proteins from heat-induced denaturation and its activity can be compared to the chaperone activity of the small heat shock protein (sHsp) in bovine milk (Guyomarc'h *et al.*, 2003; Morgan *et al.*, 2005; Singh *et al.*, 2019). Heat shock

proteins are proteins which are expressed when homeotherms (mammals and birds) are subject to thermal stress. They confer thermotolerance by reversing protein folding (denaturation) which occurs due to heat treatment (Mishra and Palai, 2014; Archana *et al.*, 2017).

1.2.3.2 Age gelation

During storage, undesirable chemical and physical reactions occur which result in age gelation, excessive sedimentation, and creaming (Anema, 2019). Cow health (such as the occurrence of mastitis), the storage history of milk and hygiene management all influence the storage stability of UHT-treated milk (Zhang *et al.*, 2018). Even though UHT-treated milk is commercially sterile, it should still be stored at relatively low temperatures, because lactolysation and Maillard reactions proceed more rapidly at increased temperatures (Anema, 2019). Age gelation of UHT-treated dairy products is a well-documented area (Datta and Deeth, 2001; Datta *et al.*, 2002; Fox and McSweeney, 2003; Prado *et al.*, 2006; Anema, 2017; Zhang *et al.*, 2018; Anema, 2019), which is caused by at least two mechanisms. It occurs when a three-dimensional network forms usually after several months of storage as a result of proteolysis, which results in milk losing its fluidity. The gel tends to start forming from the bottom of the product before extending through the pack. It is an irreversible process, and so is a shelf-life determining process. According to Anema (2019), there are no known early indicators for the onset of age gelation, either chemical or physical. However, it has been reported that there is an increase in both the particle size and the viscosity of a solution a few weeks before it gels. Anema (2019) also suggested an inverse correlation between heat energy applied and the susceptibility of the product to gelation. UHT-treated products receive less heat energy than those treated by retort

sterilisation, but they gel more frequently. Direct UHT-treated products also gel faster than indirect UHT-treated products (Manji *et al.*, 1986; Datta *et al.*, 2002). McKellar *et al.* (1984) reported that milk samples from direct UHT treatment gelled within 10 weeks when stored at 20°C, while samples treated by indirect UHT treatment gelled after 30 weeks of storage at the same temperature. Storage temperature is also important in age gelation; research has shown that increasing storage temperature (i.e., above 30°C) reduces the susceptibility of UHT-treated milk to gelation (Manji *et al.*, 1986).

Age gelation falls into two categories: non-enzymatic (physico-chemical) and enzymatic. Non-enzymatic gelation occurs beyond the shelf life of UHT-treated products which have a normal total solids concentration of protein content. Physicochemical age gelation is generally much slower than enzymatic age gelation when milk is at its natural concentration (Anema, 2019). It usually occurs after 12 months, which is longer than the usual shelf life of UHT-treated milk. It has been suggested that seasonality plays a role in physico-chemical age gelation (Graf and Bauer, 1976; Auldist *et al.*, 1996; Datta and Deeth, 2001). Several non-enzymatic gelation mechanisms have been proposed, but none have yet been universally accepted. McMahon (1996) proposed that the interaction of β-casein with κ-casein during UHT treatment forms a β-κ complex, which then dissociates from the micelle due to the breakdown of the κ-casein anchor sites. The dissociated complexes then aggregate together to form a three-dimensional gel network. Another mechanism has been more recently proposed by Anema (2017), which suggests that the casein micelles are depleted of the steric stabilisation layer of κ-casein due to UHT treatment and sediment to the bottom of the container. Once a critical concentration of this material has been reached, inter-micellar cross-linking occurs and results in age

gelation. No protein degradation occurs in physicochemical age gelation (Datta and Deeth, 2001; Nieuwenhuijse, 2003).

Enzymatic gelation is a result of heat-stable proteinases, which originate either from the plasmin system (indigenous), or psychrotrophic bacteria (endogenous) (Zhang *et al.*, 2018). These proteinases break down the protein present in the product into peptides, which destabilises the system and results in gelation.

Plasmin (EC 3.4.21.7) is an enzyme secreted from the blood, with an optimum pH of 7.5 and readily hydrolyses the caseins in the order of preference: β-casein > α_{s1}-casein >> α_{s2}-casein (Fox and McSweeney, 1996). While its activity can be beneficial to the final product (which is the case in cheese ripening), it can cause problems during storage of UHT-processed product and milk clotting (Chavan *et al.*, 2011).

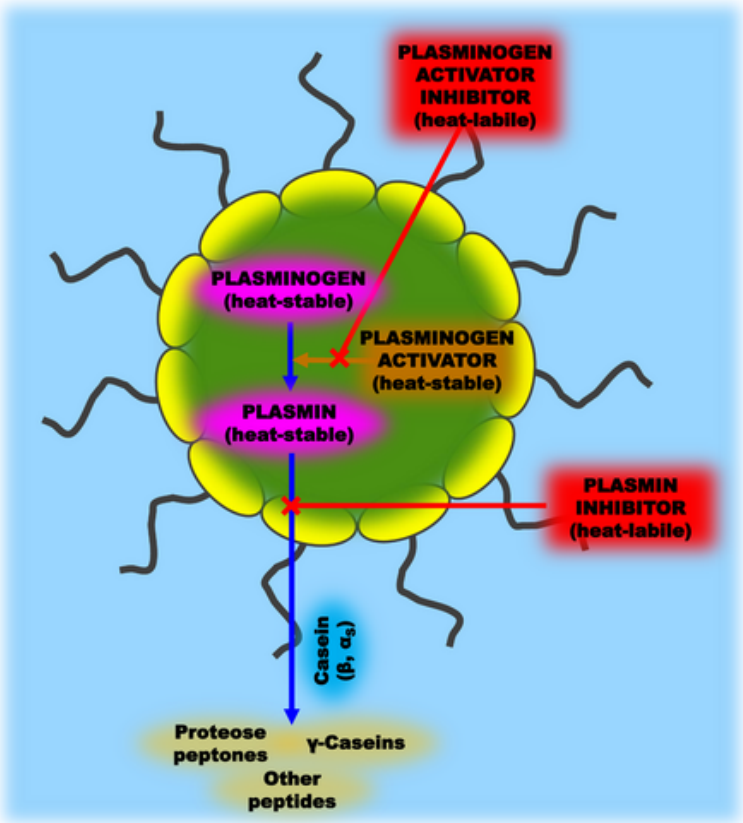


Figure 1.11: Summary of the plasmin system found in bovine milk (Anema, 2019).

Plasmin is a part of a complex system (Figure 1.11), the components of which are of varying susceptibility to heat treatment (Anema, 2019). In milk, plasmin activity is regulated by the interaction between plasminogen, which is the inactive zymogen of plasmin, plasminogen activators, and plasminogen activator inhibitors (Bastian and Brown, 1996). Plasmin, plasminogen, and plasminogen activator are all associated with the casein micelles, whereas the plasminogen activator inhibitor and plasmin inhibitor are located in the serum phase of milk (Grufferty and Fox, 1986; White *et al.*, 1995). Plasmin and plasminogen activators are heat-stable and can even survive temperatures used during UHT processing, whereas their inhibitors are more heat-labile (Alichanidis *et al.*, 1986; Lu and Nielsen, 1993; Prado *et al.*, 2006). It is possible for plasmin to be inactivated during heat treatment, resulting in minimal activity after processing, but plasminogen can then be converted to plasmin during storage, resulting in an increase in plasmin activity. The reduced activity of plasmin after UHT processing is partly due to the thermal denaturation of β-lactoglobulin, which exposes its reactive free sulphydryl (-SH) group, which then interacts with the disulphide groups that link structural subunits in the plasmin molecule (Korycha-Dahl *et al.*, 1983; Rollema and Poll, 1986). If β-lactoglobulin is acting as a plasmin inhibitor, the degree of its denaturation must not be high enough to form deposits (which may lead to plant fouling) or product degradation (Chavan *et al.*, 2011).

Indirect UHT treatment and retort sterilisation inactivate the entire plasmin system more thoroughly than direct UHT treatment due to the higher heat load experienced (Anema, 2019). Products treated using direct UHT process (and indeed HTST pasteurisation) experience lower heat loads than UHT processing and retorting, and thus have been shown to contain higher levels of proteolysis products after storage, due to the survival of plasmin and plasminogen. Newstead *et al.* (2006) determined

that the optimum conditions to reduce proteolysis due to plasmin activity is to preheat the milk at 90°C for 30 or 60 s. They also observed increased proteolysis due to plasmin activity when the preheating occurred between 80 and 90°C.

However, even with all this research, there is still doubt that plasmin causes age gelation. Zhang *et al.* (2018) proposed a four-step process by which plasmin destabilises milk and causes gelation. The first phase (penetrating phase) involves plasmin hydrolysing the most susceptible protein, β-casein, followed by α_{s1}-casein and, then α_{s2}-casein. The plasmin then enters the micelle, hydrolysing sequences which are integral to the micelle's structure. The second phase is labelled the loosening phase, which refers to the micelles increasing in size due to the loosening of its structure as a result of plasmin hydrolysing regions which are integral to the micelle structure. The third phase is the disassembling, rearrangement and aggregation phase, where the peptides and micelle fragments begin to associate and form a visible, but dispersed, gel. The solution becomes more polydisperse during this phase. The last phase is the clarification phase; in this phase, the gel and the remaining micellar structure are further hydrolysed until the solution becomes clear.

Enzymatic gelation of milk can occur due to the presence of endogenous enzymes produced by psychrotrophic bacteria. An example of this is a particular alkaline metalloprotease, belonging to the AprX family of enzymes, which has been discovered in the *Pseudomonas* species (Vithanage *et al.*, 2014; Stoeckel *et al.*, 2016b; Zhang *et al.*, 2019). As *Pseudomonas* bacteria are psychrotrophic, refrigerated storage before heat treatment enables them to proliferate (Stoeckel *et al.*, 2016a). AprX production occurs slowly at refrigerated temperatures, but dramatically increases at unrefrigerated temperatures, whereas AprX activity increases with increasing temperature, reaching is maximum between 17.5 and 20°C (Haryani *et al.*, 2003; Zhang *et al.*, 2015; Zhang

et al., 2019). It can be used as an indirect measure of the thermal storage history of the milk and hygiene management of the farm (Vithanage, 2017; Martin *et al.*, 2018). It is important to be able to rapidly detect the presence of *Pseudomonas* in raw milk as it is often responsible for milk spoilage, and the aprX gene has been proposed as a marker for the contamination of milk using polymerase chain reaction (PCR), reverse-phase high performance liquid chromatography (RP-HPLC) and (ELISA), though these have yet to be implemented (Ahn *et al.*, 1999; Dufour *et al.*, 2008; Zhang *et al.*, 2019).

1.2.3.3 Sedimentation

Sedimentation is another form of physical instability which occurs in UHT-treated products. Protein-rich material naturally accumulates as a layer at the bottom of the pack soon after processing and packaging (Gaur *et al.*, 2018; Anema, 2019). Unlike age gelation, sedimentation does not spread through the product from where it forms at the bottom of the pack, and it occurs within the first few weeks of storage, whereas age gelation does not usually occur for several months post-production (Anema, 2019). Most UHT-treated products form a small layer of sedimentation during their storage; however, it is not considered a defect unless the layer becomes excessive and forms relatively quickly after processing (Anema, 2019). Although the mechanisms for sedimentation formation are not particularly well understood, the bulk composition of the sediment formed in UHT-treated milk has been recorded (Rattray *et al.*, 1997; Gaur *et al.*, 2018). The rate of sedimentation can be estimated through Stokes' Law.

Recently, Grewal *et al.* (2017) demonstrated the possibility of predicting the propensity of a UHT-treated product to form a sediment by using attenuated total

reflectance-Fourier transform infrared (FTIR) spectroscopy and accelerated shelf-life testing. Limited research has been carried out to determine the mechanism occurring during sedimentation. Lewis *et al.* (2011) demonstrated the importance of ionic calcium and pH for the amount of sedimentation in UHT-processed milk. Gaur *et al.* (2018) analysed the composition of sediment from UHT-processed milk, which was composed of casein micelles that were depleted of κ-casein, and minimal whey protein. They also proposed a mechanism for sedimentation in UHT-processed milk; UHT treatment causes κ-casein, which form the steric stabilisation layer of the casein micelle, to dissociate from the micelles. If the milk pH is low enough, or if the ionic calcium level is high enough, the micelles will aggregate *via* calcium bridging and then sediment. According to Gaur *et al.* (2018), it is not possible to eliminate the dissociation of κ-casein entirely, but the resulting sedimentation can be reduced with an increase in pH and reducing ionic calcium, by adding calcium chelators, as ionic calcium is a very important determinant of the stability of the casein micelle (Omoarukhe *et al.*, 2010).

Analysis and testing for sedimentation involves using centrifugal forces to accelerate the separation, as predicted by Stokes' Law. This is beneficial when testing the storage stability of new products; however, it can result in a compact sediment which is not representative of what would happen over a longer timeframe, such as during natural sedimentation due to gravity (Anema, 2019). Also, particles increase in size during storage, and sedimentation due to centrifugation occurs based on the particle size at that time point.

Chalkiness is a defect commonly associated with steam-injected UHT-processed milk and is considered as an early indicator of sedimentation (Anema, 2019). Burton and Perkin (1970) reported that the placement of homogenisation during direct UHT

treatment is integral in this defect. Upstream homogenisation resulted in casein particles greater than 1 μm in size that imparted a chalky-mealy texture, which did not occur in samples which had been processed with downstream homogenisation, the theory being that the downstream homogenisation breaks up the larger casein particles formed when steam-injection occurs.

1.2.3.4 Creaming

The third type of instability observed in UHT-treated products, usually fat-containing products, is creaming. This occurs when a fat-rich layer separates from the main solution and accumulates at the top of the pack (Anema, 2019). It is a more pertinent problem in UHT-processed milk than fresh HTST pasteurised milk due to the longer storage time and increased storage temperatures. It is not considered a major defect, as the cream layer can be easily redispersed by gentle agitation. In more unusual cases, a fat plug may form at the top of the pack, or fat may accumulate in lumps which are visible during pouring. These defects are mainly a result of inadequate homogenisation (Datta *et al.*, 2002).

Like sedimentation, the factors affecting creaming can also be summarised by Stokes' Law. Larger particles and larger density differences will increase the rate of sedimentation (Anema, 2019). This process can also be sped up using centrifugation; however, using centrifugation can result in an overestimation of creaming due to temporary clustering and Brownian motion (Walstra and Oortwijn, 1975). Creaming can be controlled using homogenisation (Anema, 2019), as it disrupts the fat globules to form smaller particles of under 1 μm in size. Casein then adsorbs to the surface of the fat globule to act as an emulsifier, which increases the density of the globule. This increase in density, together with the reduced particle size, reduces the propensity of the fat to cream. Elevated storage temperatures increase the rate of fat separation (Anema, 2019).

1.2.4 Retort sterilisation

Sterilisation is a very high heat process which is designed to kill all microorganisms and bacterial spores (Walstra, 1999). In retort sterilisation, the process is designed to result in a 10-log reduction in bacteria in the milk. It is a much slower process than UHT processing; with an effective treatment time ranging between 10 and 40 min, and a temperature range of 105-120°C (Hinrichs and Atamer, 2011). More changes occur to the product during retort sterilisation compared to UHT processing, also due to the length of holding at the high temperature. These changes include Maillard browning, discolouration, development of a cooked flavour, and sometimes a sediment may also form. Thus, UHT treatment is often favourable over retort sterilisation, as there is also minimal destruction of the amino acids and vitamins present in the milk (Fox and McSweeney, 2003). However, it is still used for canned goods such as condensed milk or tinned beans etc. (Bylund, 1995). Like UHT treatment, retort sterilisation conditions largely inactivate all the components of the plasmin system (Anema, 2019).

UHT treatment and retort sterilisation have the same sterilising effect (Bylund, 1995). Both in-container batch sterilisation and continuous-flow in-container sterilisation systems, such as hydrostatic retorts, are used in the food industry (Chen *et al.*, 2008).

The milk for in-container batch sterilisation is pre-packed into its containers and is then placed into a carriage or racks in the autoclave, which is the chamber in which the sterilisation will occur, and secured (Figure 1.12). In-container-sterilised products are generally packaged in metal cans, or glass jars, as they must be able to withstand the high temperatures experienced during retort sterilisation (Hinrichs and Atamer, 2011; Takeda *et al.*, 2015). The autoclave has an increased internal pressure compared with atmospheric pressures, which allows the system to heat to the required sterilisation temperatures, which are above 100°C.

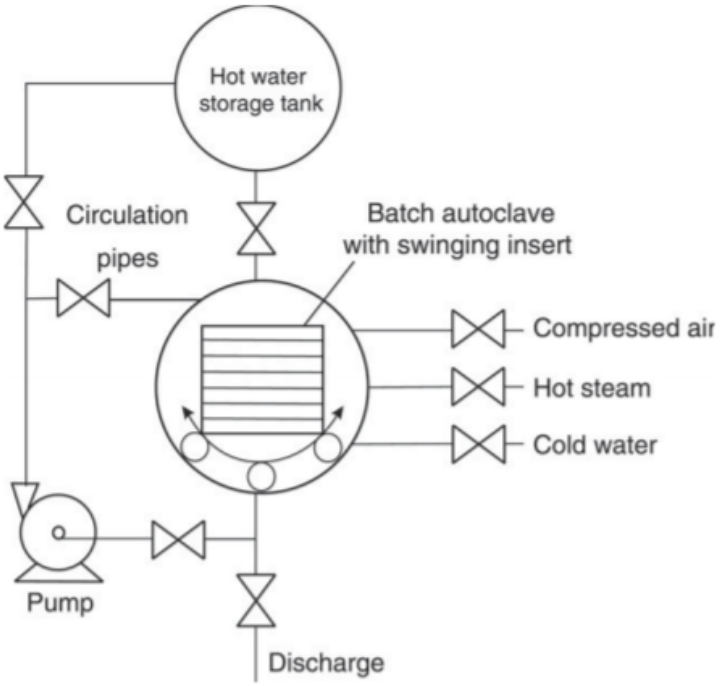


Figure 1.12: Schematic representation of a batch retort steriliser, with swinging insert (Kessler, 1996).

The chamber is then filled with hot water or condensed saturated steam, which is what heats the containers, and thus the milk (Hinrichs and Atamer, 2011). Many autoclaves allow the carriage or racks containing the product to swing or fully rotate, which improves the heat transfer (Bylund, 1995). The batch process requires a heat-up time and cooling time, between which there is a holding time at the required temperature. The containers are removed from the autoclave after the process at an approximate temperature of 35°C to allow the exterior to dry before labelling can occur (Hinrichs and Atamer, 2011).

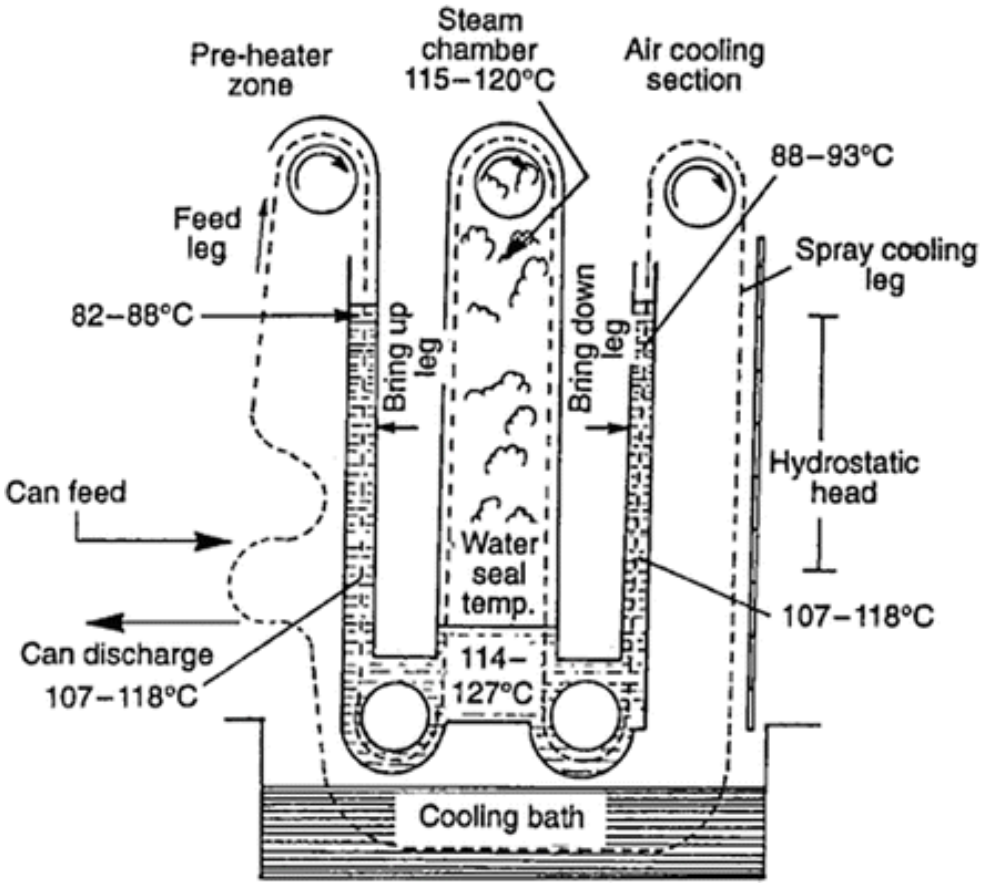


Figure 1.13: Hydrostatic steriliser used for continuous sterilisation (Holdsworth and Simpson, 2016).

In-container sterilisation can also occur using a continuously operating autoclave, known as a hydrostatic steriliser (Figure 1.13). This is generally used for large quantities (Hinrichs and Atamer, 2011). This system uses tall columns of water to counterbalance the atmospheric pressure with the processing pressure required, as it cannot be a completely isolated system like the batch autoclave. The containers are carried through the system with conveyor belts. Rotating autoclaves are another type of continuous in-container sterilisation; however, they are only suitable for canned products (Fuquay *et al.*, 2011).

1.3 Milk powders

1.3.1 Composition

As discussed, milk is quite a complex product, and is also quite heat-sensitive, so it is very important during the drying process to minimise the effect on the milk's quality. Whole milk contains between 85–90% water, but in powdered form, will only have a water content of 2–3% (Písecký *et al.*, 1997). This low moisture content minimises the possibility of bacterial growth (Bylund, 1995). Milk is also dehydrated to stabilise milk constituents (Sharma *et al.*, 2012). The transport of milk powder is much easier than its liquid counterpart due to the reduced weight and volume, which is another reason why milk powder is produced (Bylund, 1995; Sharma *et al.*, 2012). There is a wide variety of milk powder products, including but not limited to: skim milk powder, whole milk powder, filled milk powder, whey protein powder (isolates, concentrates), milk protein isolate, infant formula, coffee whiteners, and lactose (Anema, 2019). Some of these products are achieved through isolating and purifying fractions through membrane separation or through the use of chromatographic resins.

Most milk powders are produced using spray drying technology. Other technologies used for drying are fluid bed dryers (which are sometimes used in conjunction with spray dryers), roller dryers, freeze dryers, microwave dryers, and superheated steam dryers (Schuck, 2011), although some of these would be more commonly found in the general food industry, or the chemical and pharmaceutical industries.

3.1.1 Whole milk powder

The Codex Alimentarius (1999) regulation states that whole milk powder (WMP) must contain at least 42% w/w milk fat, no more than 5% w/w water, and at least 34% w/w milk protein the milk solids non-fat (SNF), the water of crystallisation of lactose is contained in the SNF fraction, not in the water content. The Codex standard also lists various food additives allowed and their limits of addition; allowed additives include stabilisers, acidity regulators, anti-caking agents, emulsifiers, and antioxidants.

3.1.2 Fat-filled milk powder

Fat-filled milk powder (FFMP) is also a fat-containing milk powder, but in which the natural milk fat fraction is partly or fully replaced by cheaper indigenous vegetable fats to produce alternative milk products perhaps intended for lower-income consumers with healthier saturated/unsaturated fat balance (Kneifel, 2003; Ejeahalaka and On, 2019; Ejeahalaka and On, 2020). Approved and suitable vegetable oils used in the production of fat-filled milk powder include soybean oil, palm oil, coconut oil and maize oil, and highly refined oils are preferable (Kneifel, 2003). Other animal fats can also be used, sometimes with additional carbohydrates being added (O'Sullivan *et al.*, 2018). To produce FFMP, a liquid skim milk concentrate is mixed with the desired fat blend, at a temperature where the fat is a liquid, usually about 50–60°C, and dried (Schmidmeier *et al.*, 2019). FFMP can then be reconstituted for direct consumption, or it can be used in the industry as an ingredient in yoghurt production, ice cream production, confectionary, and bakery products (Sharma *et al.*, 2012; O'Sullivan *et al.*,

2018). FFMP is similar to WMP in most physicochemical characteristics (Kelly *et al.*, 1999).

