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AGRICULTURE AND FOOD DEVELOPMENT AUTHORITY

Teagasc Food Research Centre,
Moorepark, Fermoy, Co. Cork, Ireland

Investigations of the complex relationships between minerals, pH and heat stability in milk protein systems

Thesis submitted to the National University of Ireland for
Degree of
Doctor of Philosophy
By

Tugce Aydogdu, B.Sc., M.Sc.

12th September 2023

Research Supervisors

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I dedicate this work with great love to my whole family and Lula

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Declaration

*Investigations of the complex relationships between minerals, pH and heat stability
in milk protein systems*

This is to certify that the work I am submitting is my own and has not been submitted for another degree, at either University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism and intellectual property. All the work described herein is the original work of the author.

Date: 12th September 2023

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*Hayatım boyunca beni destekleyen ve bana itimat eden uzak- ama yakın aileme
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Publications and Awards

Peer-reviewed publications:

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Aydogdu, T., O'Mahony, J.A., & McCarthy, N.A. (2022). Measurement of pH at high temperature in milk protein solutions. *International Dairy Journal*, 131, 105383.

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Kelleher, C.M., **Aydogdu, T.**, Murphy, K.M., O'Mahony, J.A., Kelly, A.L., O'Callaghan, D.J., & McCarthy, N.A. (2020). The effect of protein profile and preheating on denaturation of whey proteins and development of viscosity in milk protein beverages during heat treatment. *International Journal of Dairy Technology*, 73(3), 494-501.

McSweeney, D.J., **Aydogdu, T.**, Hailu, Y., O'Mahony, J.A., & McCarthy, N.A. (2022). Heat treatment of liquid ultrafiltration concentrate influences the physical and functional properties of milk protein concentrate powders. *International Dairy Journal*, 133, 105403.

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Abstract

The complex composition of milk and derivatives therefrom, mean that its stability in respect to thermal treatment, while well studied, is ever evolving. Non-fat milk powders are rich in protein, of which there may be greater than 4500 different nitrogenous compounds, often present in conjunction with milk minerals. Variations in the total concentration and individual components of proteins and minerals makes dairy science a multifaceted system from which nutritional dairy products emerge, manipulated and produced by processing factors such as temperature, pressure, dehydration, shear and filtration. At the centre of dairy processing lies the most basic element fundamental to solvent chemistry; hydrogen, used as a measure of chemical reactions, but more often taken as a control parameter for physical and sensory properties. Therefore, the objective of this thesis was to advance considerably our knowledge on milk protein and milk mineral systems with regard to three main properties; heat stability, viscosity and pH changes, which have cumulative effects on dairy processing performance and finished product quality and functionality.

A novel method of measuring pH at ultra-high temperatures was adapted from the pharmaceutical industry and used to examine the hydrogen ion concentration in dairy systems. For the first time, a non-linear decrease in skim milk pH was shown with increasing temperature from 25 to 140°C. The pH of skim milk decreased from 6.7 at 25°C to 6.1 at 140°C, with this reduction being reversible on cooling. This was not the case for milk permeate, where the pH remained low after sequential heating and cooling, due to irreversible calcium phosphate formation and precipitation. This highlights the stabilizing ability of micellar casein in skim milk against significant levels of calcium phosphate precipitation. However, while precipitation might be reduced, the addition of milk permeate to milk protein concentrate (MPC) resulted in

substantial levels of age thickening after evaporation (45% dry matter), whereas MPC with added lactose showed no age related viscosity increase. The former system also had lower pH compared to the latter.

Given the significant influence milk permeate had on viscosity of protein solutions, the heat coagulation time of commercial bulk skim milk obtained across the spring period was assessed to determine if the changes in milk composition from early lactation affected heat stability. Type B HCT-pH profiles were shown for bulk milk samples taken in February and March, compared to a type A profile for April milk, with this type A profile continuing for the remainder of the year. This is the first time that a change in HCT profile has been shown for commercial bulk milk samples; although there was no obvious difference in milk composition between any of the samples. Ultrafiltration of the skim milks was performed to obtain milk permeate fractions and was used to swap the retentate obtained in February with the permeate from April milk. This showed that a type B HCT-pH profile in February could be changed to a type A profile by simply swapping the serum phase.

The outcomes of the research in this thesis are highly applicable to the thermal processing of complex nutritional formulations (e.g., infant formulas), where mineral additions and substitutions are common practice. The ability to measure pH during UHT processing is certainly a highlight of the work presented herein. In addition, a key new finding is that macro composition, specifically protein and mineral profile, is not always a good predictor of HCT, but that the serum phase of milk has the more substantial effect on heat stability than the colloidal phase on its own. The work presented in the thesis provides novel information to both the dairy industry and academia, in terms of process control through *in-line* pH measurement, and the fundamental effects of milk serum on protein heat stability.

Introduction and Research Objectives

The stability of milk protein systems during dairy processing are strongly influenced by composition (protein content, casein: whey protein, mineral content and profile), and environmental considerations such as temperature and pH. In dairy processing, concentrated liquid protein-containing streams (e.g., skim milk and milk protein concentrates) are routinely subjected to thermal treatments such as pasteurisation, ultra high temperature treatment and preheating (e.g., during evaporation and spray drying). These thermal treatments typically vary widely in temperature and time combinations (depending on their function, ranging from microbiological control to tailoring of functionality, such as heat stability), and consequently are often associated with concomitant in process direct (e.g., protein denaturation) and indirect (e.g., alteration of pH and mineral distribution) changes in the dairy stream being processed. Such changes can cause significant increases in viscosity, reductions in flowability, often resulting in fouling and blockages of evaporators and atomisation nozzles of spray dryers. Therefore, the overall objective of the research in this thesis was to determine the chemical and physical properties of protein and mineral interactions, as influenced by pH and temperature and determine novel approaches to measure pH.

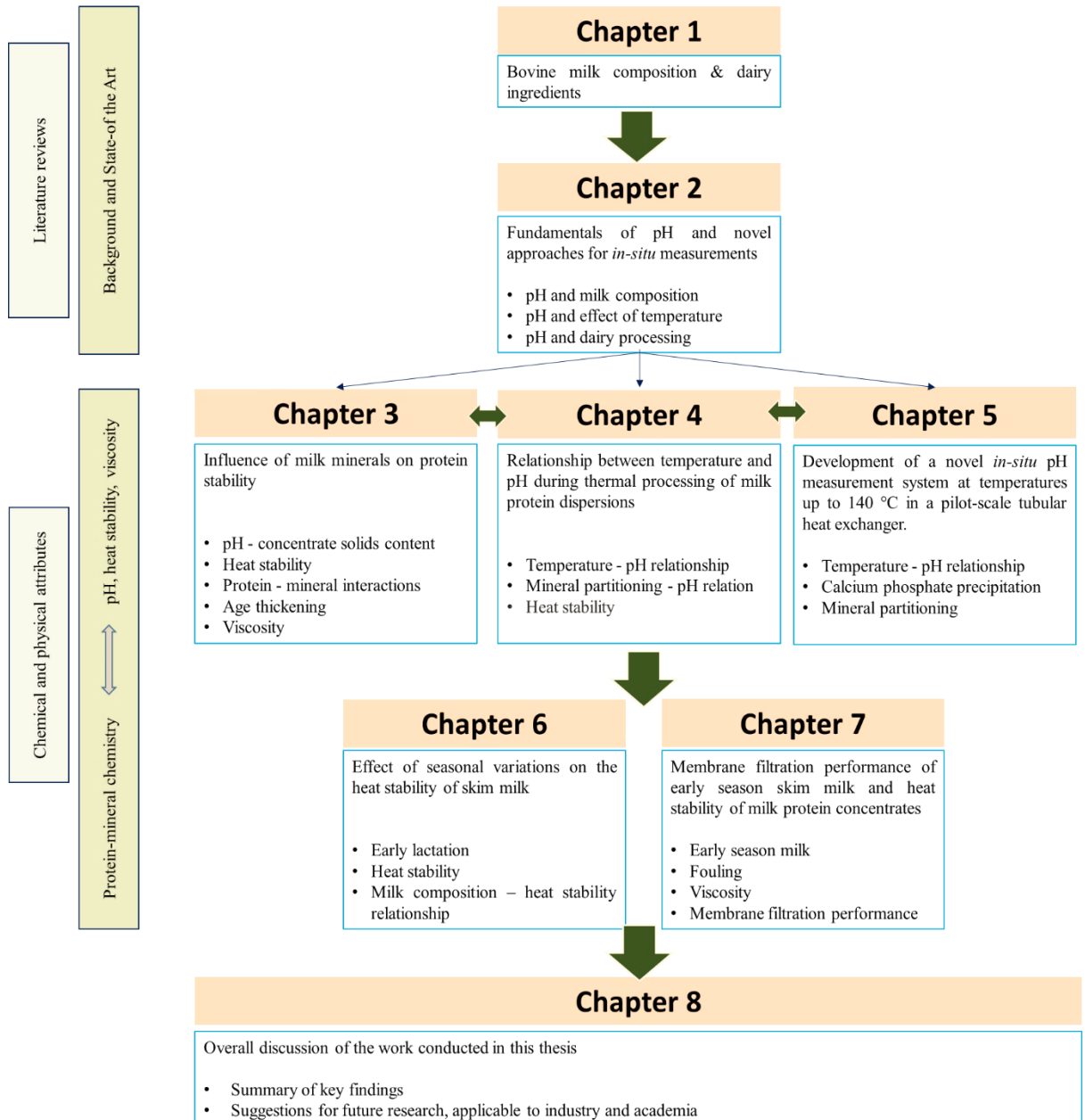
The pH changes observed in milk during ultra-high-temperature (UHT) processing are influenced not only by the macro composition, specifically protein and mineral profiles, but are also substantially affected by the serum phase of milk. The hypothesis for the research theme within the thesis stems from the suggestion that the serum phase plays a key role in determining the heat stability profile of milk and that minor changes in this phase can have enormous impacts on subsequent heat-induced properties. Additionally, it is anticipated that the relationship between temperature and

pH during thermal processing can be monitored effectively, leading to new knowledge on hydrogen ion concentration at ultra-high temperatures.

To achieve this overall objective, the specific research aims of the work were as follows:

- To review the state of the art with reference to pH measurement within the dairy industry, reviewing the effects of processing temperature and investigating the inter-relationships between total solid content, minerals, and temperature during dairy processing (Chapter 2).
- To examine the effects of milk minerals on protein-mineral interactions and calcium phosphate precipitation, in determining how milk serum minerals can influence the viscosity of milk protein-containing solutions, age thickening, and heat stability (Chapter 3).
- To investigate the relationship between temperature and pH during thermal processing of milk protein systems by monitoring pH *in-situ* at temperatures from 25 to 120°C (Chapter 4), and informed by this, to develop a pilot-scale system for rapid, high throughput *in-situ* pH analysis at 140°C for skim milk (Chapter 5).
- To investigate the effects of milk composition and physiochemical properties on heat stability of early-season milk (Chapter 6), and secondly to determine the effect of the milk serum phase on heat stability (Chapter 7). Chapter 7 also examined the effect of early season skim milk on ultrafiltration performance.

Schematic of thesis objectives and outline



Chapter 1

Milk composition and milk protein ingredients

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Declaration

This chapter was written by Tugce Aydogdu (TA) and reviewed by all co-authors.

1.1. Introduction

Despite the fact that bovine milk consists of ~87% water, it remains one of the most complex biological fluids in nature, with numerous individual components categorized as protein, fat, carbohydrate, vitamins, minerals, somatic cells and microorganisms (stemming from infection or environment), with a multitude of different products derived therefrom (Figure 1.1). Milk and milk components are traded mainly as a source of nutrition, but also find use in the pharmaceutical, cosmetic, textile and chemical industries. In 2021, a total of 149.5 million tons of whole milk were processed in the EU for a wide range of products (Table 1.1). All of these applications involve either the removal of water and/or fractionation of individual milk components with processing such as centrifugal separation, heat treatment, filtration, homogenization, evaporation and spray drying.

Table 1.1. Utilization of milk (million metric tons) and dairy products in the EU for 2022.

	Utilization of milk		Product obtained
	Skimmed milk	Whole milk	
Sub-total of process generating skimmed milk	-57.8	65.6	7.0
Butter*	-43.0	46.4	2.3
Cream	-13.9	16.3	2.5
Other fresh products	-0.9	2.9	2.3
Sub-total of process consuming skimmed milk	57.8	84.3	47.0
Drinking milk	9.7	12.7	22.5
Powder products	20.7	4.1	3.0
Concentrated milk	1.0	1.3	1.0
Acidified milk	1.7	6.0	7.7
Buttermilk	0.4	0.0	0.4
Cheese	16.9	59.2	10.4
Milk based drinks	1.0	0.6	1.8
Casein	6.3	0.5	0.2
Total	0.0	149.9	

Retrieved from Eurostart (2022).

* Differences between the amount of whole milk and finished product may be due to process lossess and average values.

The processing technologies that exist today are advanced and versatile to fulfill the need for desired and value-added dairy ingredients. However, continuous optimization and improvement is required to cater for the growing consumer health market, added complexity of products (McSweeney & O'Mahony, 2015), as well as the global climate challenge and requirement to reduce emissions. Chapter 1 provides a brief overview of some of the main bovine milk components, ingredients thereof and the effect of seasonality on milk composition.

1.2. Constituents of bovine milk

Milk is a complex and dynamic system containing macro and micronutrients, designed to provide a complete diet for the healthy growth and development of the neonate. A number of factors influence the composition of bovine milk, such as stage of lactation, diet and cow health (Haug, Høstmark, and Harstad, 2007), all of which influence the composition of fat, protein, carbohydrate, minerals and vitamins (Fox, Uniacke-Lowe, McSweeney, & O'Mahony, 2015) (Table 1.2). Containing over 85% water, milk is the sole source of hydration for the calf and provides the medium for all components, either dissolved (minerals, vitamins, carbohydrates) or suspended (proteins, fat globules, microorganisms, etc) within the system.

Table 1.2. Composition of the major bovine milk components.