1.3.2 Powder processing

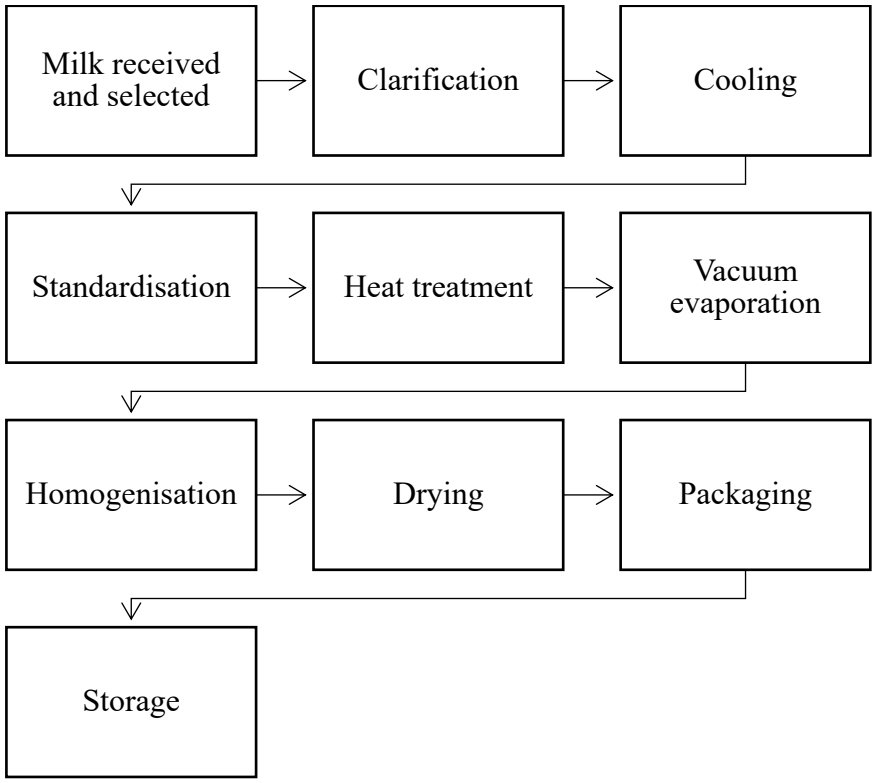


Figure 1.14: Typical process for milk powder production (Fuquay *et al.*, 2011).

1.3.2.1 Pre-processing

The process of converting liquid milk into a dry powder using a spray dryer requires the removal of almost all of the water (Písecký *et al.*, 1997). Milk used for powder production must be of very good sensory, chemical, and bacteriological quality (Schuck, 2011). The milk is first clarified when it is received, and cooled to 4°C. It is then usually pasteurised at a higher temperature than for liquid milk for general consumption, i.e., 88–95°C instead of 72-75°C for 15 to 30 s (Schuck, 2011). This is

to inactivate the majority of the lipolytic enzymes present in the milk, which would be the main causes of milk fat degradation during storage. Pathogenic bacteria and saprophytic microorganisms (i.e., yeasts) are also destroyed here, and the –SH group in β-lactoglobulin becomes reactive, which imparts an anti-oxidative effect (Chavan *et al.*, 2011; Elzoghby *et al.*, 2015). Before the milk is subjected to drying, it is imperative that the quality of the raw materials is good (Bylund, 1995); pathogens such as *Salmonella*, *Shigella*, *Escherichia coli*, and *Listeria monocytogenes* must be absent from the powder so it is imperative that they are also absent in the liquid milk (Pal *et al.*, 2016). Microfiltration or bactofugation may also be used before drying the milk to ensure there is minimal heat-resistant bacteria present (Bylund, 1995).

1.3.2.2 Evaporation and concentration

According to Písecky *et al.* (1997), there are two main stages in the removal of water from milk: vacuum evaporation, and spray drying (or less commonly, roller-drying). The first stage removes most of the free water in the system; this water is not bound to the particles of the dry solids in the system, so it is easier and faster to remove than bound water (Bylund, 1995). Schuck (2011) stated that the energy consumption during evaporation is 10–30 times lower than spray drying. The second stage, i.e., spray drying, is much more intense as the water being removed is that which exists in the capillaries and pores of the dry solids in the system.

Vacuum evaporation removes the majority of the water, resulting in a viscous milk concentrate. Evaporators are used as they are a more economical process of removing water compared with spray dryers. Evaporation involves the removal of water by bringing the liquid to its boiling point. As this is quite high for water (100°C), liquid

milk (which is heat sensitive) is evaporated under vacuum to reduce the boiling point, thus reducing the heat damage. There are a wide variety of types of evaporators available, which are used for different products and purposes; falling film evaporators are an essential part of most dairy powder production facilities (Figure 1.15) (Gourdon and Mura, 2017).

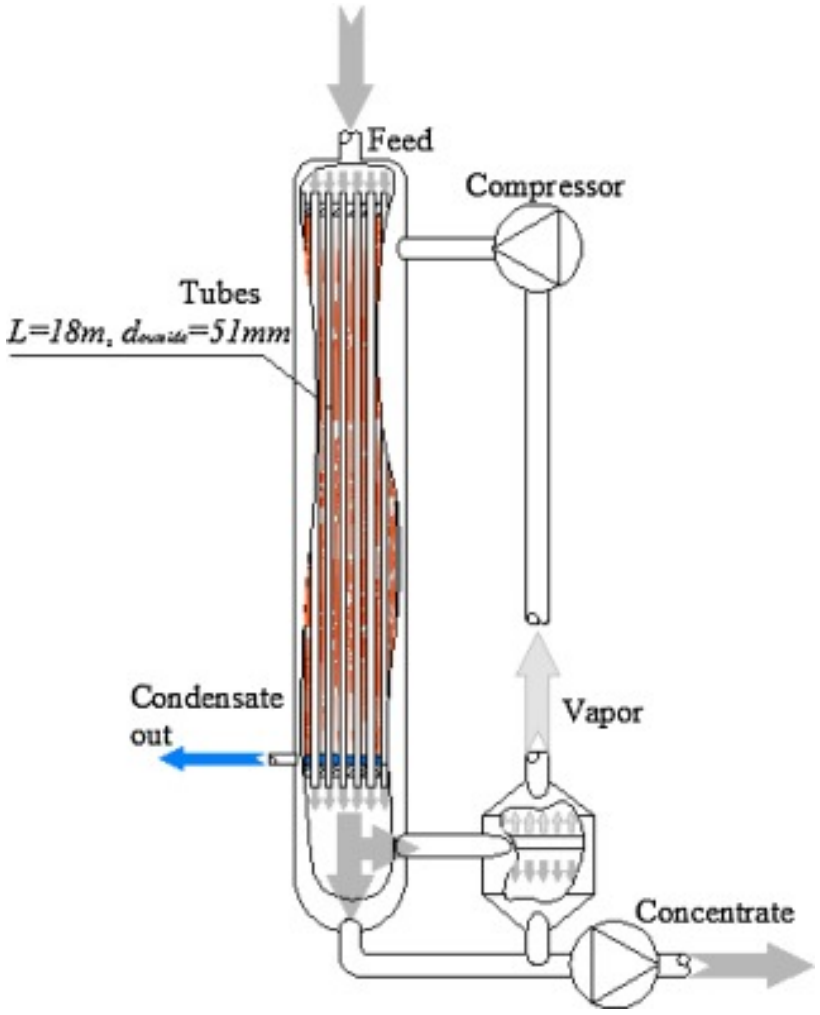


Figure 1.15: Typical falling film evaporator in the dairy industry. Most dryers have multiple stages (Gourdon and Mura, 2017).

The milk flows downwards under gravity in the inner tubes, on the walls as a thin film with a high heat transfer area, due to the high number of tubes allowing for a single-pass operation with a short retention time. The tubes can be anywhere between 4–15 m long but are only 25–60 mm in diameter. The evaporation process produces a total solids level depending on the drying processing, as follows: 45–50% total solids for spray drying, and 30–35% total solids for roller drying (Schuck, 2011).

Reverse osmosis can also be used in combination with evaporation (Schuck, 2011); sometimes it is used to replace evaporators, due to its lower running costs (Písecký *et al.*, 1997). Homogenisation may also occur after evaporation and before spray drying in high-fat milk powders, which reduces the free-fat level in the powder (Schuck, 2011).

1.3.3.3 Spray drying

Spray drying follows evaporation in the milk powder production process and it is the most frequently used technique to dry dairy products (Schuck, 2011). High heat and mass transfer rates occur, which can severely damage the quality of the powder product, unless the process is operated efficiently and correctly (Písecký *et al.*, 1997). This last stage of removal of moisture for the droplet is the most energy-intensive step of the drying process. Spray drying can be broken down into three stages: dispersion of the droplets by the atomiser into very fine droplets (resulting in a substantially increased surface-area to volume ratio, which will aid drying); droplets being dispersed in hot air which evaporates the water; and finally separation of the droplets from the hot air, which occurs through gravity (Figure 1.16) (Bylund, 1995).

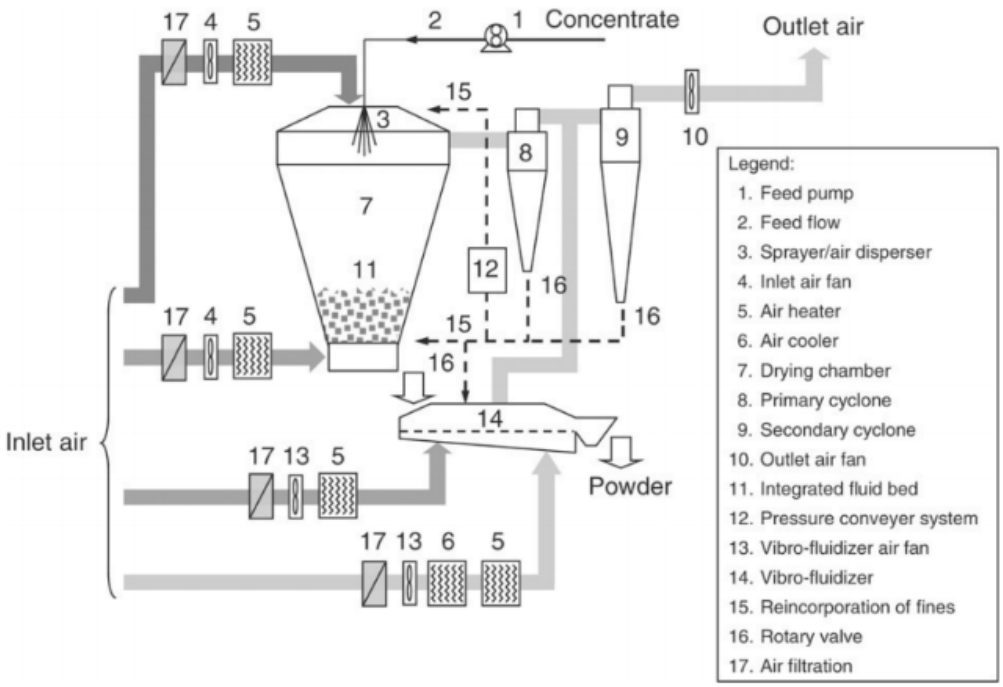


Figure 1.16: Multi-stage spray dryer (Schuck, 2011).

The concentrated milk is pumped from the evaporator to the atomiser at the top of the spray dryer main chamber. The milk is atomised and is sprayed into the chamber as a very fine mist at a temperature of approximately 80°C. The air pumped into the chamber is at a temperature of between 150 and 250°C. Evaporation occurs instantaneously at a relatively low temperature due to the small droplet size created on atomisation, which results in minimal heat damage occurring to the product (Schuck, 2011). The powder particles fall to the bottom of the chamber, where they are then pumped out using a pneumatic conveyer if it is a single-stage spray dryer. A two-stage dryer has a fluidised bed dryer in the place of the pneumatic pump, which adds an extra step of drying, as it allows for a further decrease of 2–3% of the moisture level while cooling the powder down. This step also results in larger primary particles and some agglomeration (Bylund, 1995). Three-stage drying is also possible with the

addition of the fluid bed dryer into the base of the spray dryer, as well as the external fluid bed as seen in the two-stage dryer. Three-stage drying has the main drying chamber, two smaller drying chambers, and a cooling chamber. There is usually minimal problems with regards to the microbiological quality of the final product or the maintenance of the spray drying chamber, because, even if the product comes into contact with the walls of the chamber, it is in powder form (Schuck, 2011).

Although a multi-stage drying process is preferable, a single-stage dryer is sometimes chosen due to the properties of the products, especially if they are hygroscopic, as they are too sticky at higher moisture contents. Single-stage dryers require all of the drying to occur in the spray dryer chamber, which is a disadvantage in comparison with the multi-stage dryers, as the outlet temperature must be quite high for the residence time to be sufficient for relative humidity and the powder's water activity to be in equilibrium. This will reduce the thermal efficiency (Schuck, 2011). According to Pízecký (1997), to produce a skim milk powder (SMP) from its concentrate to a final moisture content of 3.6%, an inlet temperature of 101°C is required. However, in a two-stage drying process, the inlet temperature only needs to reach 73°C to dry the powder to a moisture content of 7% in the spray dryer tower, as there is a second stage to follow which will reduce the moisture content to 3.6%. In a single stage dryer, to remove this extra 4.1% of moisture in a single stage dryer accounts for 33% of the total heat required for the process (Písecký *et al.*, 1997). Three-stage drying is suitable for high-fat products and infant formula and maltodextrins, and they dominate the dairy industry today (Schuck, 2011).

The fluidised bed dryers in the two- and three-stage dryers are a vibrating (built-in in three-stage is not vibrating) fluid bed designed to move the powder along the process, and cool the powder down while allowing it to dry out to its final moisture content.

Powder produced by a process using a fluid bed dryer is more coarse and free-flowing than a powder produced using a pneumatic conveyor system (Písecký *et al.*, 1997). The increased flowability is due to the agglomeration of particles during the atomisation step not being broken down during the fluid bed treatment. The agglomerates are broken down in the pneumatic system (Písecký *et al.*, 1997).

Instantisation occurs during drying in a multi-stage dryer (Schuck, 2011) and is based on agglomeration, which results in the improvement of rehydration characteristics of the powder. Agglomeration is the encouragement of air incorporation between particles, and occurs when the particles, which are either not yet fully dried (straight-through method), or are rewetted using steam, water or skim milk (known as rewetting), come into contact with each other during the drying process.

Viscosity is an important factor to consider when the concentrate is being atomised in the spray drying chamber (Písecký *et al.*, 1997). The denaturation of whey proteins is a major influence on viscosity at lower solids content (i.e., below 45%). This is due to the increase in voluminosity of the proteins upon denaturation (Sutariya *et al.*, 2017, Anema *et al.*, 2014). Another influence on the viscosity is the inter-particle interactions, which becomes more important as the concentration of the milk increases due to the reduction of inter-particle distance (Karlsson *et al.*, 2005).

1.3.3.4 Separation

After drying, the powder and air need to be separated, which occurs in one of three types of equipment: cyclone separator, a bag filter, or a wet scrubber (Písecký *et al.*, 1997). In a cyclone, the powder is introduced at the top of the cone, the powder falls in downward spiral to the bottom of the cone, and the air (which may still include

some fines) leaves from the top of the cyclone. Cyclone efficiency decreases with increasing cyclone size (Písecký *et al.*, 1997).

Since 2007, the European Union has required powder producers to reduce their powder losses to less than 10 mg/ Nm^3 (Westergaard and Niro, 2004). Thus, bag filters have been introduced to capture some of the residual fines escaping in the exhaust air from the cyclone. The fines caught here can be recycled or are thrown out if the powder is to be used for infant formula manufacture (Bylund, 1995). Compressed air is used intermittently on the bag filter to remove the fines stuck there, which are collected at the bottom using a hopper, and discharged through a rotary valve (Písecký *et al.*, 1997). The bag filter is kept warm to avoid microbial growth and prevent condensation.

1.3.3.5 Sifting, sorting, and importance of powder packaging

The last steps of powder production are to sift, sort, and bag off the produced powder (Písecký *et al.*, 1997). Air transport is avoided where possible as it can disrupt agglomerated particles. A sifter (generally a vibrating mesh, or a static mesh with rotating arms) separates the powder from the oversized lumps of powder that may form. There are many different containers into which the powder can be placed, but is generally packed in laminated powder bags, with an inner bag made of polyethylene. (Bylund, 1995; Písecký *et al.*, 1997). For the best preservation, milk powder moisture content must be as close to 2% as possible when at 25°C (Schuck, 2011). The packaging chosen must also protect the powder from light, insects, and general extraneous matter exposure. The most common packaging used for milk powders is a multilayer kraft paper bag with a layer of polyethylene lining (at least 25 μm in

thickness). Metals can also be used in packaging the powders, in the form of an aluminium layer or a metal barrel. The packaging is flushed with inert gases before sealing to avoid oxidative changes in the powder (Schuck, 2011). The milk powders can then be stored at ambient temperatures, but only for a limited time.

Moisture (glass transition temperature and water activity in particular) is the most important factor that must be controlled by the packaging. Milk powders are hygroscopic, which means that they readily take up moisture from the air and become sticky, and their reconstitution properties are drastically diminished. The reason for moisture absorption is due to the presence of amorphous lactose in the powder (Clark *et al.*, 2016). The rest of the components in the milk (proteins and fat globules) are embedded in this amorphous lactose, which is hygroscopic (Thomas *et al.*, 2004; Huppertz and Gazi, 2016). Lactose is the most abundant component of milk powders at approximately 38% of the total powder mass, so its physical state is important for powder technical specifications, such as particle size and flow properties (Thomas *et al.*, 2004; Thomsen *et al.*, 2005b). Lactose crystallisation is a spontaneous process which occurs when the temperature of the powder exceeds the glass transition temperature (T_g) (Schenz, 1995; Jouppila *et al.*, 1997; Thomsen *et al.*, 2005a). Water content and relative humidity is also important in lactose crystallisation as water acts as a plasticizer (Jouppila and Roos, 1994; Schenz, 1995). Crystallisation does not occur during the preparation of milk powder as the removal of water is too rapid (Huppertz and Gazi, 2016). Lactose crystallisation can be accompanied by an initiation of unwanted chemical reactions and an increase in water activity (Thomsen *et al.*, 2005b). When lactose crystallises, it can crystallise into multiple forms such as α-lactose monohydrate which is the most stable crystalline form, anhydrous β-lactose, and an anhydrous mix of α- and β-lactose (Jouppila *et al.*, 1997; Huppertz and Gazi,

2016). Lactose crystallisation can cause problems such as stickiness and caking (Downton *et al.*, 1982; Aguilera *et al.*, 1995; Listiohadi *et al.*, 2005; Paterson *et al.*, 2005). Whey protein powders include a pre-crystallisation step before drying to reduce the problems associated with lactose crystallisation post-drying (McSweeney and Fox, 2009).

1.3.3 Applications of milk powders

Milk powder is very nutritious and functional, so it has a wide variety of applications, ranging from infant formula manufacture to an ingredient in sausage production. It can also be reconstituted with clean water to give liquid milk (Schuck, 2011). In the baking industry, it can be used as an egg substitute, or to improve the water-binding capacity of a loaf of bread, while also increasing its volume (Bylund, 1995). In the dairy industry, milk powder is used in manufacture of ice cream, processed cheese, and yoghurts (Schuck, 2011). Milk powder is also utilised in the confectionary industry and for animal feed. In the confectionary industry, manufacturers take advantage of the powder's functionality to improve the flavour, taste, and texture of the product. Other miscellaneous uses include ready-meal manufacture, nutrition blends, soups, coatings, and imitation milk products (Schuck, 2011). According to Early (2012), FFMP is used in the same applications as WMP. FFMP is a commonly used ingredient in yoghurt production, coffee whiteners, and can be reconstituted as a substitute for drinking milk (Schmidmeier *et al.*, 2019).

Our search of the literature has shown that there is a lack of scientific literature regarding the use of both WMP and FFMP as a base for heat-treated dairy beverages

and it is planned that the original research section of this thesis will address this knowledge gap.

1.4 Milk salts

According to Gaucheron (2011b), milk salts accounts for about 8–9 g L^{-1} of the dry matter in bovine milk. The content is relatively constant but there can be some variation in the composition of the fraction. This can be due to a number of factors, including the breed of the cow (and its genetics), the stage of lactation, the feed consumed by the cow, the presence of an infection in the udder (mastitis), and the nutritional status of the cow (McSweeney and Fox, 2009; Cashman, 2011). The salts in milk are distributed between the soluble (diffusible) phase, and in the casein micelle (colloidal phase); this natural distribution is known as the salts equilibrium (de la Fuente, 1998).

In the soluble phase, the salts exist as ions: calcium, magnesium, potassium, and sodium are the main cations; chloride, citrate and inorganic phosphate are the major anions present in milk. Other minor salts include copper, iron, boron, zinc, lead, manganese, and iodine (McSweeney and Fox, 2009). At its natural pH of between 6.6-6.7, the aqueous phase of milk is supersaturated with calcium phosphate and has an ionic strength of approximately 80 mmol L^{-1} (Gaucheron, 2011b). The micellar fraction of milk salts has proven difficult to study according to Gaucheron (2011b) as the colloidal calcium phosphate (i.e., the calcium phosphate present in the micelle) (CCP) is difficult to study *in situ* and isolating it changes its structure. This is because CCP is integral to the casein micelle structure (Horne, 1998; Fox and McSweeney, 2003). When research requires the two phases to be separated, it is very important that the method used does not change the pH or the temperature, as both of these influence the equilibrium between the soluble and colloidal phases (McSweeney and Fox, 2009;

Fox *et al.*, 2015). In fact, the salt equilibrium is affected by many factors, and can be summarised as in Figure 1.17. Even small alterations in the distribution of salts (altering pH, temperature pressure etc.) can lead to noticeable effects in the stability of the micelle (de la Fuente, 1998).

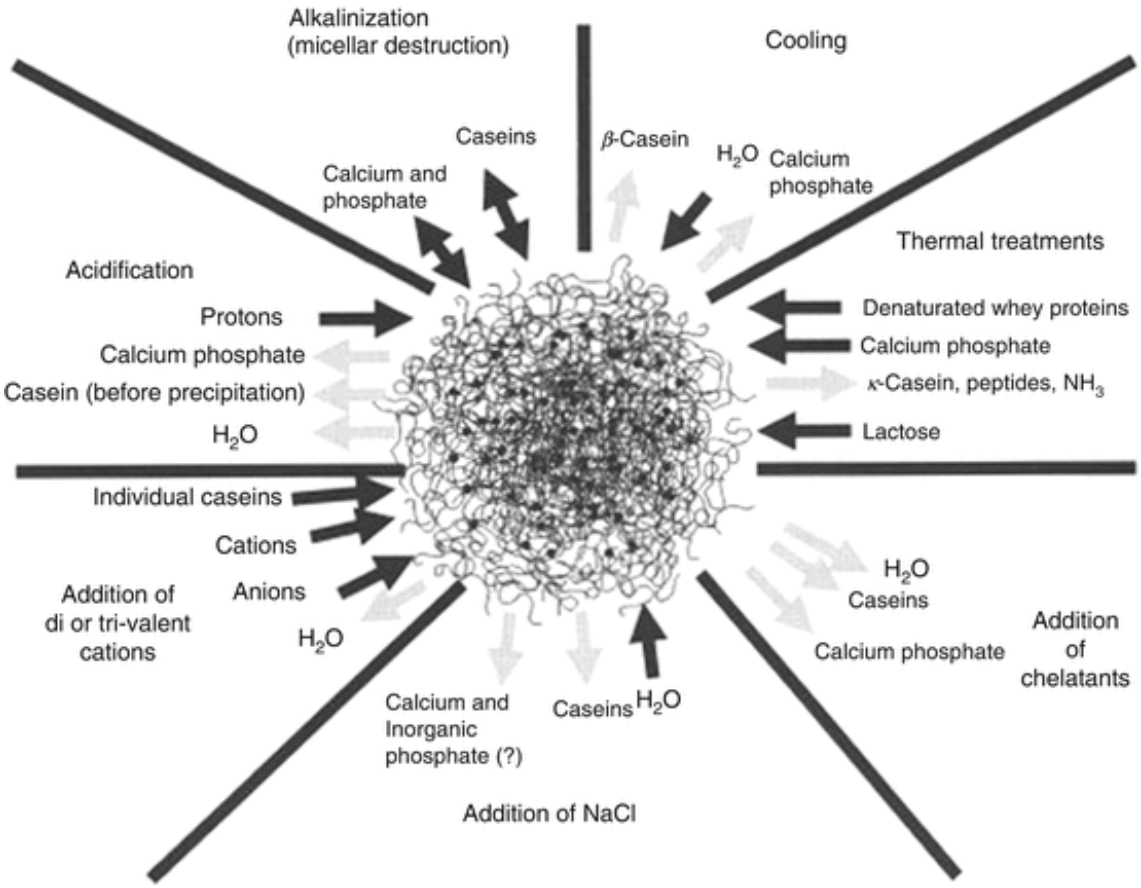


Figure 1.17: Changes occurring in the milk salts equilibrium in response to changes in processing and storage conditions (Gaucheron, 2011b).

Although the salts in milk contribute little to the overall composition of milk, they contribute significantly to the nutritional element of milk. The salts can be included under the 'mineral' fraction of milk when discussing nutrition, as in the widely accepted terminology in the field (Cashman, 2011). Sodium, calcium, potassium,

phosphorus, magnesium, and chloride are macro-elements or macro-minerals, as they are present in milk at levels greater than 0.01%. It has been shown that bovine milk is an important source of the majority of these macro-minerals in the Western diet, as well as being an important source of zinc and selenium (Gaucheron, 2011a). While the role of magnesium in various physiological processes is understood, its bioavailability has not been studied to the same degree (Cashman, 2011). Trace elements in milk include iron, copper, zinc, iodine, manganese, selenium, boron, silicon, nickel, arsenic, chromium, cobalt, molybdenum, and fluorine. The levels of these trace elements required in the diet are less than 100 mg per day (Cashman, 2011). In general, these have not been studied as thoroughly as the macro-minerals: research is lacking on copper, manganese, selenium, and fluorine in particular. Iodine is critical in foetal and childhood stages of life and is completely absorbed and metabolised in stages involving the hypothalamus, pituitary gland thyroid gland and the blood (Ahad and Ganie, 2010). The bioavailability of iron and zinc is high, but the reason for this is not yet clear. It is important to note that milk does not contain the strong inhibitors of the absorption of minerals such as polyphenols and phytate which are present in plants (Cashman, 2011).

1.4.1 Heat stability

When discussing heat stability, the main measure in milk is called the heat coagulation time (HCT), which is the time required at a certain temperature, generally 140°C (120°C is used for concentrated systems), for the formation of visible aggregates, or flecks to occur. The most influential variable of milk on its heat stability is the pH, as the inter-relationships of milk salts is greatly affected by it (McSweeney and Fox, 2009).

Table 1.4: Relationships between the concentration of milk salt constituents and the pH of milk. Adapted from McSweeney and Fox (2009).

Inversely related to pH	Directly related to pH
Titratable acidity	Colloidal inorganic calcium
Total soluble calcium	Caseinate calcium
Soluble un-ionised calcium	Colloidal inorganic phosphorus
Ionised calcium	Colloidal calcium phosphate
Soluble magnesium	Sodium
Soluble citrate	Chloride
Total phosphorus	
Soluble inorganic phosphorus	
Ester phosphorus	
Potassium	

Milk can be split into two types on the basis of heat stability; Type A (which is more common) and Type B, depending on how it behaves (Walstra, 1999). When milk is heated, the pH decreases due to calcium phosphate precipitation and formic acid production (from lactose); the rate of this pH reduction greatly influences the rate of coagulation. Walstra (1999) proposed two different reactions in milk that may cause the coagulation seen upon heating: colloidal aggregation and chemical crosslinking. Calcium ions form calcium bridges during colloidal aggregation. The aggregation is irreversible, which suggests covalent crosslinking is occurring. The resulting coagulation can be dissolved using calcium-chelating agents (such as trisodium citrate, disodium hydrogen phosphate, or sodium hexametaphosphate) (de Kort *et al.*, 2012). Less is known about chemical crosslinking, but its rate increases with a decreasing pH

and at higher temperatures (Walstra, 1999). κ-casein also has a role to play in coagulation. It is found as the "hairy layer" on the outside of the casein micelle, and acts as a steric stabilisation layer between the micelles. However, with increasing pH (especially above pH 6.7), the equilibrium at which κ-casein exists shifts, increasing its solubilisation. κ-casein and β-lactoglobulin interact at higher temperatures and so, if κ-casein is in solution when it reacts, this results in a depleted micelle. The casein micelle will aggregate when exposed to UHT treatment, which is undesirable (de Kort *et al.*, 2012).

One of the most important factors of milk's heat stability is the salt balance (Písecký *et al.*, 1997). The optimum salt balance is when the free cations (such as calcium and magnesium) exist in balance with the anions of citric acid and phosphoric acid. The balance can be improved by either adding deficient components or removing components which are present in excess. Milk is naturally supersaturated with calcium, so, to improve the heat stability, anions can be added in the form of citrate and phosphate salts, known as calcium chelators. Trisodium citrate is a common calcium chelator and is also an emulsifying salt used in the food industry, as it disrupts calcium-mediated cross-linking that may occur between proteins (Dhanraj *et al.*, 2017). Citrates are additives permitted by WHO and FAO for infant formula, as they are a natural component of milk (Písecký *et al.*, 1997). Ion exchange can be used also to improve heat stability of low-heat skim milk powder through the removal of excess calcium, although removal of over 60% of the total calcium will result in micelle disintegration (Lin *et al.*, 2006; Faka *et al.*, 2009).

A lot of research has been carried out into the use of calcium-chelating salts for improvement of heat stability (Udabage *et al.*, 2000; Omoarukhe *et al.*, 2010;

Tsioulpas *et al.*, 2010; El-Bakry *et al.*, 2011; Kaliappan and Lucey, 2011; de Kort *et al.*, 2012; Crowley *et al.*, 2014; McCarthy *et al.*, 2017; Hebishy *et al.*, 2019).

1.5 Conclusions

It is clear from our critical review of the literature, as summarise in the preceding sections, that while much original research has been carried out on ultra-high temperature (UHT) processing of dairy products, considerably less is known about the influence of retort processing on the stability of dairy beverages. Similarly, there is a lack of literature comparing the effect of these heat treatments on dairy beverages. In addition, while whole milk powder (WMP) has been extensively investigated as a stable dairy-based alternative to liquid milk, fat-filled milk powder (FFMP), a cheaper alternative to WMP, has not. Furthermore, there has also been little research performed comparing the stability of WMP-base dairy products and FFMP-based dairy products as of the writing of this thesis. Thus, the aim of the research presented was to investigate the influence of UHT and in-container sterilisation thermal treatments on the quality and stability of WMP- and FFMP-based dairy beverages, with a view to adding value to dairy ingredients.

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Objectives

Milk powders are produced as a more stable format than liquid equivalents and can be reconstituted as a substitute for liquid milk or be used across several different food sectors in the formulation of a wide variety of products, such as yoghurt, ice cream, infant formula, bakery, confectionary, clinical and medical nutritional beverages, and animal feed. Liquid dairy products are generally subjected to heat treatment before consumption, with such treatments ranging from pasteurisation (72°C x 15s; designed to destroy pathogenic bacteria), to more severe heat treatments like UHT treatment (138°C x 3s) and retort sterilisation (120°C x 20 min), designed to commercially sterilise products. These heat treatments can result in a wide variety of chemical and physical changes.

Calcium-chelating salts are sometimes used to enhance the heat stability of dairy products due to their effect on the concentration of free calcium ions (Ca^{2+}) and therefore the integrity of the casein micelle. While the micelles are very stable to heat, severe heat treatments affect the pH, among other changes, which in turn affects the electrostatic repulsion between the casein micelles, and the salts equilibrium, which can result in coagulation upon heat treatment. The chelation of the free calcium ions in the serum phase reduces the propensity of milk to coagulate when subjected to severe heat treatment.

The overall objectives of this thesis were:

1. To evaluate the stability of dairy beverages based on whole milk powder (WMP) and fat-filled milk powder (FFMP), with a range of different protein contents (2.3-5%) during heat treatment and subsequent storage;
2. To determine the important physical and chemical changes occurring in WMP- and FFMP-based beverages as affected by fat source, protein content, and heat treatment;
3. To investigate the incorporation of calcium-chelating salts on the viscosity and heat stability of reconstituted WMP and FFMP, and the influence of these salts on the colour of reconstituted low-heat skim milk powder (LH-SMP).

Chapter 2

The effect of calcium-chelating salts on the viscosity, colour and heat stability of reconstituted fat-filled milk powder and whole milk powder

Abstract

Chelating salts are often used to increase the heat stability of milk-based systems. Reconstituted whole milk powder (WMP) with milk protein concentrate (MPC) (80% protein) and fat-filled milk powder (FFMP) were prepared with MPC added to a total level of 5% protein. The influence of selected chelating salts (trisodium citrate (TSC) (5 or 20 mmol/L), disodium hydrogen phosphate (DSHP) (5 or 20 mmol/L), and sodium hexametaphosphate (SHMP) (2.5 or 10 mmol/L) on viscosity and heat stability at 140 °C was determined in these systems. Solutions of low-heat skim milk powder (LH-SMP) were also prepared with and without added chelating salts, to examine influence of the chelating salts on colour. TSC did not considerably improve or reduce the heat stability of WMP and FFMP solutions. DSHP (20 mmol/L) considerably increased the heat stability of the WMP solution between pH 6.4 and 7, but its positive effect on heat stability was less evident at pH greater than 7. The same effect was not seen for reconstituted FFMP. Both WMP and FFMP solutions containing SHMP (10 mmol/L) formed a gel within 90 s when held at 140 °C. Viscosity analysis showed that the addition of 10 mmol/L SHMP resulted in a significant increase in apparent viscosity which may be due to the increased particle-particle interactions between the casein proteins from dissociated micelles, or it may be due to the increased electrostatic repulsion between proteins on SHMP addition. Sample pH also significantly affected the apparent viscosity, as the apparent viscosity increased with increasing pH. The influence of 20 mmol/L TSC on the colour of reconstituted LH-SMP was significant. Both levels of SHMP addition resulted in a very significant difference in the colour of the LH-SMP sample. The influence of these calcium-chelating salts on solutions of WMP, FFMP, and LH-SMP make it apparent that they

improve heat stability, and influence viscosity and the colour of the samples to varying levels. Thus, it is important to be aware of their varying influence before use in dairy processing.

2.1 Introduction

In the dairy industry, salts may be used to increase specific mineral concentration (supplementation) and to increase the heat stability of milk-based beverages by chelating minerals. One of the minerals which is commonly added for fortification purposes is calcium, although it can be technologically challenging as the serum phase of milk is supersaturated with calcium (Omoarukhe *et al.*, 2010). Calcium ions are integral to the stability of the casein micelle, forming linkages between the protein molecules either as colloidal calcium phosphate (CCP) or bound directly to the caseins (Schmidt, 1982; Holt, 1992; Horne, 1998).

According to the Health Service Executive (HSE), adults require 700 mg of calcium daily to maintain bone and tooth strength (to prevent osteoporosis) and blood-clotting ability, and to regulate muscle contractions (Miller and Berner, 1989; Zemel and Miller, 2004; Hess *et al.*, 2016). There is also research to suggest an increased calcium intake results in lower blood pressure (Allender *et al.*, 1996; Bucher *et al.*, 1996; van Mierlo *et al.*, 2006). Milk and dairy products are rich sources of bioavailable calcium in the diet; however, a glass of milk (100 ml) only contains 120 mg of calcium (German *et al.*, 2009; Gaucheron, 2011). To be absorbed, calcium and phosphorus need to be present in a ratio of between 1:1 and 2:1 (Loughrill *et al.*, 2017). The calcium: phosphorus (Ca:P) ratio in breast milk is 2:1 and in bovine milk is closer to 1.4:1 (Khinchi *et al.*, 1999; Singh *et al.*, 2007; Loughrill *et al.*, 2017). The calcium salts commonly used for fortification purposes include, but are not limited to, calcium lactate, calcium gluconate, calcium lactate gluconate, calcium carbonate, calcium

phosphate, calcium chloride, and tricalcium citrate (Pírkul *et al.*, 1997; Williams *et al.*, 2005; Cerklewski, 2005; Kressel, 2010).

Calcium-chelating salts can be used to enhance the heat stability of liquid dairy products individually or in mixtures (Kaliappan and Lucey, 2011; Lewis, 2011). Heat stability is very dependent on the protein content and pH of the solution (O'Connell and Fox, 2002; de Kort *et al.*, 2012). The heat stability of reconstituted dairy powders is also dependent on the heat treatment applied during the preheating stage of manufacture (Fuquay *et al.*, 2011). pH affects the charge of proteins, the concentration of free calcium in the system, and the level of colloidal calcium phosphate (CCP) in the micelle (Walstra, 1999). Chelating agents shift the protein-mineral balance, which reduces the concentration of free calcium in the system, and sequester free calcium ions (Ca^{2+}) in solution (de Kort *et al.*, 2011; Power *et al.*, 2020). If chelating agents are used in excess, the casein micelle could become depleted of CCP, which increases the heat stability by binding free calcium in solution, inhibiting calcium bridging and aggregation, and causing the micelles to swell (increase in hydration and voluminosity) due to reduced structural rigidity (de Kort *et al.*, 2011; McCarthy *et al.*, 2017; Power *et al.*, 2020).

Common chelating salts include salts of phosphates and citrates, as well as ethylendiamine tetra acetic acid (EDTA) (de Kort *et al.*, 2011; McCarthy *et al.*, 2017). As well as binding the free calcium in the system, chelation may contribute additional effects. For example, trisodium citrate (TSC) binds calcium from the micelle, producing calcium citrate, which is found in the serum phase. Disodium hydrogen phosphate (DSHP) produces calcium phosphate in a similar way, but it precipitates from the casein micelle. Sodium hexametaphosphate (SHMP), a strong anionic polyphosphate, will bind to charged amino acids of the casein residues, as well as to

the calcium ions, due to SHMP having six calcium-binding sites with a homogeneous charge distribution, enabling SHMP to interact with casein and cations simultaneously (Mizuno and Lucey, 2007; de Kort *et al.*, 2011; Power *et al.*, 2020). This allows for the casein micelles to be cross-linked, and gelation may occur as a result (de Kort *et al.*, 2011). TSC does not allow for calcium bridges to be formed due to the formation of soluble calcium citrate complexes (Mizuno and Lucey, 2007). Phosphate and citrate salts are also used in processed cheese production, where they are used to sequester calcium and control the pH (El-Bakry *et al.*, 2011; Kaliappan and Lucey, 2011).

In this study, the influence of TSC, DSHP and SHMP on the heat stability and viscosity of milk protein beverages formulated using mixtures of whole milk powder (WMP) and milk protein concentrate (MPC), and fat-filled milk powder (FFMP) and MPC was examined. The influence of these calcium-chelating salts on the colour of reconstituted low-heat skim milk powder (LH-SMP) was also examined.

2.2 Materials and methods

2.2.1 Base materials

Fat-filled milk (FFMP), whole milk (WMP) and milk protein concentrate (MPC 80) powders were supplied by local dairy ingredient companies based in Ireland. Low heat skim milk powder (LH-SMP) was supplied by Uelzena eG (Uelzen, Germany). Trisodium citrate (TSC) (CAS number: 6132-04-3) was supplied by Sigma Aldrich (Arklow, Co. Wicklow, Ireland), disodium hydrogen phosphate (DSHP) (CAS number: 7558-79-4) was supplied by Merck (Darmstadt, 64293, Germany) and sodium hexametaphosphate (SHMP) (CAS number: 68915-31-1) was supplied by Alfa Aesar (Lancashire, United Kingdom). Ultrapure water was used for reconstitution of all powders.

Table 2.1: Compositional analysis of WMP and FFMP, provided by suppliers.

	WMP (%)	**FFMP (%)**	**MPC 80 (%)**
Moisture	3.0	3.0	5.2
Protein	25.2	25.7	83.7
Fat	26.7	25.4	0.9
Lactose	39.3	40.3	1.4
Ash	5.8	5.6	6.8

2.2.2 Reconstitution

Milk powders were reconstituted to 5% protein using ultrapure water at 44°C. The solutions were mixed using a Silverson L5M-A high shear mixer (Silverson Machines Inc., East Longmeadow, MA 01028) at 3000 rpm for 30 min. The solutions were then transferred to a water bath at 50°C and stirred using overhead stirrers for 3 h, removed and divided evenly into 10 beakers (50 ml each) and cooled to room temperature, while constantly stirring using magnetic stirrers. The pH of the solutions was standardised to values in the range 6.4-7.6, increasing in units of 0.1 from pH 6.4 to pH 7.0, then in intervals of 0.2 pH units from pH 7.0 to pH 7.6 using 1 N and 2 N HCl and NaOH solutions and placed in a cold room (4°C) overnight, mixing using magnetic stirrers on a magnetic stirring plate to allow for total powder rehydration. The next morning, the solutions were adjusted to room temperature in a 30°C water bath, and pH was readjusted if necessary.

The solutions prepared are as outlined in Figure 2.1, with the control solutions being reconstituted using the same method, but without salt addition. The salt addition is based on work by de Kort *et al.* (2009) who based their salt addition on milliequivalents in order to add a similar amount of charges to all samples.

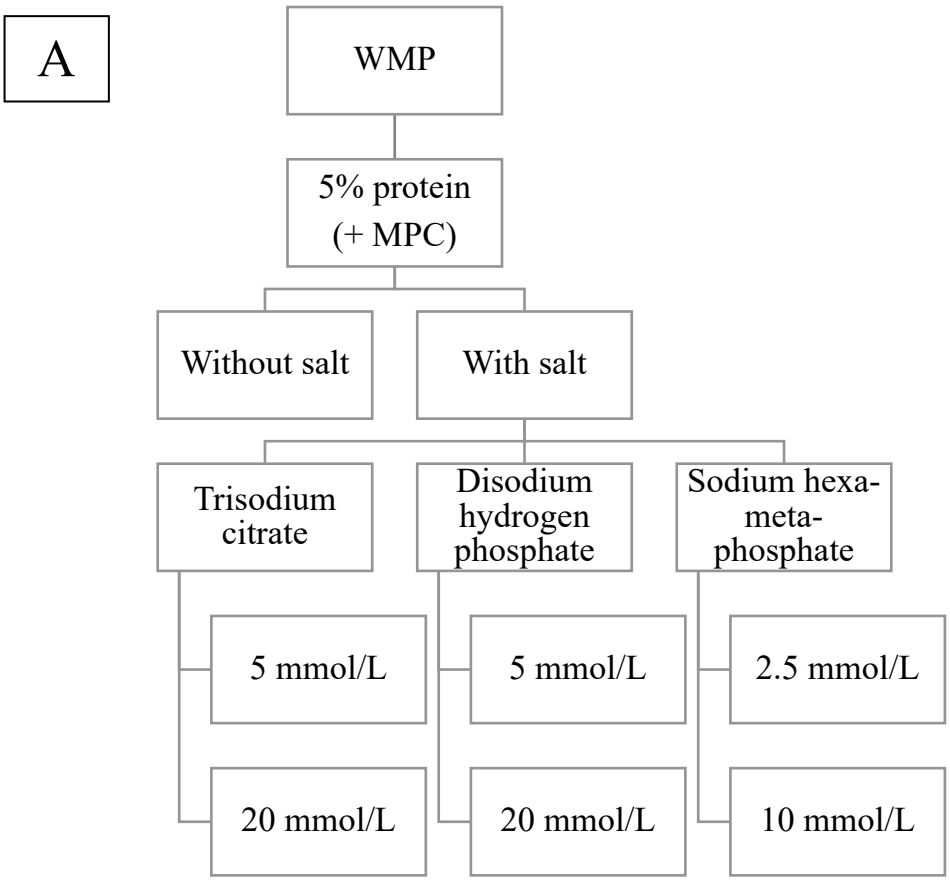


Figure 2.1: Preparation of samples. **A:** WMP-based solutions; **B:** FFMP-based solutions.

B

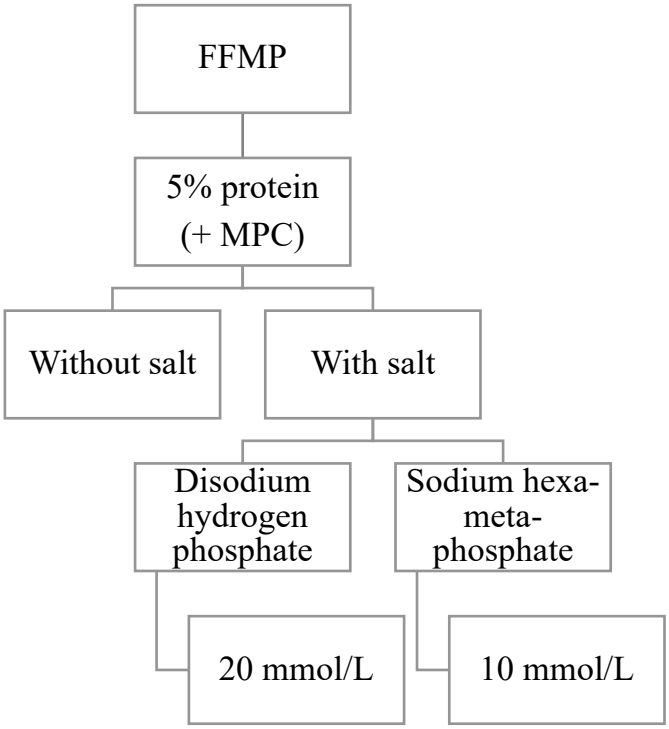


Figure 2.1 (cont.)

2.2.3 Heat stability

Heat stability at 140°C was determined using an oil bath as per the method of Davies and White (1966). From each pH-adjusted sample, 2.5 ml was pipetted into separate glass test tubes, which were then closed with rubber stoppers and secured into the rack. The rack was then placed into the oil bath and set rocking. The test was run in triplicate on each sample, the samples were prepared in triplicate.

2.2.4 Measurement of apparent viscosity

Apparent viscosity of each sample was determined using a HAAKE Roto-Visco rotational viscometer (Thermo Electron, GmbH, Karlsruhe, Germany) at 20 ± 0.5°C using the double gap concentric cylinder and DG43 cup attachments. Samples (approximately 14 ml) were loaded into the cup after equilibration, and were equilibrated to 20 ± 0.5°C for 30 s. The shear rate was progressively increased to 1000 s^{-1} over 3 min, held at that shear rate for 3 min, and brought back down to 0 s^{-1} over 3 min. The average apparent viscosity was reported at the shear rate of 1000 s^{-1} and each sample was measured once. Each sample was prepared in duplicate.

2.2.5 Colour

The colour of the pH-adjusted samples was recorded after reconstitution using a calibrated Minolta Chroma Meter CR-400 colorimeter (Minolta Ltd., Milton Keynes, UK). The colorimeter was calibrated with a white standard tile before analysis. Low heat skim milk powder (LH-SMP) was used as a base instead of FFMP as it contains no fat, which interferes with the observation of the visual effects of salts on the solution. The same reconstitution method was used as in Section 2.2.2 and LH-SMP was used to replace FFMP. Colour was recorded using the CIE L* a* b* units (explanation of colour space in Figure 2.2), and total colour difference (ΔE) was calculated using the following equation (Kelleher *et al.*, 2019; Power *et al.*, 2020)

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2}$$

where L_0, a_0 and b_0 refer to the colour of the control samples (solutions not containing salts).

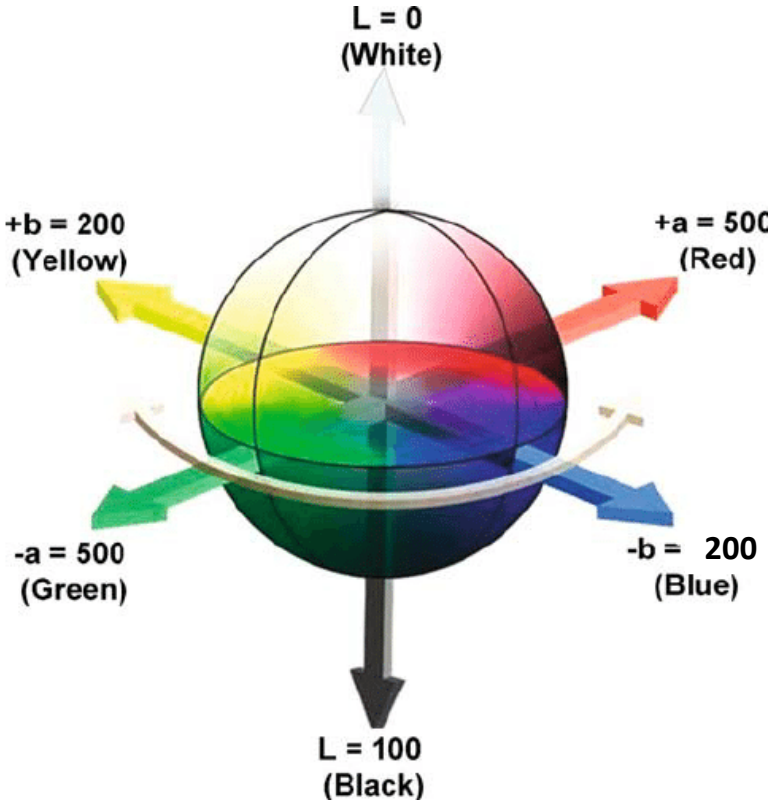


Figure 2.2: Colour space of L*a*b* colour system (Singh *et al.*, 2009).

2.2.6 Statistical data analysis

All samples were prepared in three independent trials, and all analysis was conducted in triplicate. Statistical analysis was carried out using Minitab® 19 (Minitab Ltd., Coventry, UK) using one-way analysis of variance (ANOVA) at a 95% confidence interval. The Tukey pairwise comparison test was used *post-hoc* to determine significant differences ($p < 0.05$) between mean values for different salt addition levels.

2.3 Results and discussion

2.3.1 Heat stability

The heat stability of milk falls into two main profiles: Type A and Type B, as seen in Figure 2.3 below. Type A milk is the dominant type in most countries, including Ireland (Fox and McSweeney, 2003). The control samples of both WMP and FFMP followed the Type A profile (Figure 2.4) but deviated from it when the salts were added. The magnitude of the change in profile increased with the increasing addition level of salts. O'Connell and Fox (2002) noted that the natural variation which occurs in milk does not correlate well with variation in concentration of individual milk salts.