Principal constituent	Range (%)	Mean value (%)
Water	85.5 - 89.5	87
Total solids	10.5 - 14.5	13.3
Fat	2.5 -6.0	3.9
Protein	2.9-5.0	3.4
Lactose	3.6-5.5	4.8
Minerals	0.6-0.9	0.8

Adapted from Ann Augustin, Oliver, and Hemar (2011); Laval and Pak (1995); Li, Ye, and Singh (2019).

Global milk production is based on two main systems of converting nutrients from dietary fodder/forage into milk solids. These systems are based on either year-round calving, where cows are housed indoors and fed a mixed ration diet, or the second method, established on an outdoor pasture-based feeding system, where the majority of calving takes place in early spring. Countries such as New Zealand, Ireland and Australia have mainly adopted a seasonal milk production system. In these countries, milk production is significantly shaped around the period between early Spring to late summer, which largely overlaps with the grass-growing season (O'Brien & Guinee, 2022). The ratio of each constituent might vary with regard to stage of lactation, age, breed, etc. Table 1.3 shows the effect of seasonal milk production on the major milk components. Milk seasonality can be described as the variation in key compositional components and its quality attributes in relation to process ability.

Table 1.3. Seasonal variation difference between early and late season milk in the proportions of major milk components.

	Season	Country	Fat (%)	Protein (%)	Lactose (%)
O'Brien, Mehra, Connolly, and Harrington (1999)	June-October 1993	Ireland	3.0-3.9	3.0-3.8	4.2-4.6
Chen, Lewis, and Grandison (2014)	August 2011 to October 2012 (30% outdoor grazing)	UK	3.6-4.7	2.9-3.6	4.5-4.7
Lin, O'Mahony, Kelly, and Guinee (2017)	March 2014 to November 2014 and January 2015 to February 2015	Ireland	3.6-3.7	3.4-3.9	4.7-5.1
Li, Ye, and Singh (2019)	August 2016 to May 2018.	New Zealand	4.6-5.6	3.4-4.7	5.0-4.8

1.2.1. Protein composition

The protein content of bovine milk can be divided into two major fractions. The main milk protein fraction, known as casein, makes up approximately 80% of the total nitrogenous protein content in bovine milk, while the remaining 20% consists of whey proteins, non-protein nitrogen and free amino acids (Goulding, Fox, & O'Mahony, 2020). Caseins and whey proteins can be separated by adjusting the pH to 4.6 and allowing caseins to precipitate at their isoelectric point (Lucey, 2016). Caseins can be sub-divided into α s1-, α s2-, β - and κ -caseins (Fox, McSweeney, & Paul, 1998) and can be classified as amphiphilic proteins with both hydrophobic and hydrophilic domains (Carr, 1999). They lack a well-defined secondary structure, are heat-stable, and contain esterified phosphate groups via serine and threonine amino acids.

α s1-Casein has five genetic variants (A, B, C, D and E) and contains ~199 amino acid residues, depending on the polymorphic genetic variant, with a molecular weight of 24 kDa in monomeric form. It can also form polymeric units, the sizes of which are dependent on pH, ionic strength and protein concentration (Swaisgood, 2003). α s1-Casein is the main casein in bovine milk, representing ~40% of total casein and contains 8 moles of phosphorous per mole of protein at pH 7, but can also contain 9 moles depending on the genetic variant and can bind up to 8 moles of calcium (Walstra, Jenness, & Badings, 1984). It contains three hydrophobic regions and a highly charged polar region, where the majority of the phosphate groups are located (Walstra et al., 1984). α s1-Casein has no cysteine residue and therefore cannot form disulphide linkages, but contains 17 proline residues (Walstra et al., 1984). The polypeptide chain of α s2-casein is comprised of 207 amino acid residues and represents ~10% of total bovine casein. It has the most phosphate groups within the casein family, containing between 10 and 13 moles of phosphorous per mole, with four main genetic

variants (Swaisgood, 2003). It contains two cysteine residues and therefore can form disulphide linkages.

β -Casein in bovine milk makes-up ~35% of the total casein and has 209 amino acid residues, with a molecular weight of ~24 kDa (Farkye & Shah, 2014). Similar to α_{s1} -casein it has no sulphydryl groups, making disulphide bonds impossible. There are a number of genetic variants for β -casein and it contains 5 moles of phosphorous per mole of protein. The conformation of β -casein is dependent on temperature, concentration and ionic strength, and it usually exists in the form of monomeric subunits below 4°C, while above this temperature, it can aggregate into large polymeric units (Krishna et al., 2021). This aggregation can occur in the presence or absence of ionic calcium at temperatures greater than 8.5°C.

Monomeric κ -casein (19 kDa) has two cysteine residues which form an intramolecular disulphide bond (Walstra & Jenness, 1984) and has two genetic variants, denoted A and B. κ -Casein can form polymers with itself and with other proteins during heat treatment which is particularly pertinent for dairy processing. Unlike the other casein fractions, κ -casein is glycosylated at the C-terminus and does not contain any phosphoserine cluster (O'Regan, Ennis, & Mulvihill, 2009). Therefore, κ -casein has the ability to remain soluble in the presence of ionic calcium, and supports the stabilization of other caseins from calcium-induced precipitation (Fox & McSweeney, 1998).

The casein fractions described above are mainly present in milk as spherical particles known as micelles, with an approximate diameter of 180 nm (Dalglish & Corredig, 2012), but can exist as much smaller (40 nm) or larger particles (600 nm) (de Kruif, Huppertz, Urban, & Petukhov, 2012). The unique structure of micelles in milk, exists to suspend sufficient calcium and phosphorous needed by the neonate.

The mineral composition of the micelle contains calcium, magnesium, phosphate and citrate, known collectively as colloidal calcium phosphate (CCP) (Fox & McSweeney, 1998). Micelles are highly hydrated, containing 2-4 g water per g of protein (Liu, Dunstan, & Martin, 2012). The exact casein micelle structure is still one of debate, but are essentially held together by hydrogen, hydrophobic and electrostatic charges. In the nanocluster model, phosphorylated α_{s1} -, α_{s2} -, and β -casein can bind to CCP, thereby forming a stabilizing protein shell. The micelle surface is largely covered by κ -casein molecules, which provides intermicellar electrostatic and steric repulsion (de Kruif & Zhulina, 1996). Both α_{s1} - and α_{s2} -casein contain more than one phosphate centre and can thus act to link between nanoclusters (de Kruif & Holt, 2003). This cross-linking allows nanoclusters to associate and form casein micelles.

β -Lactoglobulin consists of 162 amino acids with a molecular mass of 18 kDa and is the main whey protein present in bovine milk (~10%). β -Lactoglobulin can form four intramolecular disulphide bridges and has one free sulphydryl group, capable of forming one intermolecular disulphide linkage (Fox et al., 1998). α -Lactalbumin makes-up ~4% of the total bovine milk protein, having a relatively low molecular mass of ~14 kDa, with an isoelectric point of 4.8. It consists of 123 amino acid residues, which includes four tryptophan residues and a high proportion of essential amino acids (cysteine, isoleucine, leucine and lysine; Brew, 2003). The structure of α -lactalbumin is stabilised by four disulphide bonds and by the association of ionic calcium, which connects two domains - a large α -helix and a smaller β -sheet, while it can also bind Mg^{2+} , Mn^{2+} , K^+ and Zn^{2+} (Barbana & Pérez, 2011). Under a variety of mild denaturing conditions, α -lactalbumin adopts a partially structured conformation termed as 'molten globule' (Chang, Bulychev, & Li, 2000). The structure of α -lactalbumin molten globule is characterized by a high degree of native-like secondary structure

and a fluctuated tertiary fold. Depletion of ionic calcium from α -lactalbumin leads to a change from the native state to the molten globule state.

A number of other notable proteins are lactoferrin, immunoglobulins and bovine serum albumin. These whey proteins are present in relatively low amounts but are essential for neonatal nutrition, each having unique structure and function. However, from a dairy processing perspective, their role is not quite as influential, mainly due to their low concentrations in mature bovine milk. There are some commercial processes for isolating and concentrating lactoferrin and immunoglobulins, but these processes require large starting volumes of milk or milk derivatives and their applications are niche.

Non-protein nitrogen in bovine milk refers to a number of nitrogenous compounds that are soluble in 12% trichloroacetic acid (TCA) (Fox & McSweeney, 1998). NPN compounds include peptides, urea, uric acid, ammonia, creatinine, creatine, nucleic acids, free amino acids (e.g., taurine and glutamic acid), carnitine, choline, amino sugars and alcohols, low-molecular weight peptide-hormones and other biologically active compounds such as growth factors (Atkinson, Alston-Mills, Lonnerdal & Neville 1995).

1.2.2. A brief overview of the milk mineral system

The salt system in milk is designed to deliver a complete balance of minerals for correct physiological function of the neonate. Milk minerals can be divided, based on their concentration, into macro or minor minerals, with one group no less important than the other from a nutritional perspective (Table 1.4). Aside from their nutritional importance, and although present in relatively low amounts compared to protein, carbohydrate and fat, minerals are incredibly important for product functionality. Milk

minerals are present as insoluble, soluble, ionic and bound states, all of which is ideally balanced in milk within the mammary gland. The typical mineral concentration in skim milk is between 8 to 9 g/L, with the mineral profile depending on seasonality, stage of lactation, diet and health status of the cow (Gaucheron, 2005). As well as macro and minor minerals, the mineral balance can be distinguished based on those associated with micellar casein and those present in the serum phase. The calcium and phosphate content of milk is highly correlated with the level of casein, since much of the calcium and phosphate is associated with the micelle (Lucey & Horne, 2009). Compared to their total concentration in milk, calcium, magnesium and phosphate ions are intimately associated with the casein micelles at levels of 70, 30 and 50%, respectively.

Table 1.4. Mineral distribution of bovine whole milk, with the concentration range obtained from various sources including (Fox et al. (2015); Gaucheron (2005); Curtiss Hunt and Meacham (2001); Hunt and Nielsen (2009))

Mineral	Total concentration
<i>Major minerals (mmol/kg)</i>	
Calcium	26-32
Magnesium	4-6
Phosphate (total)	30-32
Citrate	9-11
Potassium	31-43
Chloride	22-34
Sodium	15-28
<i>Minor minerals (µg/L)</i>	
Zinc	3500-3900
Copper	90-200
Iron	200-500
Manganese	20-30
Iron	200-500
Selenium	17-23
Molybdenum	73-97
Cobalt	1

The majority of calcium is complexed as either calcium phosphate or as calcium ions bound to phosphoserine residues on casein proteins (Holt et al., 1986). However, in the aqueous phase of milk, calcium ions can also associate with anions such as, citrate, inorganic phosphate and chloride to form non-micellar salts (Cashman, 2011). As the distribution of ions and salts between the micellar and the aqueous phase of milk is in dynamic equilibrium, calcium phosphate plays an important role in the integrity and structure of the micelle. The average phosphorus content in bovine milk is ~ 890 mg/L, but varies throughout lactation. Phosphorus in cow's milk occurs as inorganic and organic phosphate and is distributed among a number of compositional components such as, lipids, phospholipids, casein, small soluble organic esters and soluble and colloidal inorganic salts (Fox et al., 2015). The majority of the total phosphorus in milk exists as inorganic phosphate (~80%) in cow's milk and the remaining ~20% exists as organic phosphate which is esterified to casein (Cashman, 2011). The organic phosphate is mainly present as soluble free phosphate ions (H_2PO_4^- , HPO_4^{2-}), whereas inorganic phosphate is associated with casein micelles in the form of calcium phosphate (de Kruif, Huppertz, Urban, & Petukhov, 2012). One mineral present in the highest quantity in milk, potassium (Table 1.4), is nearly all located within the serum phase; similarly, sodium is present nearly entirely as soluble ions or salts of chlorides, phosphates, citrates and bicarbonates (Singh, McCarthy, & Lucey, 1997).

1.2.3. *Lactose and vitamins*

Lactose, the major carbohydrate of bovine milk is a reducing disaccharide sugar, containing glucose and galactose linked together via a glycosidic bond (Fox, 2009), with a concentration (anhydrous lactose) of about 4.8%, w/w, in milk.

However, its level in milk is heavily influenced by stage of lactation (see Section 1.2.4) (Deeth, 2009). The commercial production of lactose is most commonly attained as a byproduct of cheese whey processing, where, after retention of protein by ultrafiltration the whey permeate is evaporated, cooled and the lactose allowed to crystallize in crystallization tanks under gentle agitation. The solids content of the permeate after evaporation and the subsequent cooling rate affects lactose crystal formation, particularly crystal size (Fox, 2009). There are two main structural types of lactose crystals that can be obtained, hydrate (α -lactose) or anhydrous (β -lactose), and in solution a mixture of both is present. The ratio of α - to β -lactose is dependent on lactose concentration and temperature. (Holt & Fox, 1985). The solubility of lactose and its rate of dissolution is complex with many factors affecting its state in water. α -Lactose is less soluble in water and so at saturation it converts to β -lactose, which then automatically results in a reduction in saturation, meaning that additional α -lactose can dissolve, with the process continuing until equilibrium is reached. Crystallization of lactose is usually in the α -lactose monohydrate form; however, if crystallization occurs at $>93.5^{\circ}\text{C}$, then anhydrous β -lactose crystals form. It is important to note that during water removal by spray drying, the rate of dehydration is faster than the time required for crystallization to occur and so amorphous lactose (known as the glassy state) is produced. The crystallization of lactose in dairy powders is generally detrimental to product quality and greatly affects powder functionality (e.g., contributing to caking and stickiness). As lactose is a reducing sugar it can contribute to Maillard reactions (non-enzymatic browning) with amino groups of proteins, peptides, and free amino acids, and thereby reduce the nutritional quality of protein (Berg, 1993).

Water- and fat-soluble vitamins are present in the aqueous phase and fat globules of milk, respectively (MacGibbon, 2020) Table 1.5 shows the water- and fat-

soluble vitamin composition of milk, adapted from Mehta (2015) and Haroon et al. (1982). The most abundant water-soluble vitamins in milk are riboflavin (B2), pantothenate (B5), niacin (B3) and thiamine (B1); with the exception of vitamin C, water-soluble vitamins are generally stable to relatively high thermal processing temperatures, with the exception of sterilisation (Fox et al., 2015). Variability in terms of water-soluble vitamin concentration can be influenced by bovine diet, as previously shown by Magan et al. (2019) where they highlighted that vitamin B1, B2 and B7 were significantly higher in milk from cows on a pasture base system, as opposed to milk produced from an indoor concentrate diet.