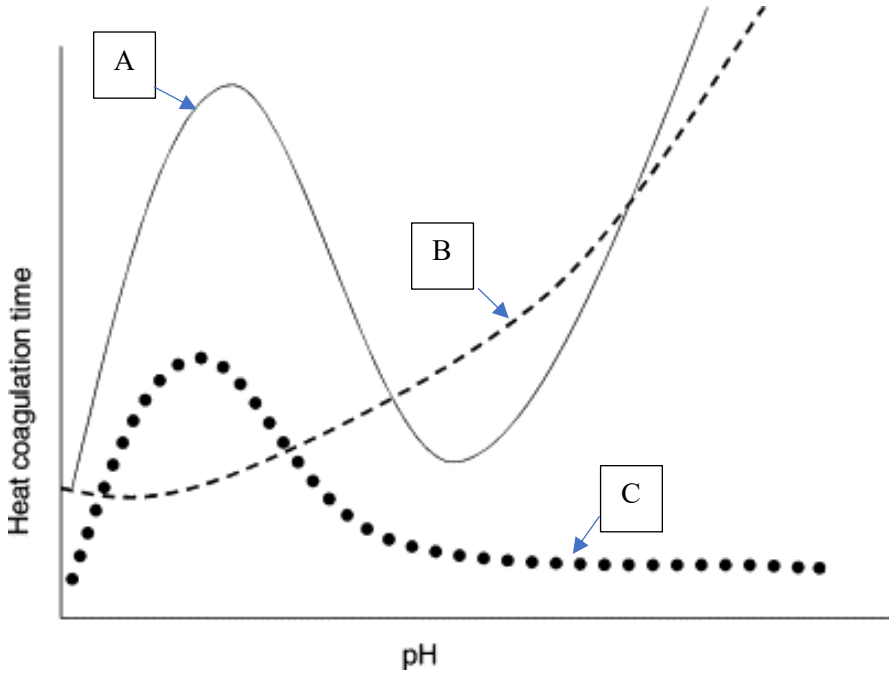


Figure 2.3: HCT-pH profiles of different milk types. **A:** Type A milk at 140 °C; **B:** Type B milk at 140 °C; **C:** Concentrated milk at 120 °C (Fox and Kelly 2004).

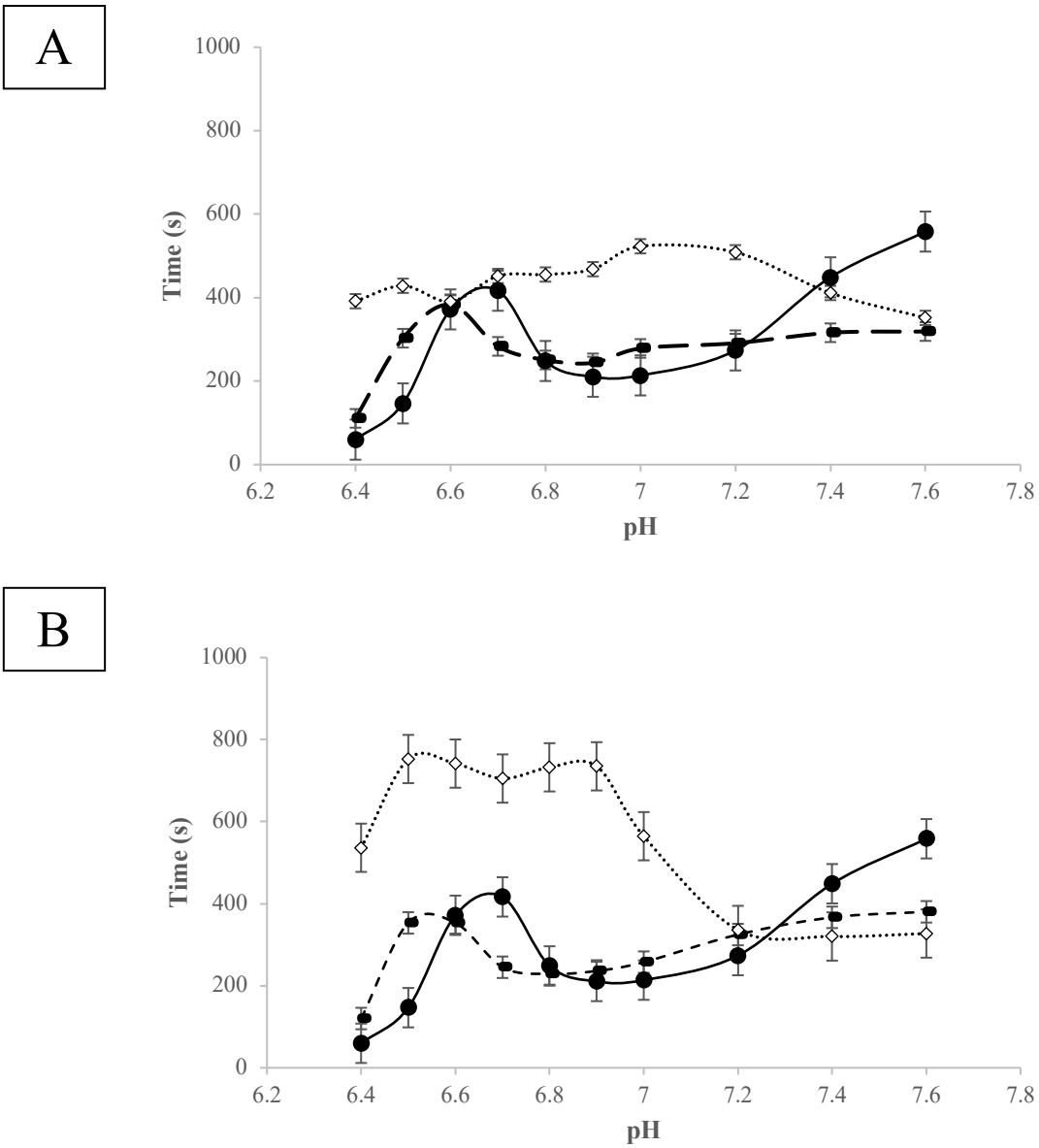


Figure 2.4: Heat stability profiles (at 140°C) of solutions as affected by addition of calcium-chelating salts. **A**: WMP-MPC control (—●—), WMP-MPC with TSC (5 mmol/L) (—■—), WMP-MPC with TSC (20 mmol/L) (···◇···); **B**: WMP-MPC control (—●—), WMP-MPC with DSHP (5 mmol/L) (—■—), WMP-MPC with DSHP (20 mmol/L) (···◇···); **C**: WMP-MPC control (—●—), WMP-MPC with SHMP (2.5 mmol/L) (–▲–), WMP-MPC with SHMP (10 mmol/L) (--◇--). Results are the means of data from three independent trials. Error bars represent the standard error.

C

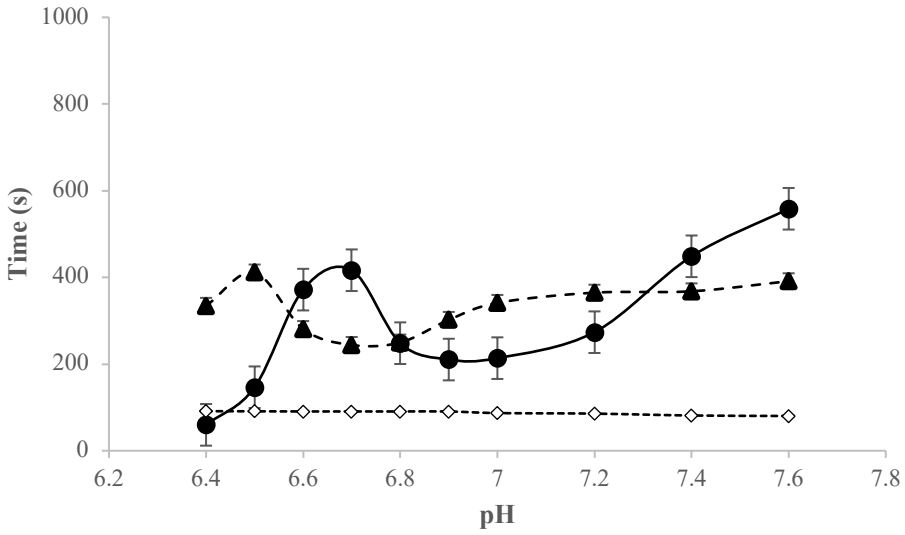


Figure 2.4 (cont.)

The addition of TSC increased heat stability of WMP when added at 20 mmol/L between pH 6.8 and pH 7.4, but did not enhance heat stability when added at 5 mmol/L. The addition of 5 mmol/L TSC shifted the heat stability peak from pH 6.7 to pH 6.6 and reduced heat stability at pH greater than 7.2 (Figure 2.4).

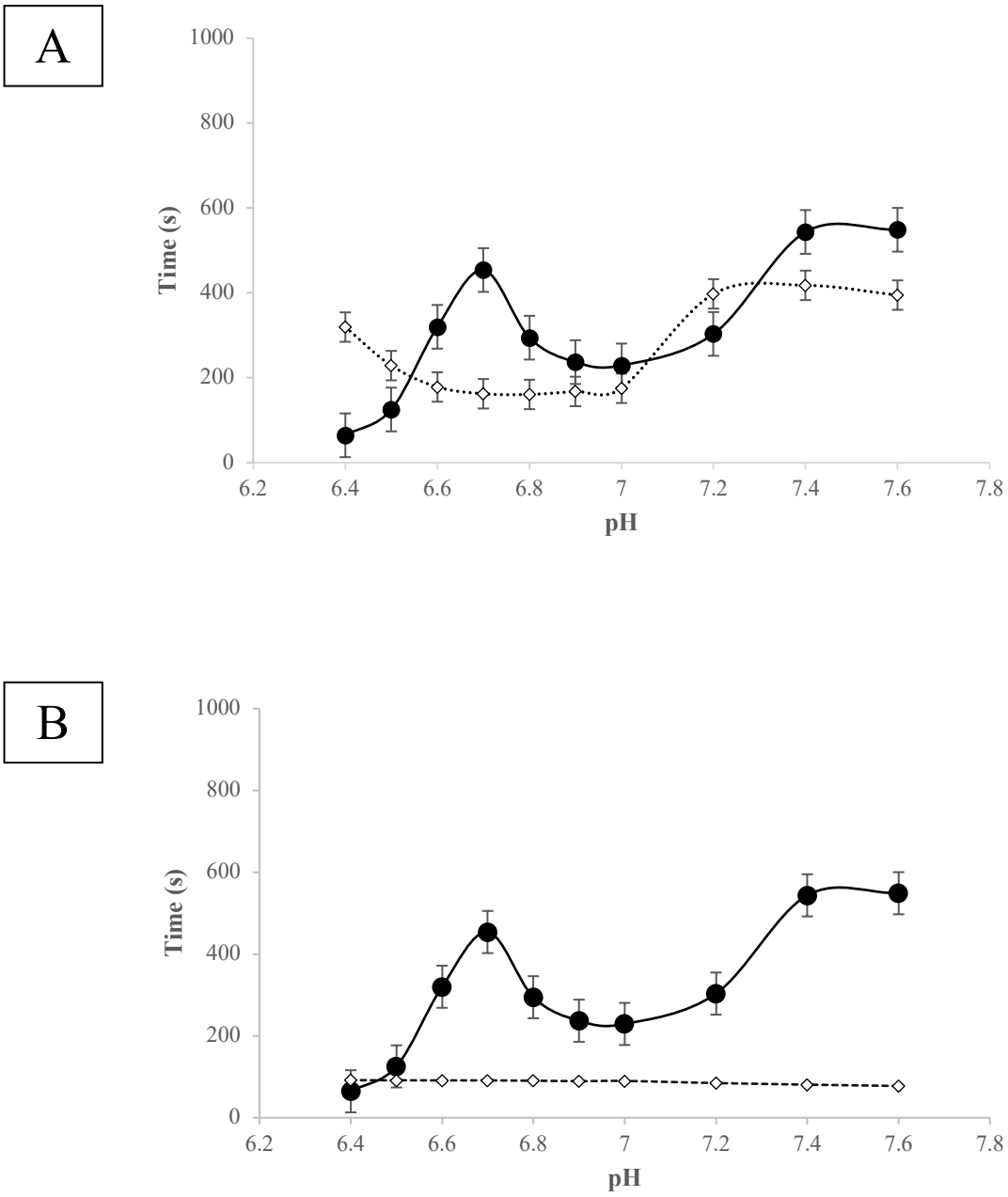


Figure 2.5: Heat stability profiles (at 140°C) of solutions as affected by salt additions. **A**: FFMP-MPC control (—●—), FFMP-MPC with DSHP (20 mmol/L) (···◇···); **B**: FFMP-MPC control (—●—), FFMP-MPC with SHMP (10 mmol/L) (--◇--). Results are the means of data from three independent trials. Error bars represent the standard error.

DSHP added at 20 mmol/L was the most effective (at pH values less than 7.0) at increasing the heat stability of WMP, although there is a dramatic reduction in heat

stability at pH greater than 7.0 (Figure 2.4). This may be due to over-chelation of CCP, which dramatically reduces heat stability of a milk system at pH greater than 7.0, as CCP is very important for micelle integrity, and its removal destabilises the micelle (Rose, 1962; Fox and Hoynes, 1975; Fox and McSweeney, 2003). Addition of DSHP to FFMP had the opposite effect than to its addition to WMP as it reduced heat stability between pH 6.6 and pH 7.0, above which it increased the heat stability (Figure 2.5).

For both the WMP and FFMP samples, SHMP added at 10 mmol/L considerably decreased heat stability and a coagulum formed within 90 s, in contrast with the flecking seen in the other samples (Figure 2.6). This difference in heat stability in comparison with DSHP and TSC may be due to factors other than its chelating capacity, as SHMP also has the ability to induce cross-linking between the casein micelles (de Kort *et al.*, 2012). There was a noticeable colour difference between the samples containing DSHP and SHMP (Figure 2.6); however, this was perhaps simply due to the difference in holding time, as the samples were removed once coagulation had occurred.

A



B

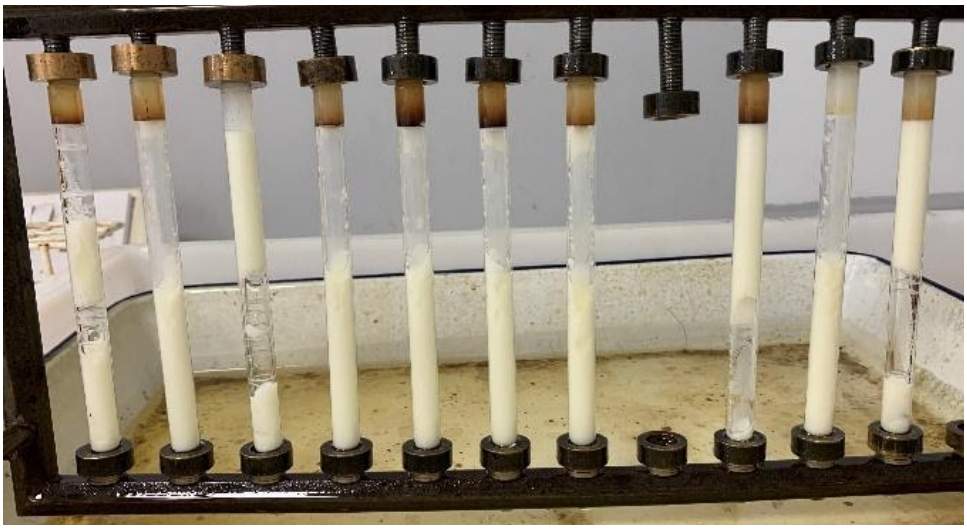


Figure 2.6: Influence of salts on heat stability of WMP. L-R: pH 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.2, 7.4, 7.6. **A:** WMP with DSHP (5 mmol/L). **B:** WMP with SHMP (10 mmol/L).

Addition of phosphate salts (such as DSHP) increases heat stability due to their chelating effect and their buffering capacity (Wang *et al.*, 2016). Citrates are even more important for heat stability, due to its higher solubility at increased temperatures (O'Connell and Fox, 2002).

Tsioulpas *et al.* (2010) reported that the addition of SHMP, TSC, and DSHP into raw skimmed bovine milk resulted in a concentration-dependent decrease in ionic calcium level.

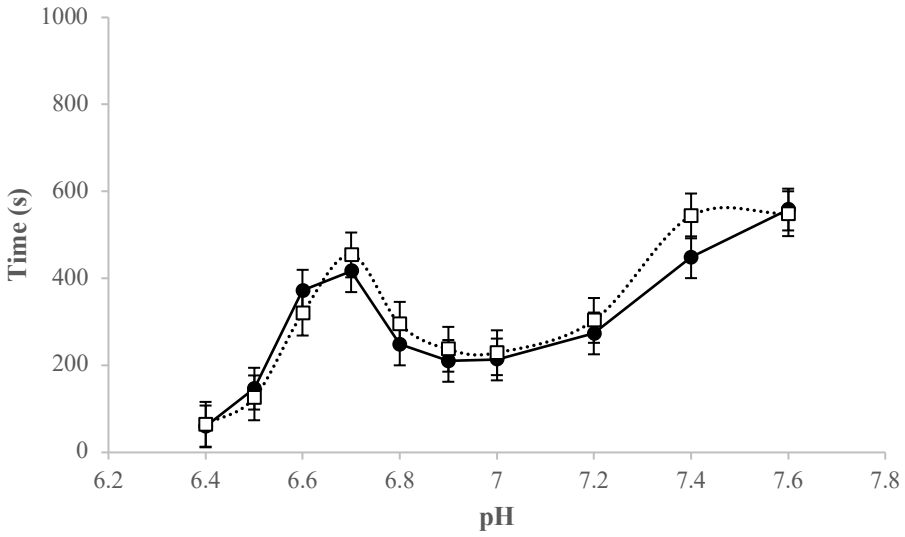


Figure 2.7: Heat stability profiles (at 140°C) of solutions of WMP-MPC (—●—) and FFMP-MPC (····□····).

2.3.2 Apparent viscosity

Viscosity of the samples increased as the pH value increased. As the pH of casein protein solutions moves away from the isoelectric point of casein (pH 4.6), the negative charge on the casein proteins increases, which increases repulsion between the molecules, thereby increasing viscosity. The isoelectric point (pI) of a protein is the pH at which the net charge on that protein is zero (Kelly *et al.*, 2009; Novák and Havlíček, 2016). At pH values less than the pI, casein has a net positive charge, and, at pH values greater than the pI, casein has a net negative charge (Figure 2.8).

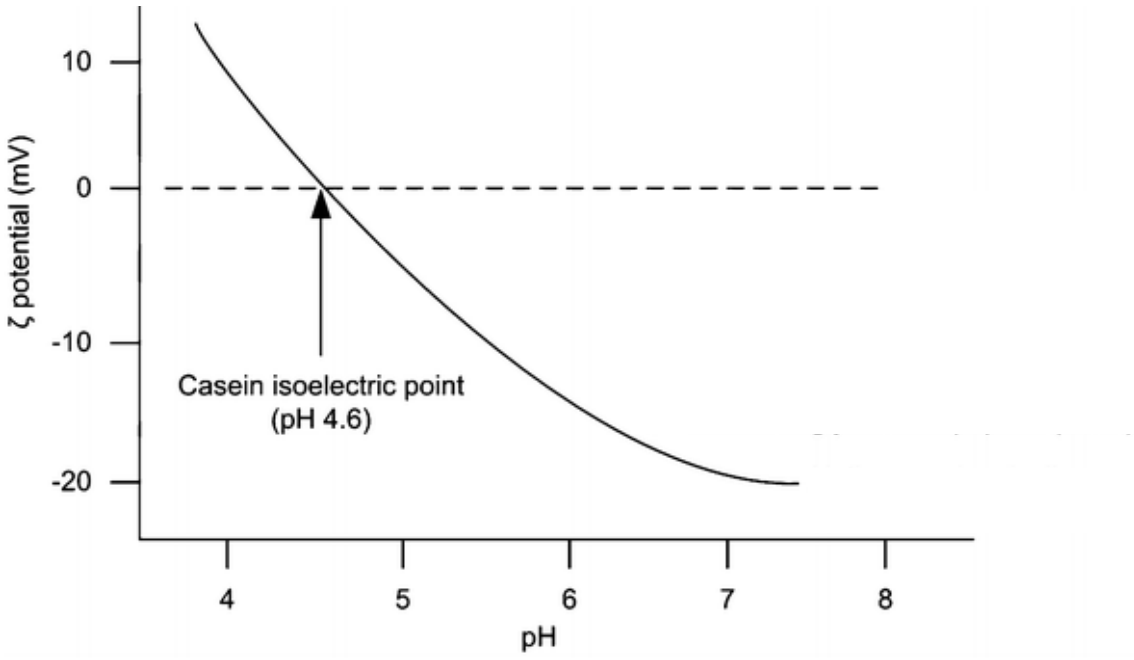


Figure 2.8: Effect of pH on the charge of casein micelles (Carr and Golding, 2015).

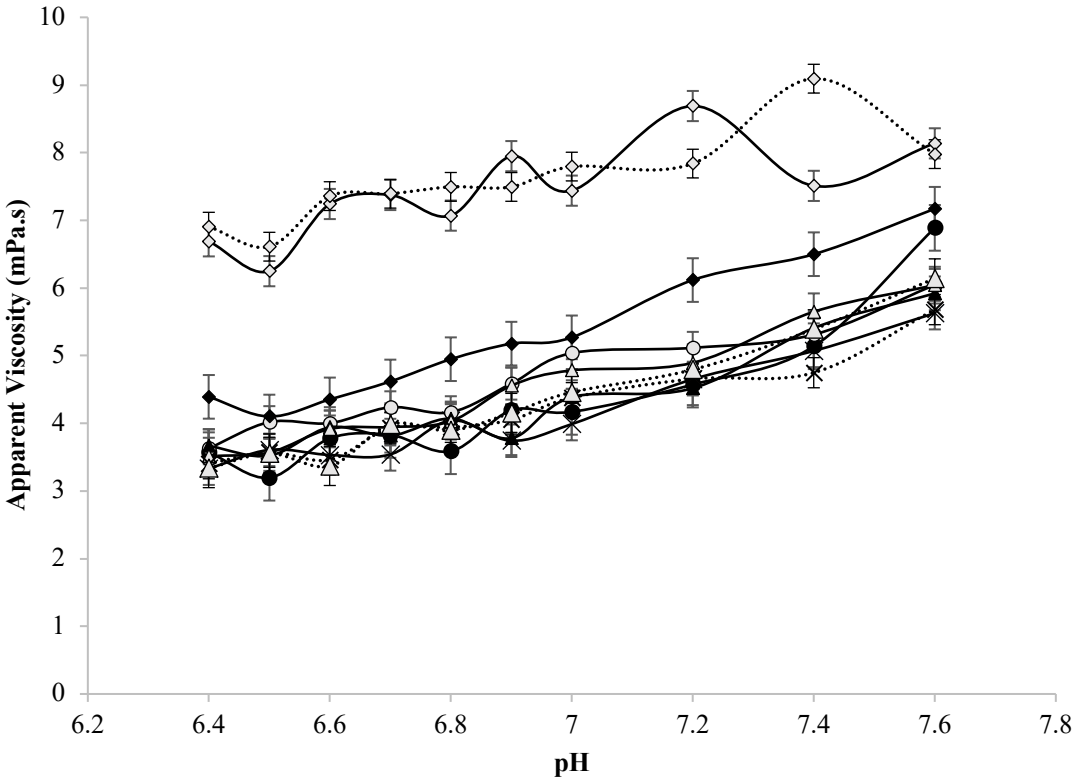


Figure 2.9: Influence of salt type and concentration on apparent viscosity of milk protein solutions prepared as follows: WMP-MPC control (—×—); WMP-MPC with TSC (5 mmol/L) (—●—); WMP-MPC with TSC (20 mmol/L) (—○—); WMP-MPC with DSHP (5 mmol/L) (—▲—); WMP-MPC with DSHP (20 mmol/L) (—△—); WMP-MPC with SHMP (2.5 mmol/L) (—◆—); WMP-MPC with SHMP (10 mmol/L) (—◇—); FFMP-MPC control (···×···); FFMP-MPC with DSHP (20 mmol/L) (···△···); FFMP-MPC with SHMP (10 mmol/L) (···◇···). Results are the means of data from two independent trials.

At pH 6.4, the viscosity of the WMP and FFMP solutions containing SHMP at 10 mmol/L were significantly different to the rest of the samples ($p < 0.05$) (Figure 2.9). However, at pH 7.6, the apparent viscosity of all of the samples did not significantly differ from each other ($p > 0.05$). The viscosity of all the WMP samples increased significantly ($p < 0.05$) as the pH increased, except for the WMP solutions containing 5 mmol/L TSC and 10 mmol/L SHMP. For the samples containing FFMP, only the

viscosity of the sample containing 20 mmol/L DSHP increased significantly between pH 6.4 and 7.6. There was no significant difference at pH 6.4 or pH 7.6 between FFMP and WMP controls, between the samples containing DSHP at 20 mmol/L, or between the samples containing SHMP at 10 mmol/L ($p > 0.05$). Therefore, the fat source apparently did not influence the effects of chelating salts on viscosity.