Fat-soluble vitamin A, particularly in the form of β -carotene, and vitamin D are most abundant in milk, with concentrations of vitamin E and vitamin K substantially lower. Concentrations of fat-soluble vitamins are more variable in different dairy products as increasing concentration of fat will consequently increase the content of fat-soluble vitamins in products such as cream or butter, while vitamin supplementation would be required in low-fat products.

Table 1.5. Average water- and fat-soluble vitamin composition of bovine whole milk.

Vitamin	Average concentration ($\mu\text{g L}^{-1}$)	Average concentration (mg L^{-1})
<i>Water-soluble</i>		
Thiamine (B1)	400	
Riboflavin (B2)	1700	
Niacin (B3)	1000	
Pantothenate (B5)	3500	
Pyridoxine (B6)	600	
Biotin (B7)	30	
Folate (B9)	50	
Cobalamin (B12)	5	
Vitamin C		20
<i>Fat-soluble</i>		
Retinol (A)	400	
Calciferol (D)	1.0	
Phylloquinone (K)	5	
Tocopherol (E)		10

Adapted from Mehta (2015) and Haroon et al. (1982).

1.2.4. Influence of seasonality on milk composition

The influence of seasonality on pasture based milk production systems is more a result of stage of lactation than diet or other external factors. On a pasture-based system, calving takes place over a narrow window, usually in late winter and early spring. Therefore, the composition of the national milk pool is generally following a trend in-line with lactation, as opposed to indoor feeding systems such as that in the

UK, continental Europe and the USA where calving is more likely to take place year-round, and therefore the composition of the milk pool is of a more consistent nature. The effect of seasonality commences post-partum where the initial milk, colostrum, is fed to the calf, before the subsequent milk is collected for processing and human consumption. Colostrum has high total solids content, with high levels of whey proteins (such as lactoferrin and immunoglobulins) and fat, but low levels of lactose (McGrath *et al.*, 2016). After the initial colostrum phase, milk yield initially increases and then remains steady for ~12 weeks before a gradual decline in milk volume occurs. This decline is in contrast to an increase in fat and protein content, with lactose content remaining constant (Table 1.6). A decline in lactose content does not commence until late lactation and is often used as a measure of milk quality by processors.

Table 1.6. Changes in major compositional components of Holstein-Friesian milk throughout lactation from a seasonal grass-based production system.

%	Early-lactation	Mid-lactation	Late-lactation
Total solids	13.60	13.56	14.58
Protein	3.33	3.51	3.89
Fat	4.56	4.46	4.90
Lactose	4.98	4.92	4.75
Casein	2.66	2.78	3.31
Whey	0.48	0.54	0.65

Adapted from Guinee and O'Callaghan (2013)

1.3. Thermal treatment and heat stability of milk

The heat stability of milk is determined by its compositional components and the temperature and holding time of treatment. Poor microbial quality can have a detrimental effect on milk heat stability, by reducing pH as a result of microbial activity-derived acid. There are a wide variety of time-temperature combinations for heat treatment across multiple processes, each with different objectives, with the

choice mainly determined by the extent of microbial deactivation required (Table 1.7), the extent of protein denaturation/aggregation achieved, the survival of key nutritional components (e.g., vitamins) and the extent of sensory change (colour and flavour).

Table 1.7. Heat treatments commonly used in the dairy industry.

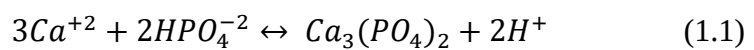
Heating regime	Conditions	Objective
Thermization	65°C × 15 min	Lipases/proteinases producing vegetative cells of spoilage psychrotrophs
Pasteurization		
Low temperature long time	63°C × 30 min	Non-spore-forming pathogens, psychrotrophic spoilage bacteria
High temperature short time	72°C × 15 s	
Production of specific products	85-90°C × 5-15 min	Pre-treatment for yoghurts and protein co-precipitates
Forewarming	90°C × 2-10 min	Preparatory step for sterilization
Ultra-pasteurized	120°C × 20 s	
UHT	130-140°C × 3-5 s	All non-spore-forming bacteria and all spores except highly heat-resistant spores
In-container	110-115°C × 10-20 min	

Adapted from Grandison, Youravong, and Lewis (2000).

Bovine milks can be grouped based on their stability when heated at 140°C across a pH range of 5.8 to 7.4 and can be designated as having either a type A or type B heat coagulation time (HCT) profile. Type A milk is the most common profile and shows an increasing HCT with increasing pH from 5.8 to 6.6, followed by a decrease in HCT between pH 6.8 and 7.1. The exact pH of HCT decrease in this region can vary between cows but is caused by a dissociation of κ -casein from the casein micelle, causing calcium-induced aggregation and coagulation. The HCT usually increases again at pH 7.2 and although κ -casein remains dissociated from the micelle, at this pH

the proteins remain heat stable for an extended period, due to an increase in electrostatic repulsion (i.e., further from the isoelectric point). Type B HCT profiles display increasing heat stability with increasing pH and can occur quite frequently in individual cows. Studies have shown HCT profile of milk obtained from individual cows to change over lactation but without any clear factor identified as the main cause (Loveday, Weeks, Luo, & Cakebread, 2021). Like Type A milks, milks displaying type B profiles also display κ -casein dissociation in the pH range 6.8-7.1, but without a clear decrease in heat stability. Previously, Tessier and Rose (1964) showed that increasing levels of κ -casein in milk could alter the HCT profile from a type A to a type B. Interestingly, the literature has not shown a type B HCT profile for commercial bulk milk samples.

Heat treatment also affects the mineral balance of milk. During heat treatment, the solubility of ionic calcium and phosphate decreases and heat-induced CCP forms with a concomitant shift towards more acidic pH, as shown in Eq. 1.1 (Lewis & Heppell, 2000; Lucey & Horne, 2009).



Under heating conditions at temperature less than 95°C, the changes in acidity, soluble calcium and phosphorous are mainly reversible once cooled (Anema, 2009), whereas, after high heat treatment at >95°C, the reaction is only partially reversible (Fox et al., 2015). Calcium phosphate deposit formation on the surface of heat exchangers and within the calandria of evaporators are a common issue in dairy processing (Rosmaninho & Melo, 2006; Visser & Jeurink, 1997). Fouling can be classified as type A (protein) or type B (mineral) fouling (Bansal & Chen, 2006), (Not

to be confused with type A and B HCT profiles). Under pasteurization conditions a type A deposit can form, containing 50-60% protein, 30-35% minerals and some fat (4-8%); however, in cases of thermal treatments at $>110^{\circ}\text{C}$, minerals precipitate as a type B deposit, mainly composed of calcium phosphate (Jeurnink & Brinkman, 1994).

1.4. Dairy-based ingredients

In general, there are a number of dairy product categories that are readily identifiable, namely, fresh and shelf-stable liquid milk, butter, cream and cheese; however, there are a multitude of sub-ingredients that are produced from whole milk (Lopez, 2021), as shown in Figure 1.1. OECD-FAO data shows that dairy powders, such as whole milk powder (WMP) and skim milk powder (SMP), are the most traded agricultural commodities worldwide, in contrast to fresh liquid dairy products (OECD, 2016). The main reason for the high trade in dehydrated products has been the requirement for convenient, low-cost transportation and shelf-stable storage of dairy products (OECD et al., 2016) as opposed to fresh dairy products, which are more prone to spoilage and have considerably more limited shelf life. Increasing international demand for high protein dairy ingredients has brought about significant growth in the dairy industry. Concentration by vacuum assisted evaporation and spray drying has allowed economical preservation of milk and milk products for global trade, although it is the valorisation of milk by membrane filtration that has allowed the greatest range of new protein ingredients to be produced (Daufin et al., 2001; Kumar et al., 2013).

Membrane filtration has allowed the conversion of what was once waste streams from cheese and acid casein production in to highly valuable ingredients and products. Filtration has facilitated the production of milk protein and whey protein

concentrates, micellar casein concentrates, in addition to more high-end ingredients such as enriched individual casein and whey protein fractions for specific nutritional purposes. Their existence is possible through technological advances such as ultrafiltration (Dash et al., 2022), diafiltration (DF), microfiltration, ion exchange, vacuum-assisted evaporation and drying (Agarwal, Beausire, Patel, & Patel, 2015; Sutariya, 2015). These technologies allow for selective fractionation and enrichment of milk proteins and depletion of milk serum components such as lactose, water and minerals (Hazlett, Schmidmeier, & O'Mahony, 2021). Although, high protein concentrates can be associated with processing difficulties, primarily attributed to high viscosity, leading to fouling of processing equipment, such as obstruction of membrane pores during filtration, product build-up in falling film evaporators, blocking of high pressure spray nozzles. Understanding the interactions between composition and processing conditions during evaporation and spray drying allow process optimization and negates unwanted downtime (McSweeney & O'Mahony, 2015). Controlling rheological properties and time-dependent behaviour of liquid or reconstituted dairy concentrates are integral parts of dairy processing (Carr, Southward, & Creamer, 2003; Trinh, 2006). The overall composition of milk as feed material has a significant impact on viscosity during unit operations, particularly the protein content, protein type, and denaturation level (Ho et al., 2018; Karlsson, Ipsen, Schrader, & Ardö, 2005; Kelleher et al., 2020; Singh, Chandrapala, Udabage, McKinnon, & Augustin, 2015) together with the lactose content and mineral composition (Bienvenue, Jiménez-Flores, & Singh, 2003; Tsermoula et al., 2021).

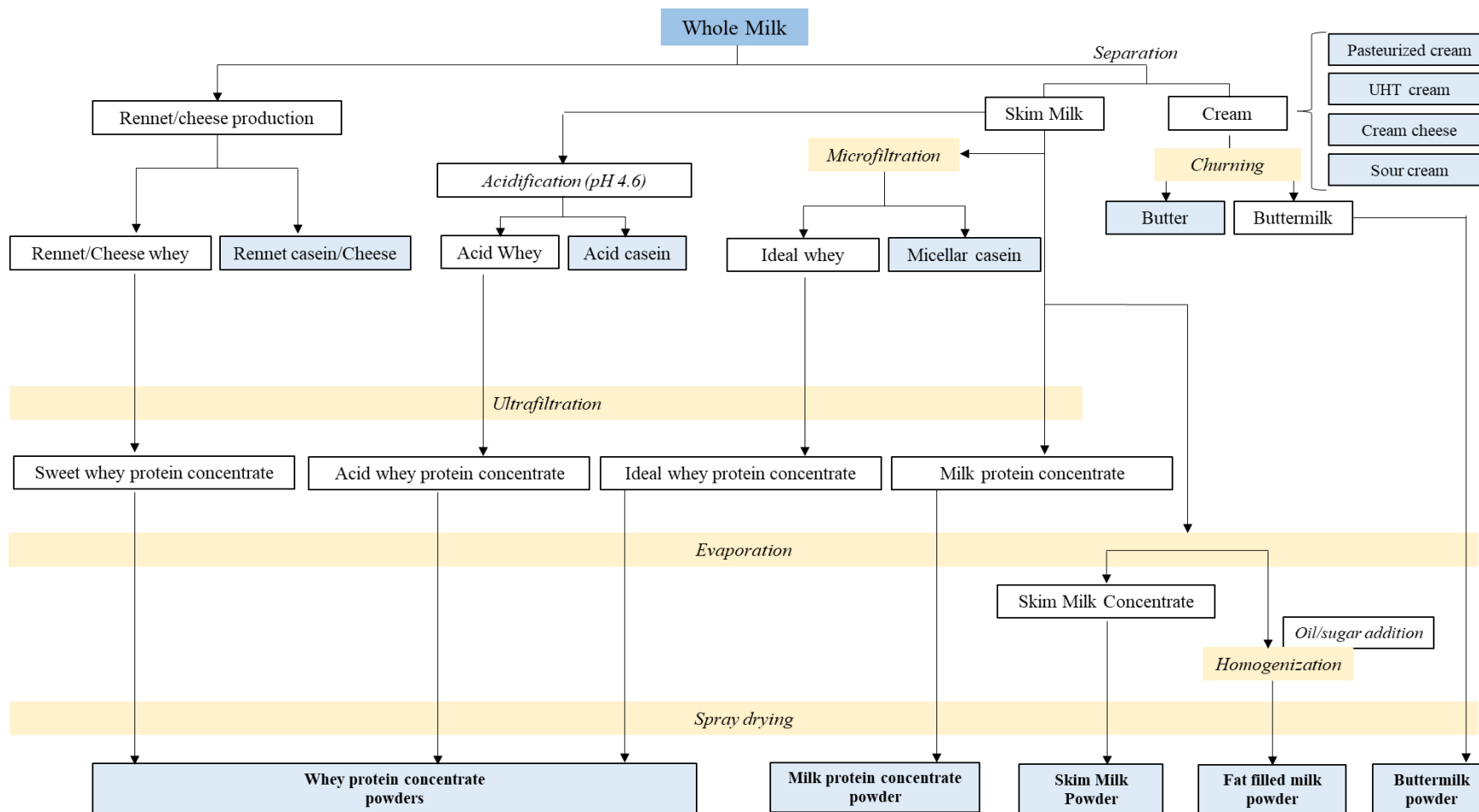


Figure 1.1. Schematic representation of a selection of dairy products and ingredients

1.4.1. High protein ingredients

Milk protein concentrates, isolates and milk permeate

Milk protein concentrates (MPCs) and isolates (MPI) contain the entire complement of dairy proteins present in the same ratio as found in skim milk. MPC ingredients are available in protein concentrations ranging from 42 to 85%, w/w, dry matter basis (e.g., MPC85) (Meena, Singh, Panjagari, & Arora, 2017). A typical manufacturing process for MPC involves ultrafiltration (Dash et al., 2022) of pasteurized skim milk using membranes with molecular weight cut-off of 5-10 kDa. This allows the retention of micellar casein, non-micellar casein, α -lactalbumin, β -lactoglobulin and other minor whey proteins while depleting the system of lactose, serum minerals and NPN (urea, free amino acids, etc.). Choosing the correct molecular weight cut-off (MWCO) for filtration is important, so as not to lose intact proteins, but also to allow a high permeate flux and efficient processing. To wash out more low molecular weight material, additional diafiltration (DF) steps are needed in order to produce MPC with a protein content greater than 70%, w/w, dry matter (Havea, 2006). The retained stream after the UF processing is subjected to pre-heat treatment followed by evaporation and spray drying. As MPC ingredients contain casein in the form of micelles, there is a significant quantity of calcium and phosphate co-enriched with the casein proteins (O'Kennedy & Mounsey, 2009). This makes MPC products highly desirable for many nutritional applications; indeed, MPCs are widely used in follow-on infant formulas, medical nutrition, cheese, yogurt, coffee creams, ice-cream, nutraceuticals, therapeutics and sports nutrition (Sunkesula, Kommineni, Meletharayil, Marella, & Metzger, 2021). The ease of creating MPC ingredients with diverse protein to lactose ratios allows for the production of tailored compositional and functional attributes for end-products (De Castro-Morel & Harper, 2002; Sikand,

Tong, & Walker, 2013). According to the American Dairy Products Institute, MPC should contain 5–6% moisture, 1.25–2.5% fat, 40–85% protein, 8–10% ash and 8–52% lactose (w/w).