For the addition of TSC and DSHP at both levels, there was a small increase in the apparent viscosity, which can be attributed to the reduced calcium-ion activity resulting in the casein micelles swelling in size (de Kort *et al.*, 2011). The largest increase in viscosity was seen in the addition of SHMP at 10 mmol/L, which was also observed by de Kort *et al.* (2011) and is most likely due to the cross-linking effect of SHMP (Mizuno and Lucey, 2007).

The higher viscosity of the WMP sample containing the lower concentration of SHMP compared with the non-SHMP-containing samples is also indicative of the effect of casein protein cross-linking on viscosity. The increase in viscosity could also be explained by the swelling of the micelle and increase of electrostatic repulsion, as reported by McCarthy *et al.* (2017). Those authors also reported that a greater addition of SHMP will eventually result in the dissociation of the micelle and a decrease in viscosity. Moderate dissolution of CCP from the micelle is reversible and does not affect the casein micelle, but, if the dissolution is too great, this results in irreversible changes as evidenced by a reduction in casein micelle size (Udabage *et al.*, 2000). Micellar dissociation is a cooperative process that does not occur until a critical level of calcium has been chelated (Pitkowski *et al.*, 2008).

Walstra and Jenness (1984) stated that the primary contributor to viscosity in milk is the casein micelles; dissociation of micelles results in an increase in interaction with

each other, which increases the viscosity. It can be suggested that addition of SHMP dissociated the micelles to the greatest extent as it resulted in the largest increase in viscosity, for both the WMP and the FFMP samples. This is in agreement with the conclusions of de Kort *et al.* (2011), who showed that the highest viscosity of micellar casein dispersions was recorded on the addition of SHMP, in comparison with TSC, DSHP, sodium phytate, and disodium uridine monophosphate. Tsioulpas *et al.* (2010) found that SHMP addition increased calcium micelle size which may account for the increase in viscosity; however, data for casein micelle size was not recorded in this experiment.

2.3.3 Colour

As the fat of LH-SMP is negligibly low (the Codex Alimentarius regulations (1999) dictate that the maximum milk fat permitted in skim milk powder (SMP) is 1%), the most abundant light-scattering particles in such solutions are casein micelles, and so this system was used to evaluate the dissociating effect of each salt on the proteins in the samples. Chelating salts dissociate the micelle which can affect the colour of solutions, depending on what they contain (de Kruif *et al.*, 2012; Thomar and Nicolai, 2015; Li *et al.*, 2018; Silva *et al.*, 2018). The reduction in whiteness (L* value) as the salt levels increased was due to swelling of the micelles, as reported by McCarthy *et al.* (2017) and Power *et al.* (2020). The reduction in L* value may be due to the liberation of the CCP due to the chelating salts, which leads to the disruption of the micelles as also suggested by the visual decrease in turbidity of the LH-SMP samples, which was in agreement with McCarthy *et al.* (2017).

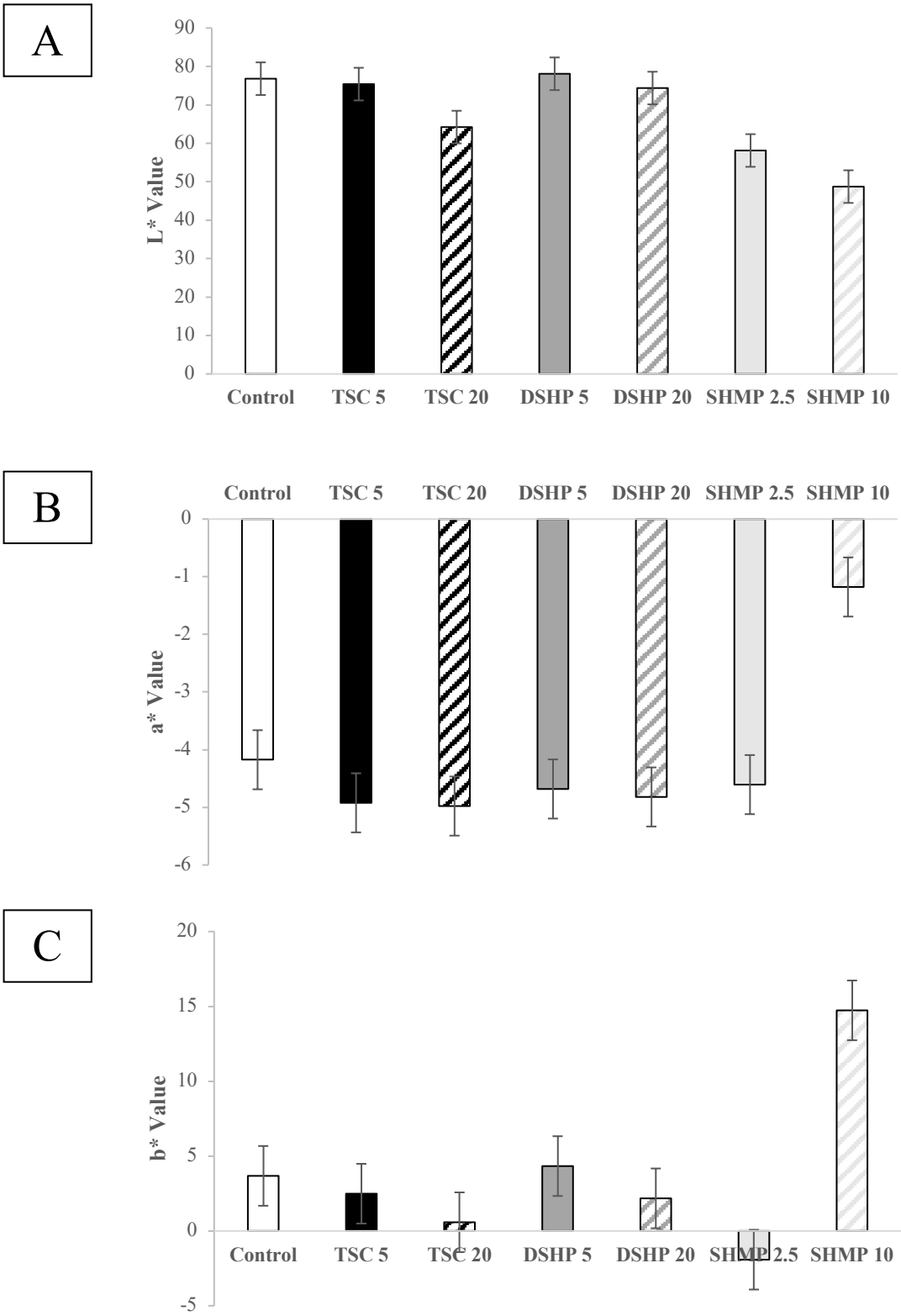


Figure 2.10: L*, a*, and b* colour values of LH-SMP solutions as affected by salt addition. **A:** L* values; **B:** a* values; **C:** b* values. The results are the average of three independent trials.

A



B



C



Figure 2.11: Visual appearance upon salt addition. **A:** (L-R) LH-SMP control, LH-SMP with TSC (5 mmol/L), LH-SMP with TSC (20 mmol/L); **B:** (L-R) LH-SMP control, LH-SMP with DSHP (5 mmol/L), LH-SMP with DSHP (20 mmol/L); **C:** (L-R) LH-SMP control, LH-SMP with SHMP (2.5 mmol/L), LH-SMP with SHMP (10 mmol/L).

Table 2.2: Total colour differences for LH-SMP solutions as influenced by calcium-chelating salt type and addition level. Means which do not share a superscript are significantly different.

Solution	Colour difference (ΔE) ± SD
LH-SMP (control)	0^{a}
LH-SMP + TSC (5 mmol/L)	4.45 ± 2.23^{a}
LH-SMP + TSC (20 mmol/L)	16.7 ± 3.12^{b}
LH-SMP + DSHP (5 mmol/L)	1.43 ± 0.513^{a}
LH-SMP + DSHP (20 mmol/L)	4.89 ± 1.455^{a}
LH-SMP + SHMP (2.5 mmol/L)	29.4 ± 6.79^{c}
LH-SMP + SHMP (10 mmol/L)	32.6 ± 1.73^{c}

DSHP did not significantly ($p > 0.05$) affect the colour of reconstituted LH-SMP. The addition of 5 mmol/L of TSC did not significantly change the colour of the LH-SMP solution; however, there was a significant difference between the colour of the control LH-SMP solution and the LH-SMP solution containing 20 mmol/L of TSC ($p < 0.05$). SHMP had the most significant effect of all salts on the colour of the LH-SMP sample ($p < 0.01$). According to these results, the turbidity was affected in decreasing order of SHMP>TSC>DSHP, which is consistent with the results of Mizuno and Lucey (2005) and de Kort *et al.* (2011).

The influence of SHMP on the colour of the LH-SMP samples suggests that dissociation of the micelles had occurred. However, the viscosity results, in addition to previous research, suggested that the dramatic increase of viscosity in the presence of SHMP is due to the cross-linking that occurs in the presence of SHMP (Mizuno and Lucey, 2007; de Kort *et al.*, 2011). When calcium is chelated, the micelles disperse,

and there is no calcium available to form cross-links (Kaliappan and Lucey, 2011). It is possible that, even though the particle size was reduced, there was an increase in particle-particle interactions and thus an increase in resistance to flow and an increase in viscosity (Hill and Carrington, 2006; Pandalaneni *et al.*, 2018). Another possibility which may explain the increase in viscosity and the micelle dissociation is that introduction of high levels of SHMP also introduced multiple negative charges into the system, increasing the electrostatic repulsion which dispersed the caseins and may reduce movement (Kaliappan and Lucey, 2011).

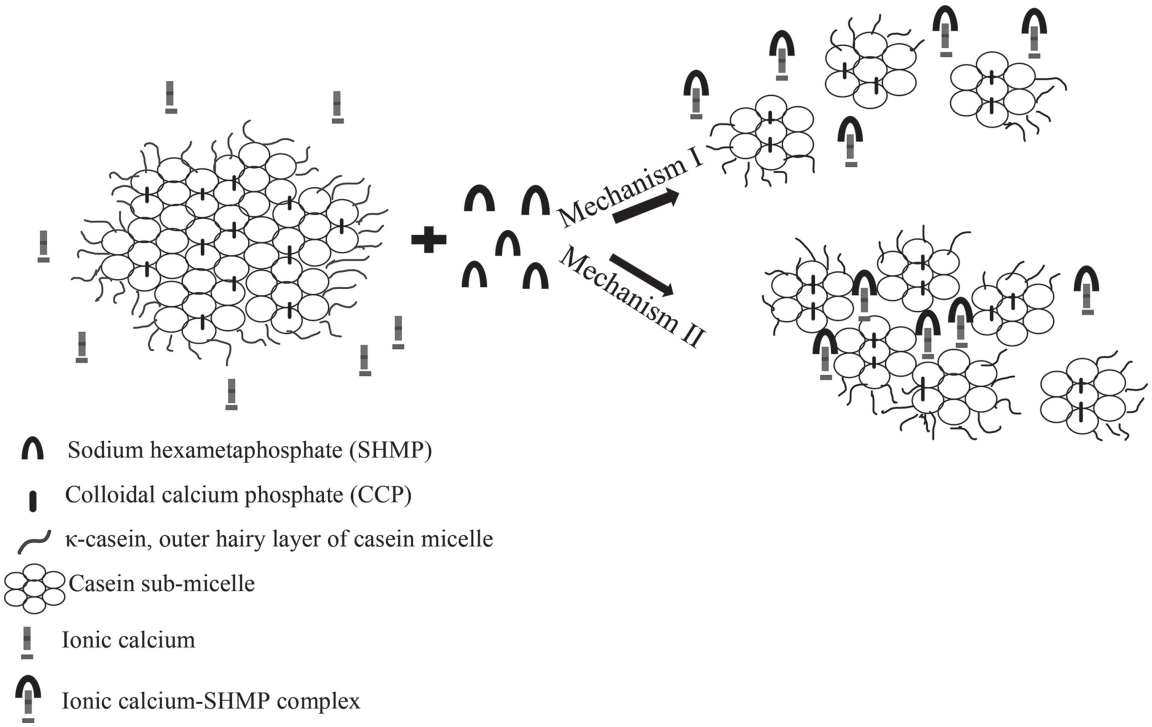


Figure 2.12: Illustration of possible interactions between SHMP and casein micelles. Mechanism I: at low concentrations of SHMP, casein micelle dissociation occurs; Mechanism II: at high concentrations of SHMP, formation of calcium-casein phosphate complexes occurs (McCarthy *et al.*, 2017; Pandalaneni *et al.*, 2018).

2.5 Conclusion

Salt addition in dairy processing is commonly practised to enhance heat stability and for fortification purposes, with the exact choice of salt type and addition level influencing processing, stability and quality. Calcium-chelating salts are added to sequester the free calcium present in a system, whose presence negatively impacts the heat stability of milk. As most milk products undergo some form of heat treatment during processing, the influence of calcium-chelating salts on heat stability is an important factor to account for when formulating such products. It is clear from the results of this study that chelating salts influenced viscosity, colour, and heat stability of the samples in a variety of ways.

DSHP and TSC addition did not significantly affect the viscosity when compared with the control for both FFMP- and WMP-based samples. Viscosity was most affected by the addition of 10 mmol/L of SHMP to both the FFMP- and WMP-based samples. This is possibly due to the cross-links formed only by SHMP and not by DSHP or TSC. However, the colour results of the SHMP suggested the casein micelles are being dissociated by SHMP addition, which would not allow cross-linking to occur. Therefore, the increase in viscosity could be due to the larger number of smaller sized particle (dissociated micelles), which would increase the amount of particle-particle interactions. The increase in viscosity may also be due to the addition of more negative charges into the system which results in dissociation of the micelles.

SHMP had the most pronounced effect on the colour of reconstituted LH-SMP at both levels of addition. This is due to the over-chelation of the calcium from the micelles, reducing their stability and resulting in micelle dissociation. Addition of TSC at 5 mmol/L and DSHP at both levels and did not result in any significant change in colour,

but there was a significant difference in colour upon addition of 20 mmol/L of TSC. Trisodium citrate also dissociates the casein micelles, although to a lesser extent than the effect of SHMP.

At pH less than 7, the addition of DHSP at 20 mmol/L increased the heat stability of the system the most in the WMP solution; however, the same effect was not observed in the FFMP samples. The addition of 20 mmol/L TSC did not result in any major change in heat stability, but it did increase heat stability between pH 6.8 and pH 7.2. High levels of addition of SHMP resulted in the coagulation of the samples within 90 s in the oil bath.

More work needs to be carried out on the addition of SHMP to study its effect in detail, in particular its influence on cross-linking and casein micelle stability.

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Chapter 3

The influence of UHT processing and retort sterilisation on storage stability of reconstituted milk powders

Abstract

The effect of different heat treatment (ultra-high heat treatment (UHT) and retort sterilisation), fat source (whole milk powder (WMP) and fat-filled milk powder (FFMP)), and protein contents on the stability of milk beverages were examined. The protein contents examined were 2.3, 3.3, and 5%, the latter of which were fortified with milk protein concentrate (MPC 80; 80% protein). Heat treatment significantly affected colour, sedimentation (in the WMP-based samples), particle size in samples containing 2.3 and 3.3% protein and apparent viscosity (at 270 d). There was no significant difference in pH (at 270 d), or creaming between any samples, and it did not result in any significant difference in the sedimentation in FFMP-based samples. The fat source significantly affected the b* values of samples, sedimentation in the UHT-treated samples, particle size for the majority of the samples, and the apparent viscosity for most of the samples. The fat source did not significantly affect the pH (at 270 d), creaming of any samples, the viscosity of the 5% protein samples, or the L* or a* values of any of the samples, or the b* values of the higher protein samples. The fat source also did not significantly affect the sedimentation in retort-sterilised samples, or average particle size in the higher protein content samples. Protein content significantly affected the viscosity for all sample types, the average particle size of all solutions containing 5% protein, the pH (at 270 d) of some samples, the creaming, and sedimentation for most of the UHT-treated samples. Protein content did not significantly affect the colour of any samples except for UWMP2.3. In conclusion, heat treatment, fat source, and protein content influenced the stability of the final product.

3.1 Introduction

Heat treatment of milk was first attempted in 1810 by Nicolas Appert (O'Connell and Fox, 2002) as a form of preservation. Today, any milk on the market is subject to at least one form of heat treatment, which is used not only to reduce or eliminate spoilage bacteria and pathogens, but as an integral step in the production of selected products, such as yoghurt. Milk is naturally relatively heat stable, due to the structure and physicochemical properties of the caseins (O'Connell and Fox, 2002); however, challenges can arise during processing and storage. Sterilisation methods include retort sterilisation and UHT processing with the main differences between milk processed by these methods being in its appearance and protein quality, while they both have similar microbial inactivation effects (Bylund, 1995; Takeda *et al.*, 2015).

UHT treatment of milk generally involves temperatures in the range 135-150°C for 2-20 s, followed by aseptic packaging (Hinrichs and Atamer, 2011). This short intense application of heat, accompanied by aseptic packaging, ensures commercial sterility without a substantial loss of nutritional or sensory quality, especially when compared with retort sterilisation (Newstead *et al.*, 2006; Popov-Raljić *et al.*, 2008; Deeth and Datta, 2011; Anema, 2017; Anema, 2019). The demand for UHT-treated milk is increasing due to its long shelf life (at least six months), and the fact that it does not require refrigeration during transport or storage, which makes it very suited for developing and more tropical countries (Zhang *et al.*, 2018). A common challenge with UHT-treated milk-based beverages is gelation, which is due to residual enzyme activity even after UHT treatment (Datta and Deeth, 2001; Newstead *et al.*, 2006; Chavan *et al.*, 2011; Anema, 2017; Anema, 2019). This is a well-documented area;

however, the exact mechanism responsible for age gelation is still not entirely understood. Sedimentation and creaming (fat separation) can also occur during storage of UHT-treated milk (Dalgleish, 1992; Grewal *et al.*, 2017; D'Incecco *et al.*, 2018; Gaur *et al.*, 2018; Anema, 2019).

Retort sterilisation is another form of heat treatment used in milk processing, which is designed to result in a 10-log reduction in the number of bacteria in milk, with destruction of bacterial spores (Walstra, 1999; Hinrichs and Atamer, 2011). It is a slower process, applied at a lower temperature than UHT, with time-temperature combinations of 110-20°C for 10-40 min, applied to products in an in-container format. The resulting products are quite different to those processed using UHT treatment and this is mainly due to the Maillard reaction, the rate and extent of which depends on the exact temperature and duration of heating (van Boekel, 1998; Takeda *et al.*, 2015).

In this chapter, reconstituted milk powders were subjected to two high heat treatments (UHT and retort sterilisation) at various protein contents, and their stability was determined over nine months following processing. The source of fat was also compared, as the fat in WMP is from bovine milk, whereas the fat in the FFMP is plant-based. The colour, particle size, emulsion stability, apparent viscosity, and pH were monitored throughout storage to determine the influence of fat source and type on quality of resulting sterilised milk-based beverages.

3.2 Materials and methods

3.2.1 Milk powders

Fat-filled milk powder (FFMP), whole milk powder (WMP) and milk protein concentrate (MPC 80) powder were supplied by local dairy ingredient companies based in Ireland. Compositional analysis was carried out by the suppliers and the results can be found in Table 2.1 in Section 2.2.1.

3.2.2 Reconstitution

Powders were reconstituted to the target protein content in deionised water at 44°C. The powder was dispersed in the water using a Silverson L5M-A high shear mixer (Silverson Machines Inc., East Longmeadow, MA 01028) at 3000 rpm until all of the powder was visibly dispersed. The dispersion was then cooled to room temperature using an ice bath and the pH of the solution was measured and adjusted to pH 6.7 using a caustic solution and maintained at 4°C overnight with gentle mixing using an overhead stirrer to facilitate complete rehydration. After overnight storage, the solutions were heated in a water bath to 50°C before homogenisation. There were 12 samples in total per trial, and the trials were performed in triplicate without variation in process or sample composition. The 12 samples are listed in Table 3.1.

Table 3.1: Detailed description of samples prepared for each trial. Note: the only difference in composition between the 3.3% and 5% protein samples was the addition of MPC 80.

Sample number	Protein content	Powders used	Heat Treatment	Code
1	2.3	WMP	UHT	UWMP2.3
2	3.3	WMP		UWMP3.3
3	5	WMP + MPC		UWMP5
4	2.3	FFMP		UFFMP2.3
5	3.3	FFMP		UFFMP3.3
6	5	FFMP + MPC		UFFMP5
7	2.3	WMP	Retort	RWMP2.3
8	3.3	WMP		RWMP3.3
9	5	WMP + MPC		RWMP5
10	2.3	FFMP		RFFMP2.3
11	3.3	FFMP		RFFMP3.3
12	5	FFMP + MPC		RFFMP5

3.2.3 UHT and retort sterilisation

Homogenisation of the solutions was performed before heat treatment, using an APV Lab1000 homogeniser (SPX FLOW Technology, Denmark) at 180 bar. The samples were then split for heat treatment. UHT processing was performed using a pilot scale indirect UHT plant (MicroThermics HTST/UHT Lab Electric Model 25DH, MicroThermics, Inc. North Carolina, US). The solutions were preheated at 90°C for 30 s, followed by final heat treatment at 138°C for 3-4 s. The solutions were then

cooled to 10-20°C and packaged in sterile plastic containers under a laminar flow clean-fill hood. The flow rate for this process was 1 L/min.

Retort sterilisation was performed using a Surdry rotary retort (APR-95-SV, Surdry, Abadiano, Vizcaya, Spain) in rotational full immersion mode. The samples were pre-filled in glass jars with roll-on roll-off lids. The jars were then loaded into the rack and fitted into the retort. The jars were heated at 120°C for 20 min, and held for 20 min, followed by 20 min of cooling to approximately 30°C.

Both UHT treated and retort sterilised samples were stored at room temperature in the dark and monitored during storage. This procedure was followed for all three trials.



Figure 3.1: Images of containers used in trials. **A**: Plastic sterile containers used for UHT samples. **B:** Glass jars used for retort samples.

3.2.4 Colour

The colour of the samples was measured using a calibrated Minolta Chroma Meter CR-400 colorimeter (Minolta Ltd., Milton Keynes, UK). The colorimeter was calibrated with a white standard tile before analysis and the sample was poured into and measured in a quartz cuvette. Each sample was measured five times, from which the average was calculated. Colour was recorded using the CIE L* a* b* units and total colour difference (ΔE) was calculated using the following equation (Kelleher *et al.*, 2019; Power *et al.*, 2020):

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2}$$

where L_0, a_0 and b_0 denote the colour of the samples at 0 d.

Colour was measured for each trial, after which the average was calculated from the three trials.

3.2.5 pH

The pH of the all samples was measured and recorded using a pH meter. The pH meter was calibrated using standard pH 4 and pH 7 solutions from Lennox (Naas Road, Dublin, Ireland).

3.2.6 Emulsion and storage stability

An analytical centrifuge (LUMiSizer, L.U.M. GmBH, Berlin, Germany) was used to measure the emulsion stability of the samples and to predict its shelf life with respect to physical stability. Aliquots (0.4 ml) were placed in polycarbonate cells (PC 110-131XX; L.U.M. GmBH, Berlin, Germany) and centrifuged at 3500 rpm for 5 h and 50

min at 20°C. Each sample was measured in triplicate every month. This was performed for each trial, and the average values were calculated.

3.2.7 Determination of particle size distribution

The particle size of the samples was measured using a Malvern Mastersizer (Mastersizer 3000; Malvern Instruments Ltd., Worcestershire UK) static light-scattering instrument equipped with a Hydro MV wet cell. The refractive index for the dispersant and the particles chosen were 1.33 and 1.46, respectively (McCarthy *et al.*, 2012). Laser obscuration was approximately 12%. Each sample was measured in triplicate every month and this analysis was performed for each trial, after which the average for each month was calculated. The average volume mean diameter (D_{50}) is used as the average particle size (Avsar, 2010).

3.2.8 Measurement of apparent viscosity

Apparent viscosity of each sample was determined using a HAAKE Roto-Visco rotational viscometer (Thermo Electron, GmbH, Karlsruhe, Germany) at 20 ± 0.5°C using the double gap concentric cylinder and DG43 cup attachments. Samples were measured as per Section 2.2.4. Each sample was measured once, and an average of each sample was taken across the three trials.

3.2.9 Protein profiling by electrophoresis

Protein profile and hydrolysis were observed by performing electrophoretic analysis on samples which had been held in frozen storage (-20°C). The loading volumes used

for urea-PAGE and SDS-PAGE were 6 and 10 μL respectively. The staining method followed for the urea-PAGE gels was as described by Blakesley and Boezi (1977). SDS-PAGE was performed on Bio-Rad Mini-PROTEAN® TGX™ pre-cast mini-gels and samples (5 μL) were loaded under reducing and non-reducing conditions. The method followed was as described by Andrews (1986) and the SDS-PAGE gels were destained as described by Anema (2017). Representative gels are shown in Section 3.7.

3.2.10 Statistical data analysis

All samples were prepared in three independent trials and statistical analysis was carried out using Minitab® 19 (Minitab Ltd., Coventry, UK) using one-way analysis of variance (ANOVA) at a 95% confidence interval. The Tukey *post-hoc* pairwise comparison test was used to determine significant differences ($p < 0.05$) between mean values for different protein contents.