Whey protein concentrates

Whey, a by-product from the manufacture of rennet, cheese or acid casein was once discarded or used as animal feed. It primarily consists of water, whey proteins (β -lactoglobulin, α -lactalbumin, bovine serum albumin, immunoglobulins and other minor nitrogen components), lactose and minerals. Liquid whey is ~6-7% total solids, with 0.7-1.0% protein, ~4.5% lactose, minerals and residual fat (McDonough, Hargrove, Mattingly, Posati, & Alford, 1974), with the specific composition of whey varying based on the manufacturing method (Figure 1.1). Whey from cheese differs depending on cheese variety, while acid whey differs depending on its origin, i.e., from acid casein production or Greek style yogurt (de la Fuente, Hemar, Tamehana, Munro, & Singh, 2002). Whey obtained from rennet coagulation involves cleavage of κ -casein, therefore it has micro-peptide content in the resulting whey stream (Fox, Guinee, Cogan, & McSweeney, 2017). Cheese whey powders are produced by first concentrating using evaporation or reverse osmosis followed by spray drying, and contain ~10-15% protein in the final powder. Since whey powders contain a high lactose content, care must be taken during drying to prevent crystallization, similarly during storage, atmospheric conditions must be controlled. In some cases, controlled crystallization takes place prior to drying, to prevent product quality issues. Applications include sausages, baked goods, soups and salad dressings. With the valorisation of whey in the food and beverage industry, more than 50% of the volume produced from cheese manufacture is further processed to create additional value.

Ultrafiltration, as described above in the production of MPC, is used to concentrate protein and remove lactose, NPN, soluble minerals and water (Marcelo & Rizvi, 2008). Similar to MPC powder, WPC ingredients are produced with protein contents typically ranging from 35-80%, w/w, and whey protein isolates at >90%, w/w (Morr, 1985).

Micellar casein concentrate

Micellar casein concentrate (MCC) is produced by microfiltration of skim milk using 0.1-0.2 μm molecular weight cut-off membranes, which allow the permeation of whey proteins, lactose, non-casein bound minerals, vitamins and water through the membrane, retaining and concentrating the micellar casein fraction of the milk (Hammam, Martínez-Montegudo, & Metzger, 2021). Membranes used for MCC production vary, with spiral wound polymeric membranes most common in industry, although ceramic membranes are also used. The advantage of spiral wound membranes is their large surface area to overall size compared ceramic membranes, along with their lower capital and operating costs. Numerous studies have examined efficient means of producing MCC; however, even after several rounds of diafiltration, MCC powders can contain significant levels of whey protein, often with a casein to whey protein ratio of 90:10. Several studies examined the optimum membrane selection and processing conditions to obtain higher levels of whey protein depletion, with up to 95% rejection. Beckman and Barbano (2013) investigated the effect of concentration factor on the removal of serum proteins from skim milk during microfiltration at 50°C using a 0.3 μm pore size spiral-wound polymeric polyvinylidene fluoride membrane and reported that as concentration factor increased, the percentage of serum protein removed from skim milk increased using a single-

stage feed-and-bleed MF system. Adams and Barbano (2013) examined the use of isoflux ceramic MF membranes with 0.14 μm MWCO to deplete serum proteins from skim milk and reported significantly lower removal of protein compared to theoretical values. A study by Subhir, McSweeney, Fenelon, Magan, and Tobin (2022) assessed the partitioning of serum proteins in skim milk using 0.1 μm MWCO ceramic membranes, and highlighted the challenges of correctly determining nitrogen fractions. The authors suggested that a combination of quantitative analysis (Kjeldahl) and qualitative analysis (HPLC/SDS-PAGE) provides better insights into the true partition efficiency of serum proteins during MF. Factors, such as foulants (concentration polarization and membrane blocking) and whey protein aggregation can all hinder permeation of serum proteins. Notwithstanding the challenges experienced with production of MCC, there are a number of applications for MCC ingredients, such as Greek-style yogurt, cheese, processed cheese, nutritional beverages and even cosmetics.

Caseinates

Caseinates are produced when skim milk is acidified to the isoelectric point of casein at $\sim\text{pH}$ 4.6 and casein is allowed to precipitate. While different acidulants may be used, hydrochloric acid is the most commonly used, being added to tempered skim milk at $\sim 30^\circ\text{C}$ and gently agitated. Once precipitated, the aggregated casein is separated from the acid whey using a decanter, where the acid casein may be washed with water several times to remove any residual lactose and other serum components. Once washed, the acid casein is dispersed in water and re-neutralised using sodium or calcium hydroxide to produce sodium and calcium caseinate, respectively (Carr & Golding, 2016). The re-dispersed casein is usually spray dried at $\sim 18\text{-}20\%$ TS and has

numerous technological applications (e.g., emulsification) and represents an excellent source of amino acids (Carr & Golding, 2016). Caseinate powders contain approximately 92% protein, ~4% ash and about 4% moisture. Applications in the food, cosmetic, paint and pharmaceutical industries mean that caseinates are highly versatile ingredients, due to the ease with which their functional properties can be manipulated.

1.4.2. Concentration of skim milk and high protein systems

Water removal prior to spray drying is an essential step in dairy powder production, considering water evaporation in the dryer is essentially non-recoverable and so very energy intensive (Patil, Tanguy, Floch-Fouéré, Jeantet, & Murphy, 2021). Therefore, vacuum assisted evaporation is much used, economical means of increasing total solid content prior to drying. The precise process used for skim milk powder production is actually determined by the heat classification of the final powder, low, medium or high heat SMP (Patel, Anema, Holroyd, Singh, & Creamer, 2007). The heat classification is an indicator of whey protein denaturation using the whey protein nitrogen index (WPNI) method and ranges from >6.0 mg/g powder for low, 6.0–1.5 mg/g powder for medium and <1.5 mg/g powder for high heat (Sharma, Jana, & Chavan, 2012). Low heat skim milk powder, while obviously possible, can be difficult to manufacture due to the low pasteurization temperature required to avoid significant denaturation. This is also made problematic by the sequential heating processes during production, i.e., pasteurization, cream separation, heat load in each effect of the evaporator and pre-heating on the way to the dryer. The more commonly produced heat classification of SMP is medium heat, usually requiring a higher heat treatment (e.g., 100°C for 0.5 to 1 min). Medium heat SMP is used in a wide range of applications such as infant formulas, fat filled milk powders, for yogurt manufacture

and tea and coffee whitening, etc. High heat SMP, as the name suggests, is subject to severe heat treatment temperatures in process (e.g., $\sim 120^{\circ}\text{C}$ for >3 min), where heat stability is essential for end-product use, such as recombined evaporated milk (Sharma et al., 2012). The level of heat treatment has consequences for evaporative capacity during concentration; for the most part, skim milk can be evaporated from 9 to 50-55% TS, but is dependent on the protein content of the milk, with higher protein content reducing the solids content achievable. This can be highly relevant at the end of the lactation season where protein content can increase significantly.

Reducing the protein content for standardised SMP by adding either lactose or milk permeate can allow for higher solids to be obtained, compared to non-standardized milk. However, there can be considerable differences in the evaporation capacity, as milk permeate addition has been shown to result in higher viscosity in concentrates compared to lactose addition. Bienvenue et al. (2003) showed that the mineral content of skim milk has a significant effect on viscosity and the age-thickening properties post-evaporation, with higher mineral content associated with higher viscosity. Murphy et al. (2018) reported that protein standardisation of skim milk with milk permeate resulted in higher viscosity in skim milk concentrates, compared with protein standardisation with lactose.

Similar to skim milk, high protein products are almost always evaporated prior to drying but unlike skim milk, such products have a significantly lower mineral and lactose content. One other important consideration when evaporating high protein streams is to consider the protein profile of the product, for instance, MPC is likely to be more heat stable than WPC's, where $\sim 80\%$ of the total protein is micellar casein. Therefore, liquid MPC streams can be heat treated to 120°C to impart desired end-user functionality. This is in comparison to WPCs, where significant denaturation can

occur at temperatures greater than 65°C ,and result in immediate gelation of the product within the evaporator. Contrastingly, WPI liquid retentate streams often undergo nanofiltration, after ultrafiltration, to remove additional water before being sent to the dryer with no vacuum assisted evaporation needed. For MPC, the achievable solids content from the evaporator can be as high 30% dry matter; for instance, an evaporated MPC80 stream at 30% TS contains ~24% protein in the concentrate. This is considerably higher than the 17.5% protein in skim milk concentrates (i.e., at ~50% total solids); however, the reduced mineral content in the MPC does contribute to a lower viscosity.

1.5. Conclusion

This chapter provided important fundamental background on milk composition and the effects that a seasonal milk production system have on milk composition, heat stability and processability. However, a number of factors remain unknown, or poorly understood, in relation to milk composition and its influence on dairy processing, particularly the role of soluble minerals and the effect of pH on protein stability and product viscosity during heat treatment. Moreover, with a large and growing range of dehydrated dairy products and ingredients originating from whole milk, the need to create heat stable milk protein ingredients for nutritional product applications is expanding. Building on this, the next chapter in the thesis will discuss the key considerations in relation to pH measurement, and investigate how new technologies might allow greater control of pH during thermal dairy processing.

1.6. Acknowledgments

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Chapter 2

pH, the Fundamentals for Milk and Dairy Processing – A Review

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Declaration

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2.1. Abstract

The ability to measure and capture real-time unit operational data has significant benefits during dairy processing, whether it is the basics, such as measuring temperature, pressure, and flow rates, or more recent developments in the case of in-line viscosity and product-compositional measurements. This rapid data collection has helped increase profitability by reducing energy costs, minimizing product loss, and allowing automated control. Advances in technology have allowed for in-line measurements of the composition and some physical attributes such as particle size and viscosity; however, an attribute that spans both compositional and physical attributes is pH, directly influenced by composition but also environments, such as temperature and total solid content. pH is measured for a plethora of reasons, such as a measure of milk quality (microbial spoilage), acidification of casein, cheese production, maintaining optimum conditions during protein hydrolysis, etc. However, very little is published on the fundamentals of pH and pH measurement in dairy processing; rather, it is usually a cause-and-effect phenomenon. This review visits one of the oldest analytical considerations in the dairy industry and re-examines how it is affected by product composition and processing conditions.

2.2. Introduction

One of the most critical control parameters within the food, pharmaceutical, cosmetic, electrochemical, paper, and textile industries, to name a few, is the monitoring and measurement of pH (Karastogianni et al., 2016; Orouji et al., 2022; Salvo et al., 2018). In the dairy industry, pH is often the first indication of microbiological spoilage but is more often manipulated to create and produce an array of dairy products, including cheese, acid casein/whey, yogurt, fermented beverages, and protein hydrolysates. There are several attributes associated with fluids, such as density, pressure, compressibility, and viscosity, all of which are affected by environmental conditions and compositional attributes. A biological fluid such as milk is defined by the aforementioned properties; however, dairy processing compounds the complexity to which milk properties are subject. Water, the main constituent of bovine milk (~87%, w/w), contains both dissolved and suspended components, all designed to act as a source of hydration and nutrient delivery for the neonate. pH is a dynamic parameter that changes constantly during milk processing, whether due to temperature, pressure, total solid content/water removal, or microbial activity. For the most part, pH can only be measured in an aqueous medium (continuous phase comprised of water) and so cannot be measured in pure oils or alcohols. In the dairy industry, pH is usually adjusted or controlled by the addition of mineral acids and bases or through the addition of microbial cultures.

Since pH has been used universally in food physics and chemistry for well over a century, not much thought is given to its actual meaning, but rather pH is only associated with a certain outcome or desired product. Therefore, this review covers the fundamental understanding of pH, its measurement, and how it changes during milk processing, emphasizing the interrelationships between total solid content,

minerals, and temperature. The review also offers insights into some of the new developments in pH data collection of specific relevance to the dairy industry.

2.3. Understanding pH

The quantitative determination of pH is perhaps one of the oldest and most commonly used analytical methods in the dairy industry. The term “pH” is an abbreviation of the power of hydrogen or potential of hydrogen introduced by the Danish scientist Søren Peder Lauritz Sørensen in the early 20th century. pH is defined as a measure of the hydrogen ion activity, an ‘effective concentration’ in an aqueous solution representing the moles of hydrogen ion (H^+) per liter of solution for dilute systems at a given temperature. Mathematically, pH and pOH (power of hydroxide ion) are defined as the negative logarithm to the base 10 of H^+ or OH^- ion concentrations at 25 °C ((2.1)–(2.3)).

$$pH = -\log [H^+] \quad (2.1)$$

$$pOH = -\log [OH^-] \quad (1.2)$$

$$pH + pOH = 14 \quad (2.2)$$

As mentioned previously, pH is, for the most part, measured in aqueous solutions and is based on the dissociation of water (H_2O) into equal concentrations of hydrogen and hydroxyl (OH^-) ions.



The dissociation constant for this reaction is expressed as K_w and is the auto-ionization or auto-dissociation to form H^+ and OH^- ions, equal to 10^{-14} at 25 °C (2.5).

This relationship may be expressed as:

$$[\text{H}^+][\text{OH}^-] = K_w = 10^{-14} \quad (2.4)$$

Since the concentration of H^+ and OH^- ions are equal in pure water, it is referred to as pH neutral, giving an equal concentration of H^+ and OH^- ions (i.e., 10^{-7} mol/L). Therefore, to simplify the use of H^+ and OH^- ion concentration, pH may be defined as the negative logarithm of $[\text{H}^+]$ ((2.6) and (2.7)).

$$\text{pH} = -\log[\text{H}^+] \quad (2.5)$$

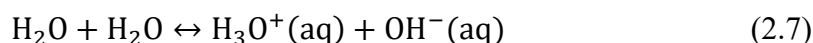
$$[\text{H}^+] = 10^{-\text{pH}} \quad (2.6)$$

Therefore, a convenient scale for pH was developed based on the dissociation of water (H_2O) and ranged from 0 to 14 (Table 2.1). However, it must be mentioned that at high concentrations, acids and bases can expand outside the typical pH scale of 0–14 (further details provided in Section 2.4).

Table 2.1 The pH scale based on H^+ and OH^- concentration.

	pH	H^+ Concentration (mol/L)	OH^- Concentration (mol/L)
Acidic	0	1.0	0.00000000000001
	1	0.1	0.00000000000001
	2	0.01	0.00000000000001
	3	0.001	0.00000000000001
	4	0.0001	0.00000000000001
	5	0.00001	0.00000000000001
	6	0.000001	0.0000000001
Neutral	7	0.0000001	0.0000001
Alkaline	8	0.00000001	0.000001
	9	0.000000001	0.00001
	10	0.0000000001	0.0001
	11	0.00000000001	0.001
	12	0.000000000001	0.01
	13	0.00000000000001	0.1
	14	0.000000000000001	1.0

In nature, the description of a singular atomic hydrogen ion (H^+) is not entirely accurate. Free H^+ ions are extremely reactive and therefore remain free for an exceptionally short period in aqueous solutions. The proton (H^+) cannot exist alone in water, as this form of hydrogen has one proton and only one electron; therefore, the electric field near the singular proton is exceptionally strong, and so the proton is hydrated, forming a hydronium ion (H_3O^+), see Section 2.1. Thus, the use of the term hydrogen ion does not refer to the atomic H^+ ion but to its hydrated form (Martinsen & Grimnes, 2011) (2.8). In most cases, the hydronium ion is further solvated by water molecules in clusters such as $H_5O_2^+$ and $H_9O_4^+$ (Calio et al., 2021).



2.3.1. The Hydrogen Ion

Hydrogen, with an atomic number of 1, is the simplest of all atoms containing a single proton and a single electron (Yang, 2016), while the simplest molecule is H_2 , a molecular analog of atomic hydrogen consisting of two protons and one electron. Hydrogen is the most abundant element in the universe, comprising ~75% of all matter. Hydrogen ions are the foundation of all aqueous acid-base reactions and are involved in countless biological functions, catalysis reactions, and protonation. Despite its importance in natural sciences, it is near impossible to measure H^+ ions due to their highly reactive nature. This reactivity makes it one of the most essential elements in nature.

The term hydrogen ion refers to the hydrogen ion present in aqueous solutions, in which it exists as the combined molecule (H_3O^+). Hydrogen has three known isotopes observed in nature, existing as either protium, deuterium, or tritium. The protium form is the most common isotope comprising 99.98% of all hydrogen, which

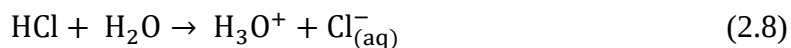
consists of only one single proton and electron (Bleam, 2017). Deuterium makes up only 0.0026–0.0184% of all hydrogen that exists on Earth and consists of one electron, and within its nucleus contains one proton and one neutron(Katz). Water enriched in molecules that contain deuterium is known as heavy water and is used in nuclear reactors as a coolant (Carroll, 2020; Iglesias & Barber, 2001). Tritium, the radioactive isotope of hydrogen, contains one proton and two neutrons in its nucleus. An insignificant amount of tritium occurs naturally due to its interaction with atmospheric gases.

The pH scale defines the acidity or basicity of a “dilute aqueous solution, in which the solvent is water,” and the hydrogen ions can move about freely in the solution. Therefore, it is strictly applicable and correlated to the “hydrogen ion activity” and not to other ions that might exist in the solution. If measurements are to be performed in non-aqueous liquid and aqueous–organic mixed solvents, the traditional pH measurement loses its ability to respond to H^+ ions due to the dehydration of the electrode and consequent signal drifting (Rondinini, 2002). Measurement of hydrogen in non-aqueous solvents can be conducted with electrodes that contain a non-aqueous filling solution (i.e., saturated lithium chloride in ethanol for non-polar solvents or acetic acid for polar solvents).

2.3.2. Acid -Base Reactions

The transfer of hydrogen ions in an acid–base reaction is referred to as proton transfer (2.9). The acid is the H^+ donor, and the base is the H^+ acceptor. K_a and K_b are correlated through the ionic constant of water (K_w) (2.10). Under the same conditions, weak acids have a higher pH value than strong acids.

Acids and bases are classified based on their ionization level in an aqueous solution, where ionization is a measure of the tendency of an atom to resist the loss of electrons.



$$K_w = [\text{H}_3\text{O}^+] [\text{OH}^-] = 1 \times 10^{-14} \quad (2.9)$$

Weak acids (e.g., acetic acid, phosphoric acid, and hydrofluoric acid) and bases (e.g., ammonia, sodium bicarbonate) do not fully ionize in water compared to strong acids (e.g., hydrochloric acid, nitric acid, and perchloric acid) and bases (e.g., sodium hydroxide, potassium hydroxide). Therefore, the equilibrium constant becomes a critical parameter in order to calculate the pH (Butler, 1998; Streng et al., 1984). The equilibrium constant values of some commonly used acids in dairy science can be seen in Table 2.2.

Table 2.2. Equilibrium constant values of some strong and weak acids commonly used in dairy processing.

Acid processing					
Acid		Chemical Formula	pK _{a1}	pK _{a2}	pK _{a3}
Strong acid	Hydrochloric Acid	HCl	-7.0	-	-
	Phosphoric Acid	H ₃ PO ₄	2.1	7.1	12.4
Weak acids	Citric Acid	HOC(CO ₂ H)(CH ₂ CO ₂ H) ₂	3.1	4.8	6.4
	Lactic Acid	CH ₃ CH(OH)COOH	3.9	-	-
	Acetic Acid	CH ₃ COOH	4.8	-	-

The ability of acids to become deprotonated (donating a hydrogen ion) at a particular pH value is determined by their H⁺ dissociation constant (pK_a) (Upreti et al., 2006). pH, pK_a, and pK_b are closely related, where the pK_a and pK_b values are a quantitative measure of the acid and base strength ((2.11)–(2.13)), both representing the pH value required for the system to be able to donate or accept a proton.

$$pK_a = -\log[K_a] \quad (2.10)$$

$$pK_b = -\log[K_b] \quad (2.11)$$

$$pK_a + pK_b = 14 \quad (2.12)$$

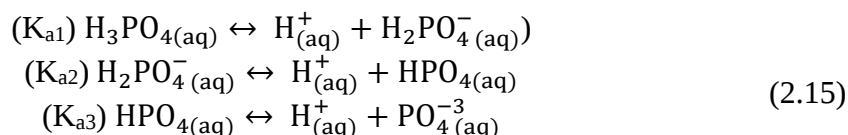
The pK_a and pK_b values are described by the Henderson–Hasselbalch Equations (2.14) and (2.15).

$$pH = pK_a + \log\left(\frac{\text{conjugate base}}{\text{weak acid}}\right) \text{ (for weak acid)} \quad (2.13)$$

$$pOH = pK_b + \log\left(\frac{\text{conjugate acid}}{\text{weak base}}\right) \text{ (for weak base)} \quad (2.14)$$

Polyprotic acids and bases are able to donate or accept more than one proton per molecule (Khan et al., 2019). Phosphoric acid is classified as a polyprotic acid and has

three ionization stages and therefore has three equilibrium constants described as K_{a1} , K_{a2} , and K_{a3} (2.16).



It is important to understand the mechanism of polyprotic acids, such as the calcium salt of phosphoric acid (tricalcium phosphate), as this is a key component of milk and particularly of the casein micelle and becomes even more important during milk processing and product manufacture (Holt, 1997; Lucey & Horne, 2009).

2.3.3. *The pH-Temperature Relationship*

Temperature is known to have a strong effect on the chemical equilibrium and concomitantly affects the equilibrium constant (K). The equilibrium constant depends on temperature, ionic strength, and the dielectric constant of the solvent. Increasing the temperature of the system results in increased molecular vibration, ion activity, and decreased propensity for hydrogen bond formation (i.e., more free H^+ ions). Studies have applied mathematical modeling in order to calculate the temperature dependence of the pK_a values (Ayyampalayam, 2004; Reijenga et al., 2013).

In thermodynamics, the equilibrium constant is related to the rate of the free energy change of the reaction in relation to temperature, which is represented by Van 't Hoff's Equation (2.17). R represents the gas constant, and ΔH represents the enthalpy change, which can be calculated from the change in Gibb's free energy (ΔG) by calculating the difference between the enthalpy (H) and entropy (S) (-4:). By

integrating Van 't Hoff's Equation (2.18), one can obtain the temperature dependence of the pK_a (2.19).

$$\Delta G = \Delta H - T\Delta S = -RT\ln K \quad (2.16)$$

$$\frac{d \ln K}{d(1/T)} = -\frac{\Delta H}{R} \quad (2.17)$$

$$pK_a = \frac{\Delta H}{2.303RT} \quad (2.18)$$

Therefore, measuring pH, essentially a measure of the hydrogen ion activity, is only relevant when combined with the temperature at which it was measured.

2.3.4. Hydrogen Ion Activity and pH in Non-Aqueous Solutions

For most applications, hydrogen ion concentration (molality: mol/kg solvent) is used without specifically mentioning ion activity. Hydrogen ion activity (a_{H^+}) is defined by both the concentration of hydrogen ions and the activity coefficient (γ_{H^+}), as shown in (2.20).

$$a_{H^+} = \gamma_{H^+} \times [H^+] \quad (2.19)$$

For dilute solutions, the hydrogen ion activity (a_{H^+}) and hydrogen ion concentration ($[H^+]$) are almost equal, but this is not the case in concentrated systems or high ionic environments, as the activity coefficient changes depending on the ionic strength, temperature, dielectric constant, ion charge, ion size, and solvent density (Bolan & Kandaswamy, 2005).

$$I = 1/2 \sum c_i z_i^2 \quad (2.20)$$

Therefore, one must know the ionic strength of the solution, which is determined as follows: where c_i is the concentration of each ion present (in moles per liter) and z_i represents its charge. The influence of salts presents in a solution of which the pH value is measured is called the salt effect. This salt effect is denoted by the symbol γ_{xH^+} and is defined as: where I is the ionic strength of the system and is defined as shown in Equation (2.21).

$$\log \gamma_H^x = A z_j^2 \frac{\sqrt{I}}{1 + B a_0 \sqrt{I}} \quad (2.21)$$

For aqueous solutions at 25 °C, $A = 0.51 \text{ mol}^{-1}/2\text{dm}^{3/2}$ and $B = 3.29 \text{ nm}^{-1} \text{ mol}^{-1}/2\text{dm}^{3/2}$ (2.22 and 2.23)

$$\log \gamma_H^x = \frac{-0.5\sqrt{I}}{1 + 3\sqrt{I}} \quad (2.22)$$

In addition, it is worth mentioning that strong acids and bases do not fully dissociate in water at high concentrations. This is applicable during wet chemistry methods or for cleaning/sterilization-in-place (CIP and SIP) procedures within the dairy industry. For example, the pH of 12 M HCl (concentrated hydrochloric acid) is calculated to be $-\log(12) = -1.08$, although most glass pH probes are incapable of accurately measuring this ion concentration. Furthermore, 12 M HCl may not fully dissociate in aqueous solutions, and therefore, the absolute pH may be higher than the pH theoretically calculated from acid molarity.

The definition of pH and its measurement is well-developed for aqueous solutions; however, the determination of pH in non-aqueous solutions is less defined. Numerous functions, theories, and calculations have been developed over the years to indicate the acidity/basicity of non-aqueous or strongly acidic solutions (Himmel et al., 2010). Himmel et al. proposed a unified Brønsted acidity scale following on from initial studies by Ugo et al. and Katritzky et al., who investigated acid–base equilibria in organic solvents. This was based on the absolute chemical potential of protons in any medium, which allows us to directly compare acidities in different media and give a thermodynamically meaningful definition of super-acidity. Himmel et al. established a new unified Brønsted acidity scale at 1 bar and 298.15 K. Following the origin of the unified acidity scale, the scale has been extended for several solvent–water mixtures. Several studies (Deleebeeck et al., 2021; Heering et al., 2020; Kahlert & Leito, 2019; Lainela et al., 2021) have investigated the new concept of a unified pH scale in non-aqueous, non-hydrogen bond donor solvents, such as in ethanol, methanol, acetonitrile, and also when mixed with water and saline solutions. Recently, a large European research group (Radtke et al., 2021) developed and validated reliable and universally applicable measurement procedures for determining absolute pH. In dairy processing, the measurement of absolute pH may find relevance in systems with high alcohol content, such as in cream liqueurs, where the ethanol content can be as high as 20% (v/v) in the finished product. However, this may be higher during production where the ethanol content can be as high as 50% (v/v) prior to oil and sugar addition (Erxleben et al., 2021).