3.3 Results and discussion

The results indicate that the type of heat treatment, the protein content, and the fat source all significantly influenced the stability and the properties of the samples. Directly post-processing, there were colour differences between samples, which were studied by colourimetry, and these were monitored during storage (Section 3.3.1). No gelation was observed in any samples at nine months of storage. Age gelation is a common issue which is encountered in UHT products; however, the lack of gelation may be due to the fat content of the products; Chavan *et al.* (2011) attributed this to the reduced plasmin and exogenous enzyme action in whole milk and fat hindering the access of the enzymes to their casein substrates. However, non-enzymatic age gelation could still occur after 12 months (McMahon, 1996; Anema, 2019).

3.3.1 Colour

L* values of the UHT-treated samples were higher than those of the retort-sterilised samples. This lower L* value in the retort-treated samples is due to increased heat load to which the samples were exposed (120°C for 20 min versus 138°C for 3 s). This increased the extent of the Maillard reaction in the retort samples (Anema, 2019). The Maillard reaction is a non-enzymatic reaction which occurs at high temperatures (i.e., above 100°C) involving amino groups and reducing sugars, such as lactose. This results in the production of aromatic compounds as well as brown polymers, which impart a darker colour (i.e., reduced L* value) on the product when present (Bylund, 1995; Nursten, 2005).

The L* values observed indicated that overall, the retort-sterilised samples were less bright than the UHT-treated samples. The negative a* values observed in the samples indicated that they were more green than red in appearance. The positive b* values indicated the samples were more yellow than blue. According to Dufossé and Galaup (2010), the main components of milk influencing the a* and b* values are riboflavin (green), β-carotene (yellow), and lutein (yellow). Riboflavin is a natural photosensitiser, which means that it absorbs light energy, generates reactive oxygen species and improves the degradation of organic compounds (Meng *et al.*, 2019). Carotenoids are degraded by high temperatures (Dufossé and Galaup, 2010); thus, one could expect that samples post-heat treatment would be less yellow than their unprocessed counterparts. However, the Maillard reaction (non-enzymatic browning) may mask this effect.

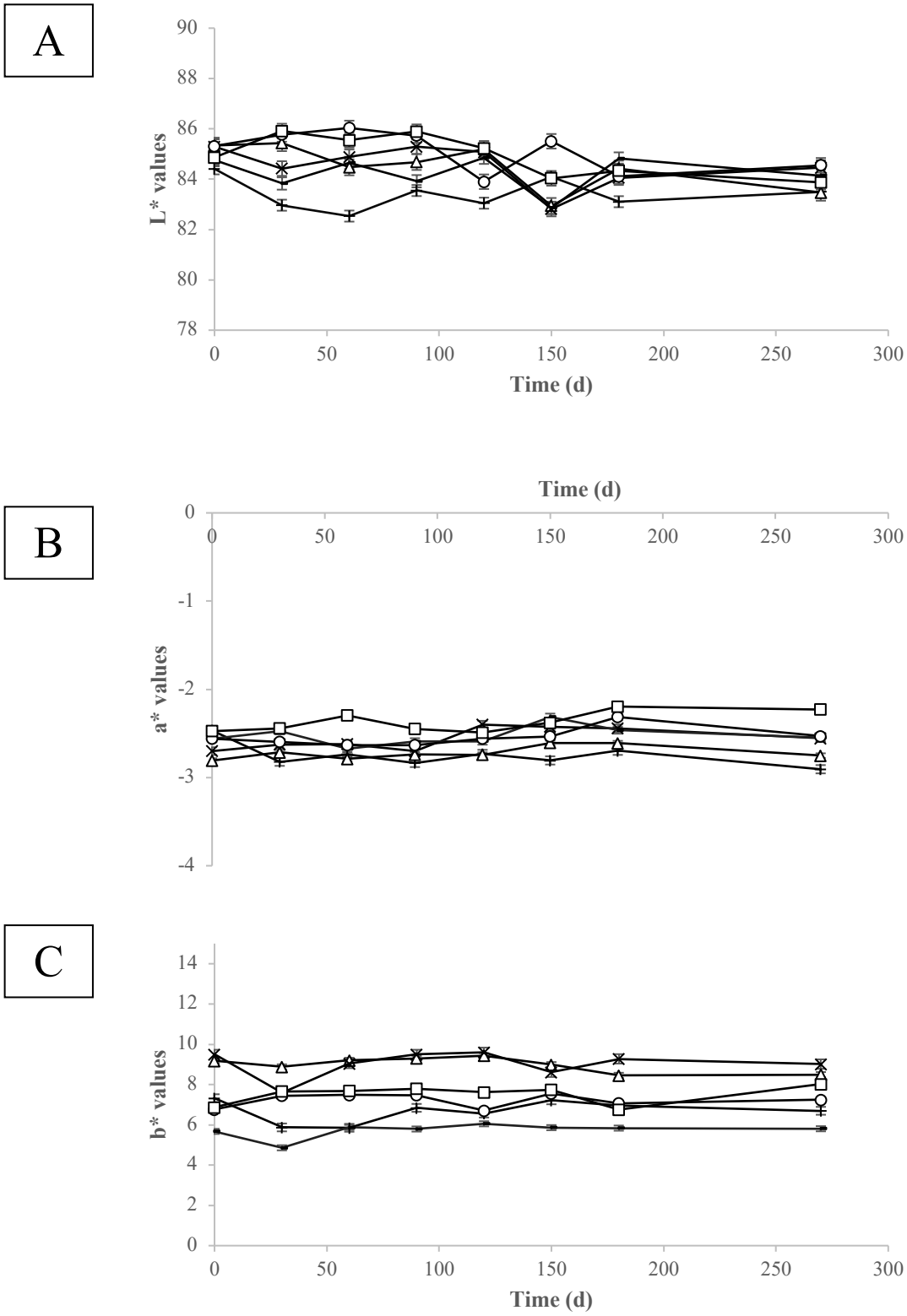


Figure 3.2: L*a*b* values of UHT-treated samples. **A**: L* values; **B**: a* values; **C**: b* values. UWMP2.3 (—+—), UWMP3.3 (—△—), UWMP5 (—×—), UFFMP2.3 (——), UFFMP3.3 (—○—), UFFMP5 (—□—). The results are the average of three independent trials.

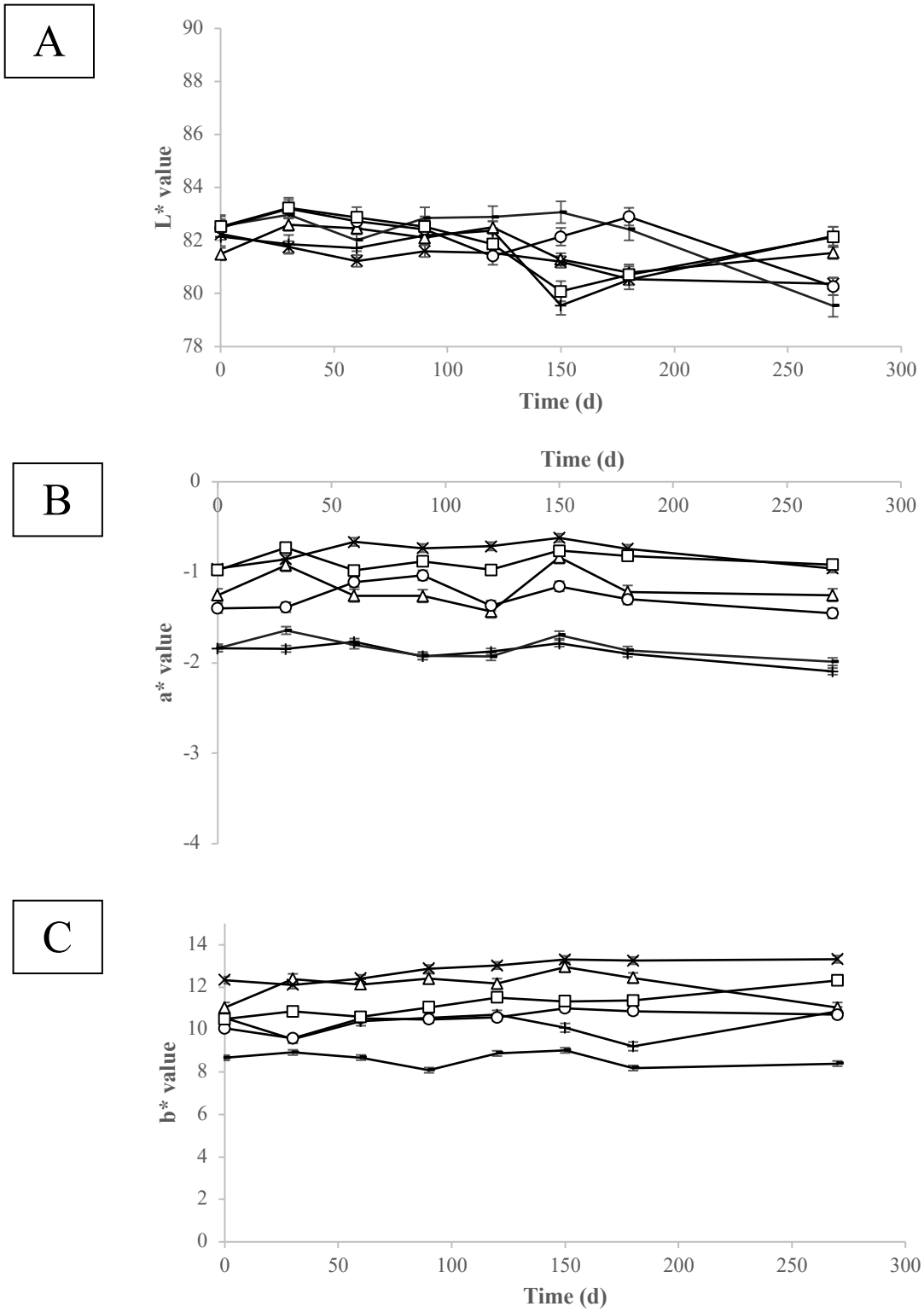


Figure 3.3: L*a*b* values of retort-sterilised samples. **A**: Retort L* values; **B**: Retort a* values; **C**: Retort b* values. RWMP2.3 (—+—), RWMP3.3 (—△—), RWMP5 (—×—), RFFMP2.3 (——), RFFMP3.3 (—○—), RFFMP5 (—□—). The results are the average of three independent trials.

Fat source influenced the colour of the samples, with the initial L* value being lower in reconstituted WMP samples compared with their FFMP counterparts. The colour of the milk fat depends on its content of carotene (Bylund, 1995; Dufossé and Galaup, 2010). While the source of fat did not result in a significant difference between the L* values or between a* values of the samples ($p > 0.05$), the b* values were significantly different between the corresponding samples (for example: UWMP2.3 and UFFMP2.3) ($p < 0.05$), except for between RWMP3.3 and RFFMP3.3 and between RWMP5 and RFFMP5 ($p > 0.05$). The differences observed between the b* value for the majority of the samples may be attributed to the presence of β-carotene in bovine milk fat (Parodi, 2009). The b* value is used most frequently to study the difference in animal fat colour as this reflects the carotenoid concentration (Strachan *et al.*, 1993; Moloney *et al.*, 2013).

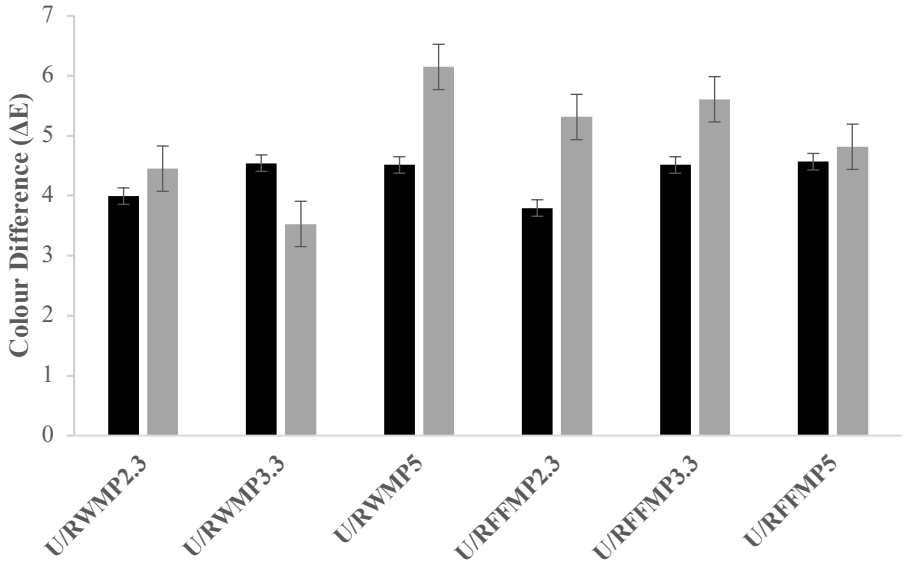


Figure 3.4: Total colour difference (ΔE) between heat treatments on 0 d and 270 d. Each bar represents the difference between each sample as treated by UHT and retort sterilisation. 0 d: (■); 270 d: (■).

The colour of all of the samples was consistent over time as there was no significant difference between the colour of each sample at 0 and 270 d ($p > 0.05$). In contrast, there has been reported to be a reduction in L* in UHT products over time, which is attributed to the degradation of tryptophan (Anandh *et al.*, 2014). Sunds *et al.* (2018) examined the effect of temperature on the rate of the Maillard reaction in UHT-treated samples, and reported that noticeable colour changes (due to the Maillard reaction) only occurred if the sample storage temperature was above 30°C. As the samples in this trial were stored at room temperature, this progression of the Maillard reaction was not observed.

Heat treatment significantly influenced the colour of the samples as measured on 0 d. The L* values in all corresponding samples (for example UWMP2.3 and RWMP2.3) were significantly different ($p < 0.05$), except there was no significant difference between UFFMP5 and RFFMP5 ($p > 0.05$). Heat treatment did not result in a significant difference in the a* values between the corresponding samples ($p > 0.05$), the exception being between samples UWMP5 and RWMP5, where there was a significant difference ($p < 0.05$). Heat treatment also resulted in a significant difference in the b* value between corresponding samples ($p < 0.05$) except for between UWMP3.3 and RWMP3.3. According to Burton (1959), in both UHT processing and retort sterilisation, liquid milk become whiter in appearance, whereas Maillard browning only occurs in in-container sterilisation.

Since both the casein micelles and fat globules deflect light in milk, the influence of solely the protein content cannot be accurately observed in these samples (Bylund, 1995; Dufossé and Galaup, 2010; Dimitrova, 2014). Protein content did not result in significant differences between most of the samples ($p > 0.05$), except for between the

UHT-treated WMP-based samples, where the L* and b* values of the UWMP2.3 were significantly different to the other two samples.

3.3.2 pH

Heat treatment did not significantly affect the pH of the samples as there was no significant difference between the pH of the samples at 270 d which were processed using the different heat treatments (e.g., no significant difference between UWMP2.3 and RWMP2.3; $p > 0.05$). There was no significant difference in pH between the WMP samples at the end of the storage trial. The pH of UFFMP5 was significantly different to UFFMP2.3 and UFFMP3.3 at 270 d. For the retort-sterilised FFMP samples, the pH of RFFMP2.3 was significantly different to the pH of RFFMP3.3 and RFFMP5.

The fat source did not result in a significant difference ($p > 0.05$) in pH between the corresponding samples (e.g., no significant difference between UWMP2.3 and UFFMP2.3).

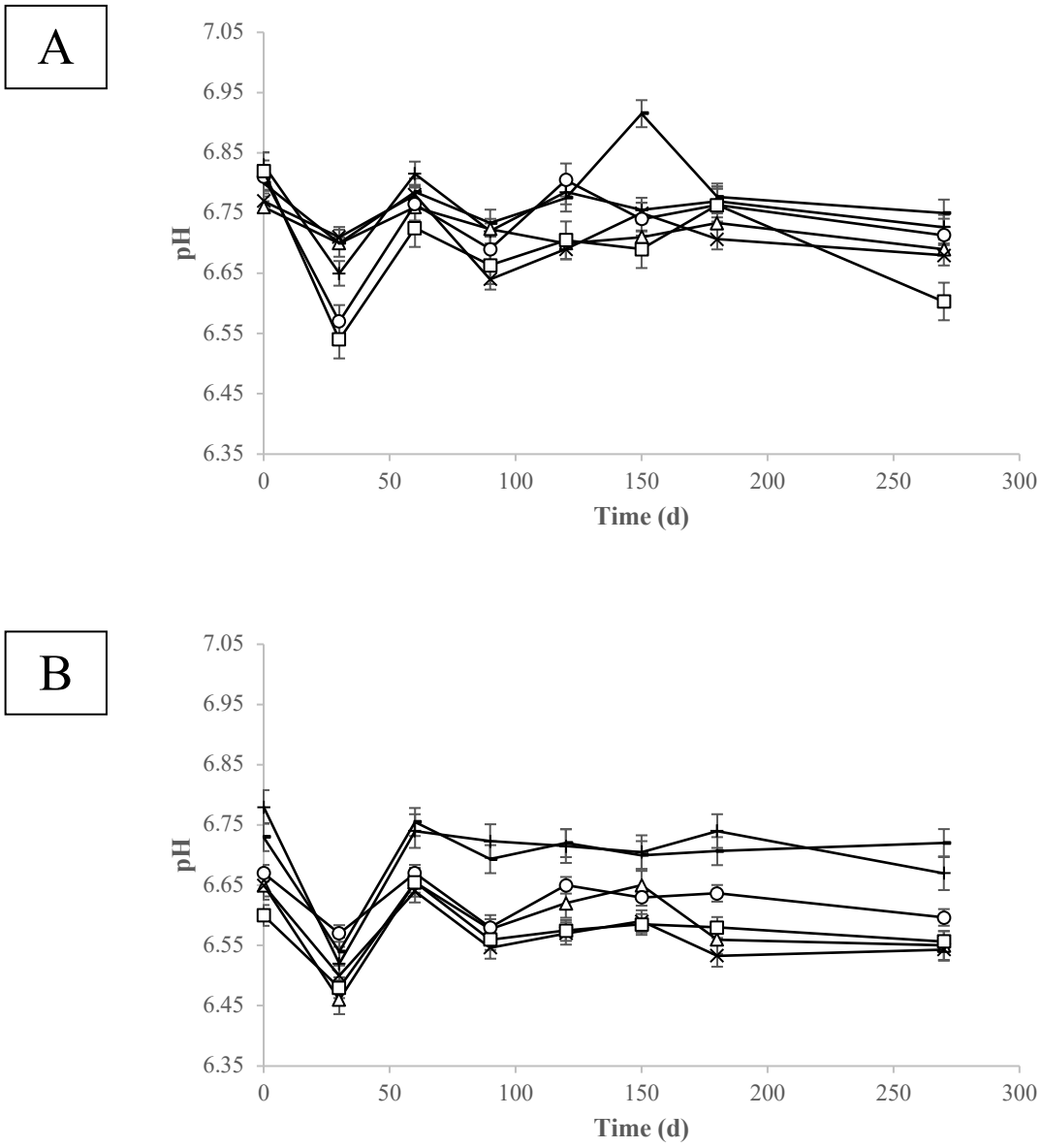


Figure 3.5: pH of UHT and retort samples during storage. **A**: UHT samples pH values; **B**: Retort samples pH values. U/RWMP2.3 (—+—), U/RWMP3.3 (—△—), U/RWMP5 (—×—), U/RFFMP2.3 (——), U/RFFMP3.3 (—○—), U/RFFMP5 (—□—). The results are the average of data from three independent trials.

3.3.3 Emulsion and storage stability

The integral transmission of a solution represents the separation occurring in the sample, which includes both creaming and sedimentation (O'Sullivan *et al.*, 2018). In this study, there was a trend of reduced transmission and, thus, higher stability in samples with higher protein content. Upon homogenisation, milk proteins adsorb to the surface of the newly formed smaller milk fat globules (McCarthy *et al.*, 2012). As protein content reduced, the transmission of the solution increased. This is due to the reduced protein available for fat emulsification. As a result, the samples with reduced protein content showed increased transmission, which represents the separation occurring in the sample due to sedimentation and creaming (O'Sullivan *et al.*, 2018).

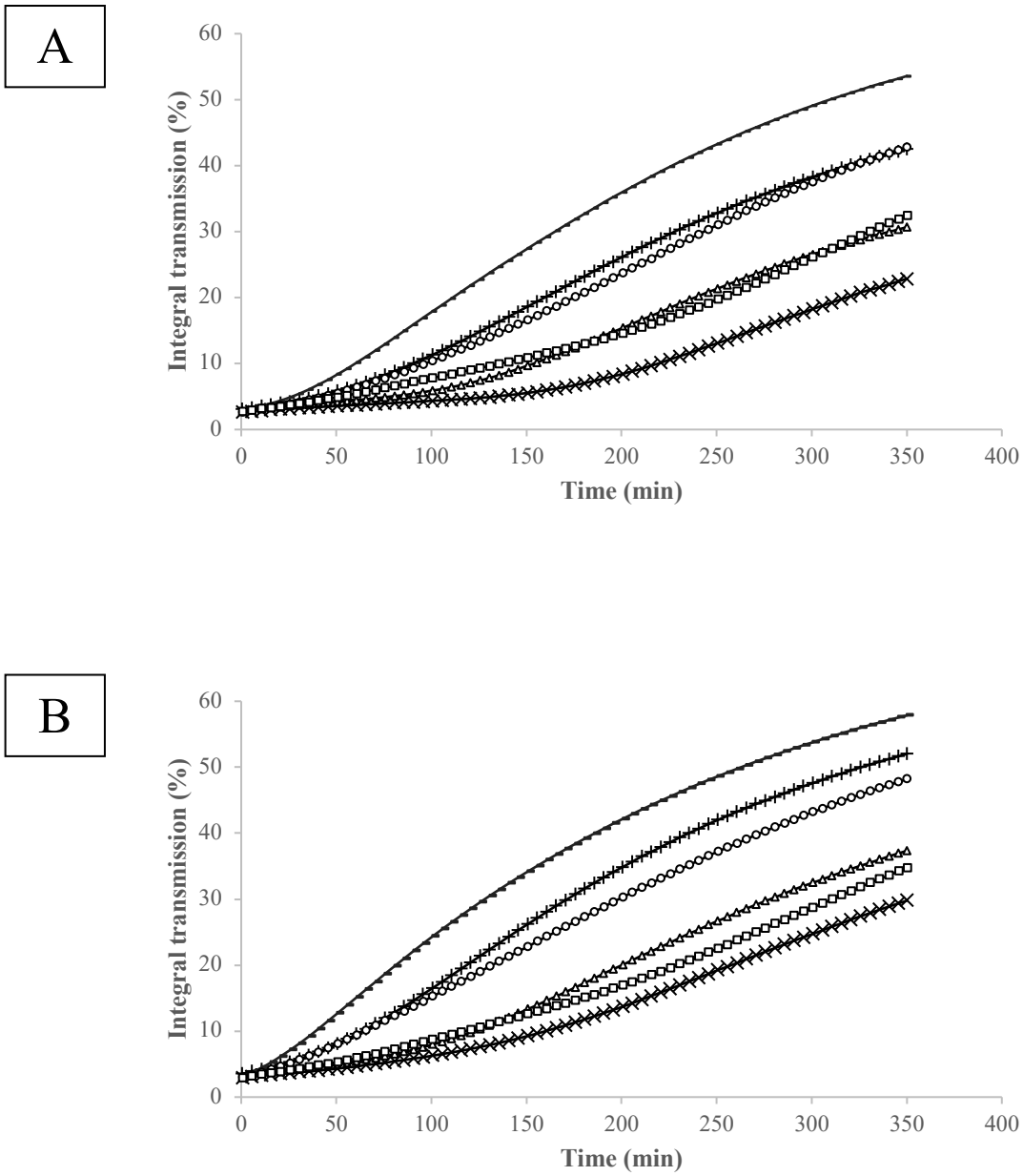


Figure 3.6: Integral transmission of UHT samples during storage. **A**: 0 d; **B**: 270 d. UWMP2.3 (—+—), UWMP3.3 (—△—), UWMP5 (—×—), UFFMP2.3 (——), UFFMP3.3 (—○—), UFFMP5 (—□—). The results are the average of data from three independent trials.

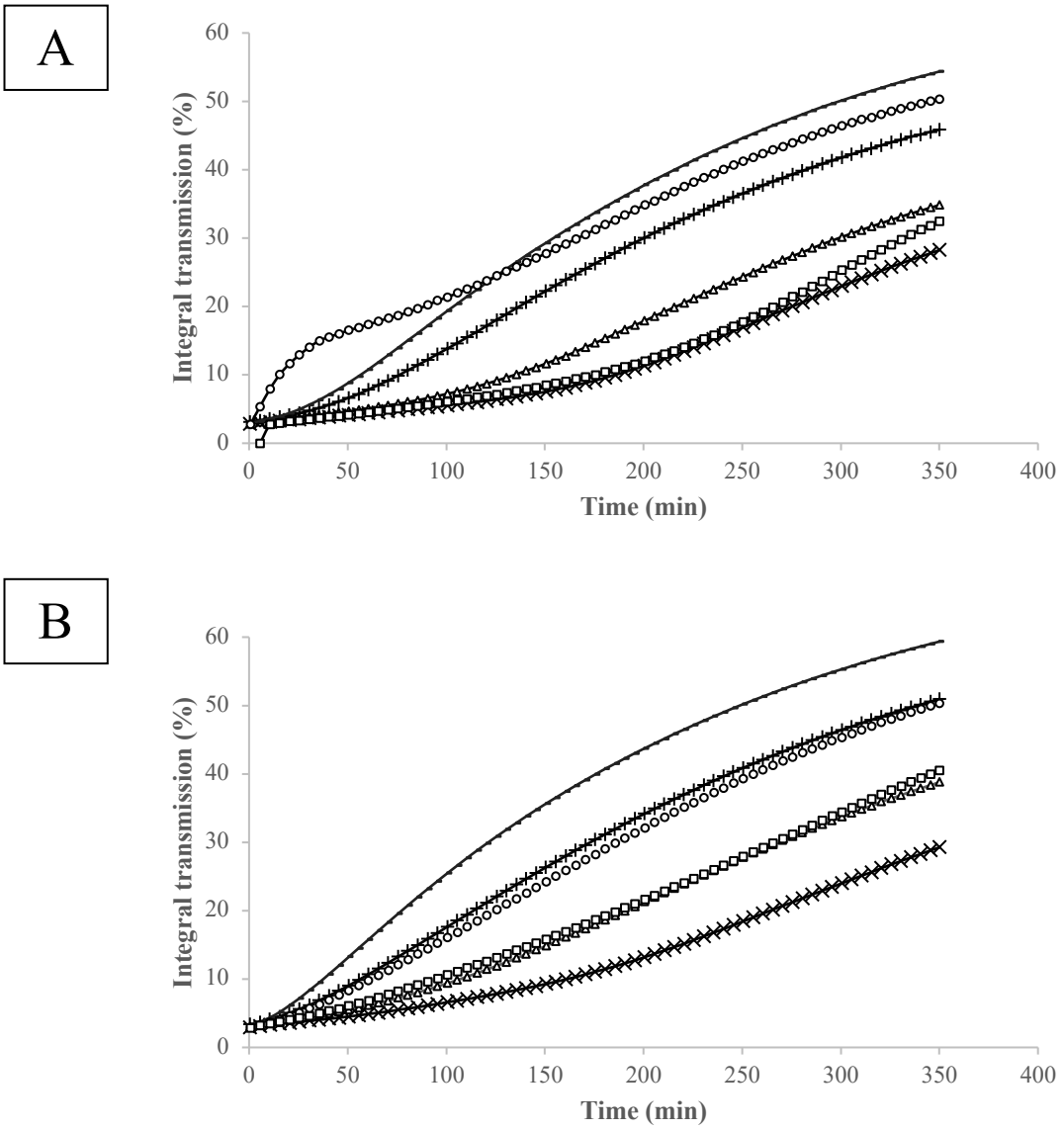


Figure 3.7: Integral transmission of retort samples during storage. **A**: 0 d; **B**: 270 d. RWMP2.2 (—+—), RWMP3.3 (—△—), RWMP5 (—×—), RFFMP2.2 (——), RFFMP3.3 (—○—), RFFMP5 (—□—). The results are the average of data from three independent trials.

A

Table 3.2: Concentrated layer formed during centrifugation. **A**: Creaming layer; **B**: Sedimentation layer.

SAMPLE	CREAMING ± SD (MM)					
	0 d	**30 d**	**60 d**	**90 d**	**180 d**	**270 d**
UWMP2.3	0.78 ± 0.11	0.65 ± 0.24	0.82 ± 0.26	0.91 ± 0.09	1.00 ± 0.20	0.83 ± 0.36
UWMP3.3	1.66 ± 0.57	1.43 ± 0.87	2.76 ± 0.42	2.68 ± 0.58	2.38 ± 0.99	2.31 ± 1.35
UWMP5	2.20 ± 0.35	2.40 ± 0.78	2.95 ± 0.12	3.29 ± 0.47	3.58 ± 0.37	3.28 ± 0.30
UFFMP2.3	0.80 ± 0.10	0.72 ± 0.23	1.22 ± 0.73	1.00 ± 0.17	1.25 ± 0.27	0.89 ± 0.11
UFFMP3.3	1.18 ± 0.14	1.52 ± 0.17	1.99 ± 0.66	2.11 ± 0.48	1.62 ± 0.57	1.34 ± 0.31
UFFMP5	1.79 ± 0.75	2.65± 0.46	2.76 ± 0.32	2.84 ± 0.22	2.24 ± 0.91	3.18 ± 0.81
RWMP2.3	0.86 ± 0.13	0.79 ± 0.28	0.88 ± 0.09	1.13 ± 0.48	0.90 ± 0.53	1.04 ± 0.08
RWMP3.3	1.66 ± 0.33	2.29 ± 0.41	2.33 ± 0.36	2.20 ± 0.51	2.39 ± 0.19	2.18 ± 0.51
RWMP5	2.30 ± 0.45	2.44 ± 0.31	2.84 ± 0.60	2.76 ± 0.65	3.07 ± 0.11	2.87 ± 0.37
RFFMP2.3	0.85 ± 0.30	1.04 ± 0.34	0.95 ± 0.10	1.14 ± 0.50	1.05 ± 0.10	1.15 ± 0.57
RFFMP3.3	1.41 ± 0.37	1.11 ± 0.29	1.71 ± 0.16	2.07 ± 0.33	1.60 ± 0.45	1.53 ± 0.34
RFFMP5	2.28 ± 0.68	2.35 ± 0.15	2.50 ± 0.30	2.68 ± 0.37	2.76 ± 0.64	2.50 ± 0.56

B

Table 3.2 (cont.)

SAMPLE	SEDIMENTATION ± SD (MM)					
	0 d	30 d	60 d	90 d	180 d	270 d
UWMP2.3	1.07 ± 0.05	0.86 ± 0.26	0.93 ± 0.35	1.17 ± 0.39	1.26 ± 0.12	1.4 ± 0.13
UWMP3.3	1.49 ± 0.35	1.21 ± 0.49	1.71 ± 0.32	1.86 ± 0.47	1.63 ± 0.31	1.91 ± 0.30
UWMP5	1.62 ± 0.26	1.38 ± 0.35	1.40 ± 0.12	2.43 ± 1.16	1.38 ± 0.35	1.68± 0.33
UFFMP2.3	0.79 ± 0.08	1.00 ± 0.27	1.01 ± 0.20	0.96 ± 0.41	1.11 ± 0.12	1.21 ± 0.08
UFFMP3.3	0.79 ± 0.36	1.06 ± 0.19	0.98 ± 0.12	1.11 ± 0.30	1.02 ± 0.13	1.06 ± 0.12
UFFMP5	1.25 ± 0.18	1.15 ± 0.17	1.18 ± 0.15	1.15 ± 0.64	1.12 ± 0.12	1.04 ± 0.08
RWMP2.3	0.99 ± 0.05	0.76 ± 0.29	0.94 ± 0.11	0.92 ± 0.16	1.27 ± 0.07	1.25 ± 0.24
RWMP3.3	1.19 ± 0.11	1.32 ± 0.25	1.12 ± 0.21	1.38± 0.55	1.12 ± 0.14	1.16± 0.20
RWMP5	1.03 ± 0.37	1.42 ± 0.31	1.29 ± 0.18	1.57 ± 0.81	1.31 ± 0.13	1.34 ± 0.26
RFFMP2.3	0.87 ± 0.16	0.90 ± 0.18	0.87 ± 0.16	0.88 ± 0.34	1.11 ± 0.04	1.14 ± 0.10
RFFMP3.3	1.42 ± 1.25	0.86 ± 0.24	0.94 ± 0.12	1.04 ± 0.27	0.92 ± 0.18	0.97 ± 0.24
RFFMP5	1.01 ± 0.24	0.95 ± 0.22	0.98 ± 0.12	1.29 ± 0.36	1.07 ± 0.28	0.95 ± 0.17

At 0 d, heat treatment had a significant effect on the sedimentation in samples containing WMP ($p < 0.05$), but not on the samples containing FFMP ($p > 0.05$). Heat treatment did not significantly affect ($p > 0.05$) the creaming in the samples. At 0 d, the difference in sedimentation between samples containing 2.3 and 3.3% protein was significant ($p < 0.05$) in samples containing WMP, but not in samples containing FFMP ($p > 0.05$). The difference in sedimentation between samples containing 3.3 and 5% protein was not significant ($p > 0.05$) except between the samples UFFMP3.3 and UFFMP5 ($p < 0.05$). The difference in sedimentation between samples containing 2.3 and 5% protein was significant in samples which have been processed by UHT ($p < 0.05$), but not in samples that have been retort-sterilised.

The highest stability (i.e., lowest transmission), regardless of heat treatment, was observed in WMP5, and the lowest stability, again, regardless of heat treatment, was seen in FFMP2.3.

The trend observed for stability of both the UHT and retort samples can be summarised as follows: WMP5>WMP3.3=FFMP5>FFMP3.3=WMP2.3>FFMP2.3. This trend was consistent both at the start and the end of the storage period. The stability can also be seen for samples WMP5 and FFMP2.3 in Figures 3.8 and 3.9, respectively, which showed a substantially lower transmission for the more stable samples.

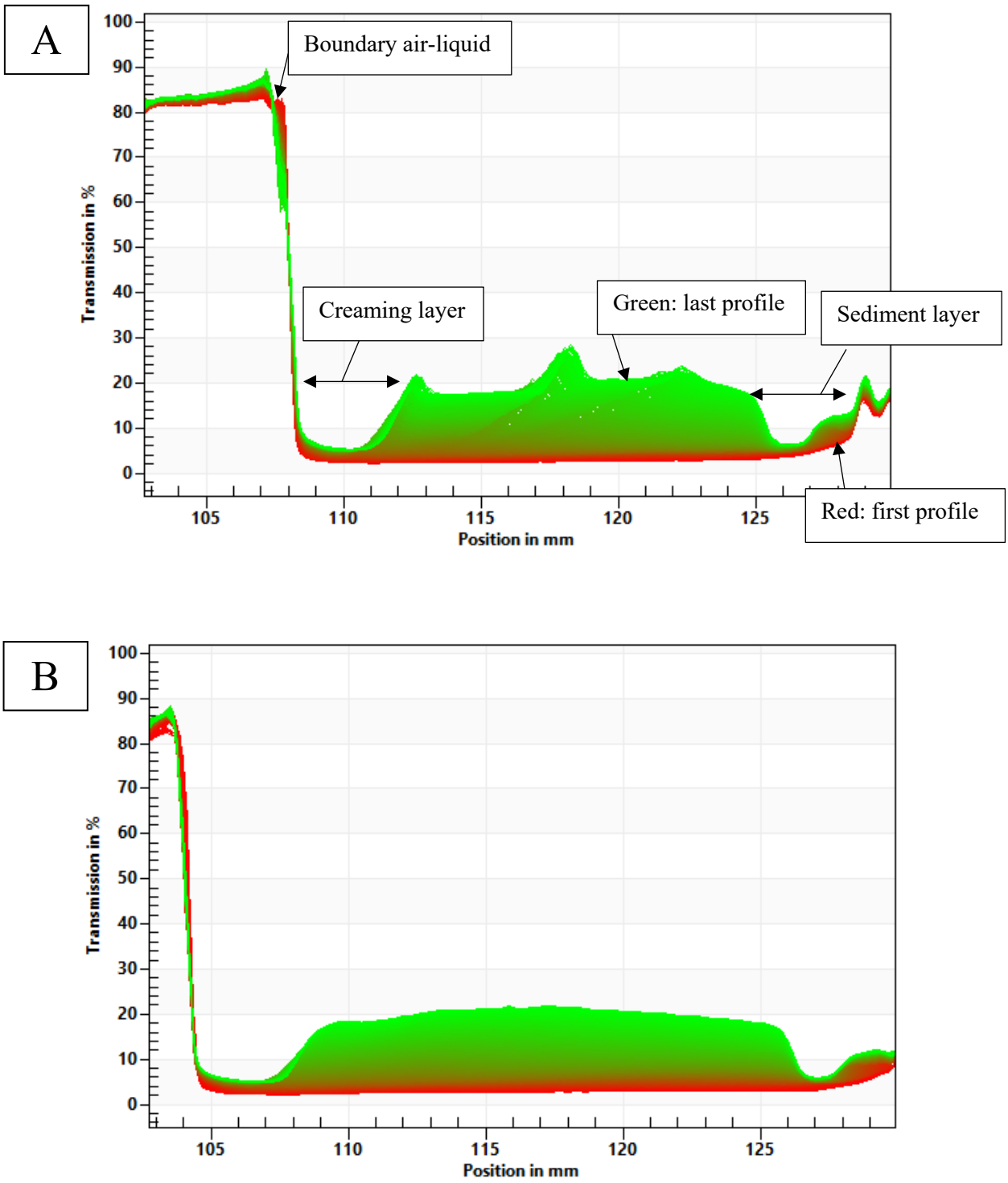


Figure 3.8: Transmission profile of UWMP5 during storage as an example of a stable sample. **A**: 0 d; **B**: 270 d. The red lines represent earlier transmission profiles, and green lines represent later transmission profiles.

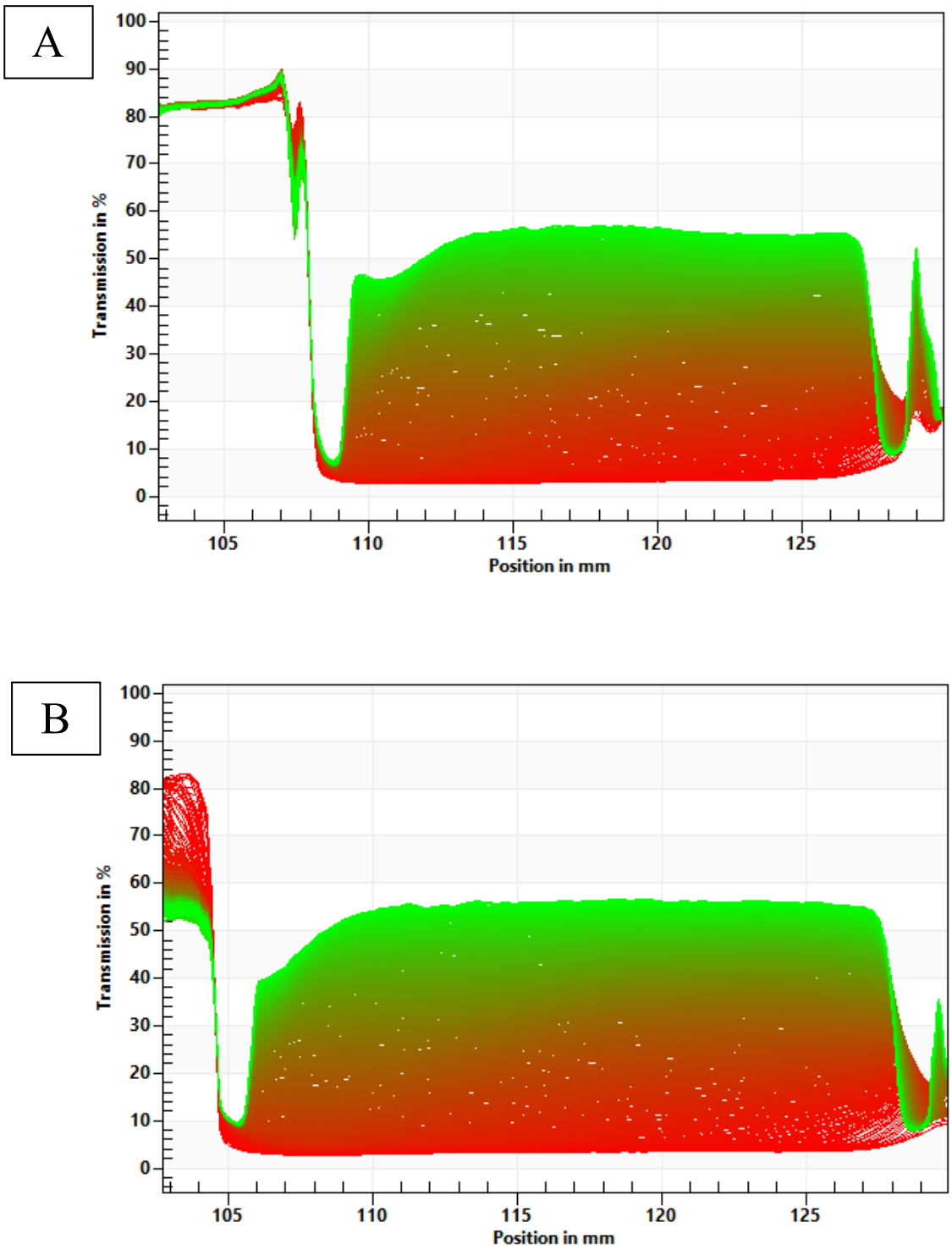


Figure 3.9: Transmission profile of UFFMP2.3 during storage as an example of an unstable sample. **A:** 0 d; **B:** 270 d. The red lines represent earlier transmission profiles, and green lines represent later transmission profiles.

The fat source influenced the stability of the samples, with FFMP samples generally displaying lower stability than WMP samples. While FFMP2.3 had the lowest stability both at the beginning and the end of storage, the stability of FFMP3.3 and WMP5 were equivalent to that of WMP2.3 and WMP3.3, respectively.

Ivanova and Vlaseva (2011) demonstrated that the replacement of milk fat with vegetable oil requires an emulsifier (such as glyceryl monostearate) to achieve an emulsion stability. A lower emulsion stability was observed in the FFMP samples. It is known that milk proteins in their natural state possess good emulsification properties (Braun *et al.*, 2019). In bovine milk, the milk fat globule membrane is a very important natural emulsifier which protects the fat globules from physical destabilisation (coalescence) and oxidation (Le *et al.*, 2009). Fat-filled milk powder, as its fat fraction is sourced from vegetables, does not contain this natural emulsifier and is thus potentially less stable, unless there was addition of emulsifier during the production of the powder, or the protein present acts as an effective emulsifier. This is also seen in the fat globule size measurement (Section 3.3.4) as the FFMP-based solutions showed signs of increased globule size due to their relative instability. At 0 d, creaming was not significantly affected by fat source ($p > 0.05$); however, sedimentation was significantly affected by the fat source ($p < 0.05$), with the samples containing 2.3 and 3.3% protein being affected more significantly ($p < 0.01$).

At 0 d, the difference in creaming between samples containing 2.3 and 3.3% protein is significant ($p < 0.05$), and the difference in creaming between samples containing 3.3 and 5% protein in UHT-treated samples was not significant ($p > 0.05$), whereas the difference in creaming between the same samples that went through retort sterilisation was significant ($p < 0.05$). The difference in creaming between samples containing 2.3 and 5% protein was significant, regardless of fat source and heat

treatment. According to McCarthy *et al.* (2012) the increased creaming, coalescence and flocculation that occurs at a reduced protein content is due to the insufficient amount of protein available to form the recombined MFGM of the newly formed smaller fat globules after homogenisation.

3.3.4 Determination of particle size distribution

The fat globules in raw milk range in size from 200 nm to 15 μm (Argov-Argaman, 2019) and in this study, all samples were subjected to the same homogenisation treatment. There was a significant difference ($p < 0.05$) in the volume mean diameter (D_{50}) between UHT- and retort-treated samples containing 2.3 and 3.3% protein. However, there was no significant difference ($p > 0.05$) between the volume mean diameter samples containing 5% protein, regardless of heat treatment.

At 0 d, the difference in the D_{50} value was significant between samples containing 2.3 and 5% protein, and between samples containing 3.3 and 5% protein ($p < 0.05$), regardless of fat source or heat treatment. Between the samples containing 2.3 and 3.3% protein, there was no significant difference in the D_{50} value ($p > 0.05$), except between UWMP2.3 and UWMP3.3.

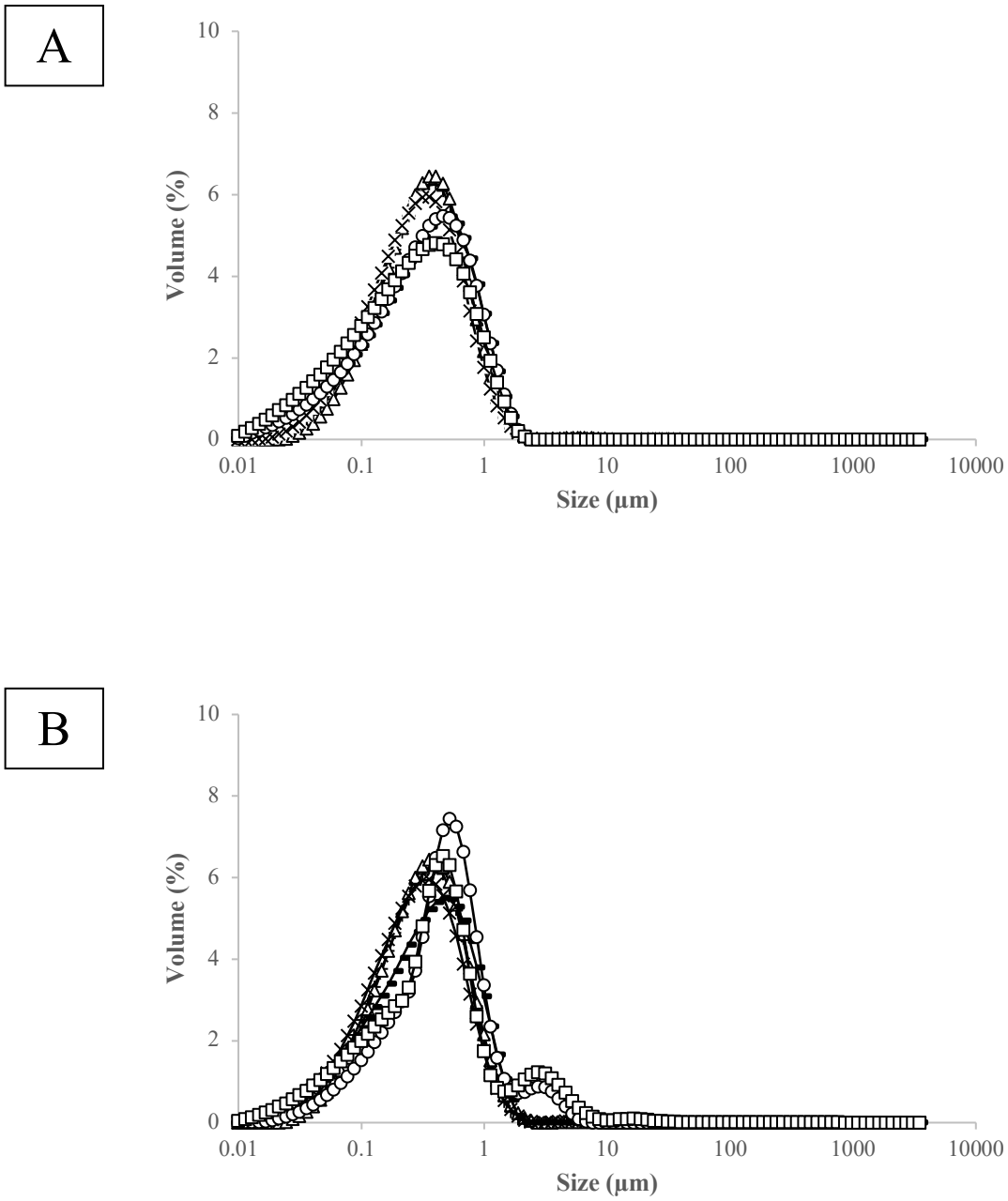


Figure 3.10: Change in particle size (µm) in UHT samples during storage. **A**: 0 d; **B**: 270 d. UWMP2.3 (—+—), UWMP3.3 (—△—), UWMP5 (—×—), UFFMP2.3 (——), UFFMP3.3 (—○—), UFFMP5 (—□—). The results are the average of data from three independent trials.