2.4. Measuring pH

2.4.1 Measurement Approaches and Types of Probes

There is a large range of approaches available for measuring pH, ranging from easy, inexpensive methods such as colorimetric analysis to glass and titanium probes capable of withstanding severe environmental conditions. Colorimetric methods, using indicator reagents or pH test strips, were widely used prior to the development of the metal electrode and potentiometric/electrochemical measurements of pH using glass electrodes (Galster, 1991; Webster, 2003; Yuqing et al., 2005). Measurements conducted using indicator reagents (e.g., phenolphthalein, ethyl red, methyl red) provide rapid, reproducible, and inexpensive measurements (Johnson & King, 1951); however, the measurements obtained are usually only approximate values (Allan & Heacock, 2017). Colorimetric pH methods are prone to inaccuracies when used in milk systems, as measurements can be influenced by the concentration and type of protein and their isoelectric point (Webster, 2003). Therefore, most laboratory pH meters used today are potentiometric sensors consisting of single combination electrodes, also known as ion-sensitive membranes, which can be prepared as either solid, liquid, or specific to the analyte (Herber et al., 2003). They operate based on the electrical potential difference ($\Delta\phi$) principle between the interface (liquid, solid, or other specific type) and analyte, with an ideal Nernstian response (Herber et al., 2003; Horváth & Horvai, 2005). Based on the Nernst Equation (2.24), R is the gas constant, T is the absolute temperature, F is the Faraday constant, and concentrations (c_i) are noted in terms of H^+ ion activity of inner and outer solutions (a_i) (-4:).

$$\Delta\phi = \left(\frac{RT}{F}\right) \ln \frac{a_{\text{inner}}}{a_{\text{outer}}} \quad (2.23)$$

Glass membrane electrodes are classified as solid electrodes responsive to changes in H^+ activity and may consist of a combined reference and pH electrode.

Compared to colorimetric methods, commercially available glass electrodes give a good linear response in the pH range 2 to 9, with a short pH response time up to pH 14, give good reproducibility, and are durable (Hashimoto et al., 2019; Kohler et al., 2005). Glass probes are most suited to laboratory use and given their brittle nature and relatively small operation temperature range, there are limitations when used in the food, beverage and biomedical industry (Yuqing et al., 2005). However, with developing technology, there are several new and innovative designs such as microelectrodes (Huang et al., 2014; Yamamoto et al., 2003), disposable electrode tubes (Guinovart et al., 2014; Nyein et al., 2016) and needle-type pH electrodes, gel-filled electrodes, solid-state electrodes, ion selective electrodes (ISE), and epoxy body electrodes. Hashimoto et al. (Hashimoto et al., 2016) recently developed a novel metal-oxide-coated stainless steel pH sensor with a pH sensitivity of 88–100%, pH repeatability of 0.1–0.6 pH units, and an initial pH response time of ~1 s which is significantly shorter than commercial glass electrodes (typical response time of 14 s).

2.5. Monitoring pH

2.5.1 Off-Line and At-Line Measurements

Off-line analysis is commonly used for most analytical methods and particularly for pH measurements, whereby a sub-sample or aliquot of product is removed from the production process and analyzed independently of the manufacturing process. A disadvantage of off-line analysis is the inevitable time delay, which may allow significant changes to occur between sampling and pH analysis, such as fluctuations in temperature, pressure, microbial contamination, or time-related changes in mineral equilibrium (e.g., calcium phosphate $\rightleftharpoons$ ionic calcium).

At-line analysis is similar to off-line measurements; the sample is removed from the process but is measured close to the manufacturing line (Figure 2.1). There is little time delay between sampling and analyses, although changes may still occur to the sample, similar to off-line where temperature and other environmental factors may change. For most applications, at-line analysis offers a suitable compromise where in-situ measurements of pH are not feasible.



Figure 2.1 Schematic representation of off-line, at-line, on-line, and in-line approaches to measuring pH in dairy processing.

2.5.2 In-Line/In-Situ Measurements

In-line or *in-situ* measurements take place directly in the process and monitor product properties in real-time (Figure 2.1), such as dissolved CO₂, conductivity, pH, temperature, etc. (Munir et al., 2017; O'Shea et al., 2019; Tajammal Munir et al., 2015). In-situ pH monitoring is ubiquitous in the dairy industry for applications such as cheese and yogurt manufacture, monitoring fermentation properties within bioreactors, and particularly in cases of large-scale protein *hydrolysis* where pH may have to be maintained or controlled for optimum enzyme activity. However, where in-situ pH measurement becomes more difficult is during high-temperature heat treatment, particularly during thermal processing in excess of 90 °C. Essentially, the dairy industry uses off-line and at-line measurements for capturing pH data of milk

and milk derivatives, with the general exception of specific fermentation processes which make use of in-situ pH analysis, although this usually takes place at relatively low temperatures. In-situ probes should be of sanitary design, i.e., European Hygienic Engineering Design Group (EHEDG) standards or 3A design, without moving parts or seals (34). These in-situ sensors should also be compatible with repeated cleaning-in-place or sterilization-in-place regimes, while extended periods at high temperatures should not affect the sensor's performance. Regular calibration is also required using appropriate buffering solutions.

2.6. Industrial applications of in-line pH measurement under challenging environmental conditions

In-line pH sensors, designed for continuous data measurement, have been used in the biotech and pharmaceutical industries for a number of years (Bychkov et al., 2020; Palmer et al., 2001; Wen et al., 2021). However, there are a limited number of electrode systems capable of providing in-situ pH measurements at temperatures greater than 100 °C and at pressures greater than saturated water pressure. Advances in probe technology have recently allowed relatively inexpensive glass probes to be developed. Recently, Aydogdu et al. published two studies examining the pH of skim milk, milk permeate (Aydogdu et al., 2023), and milk protein solutions (Aydogdu et al., 2022) under relatively high temperatures (i.e., 120 °C and 140 °C) using a static batch type system and a continuous method where the probe was inserted in the holding section of a tubular heat exchanger. While the aforementioned studies were a first for dairy products, the pharmaceutical and geothermal industries have been measuring pH under challenging environmental conditions such as high temperature and pressure for a number of years. A report by Sanjuan et al. (Sanjuan et al., 2009)

highlighted a number of probe types and listed their capabilities and disadvantages when operating under harsh geothermal conditions. These geothermal conditions are far more severe than almost anything experienced in the dairy industry, with pH measurements required at temperatures up to ~500 °C and pressures up to 50 MPa, such as those in geothermal vents and hydrothermal fluids.

One of the most robust probes for measuring pH is the solid-state yttria-stabilized zirconia (YSZ) ceramic probe combined with an Ag-AgCl reference electrode allowing in-situ pH to be measured under the most severe environmental conditions. The pH electrode assembly is located at the tip of the sensor and housed in a titanium casing. Several studies have used variations of the YSZ probe. An early study by Inda et al. (Inda et al., 1996) showed that the YSZ probe followed an almost Nernstian response to potential as a function of pH and gave a stable pH reading within 30 s, as opposed to other probes, which can take significantly longer. At the same time, Jung and Yeon (Jung & Yeon, 2010) designed a loop system using stainless steel coated in titanium and a YSZ probe to measure the pH of coolants in pressurized water reactors and were capable of accurately measuring pH at 280 °C. A comprehensive study by Truche et al. (Truche et al., 2016) measured the pH in-situ of NaCl hydrothermal solutions at temperatures up to 280 °C and 150 bar. The pH probe was an oxygen-ion conducting ceramic sensor coupled to an Ag/AgCl reference electrode. An additional custom-made water-cooled jacket was fitted around the external extremity of the reference probes to keep the Ag/AgCl couple at room temperature and to stabilize the reference potential. Accurate pH measurements require that the potential of the reference electrode remains constant. These authors stated that room temperature measurements could not be extrapolated to experimental conditions by numerical simulations when dealing with complex and extreme systems such as those

occurring in the Earth's crust or geothermal wells. Similarly, Aydogdu (Aydogdu et al., 2023) made a similar statement for milk and milk permeates where capturing pH data from 25 up to 80 °C was not representative of pH at 140 °C when quantified by extrapolation. Limitations of YSZ sensors have been highlighted in recent years, especially dealing with sensor durability when exposed to high temperatures [72], but for the most part, this is within environments of extremes well beyond those used in dairy processing.

This probe may be relevant to the dairy industry for UHT processing systems where temperatures in excess of 140 °C can be reached, although the product is usually only held for a short period (≤ 5 s). This type of pH probe may also find more practical applications in high-temperature processing with extended holding times, such as in the use of in-container sterilization, where time–temperature combinations are in the region of 121 °C for >15 min. Previous studies by Aydogdu (Aydogdu et al., 2023; Aydogdu et al., 2022) showed the use of glass probes, while practical, relatively cheap, and ideal for laboratory applications up to 140 °C, are not suitable for in-line measurements during industrial processing due to the use of a glass housing for the probe (Figure 2.2). There are, however, more commercially available probes capable of withstanding temperatures up to 130 °C comprised of titanium or ceramic housing and may be an option for pilot–plant applications where run times are relatively short. These probes may also be used during cleaning- or sterilization-in-place for processing equipment. Implementation of analytic tools and smart sensors for real-time monitoring would enable consistent and accurate pH measurements and allow process optimization for maximum efficiency and quality.

There are many commercial suppliers offering a range of probes capable of measuring pH at high temperatures, and it is often a case of weighing up the conditions

of use, i.e., temperature, pressure, accuracy, durability to corrosion, physical abrasion, length of time spent under such conditions and ultimately cost.

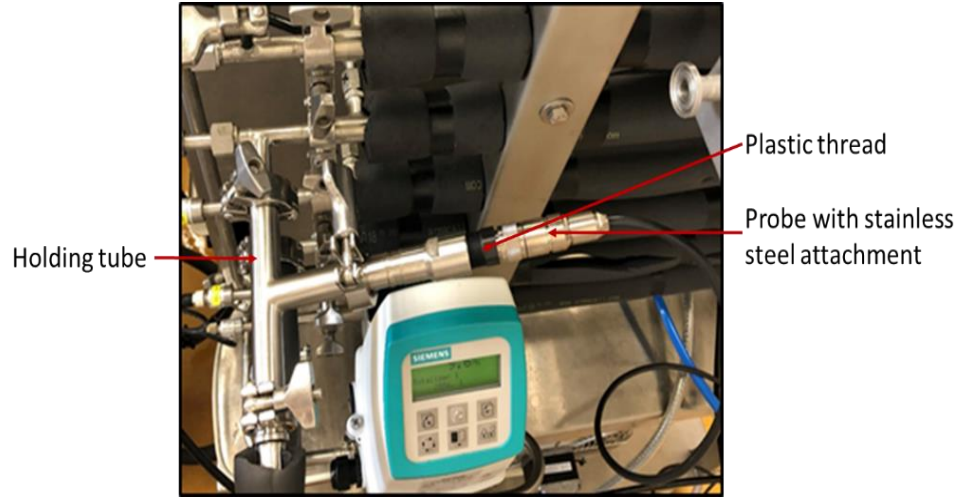


Figure 2.2. Image of an in-line pH probe inserted within the holding tube of a tubular heat exchanger (Aydogdu et al., 2023).

2.7. pH in dairy systems

The pH of milk is mainly influenced by the amino acid profile, temperature, and mineral composition. Other milk components such as lactose, lactose breakdown products, and microorganisms all play a role in milk pH but may be considered either secondary or external factors. However, one means of assessing the influence of the entire milk ecosystem on pH is by examining buffering capacity. The method involves adding a known volume of acid or base and monitoring the subsequent pH change. Van Slyke (Van Slyke, 1922) defined the buffering capacity as ((2.25) and (2.26)): where B is the number of moles of a strong base and A the number of moles of strong acid.

$$\beta = \frac{dB}{dpH} \text{ or } \beta = -\frac{dA}{dpH} \quad (2.24)$$

$$\beta = \frac{dB}{dpH} = \frac{(\text{volume of acid added}) \times (\text{normality of the acid})}{(\text{volume of the sample}) \times (\text{pH change})} \quad (2.25)$$

Upreti et al. (2006) stated that a total of 36 chemical species were relevant for modeling the buffering capacity of bovine milk and listed them as non-protein substances such as cations (i.e., Na^+ , K^+ , Ca^{2+} , Mg^{2+}), anions (i.e., phosphate, citrate, and lactate), metal ion complexes and insoluble calcium phosphate, as well as protein-bound amino acids. The association of ions with proteins, particularly caseins, occurs via electrostatic interactions between phosphoserine residues on the proteins and positively charged free ions, although phosphoserine groups mainly bind with colloidal calcium phosphate. Minerals represent a relatively small fraction of milk compared to fat, protein, lactose, and water; however, they play a vital role in the stability and configuration of casein micelles and concomitantly on the physicochemical and functional properties of dairy products (Yadav & Singh, 1970). The six major minerals in milk include Ca, Mg, K, Na, P, and Cl, which are found as complex minerals or free ions and are in a constant state of dynamic equilibrium. This dynamic equilibrium is affected by numerous environmental conditions such as pH, temperature, and solids concentration (Gaucheron, 2005). Aside from the environmental conditions that effect the mineral balance, it is the protein content, and specifically the micellar casein concentration that distinguishes the distribution between the soluble and insoluble mineral fraction. Soluble milk minerals are present as free ions or associated with counter ions and distributed throughout the aqueous phase, compared to insoluble minerals mainly associated with the micellar casein fraction (e.g., micellar calcium phosphate) (Holt, 1997; Lucey & Horne, 2009; Nieuwenhuijse & Huppertz, 2021). Approximately one-third of calcium (~9.0 mM), two-thirds of magnesium (~3.0 mM), half of the inorganic phosphate (~11.0 mM), and almost 90% of citrate (~8.0 mM) are found in the aqueous phase, while the remaining

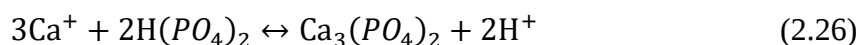
minerals are associated with the phosphorylated residues of caseins and form micellar calcium phosphate (Gaucheron, 2005; Kelleher et al., 2020).