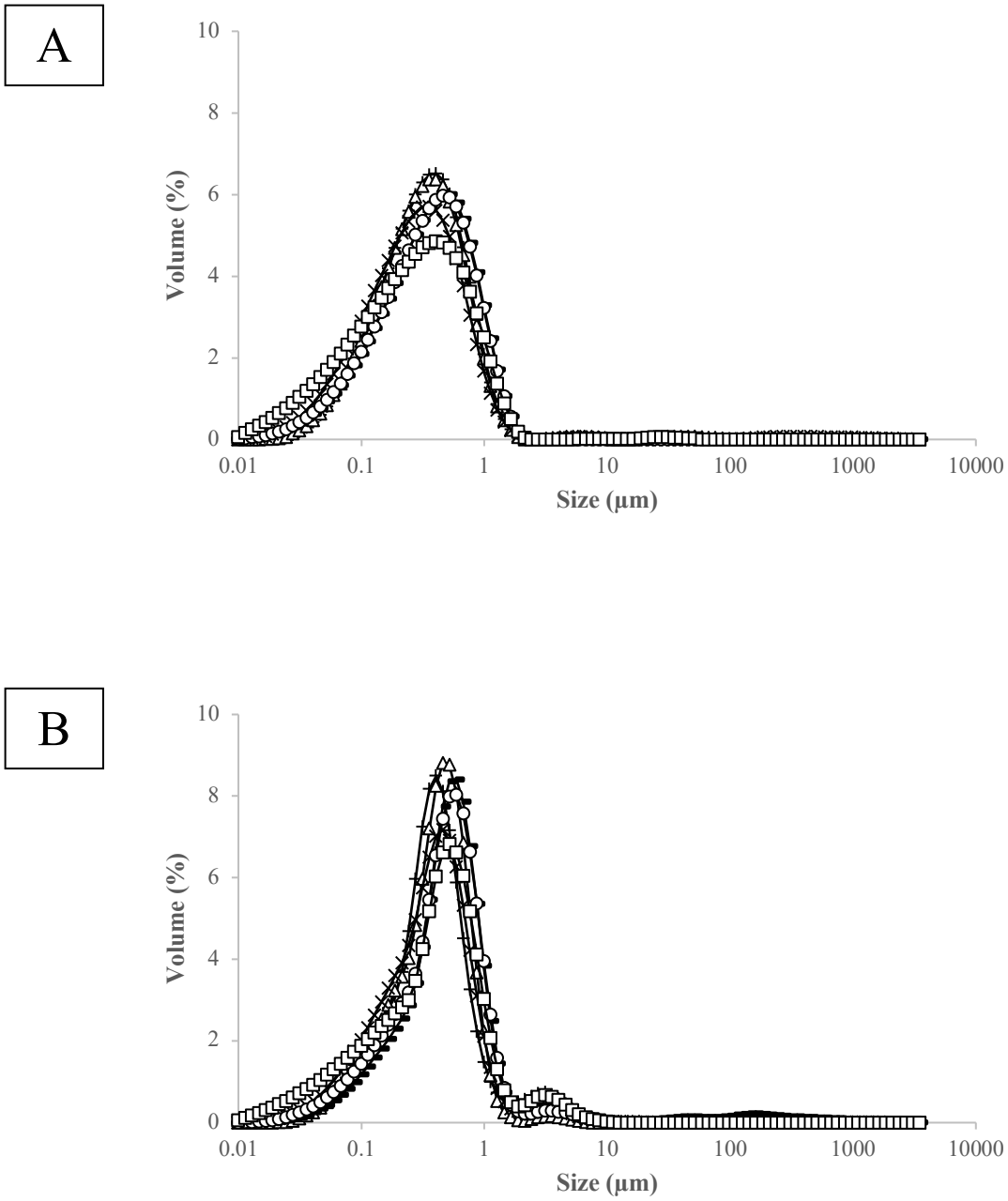


Figure 3.11: Change in particle size (µm) in retort samples during storage. **A**:0 d; **B**: 270 d. RWMP2.3 (—+—), RWMP3.3 (—△—), RWMP5 (—×—), RFFMP2.3 (——), RFFMP3.3 (—○—), RFFMP5 (—□—). The results are the average of data from three independent trials.

There was a significant difference in the D_{50} value between most of the samples containing the same protein content with different fat sources ($p < 0.05$), except for

between UWMP3.3 and UFFMP3.3, and between RWMP5 and RFFMP5 ($p > 0.05$). Storage time had a significant effect on the D_{50} value ($p < 0.05$), except for UFFMP2.3, for which there was no significant difference between the particle size measured at the start of the trials (0 d) and at the end (270 d) ($p > 0.05$). At the end of storage, a new peak was seen in the following samples: UFFMP5, UFFMP3.3, and all of the retort-treated samples, which probably influenced the D_{50} value. This new peak could be larger fat globules forming over time, either through flocculation or coalescence.

3.3.5 Measurement of viscosity

Apparent viscosity increased significantly with increasing protein content ($p < 0.05$) for all samples. The addition of 1.7% MPC-80 to WMP5 and FFMP5 had a dramatic effect on viscosity. According to Cano-Ruiz and Richter (1998), as the apparent viscosity decreases, the rate of age gelation and sedimentation increases. However, Datta and Deeth (2001) reported that an increase in apparent viscosity of over 10 mPa.s occurs at 20°C immediately before age gelation, so it is obvious from the viscosity results, as well as through visual observation, that age gelation has not occurred in these samples.

The method of heat treatment, fat source, and protein content all significantly affected the apparent viscosity of the samples ($p < 0.05$). The viscosity of the UHT solutions at 60 d (the first measurement of apparent viscosity) ranged between 2.03 and 3.46 mPa.s, increasing with increasing protein content, whereas the viscosity of the retorted samples, ranging between 2.13 and 3.8 mPa.s, increasing with increasing protein content. The same samples processed with UHT and retort sterilisation (for example UWMP2.3 and RWMP2.3) showed significant differences ($p < 0.05$) in apparent

viscosity. Between 60 and 270 d, there was a significant difference in viscosity recorded for all of the retort-sterilised samples, and for the UWMP5, UFFMP2.3, and UFFMP5.

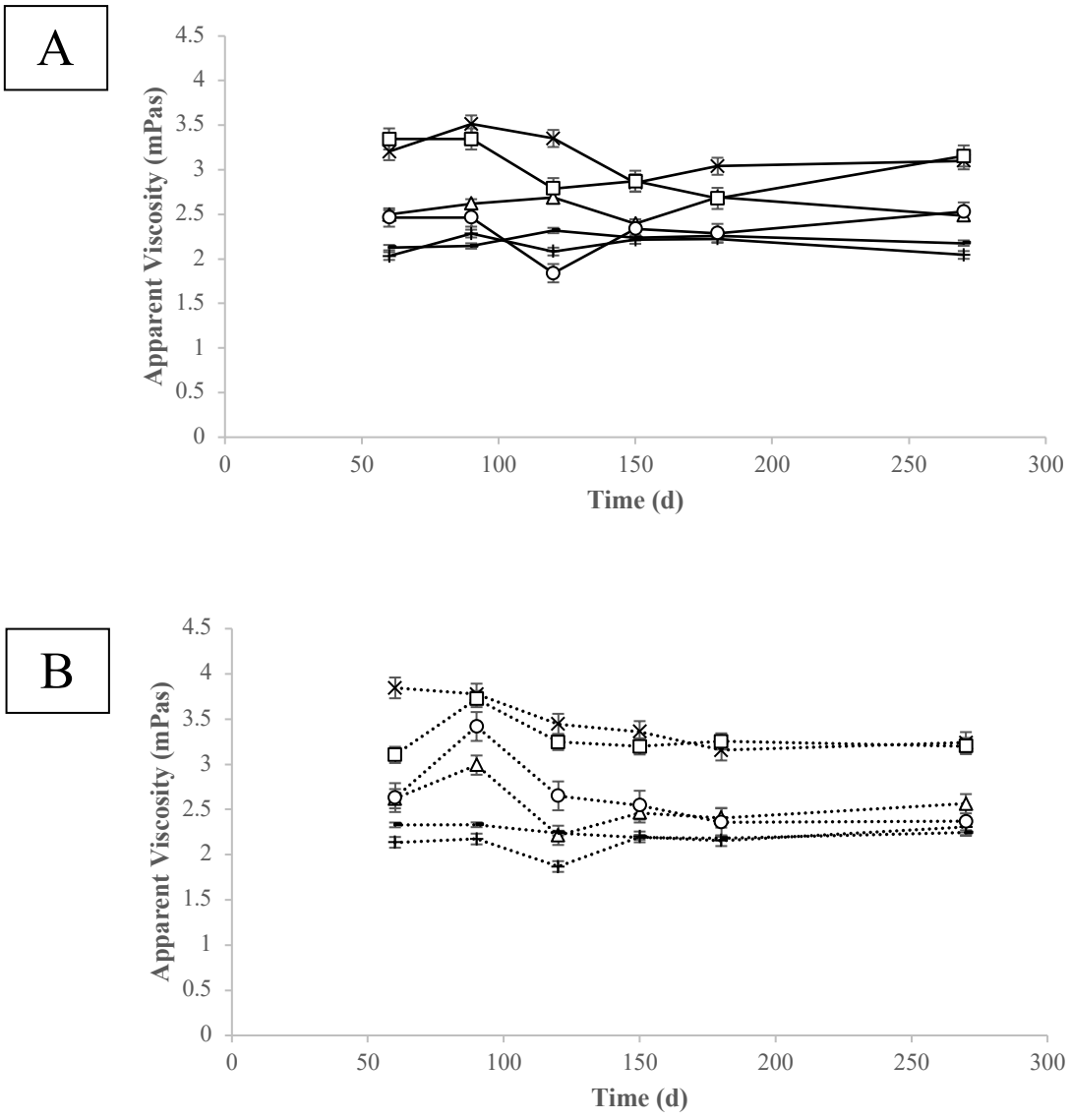


Figure 3.12: Apparent viscosity of UHT and retort samples during storage. **A**: UHT-treated samples; **B**: Retort-sterilised samples. U/RWMP2.3 (+), U/R/WMP3.3 (Δ), U/RWMP5 (×), U/RFFMP2.3 (-), U/RFFMP3.3 (○), U/RFFMP5 (□). The results are the average of data from three independent trials.

At 270 d, there was a significant difference between the viscosity of the WMP-based samples and the FFMP-based ones ($p < 0.05$), except for between UWMP5 and UFFMP5 ($p > 0.05$). In terms of the emulsion stability results, the lowest transmission was seen in all measurements for treatment WMP5, and the highest transmission was observed for FFMP2.3. The viscosity of these solutions at 270 d were 3.24 ± 0.21 and 2.24 ± 0.045 mPa.s, respectively, which were the extremes recorded at 270 d. Only the samples containing 5% protein had an apparent viscosity greater than 3 mPa.s, and they also showed the lowest transmission when measured using the Lumisizer. Protein has been often linked to emulsion stability (McClements, 2004; Kim *et al.*, 2005; Ghosh and Rousseau, 2010; McCarthy *et al.*, 2012; O'Sullivan *et al.*, 2018). As for viscosity, Stokes' Law indicates that, as the viscosity of the sample increases, the rate of particle movement (either due to creaming or sedimentation) should decrease, resulting in a more stable emulsion. This can be seen in the Lumisizer results (Section 3.3.3) and the apparent viscosity results (Section 3.3.5).

3.4 Conclusion

It is obvious from the results of this study that the heat treatments investigated produced different results and that the protein content and fat source also affected the samples. These factors are important to consider when formulating and designing a process for a new product. While FFMP is similar in composition to WMP, there was a significant difference in colour, particle size, and apparent viscosity between the reconstituted powders. This may influence the consumer's preference; however, all of the samples were stable throughout storage. The difference between the colour and the potential difference between the flavour of beverages made with FFMP as an alternate to WMP may be masked by adding flavourings and colours. Thus, FFMP can be a cost-effective alternative to WMP in the dairy industry.

UHT processing is the industry standard for sterilisation of the majority of dairy-based beverages nowadays. As it is a continuous process, it is more suitable for large-scale production than retort sterilisation. The most obvious difference between the samples of the two heat treatments was the colour: the higher heat load experience during retort sterilisation resulted in a greater occurrence of the Maillard reaction and thus a darker colour. The higher heat load also results in a loss of nutritionally significant compounds such as lysine and thiamine (not measured in this study). Retort sterilisation is also a slower process and requires more space than UHT processing. It is important in nutritional beverages to contain protein to supply necessary amino acids to the consumer and to increase satiety. While increasing the protein content of the samples did not completely destabilise the system, there was a significant increase

in viscosity, particle size, pH, creaming, and sedimentation as the protein content increased.

3.5 Appendices

3.5.1 Protein profiling by electrophoresis

The base powders were analysed using SDS-PAGE gels to determine if there was any evidence of denaturation of the whey proteins during powder production. Proteolysis during storage was examined by running the samples on urea-PAGE gels. The standard used in the urea-PAGE gels was sodium caseinate.

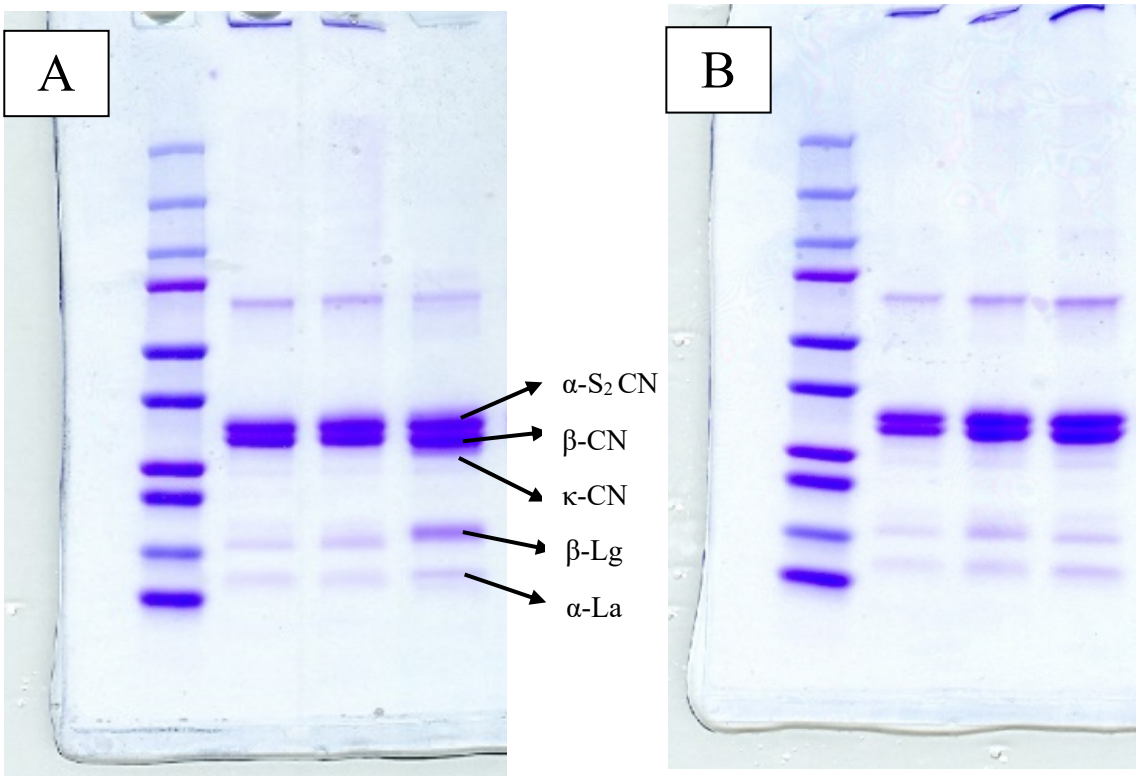


Figure 3.13: SDS-PAGE gels of base powders. **A:** Reducing conditions; **B:** Non-reducing conditions. Lane 1: Standard; Lane 2: WMP (3.5% protein); Lane 3: FFMP (3.5% protein); Lane 4: MPC (3.5% protein).

In the SDS-PAGE gels of the base powders (Figure 3.13), under reducing conditions, the β-mercaptoethanol disrupts covalent interactions which form between κ-CN and whey proteins upon heat treatment (Choudhary *et al.*, 2018). Whey protein

denaturation is indicated by reduced band definition, which can be seen in both the WMP and FFMP lanes. Thus, is can be concluded that MPC received a more severe heat treatment than WMP and FFMP as the β-lactoglobulin band is stronger in the MPC lane compared with the WMP and FFMP under reducing conditions. Other than this, the lanes are similar in pattern.

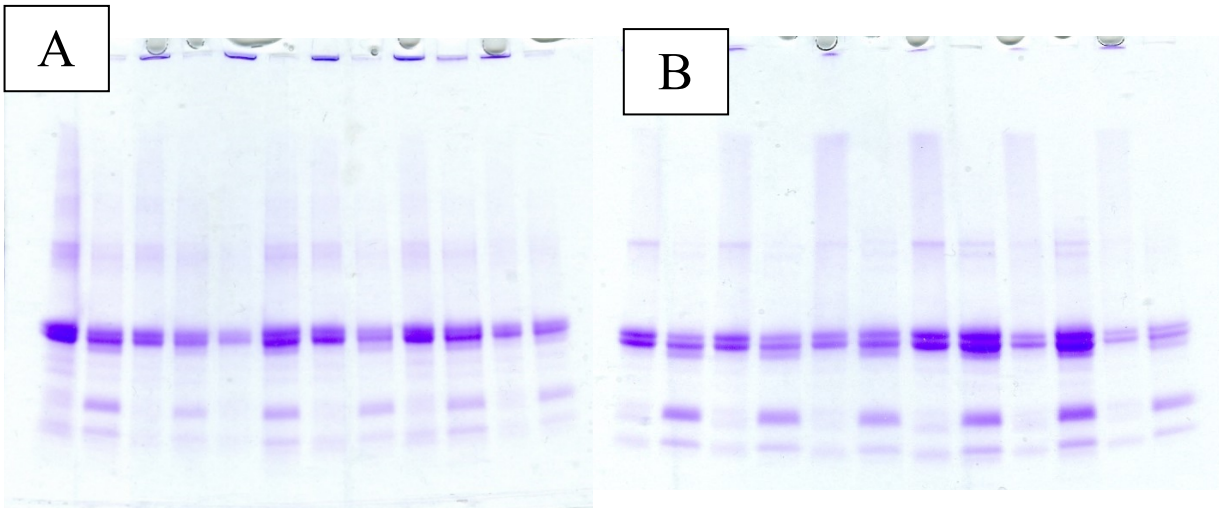


Figure 3.14: SDS-PAGE gels of samples at 0 d. **A:** Retort-sterilised samples; **B:** UHT-treated samples. Lane 1: R/UWMP2.3 (non-reducing); Lane 2: R/UWMP2.3 (reducing); Lane 3: R/UWMP3.3 (R); Lane 4: R/UWMP3.3 (NR); Lane 5: R/UWMP5 (R); Lane 6: R/UWMP5 (NR); Lane 7: R/UFFMP2.3 (non-reducing); Lane 8: R/UFFMP2.3 (reducing); Lane 9: R/UFFMP3.3 (R); Lane 10: R/UFFMP3.3 (NR); Lane 11: R/UFFMP5 (R); Lane 12: R/UFFMP5 (NR).

At 0 d (Figure 3.14), there has been some denaturation of the whey proteins in both heat treatments, as there is a difference in definition between the reducing (R) and non-reducing (NR) lanes.

In the urea-PAGE gels, faster moving bands (i.e., further down the gel) indicate more proteolysis (Figure 3.15). This is evident in the samples at 180 d, where there is more

proteolysis occurring in the UHT-treated samples than in the retort-sterilised samples. Indeed, at 0 d, there are more bands lower in the gel for the UHT-treated samples, which indicates more proteolysis. Also, the bands are stronger in the samples containing higher amounts of protein. However, the clarity of the gels was not sufficient to allow detailed conclusions to be drawn regarding proteolysis rates. Ideally, proteolysis would have been studied further by peptide profiling using Reversed-Phase High-Performance Liquid Chromatography (RPHPLC), which was not performed in this case but may be of interest for further studies.

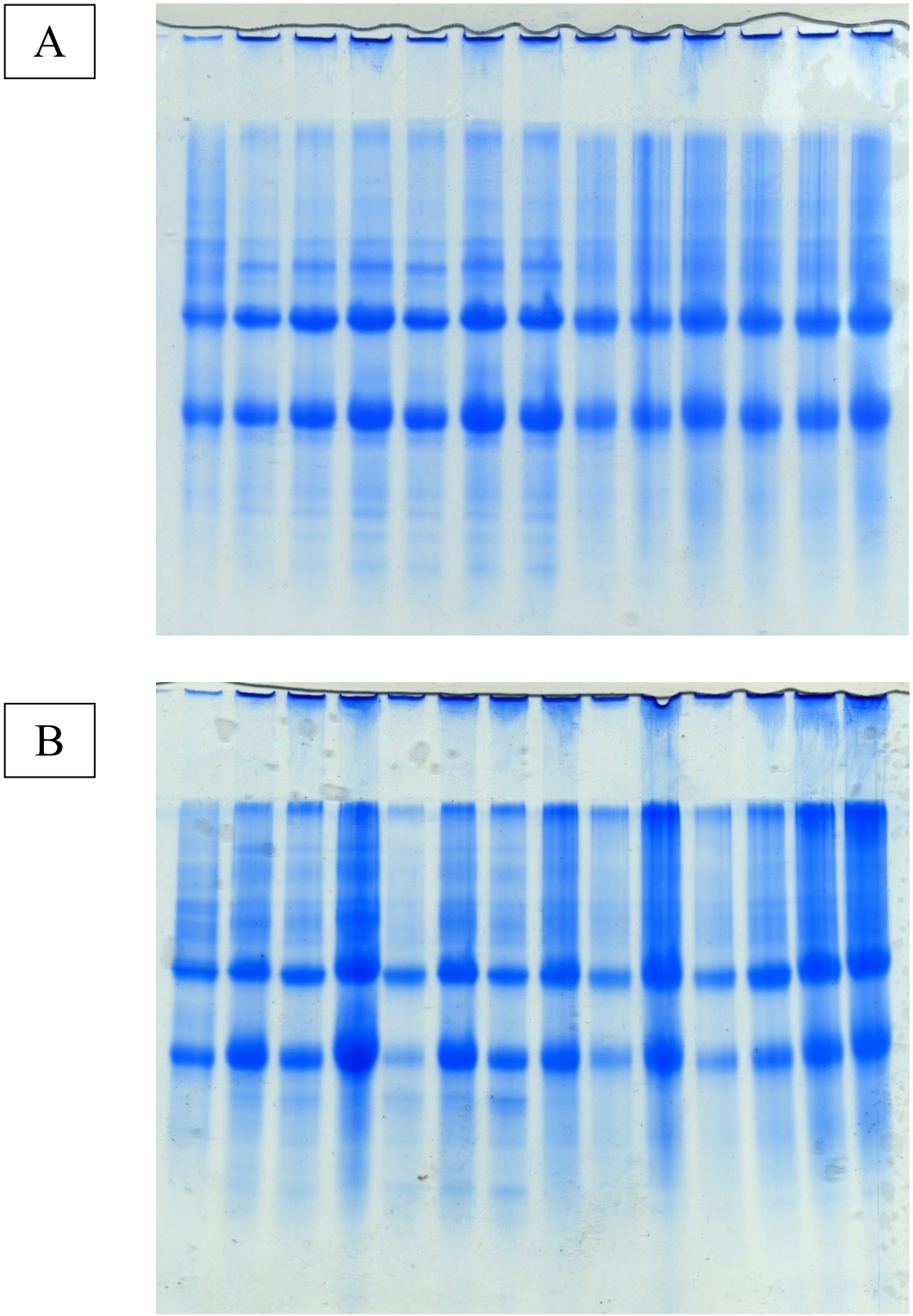


Figure 3.15: Urea-PAGE gels of samples. **A**: Samples at 0 d; **B**: Samples at 180 d. Lane 1: Standard; Lane 2: UWMP2.3; Lane 3: UWMP3.3; Lane 4: UWMP5; Lane 5: UFFMP2.3; Lane 6; UFFMP3.3; Lane 7: UFFMP5; Lane 8: RWMP2.3; Lane 9: RWMP3.3; Lane 10: RWMP5; Lane 11: RFFMP2.3; Lane 12: RFFMP3.3; Lane 13: RFFMP5; Lane 14 (only gel B): RWMP5 (repeat).

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Chapter 4

Overall conclusions and suggestions

for future work

The whole milk powder (WMP) and fat-filled milk powder (FFMP) ingredients used as base materials for the formulation of long-life, nutritional, milk-based beverages in this project were similar in composition, apart from the source of fat. They are also suitable for simple reconstitution as a liquid milk alternative. FFMP could potentially be more suitable as a base for such nutritional beverage development than WMP as manipulation of the fatty acid profile is possible through the use of various vegetable oils.

Both materials were shown to be broadly suitable for production of long-life milk protein beverages of different protein contents, using either of the most common processes for achieving long-term stability (retort sterilization and UHT treatment). None of the beverage samples analysed in this project gelled over the nine months of storage. The most significant physical effect that the heat treatment had on the samples was the colour difference. Protein content affected the viscosity of the samples, and the only implication of fat source appeared to be for product colour.

The heat stability of reconstituted WMP and FFMP were also similar without the addition of the calcium-chelating salts. The choice of chelating salt and addition level greatly affected stability, with DSHP added at 20 mmol/L significantly increasing heat stability at pH <7 in WMP samples, whereas TSC did not seem to have such an effect in either WMP or FFMP samples. Conversely, the addition of SHMP at 10 mmol/L resulted in the samples coagulating in less than 90 s in the oil bath, and this level of SHMP also significantly affected the apparent viscosity. SHMP and 20 mmol/L TSC significantly affected the colour of reconstituted low-heat skim milk powder (LH-SMP), whereas DSHP did not.

Suggestions for future research to follow up on this work could include the following:

- Further investigation into FFMP as a viable alternative to WMP in the formulation of long-life, nutritional, dairy-based beverages could involve the addition of other ingredients commonly used in nutritional beverages (such as whey protein ingredients, vitamins, minerals, stabilisers, colours and flavours) and to examine the difference in stability between the beverages;
- The mechanism by which SHMP dissociates the casein micelles should be further investigated as, in past research, the viscosity increase was attributed to the cross-linking by the SHMP; however, the LH-SMP colour results showed that the casein micelle dissociation occurred after SHMP addition, making cross-linking impossible. Thus, the viscosity increase could not be solely due to cross-linking, but perhaps another mechanism such as increased electrostatic repulsion through addition of negative charge or increased particle-particle interactions between the casein proteins from dissociated micelles.