2.7.1 Effects of solids content and temperature on milk pH during dairy processing

The removal of water in milk systems equates to an increase in the solute-to-solvent ratio, with a concomitant increase in the ionic strength of the solution. As shown in Section 2.4, the increase in ionic strength directly influences the hydrogen ion activity and, consequently, the pH. Therefore, during evaporation, the pH of milk decreases, and ionic strength increases, causing a reduction in the activity coefficient of soluble calcium and phosphate (Aydogdu et al., 2021). Aside from the increase in ionic strength, the concentration of milk often coincides with an increase in viscosity that can affect pH measurement, as the electric potential between the sample and reference can be hindered. This becomes particularly relevant in highly viscous products such as processed cheese. Concurrently heat treatment often occurs simultaneously with evaporation, and as a result, there are multiple factors to be considered when measuring the pH of milk concentrates. As described in Section 3.1, the pH–temperature relationship is described by the Nernst equation and is important for milk systems, which may be subject to thermal conditions ranging from refrigeration at 4 °C, during milk intake and storage, up to ultra-high temperatures (≥ 140 °C).

Thermal treatment of milk is common practice in the dairy industry in order to inactivate microorganisms, maximize shelf life (Dash et al., 2022), and alter product functionality (e.g., low, medium, and high heat-treated milk) (McCarthy et al., 2022; Raikos, 2010). Studies have shown that milk subjected to heat treatment result in changes in the pH and mineral equilibrium, which is correlated to the extent and

severity of the heat treatment (Boiani et al., 2018; Havea et al., 2002; Pouliot et al., 1989a; Sauer & Moraru, 2012; Schiffer et al., 2021). Heat treatment of milk at temperatures greater than 90 °C is known to cause irreversible changes to both protein and mineral properties, such as precipitation of calcium phosphate, denaturation and aggregation of whey proteins, casein de-phosphorylation, and release of non-protein nitrogen compounds (Morr, 1965; Van Dijk & Hersevoort, 1992). However, at temperatures less than 90 °C, the shift in the mineral equilibrium from soluble to insoluble is considered to be largely reversible after cooling (Pouliot et al., 1989b; Wahlgren et al., 1990). At ~25 °C, ~66% of the calcium and ~50% of the phosphate in milk are found to be associated with the micellar casein fraction (colloidal calcium phosphate). Increasing the temperature of milk causes a decrease in the soluble contents of both calcium and phosphate with a concomitant release of hydrogen, resulting in a decrease in pH (Lucey & Horne, 2009; Schmitt et al., 1993). This would mean a shift in equilibrium from left to right in Equation (2.27) below.



An early study by Brule et al. (1978) investigated the effect of heat treatment intensity on the protein-free aqueous phase of milk. In their study, milk permeate (obtained by ultrafiltration of skim milk at 20 °C) was heated to 30 °C, 60 °C, or 90 °C. Results showed that heat treatment significantly affected the precipitation of either mono-calcium phosphate or tri-calcium phosphate based on temperature and initial pH of the permeate. Increased temperature resulted in increased precipitation of calcium and phosphorus and a greater decrease in the pH as a result of hydrogen ion release. These authors suggested that the precipitation of calcium and phosphorus decreased by decreasing the initial pH of the permeate. At pH 6.6, approximately 40% of the calcium and 26% of the phosphorus was precipitated; however, when the pH was

adjusted to more acidic values, such as pH 6.0, almost no precipitation was observed. A review by Fox (Fox, 1981) on heat coagulation of milk stated that heat-induced changes are mainly a result of heat-induced acidity, as continuous neutralization delays coagulation indefinitely, regardless of all other heat-induced changes that occur.

2.7.2 *Addition of salts*

The addition of salts to milk and dairy products occurs for numerous reasons, often added as processing aids for binding free ions or added as mineral fortification to meet nutritional requirements. Thus, there is a plethora of research articles and reviews already published focusing on this subject. Primarily covering the addition of ethylenediaminetetraacetic acid (EDTA), citrates, phosphates, etc., to milk systems in order to increase heat stability, particularly in infant formula manufacture, or for protein standardization in milk through the addition of milk permeate (Augustin & Clarke, 1990; 2014; Karlsson et al., 2019; Le Ray et al., 1998; Renhe et al., 2018; Singh et al., 2021; Sweetsur & Muir, 1980; Wang et al., 2016), all of which significantly affect pH depending on concentration and type of mineral addition. Ion sequestrants, such as citrates and phosphates, are well known to influence protein stability and mineral equilibrium and can concomitantly affect pH (de Kort et al., 2011, 2012; Renhe et al., 2018). Sequestrants are often added to high-protein dairy concentrates on enhancing their subsequent rehydration and solubility properties after spray drying (de Kort et al., 2011; McCarthy et al., 2017). This is achieved by binding free divalent ions and calcium from colloidal calcium phosphate, which results in swelling and even dissociation at certain levels of the casein micelle. McCarthy et al. (McCarthy et al., 2017) showed significant changes in the pH of milk protein solutions

with the addition of salt type and concentration, with trisodium citrate increasing pH, whereas sodium dihydrogen phosphate caused a significant decrease in pH. Therefore, there was not only an effect from sequestering ions but also from the protonation or deprotonation of amino acids affecting protein electrostatic charge. Sequestrants are often referred to as emulsifying salts in the area of processed cheese manufacture, where they are added to hydrate the protein from cheese and allow hydrated proteins to sufficiently emulsify the melted fat during the cooking process, which usually takes place at temperatures between 80 °C and 100 °C (Chen & Liu, 2012). pH is adjusted to between pH 5.7 and 6.0 using either citric acid, lactic acid, or sodium hydroxide at a total solid content of ~50% (w/w). However, as shown by Guinee and O’Callaghan (Guinee & O’Callaghan, 2013), pH adjustment is often performed off-line (Section 3.2.1), where subsamples were cooled, pH adjusted, and re-measured after 24 h. Given the influence of pH on the texture and microstructure of processed cheese, in-situ pH monitoring during cooking and shearing could be particularly valuable, where hydrogen ion activity will differ significantly at high temperature in addition to the ion binding capacity of emulsifying salts.

2.8. Conclusion

Hydrogen ion concentration defines most chemical reactions, influencing the biological and physical properties of systems. From its inception in the early 20th century, the measurement of pH has remained a barometer for chemical processes. However, it is apparent that the significant level of pH monitoring in the dairy industry is usually performed at-line or off-line with a significant lack of in-situ measurements taking place. However, advances in pH measurement have increased steadily with more rapid and accurate probes available. This review has highlighted these probes

are able to withstand high pressure and temperatures and that the dairy industry could potentially avail of them. Processed cheese and infant formula applications are ideal examples of where high solids (~50%, w/w, dry matter), high-temperature processing, and the sequestering of monovalent and divalent ions is performed and where real-time in-situ monitoring of pH could provide greater in process control and product quality. Aside from the dairy industry, examining potential technologies from other industries, such as the geothermal sector, has allowed dairy scientists to examine pH in milk-based systems under challenging conditions, highlighting the benefit of using cross-sector technology.

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Chapter 3

The influence of milk minerals and lactose on heat stability and age-thickening of milk protein concentrate systems

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Declaration

This chapter was written by TA and reviewed by all co-authors. TA co-designed the study and performed all the experiments with the exception of mineral analysis, which was performed by an external laboratory (FBA Laboratories, Waterford, Ireland). QTH assisted during the pilot plant processing.

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3.1. Abstract

Reconstituted milk protein concentrate (MPC; 80% protein) was mixed with lactose (MPC-Lac) or milk permeate (MPC-Perm) to 20% dry matter (DM) before being evaporated to 45% DM and subsequently spray dried. The pH of protein solutions, measured during evaporation from 20 to 45% DM at 50 °C, decreased from pH 6.64 to pH 6.53 and from 6.1 to 5.95 for MPC-Lac and MPC-Perm, respectively. The particle size and viscosity were greater in MPC-Perm than MPC-Lac solutions after evaporation. However, the heat stability of rehydrated MPC-Perm powders (3.5% protein) were significantly higher than MPC-Lac at pH values between 6.4 and 6.8, which may be attributed to the lower calcium ion concentration in MPC-Perm than in MPC-Lac. This study highlighted the complexity of mineral addition and phase distribution in protein-standardised milk systems; whereby higher levels of mineral addition contribute to viscosity and age thickening, but not necessarily lower heat stability.

3.2. Introduction

Thermal treatment of milk protein systems can be challenging, due to protein denaturation and aggregation, and concomitant precipitation and fouling, with the extent of such effects being dependent on the specific conditions of the thermal treatment applied (Ho et al., 2019; Meena, Singh, Panjagari, & Arora, 2017; Morison & Tie, 2002). One of the main factors responsible for high viscosity of milk protein solutions during and post evaporation is the increased volume fraction of protein and the ability of casein micelles and denatured whey proteins to absorb water and other solutes strongly. While the volume fraction is mainly defined by the protein concentration, the viscosity of the continuous phase is more strongly influenced by the lactose content and mineral profile (Anema, Lowe, & Li, 2004; Jeurnink & De Kruif, 1993). However, the exact role that milk minerals play in the development of viscosity and age thickening/gelation of concentrated milk systems is still not well understood, particularly with respect to pH changes, calcium ion concentration and the distribution of colloidal and diffusible minerals. Increasing both the volume fraction and the concentration of the continuous phase of milk protein systems during evaporation are the main factors contributing to high viscosity. These factors can lead to challenges in processing efficiency (e.g., reduced heat transfer efficiency) and product quality (e.g., fleck formation) during milk powder production, resulting in shorter run times, increased down grading of product quality, increased cleaning-in-place interventions and associated use of cleaning chemicals. Colloidal calcium, magnesium and citrate are mainly associated with the casein micelles in milk, with phosphorous mainly present as inorganic phosphate (P_i), in the form of $H_2PO_4^-$ and HPO_4^{2-} , and often associates with divalent ions such as calcium (Ca^{2+}) and magnesium (Mg^{2+})

(Gaucheron, 2005; Holt, 1985; Vegarud, Langsrud, & Svenning, 2000). However, the minerals in milk are in a dynamic equilibrium during processing, with constant inter-conversion between soluble and insoluble forms. This equilibrium is affected by temperature, pH and concentration, with positively charged free ions such as Ca^{2+} and Mg^{2+} contributing to whey protein denaturation and aggregation, as they can bind to negatively charged amino acid residues (de la Fuente, 1998). Therefore, soluble minerals, in particular free ions, are understood to play an important role in the viscosity and age-thickening behaviour of skim milk concentrates. In previous work, Bienvenue, Jiménez-Flores, and Singh (2003) showed that the mineral content of skim milk has a significant effect on viscosity and age thickening properties post-evaporation, with higher mineral content associated with higher viscosity during storage. Murphy et al. (2018) reported that protein standardization of skim milk with milk permeate resulted in higher viscosity in skim milk concentrates, compared to protein standardization with lactose. This was attributed to the higher mineral content in the permeate, resulting in increased protein-mineral interactions during heat treatment.

While many studies (Dumpler, 2018; Huppertz & Timmer, 2020; Ojaniemi, Pättikangas, Jäsberg, Puhakka, & Koponen, 2020; Rosmaninho & Melo, 2006; Sikand, Tong, & Walker, 2013; Snoeren et al., 1982; Vélez-Ruiz & Barbosa-Cánovas, 1998) have focused on the role of protein-mineral interactions, calcium phosphate precipitation and fouling, a comprehensive study on the influence of milk serum minerals on milk protein concentrate (MPC) viscosity, age thickening and heat stability has been absent. Aside from a study by Lin, Kelly, O'Mahony and Guinee (2018a), where milk permeate was used as a solvent to assess some of the functional

characteristics of milk protein concentrate, there is an extensive gap in the knowledge on how serum minerals influence milk proteins, in systems similar to skim milk. The aim of the current study was to manipulate protein-mineral interactions and examine the heat stability, viscosity and agethickening in MPC solutions through the re-introduction of minerals, in the form of milk permeate, and compare its physical properties with that of a protein solution with added lactose only. The practical implications of this research are that its in-depth compositional analysis and associated functionality can be extrapolated to inform milk producers of the main factors contributing to viscosity, heat stability and possible fouling. One example may be the seasonal effects of certain milk products on process-ability, which often requires in-depth compositional analysis to determine the source of the issue.

3.3. Materials and methods

3.3.1. Materials

Milk protein concentrate (MPC) powder (80%, w/w, protein), lactose powder and liquid milk ultrafiltrate permeate were obtained from a local commercial dairy manufacturer. The MPC 80 used in this study was chosen as a high heat-treated MPC to avoid any matrix interference from non-denatured whey proteins. The ash content of MPC powder, lactose and liquid milk permeate were 7.57%, 0.21% and 9.95% (w/w, dry matter (DM)), respectively. The mineral profile of the materials was measured using inductively coupled plasma mass spectrometry (ICP-MS) and is shown in Table 3.1. For details on the mineral profile analysis see Section 3.4.1. Analytical grade sodium hydroxide and hydrochloric acid were sourced from Sigma-Aldrich (Arklow, Ireland).

3.3.2. *Processing of milk protein concentrate solutions*

Lactose powder was dissolved in water at 70°C to 36% DM and agitated for 30 min to facilitate complete solubilisation. Liquid UF milk permeate (22% DM) was evaporated using a single-effect falling film evaporator (Anhydro F1 Lab; Copenhagen, Denmark) to 36% DM. MPC powder was rehydrated to 20% DM at 50°C for 30 min using an in-line high shear mixer (YTRON-Z, 1.50FC, YTRON Process Technology GmbH, Bad Endorf, Germany) operating at an inlet and vacuum pump efficiency of 20 and 80%, respectively, before being stored overnight at 4°C under gentle agitation. Rehydrated MPC was then combined with lactose (MPC-Lac) or milk permeate (MPC-Perm) to give a protein solution with a solids content of 26% DM and a protein content of 34.9%, w/w, on a dry basis, similar to that found in skim milk. MPC-Lac and MPC-Perm solutions were then heated to 65°C and evaporated to 45% DM using the single-effect falling film evaporator operating in recirculation mode. During evaporation pH was monitored using a SC25V 12 mm combination pH sensor/meter (Yokogawa, Amersfoort, The Netherlands). Evaporated protein solutions were then dried using a single-stage spray dryer (Anhydro Laboratory Spray Dryer, SPX Flow Technology, Denmark) equipped with a two-fluid nozzle atomization system operating in counter-flow mode at inlet and outlet temperatures of 185 and 85°C, respectively. MPC-Lac and MPC-Perm powders were produced in triplicate and stored in aluminium foil bags under low humidity conditions (maximum relative humidity of 25% at 10°C) prior to further analysis.

3.3.3. Mineral analysis

Liquid samples (MPC-Lac and MPC-Perm) were taken prior to evaporation, centrifuged at $100,000 \times g$ at 25°C for 60 min (Sorvall Discovery 90SE ultracentrifuge, Kendro Laboratory Products, Asheville, NC) and the serum phase removed for mineral analysis. Powder (MPC, lactose, MPC-Lac and MPC-Perm) (~0.2 g) and liquid samples (milk permeate and serum phase of MPC-Lac and MPC-Perm) were analysed for macro (Ca, P, K, Na, and Mg) and trace elements (S, Zn, Fe, I, Mn, Cu, Mo, and Se) using inductively coupled plasma mass spectrometry (Agilent ICPMS 7700 Santa Clara, CA). Mineral analysis was carried out by FBA Laboratories Ltd. (Cappoquin, Waterford, Ireland).

3.3.4. Rheological characterization

Viscosity measurements of protein solutions (MPC-Lac and MPC-Perm) were performed on samples before and after evaporation using a controlled-stress rheometer (ARG2 Rheometer, TA Instruments, Crawley, UK), equipped with a concentric cylinder geometry at 50°C. The temperature was controlled using a Peltier system ($\pm 0.1^\circ\text{C}$). Samples were pre-sheared at a shear rate of 200 s⁻¹ for 60 s and subsequently analysed at a constant shear rate of 300 s⁻¹ over 5 min. Evaporated MPC-Perm and MPC-Lac were stored at 50°C in a water bath for 30 and 90 min in order to perform the age related viscosity measurements.

3.3.5. Particle size distribution

The particle size distribution of liquid milk protein systems before (26% DM) and after evaporation (45% DM) was determined using a laser-light diffraction unit (Mastersizer 3000, Malvern Instruments Ltd, Worcestershire, UK) equipped with a 300 reverse Fourier lens. Liquid samples were diluted to 5% DM (w/w) and analyses carried out at 20°C using a dispersion unit with sample re-circulating at 2000 rpm. Refractive index was measured for each sample prior to analysis using a benchtop refractometer (Bellingham + Stanley™ RFM340+ Refractometer, United Kingdom). Particle and dispersant (water) refractive index and absorption were set at 1.38, 1.33 and 0.001 respectively. Diluted samples were introduced to the mixing chamber and dispersed in ultra-pure water until a laser obscuration of ~5% was reached.

The particle size distribution (PSD) of the spray dried MPC-Lac and MPC-Perm powders were determined as described by Barone, O'Regan, and O'Mahony (2019), using a Malvern Mastersizer 3000, equipped with an automated Aero S dry powder dispersion unit (Malvern Instruments, Worcestershire, UK). The air pressure was set at 1.5 bar, particle refractive index and absorption were set at 1.45 and 0.01, respectively. Size measurements were recorded as the median diameter (D_{50}), mean diameter ($D_{4,3}$) and cumulative diameter (D_{90}), whereby 90% of the sample volume is represented by particles smaller than the size indicated.

3.3.6. Heat stability

Heat stability was determined using the heat coagulation time method first described by Davies and White (1966). MPC-Lac and MPC-Perm solutions (prior to

evaporation at 26% DM) and reconstituted powders (10% DM) were adjusted to pH values ranging from pH 6.2 to 7.2, either with the addition of 0.5 M NaOH or HCl solutions and samples were maintained overnight at 4°C to ensure equilibrium. Samples (2.5 g) were added to glass test tubes and the tubes were immersed in an oil bath containing mineral oil at 140°C. The heat coagulation time (HCT) was recorded as the time elapsed between immersing the sample in the oil bath and the visible detection of aggregates/flecks within the sample.

3.3.7. *Calcium ion concentration*

Ionic calcium concentration of reconstituted powders, MPC-Perm and MPC-Lac (10% DM) was determined with a calcium ion selective electrode (sensION+ 9660C, Hach Co., Loveland, Colorado, USA.) using the method described by Crowley et al. (2014). The ion-selective calcium probe was calibrated with standard calcium solutions at 0.05, 1.00, 2.5, 5.00 mM at 25°C, by diluting a 1 M standard solution of CaCl₂ in ultra-pure water. A standard curve was obtained using the linear relationship between electrical output (mV) and the logarithm of ionic calcium concentration. Prior to calcium ion measurements, the pH of MPC-Lac and MPC-Perm solutions were adjusted from pH 6.2 to 7.2 at 0.2 pH unit increments using 1 M NaOH or HCl. The pH of the samples was measured again after overnight storage and re-adjusted, if necessary. Potassium chloride (3 M) was added to CaCl₂ standard solutions and all MPC-Lac and MPC-Perm samples at 1%, v/v, and stirred for 1 min at 25°C.

3.3.8. *Zeta Potential*

Zeta potential measurements of reconstituted MPC-Perm and MPC-Lac powders were measured using a Zetasizer nano (Malvern Instruments, Worcestershire,

UK). Reconstituted MPC-Lac and MPC-Perm solutions at 10% DM were diluted (1:20) in deionized water and pH adjusted from pH 6.0 to 7.2 with the addition of NaOH or HCl (0.5 M) solutions. Protein solutions were stored overnight at 4°C to ensure pH reached equilibrium. The pH of the samples was measured after overnight storage and re-adjusted if necessary. Measurements were performed using a dispersant refractive index of 1.33, sample refractive index of 1.38 and viscosity of 0.89 mPa.s at 25°C.

3.3.9. *Statistical analysis*

The results presented are the average of at least three independent measurements carried out on two replicate trials and are reported as the mean value $\pm$ standard deviation. Analysis of variance was carried out and the Tukey test was used to determine statistically significant differences among means. An independent sample t-Test was performed in order to compare the means of two independent groups for powder particle size data. All statistical analyses were performed using SPSS software (SPSS V.18, IBM, New York, US). Statistical significance was determined at $P < 0.05$.

3.4. **Results**

3.4.1. *Mineral Composition*

The mineral composition of MPC, lactose, milk permeate and milk protein concentrate solutions (MPC-Lac and MPC-Perm) are shown in Table 3.1. MPC-Perm had a significantly ($P < 0.05$) higher level of all macro elements than MPC-Lac, which was expected due to the high innate mineral content of the permeate obtained from

milk by ultrafiltration. In MPC, calcium was present at 1938 mg 100 g⁻¹, DM, compared to 214 and 34 mg 100 g⁻¹, DM, in milk permeate and lactose, respectively. Combining MPC with milk permeate or lactose to dilute the protein content to 34.9% (w/w, DM), which was chosen to reflect the protein content typical of skim milk powder, resulted in considerable changes in mineral composition, with MPC-Perm and MPC-Lac having calcium concentrations of 1131 and 936 mg 100 g⁻¹, DM, respectively. These values were similar to those previously reported by Holland et al. (1995) and Lin, Kelly, O'Mahony and Guinee (2018b) for skim milk powder (i.e., 1280 mg 100 g⁻¹, DM) and liquid skim milk at 9.39% DM (124 mg 100 g⁻¹), respectively. Non-sedimentable calcium values in MPC-Perm and MPC-Lac solutions were 379 and 140 mg 100 g⁻¹, DM, representing 33.5 and 15.0% of total calcium, respectively.

Phosphorous followed a similar trend to that of calcium, with a concentration of 1780 mg 100 g⁻¹ in MPC80, significantly higher than in milk permeate and lactose (747 and 28 mg 100 g⁻¹ DM, respectively). The non-sedimentable phosphorous content of MPC-Perm and MPC-Lac was 486 and 160 mg 100 g⁻¹, respectively, representing 35.4 and 21.6% of total phosphorous. The levels of soluble calcium and phosphorous in MPC-Perm were similar to those presented by Fox, Uniacke-Lowe, McSweeney, and O'Mahony (2015) where 34 and 43% of the total calcium and phosphorous in milk was in the soluble phase.

Table 3.1. Mineral composition of milk protein concentrate (MPC), milk permeate and lactose powders and milk protein solutions with added lactose (MPC-Lac) or added milk permeate (MPC-Perm).

	Na	Mg	P	S	K	Ca	Mn	Fe	Cu	Zn	Mo	I
	(mg 100 g ⁻¹ , dry matter)											
MPC	138±1	90±2	1780±47	596±64	635±13	1938±8	0.04±0	0.46±0	0.16±0.01	9±0.4	0.04±0	0.08±0
Permeate	840±15	176±4	747±56	116±1	3650±24	214±7	0±0	0.02±0	0±0	0.13±0.01	0.02±0	0.06±0
Lactose	15±4	6±6	28±7	7±2	24±18	34±11	0.01±0	0.07±0	0.01±0	0.19±0	0±0	0.02±0
<i>Spray dried powders</i>												
MPC-Perm	448±18	117±7	1372±22	306±42	1862±46	1131±128 (28.2)	0.03±0	0.5±0	0.1±0.03	3.6±0.53	0.14±0.08	0.1±0
MPC-Lac	95±15	48±2	740±66	300±3	321±33	936±0 (23.4)	0.02±0	0.2±0.1	0.2±0.01	4.5±0.02	0.04±0.01	0.05±0
<i>Serum phase of MPC-Perm and MPC-Lac before evaporation (Non sedimentable)</i>												
MPC-Perm	493±9	98±3	486±1	220±12	2113±36	379±22 (9.45)	0.02±0	0.19±0.01	0.09±0.03	1.03±0.13	0.03±0	0.19±0.03
MPC-Lac	107±17	17±2	160±19	136±18	398±75	140±9 (3.49)	0.005±0	0.085±0.02	0.06±0.01	0.88±0.4	0.025±0	0.05±0.01

^a Values presented for the ingredients (100 g⁻¹, dry matter), supernatant and spray dried powders are the mean of duplicate analysis Mm.

The concentration of potassium was 635 mg 100 g⁻¹, DM, in MPC, and was significantly higher in milk permeate at 3650 mg 100 g⁻¹, and significantly lower in lactose at just 24 mg 100 g⁻¹ DM. In MPC-Perm, the potassium content was 1862 mg 100 g⁻¹, DM and was the most abundant mineral in the protein solutions, while, the total sodium content of MPC-Lac and MPC-Perm was 95 and 448 mg 100 g⁻¹ DM, respectively. These results are similar to those of Holland et al. (1995) who reported potassium and sodium concentrations of 1590 and 550 mg 100 g⁻¹ for skim milk powder. Essentially 100% of the potassium and sodium in MPC-Perm and MPC-Lac solution was present in the serum phase (Table 3.1.), while magnesium was present at the lowest concentration of the six major elements at 90, 117 and 48 mg 100 g⁻¹ in MPC80, MPC-Perm and MPC-Lac, respectively. Approximately 83% of magnesium in MPC-Perm solutions (98 mg 100 g⁻¹, DM) was non-sedimentable, similar to previous reports by Fox et al. (2015).

The total sulphur content in the MPC (596 mg 100 g⁻¹, DM) was significantly higher than in the milk permeate (116 mg 100 g⁻¹, dry matter) or lactose (7 mg 100 g⁻¹, DM), which is probably due to its association and integral role in the structure of amino acids, with MPC-Lac and MPC-Perm containing 306 and 300 mg 100 g⁻¹, DM sulphur, respectively. The non-sedimentable sulphur content of the MPC-Lac and MPC-Perm was 136 and 220 mg 100 g⁻¹, respectively. However, the reason for the large difference between the non-sedimentable sulphur content in MPC-Perm and MPC-Lac, compared to milk permeate is due to the presence of whey proteins in the serum phases of the ultra-centrifuged samples. Amino acids, particularly cysteine and methionine, are extremely rich in sulphur (Flynn & Power, 1985).

Trace elements, such as zinc and iron, were present at higher concentrations in MPC than in milk permeate or lactose (Table 3.1.). MPC-Perm contained 3.6 mg 100 g⁻¹

zinc and 0.5 mg 100 g⁻¹ of iron, while MPC-Lac contained 4.5 and 0.2 mg 100 g⁻¹ DM of zinc and iron, respectively; these values for zinc and iron in MPC-Perm were comparable to those reported recently by Gulati et al. (2018) for skim milk. The non-sedimentable proportion of MPC-Perm and MPC-Lac had 28 and 20% of zinc, respectively, with these values slightly higher than previous studies by Gulati et al. (2018), who reported that ~10% of total zinc was soluble. Blakeborough, Salter, and Gurr (1983) found that almost all of the zinc (~95%) present in bovine milk is associated with micellar casein and the remaining 5% is present as complexes with other low molecular weight compounds, such as citrate. The proportion of non-sedimentable iron in MPC-Perm and MPC-Lac was 62 and 58%, respectively, indicating that significant proportions are associated with the casein micelle. Other trace elements, such as copper, iodine, molybdenum and selenium levels were present in MPC-Perm at levels similar to those previously shown for skim milk by Gulati et al. (2019).

3.4.2. *Changes in pH as a function of total solids*

The pH of MPC-Perm and MPC-Lac decreased as the total solids content increased from 27 to 45% (w/w, DM) during evaporation (Figure 3.1). The pH of MPC-Lac decreased from 6.65 to 6.54, while the pH of MPC-Perm decreased more extensively from 6.12 to 5.95 at 65°C. Previously, Anema (2009) showed a decrease in pH from 6.5 to 6.2 for low heat skim milk on increasing total solids from 9.8 to 28.8% (w/w, DM) at 50°C. Change in pH is strongly correlated with the solubility of colloidal calcium phosphate, which is influenced by changes in mineral equilibria, temperature, and hydrophobic and electrostatic interactions. Evaporation at 65°C

causes a decrease in the solubility of CCP with concomitant release of hydrogen ions (H^+) ions causing a slight decrease in pH (Pouliot, Boulet, and Paquin, 1989).

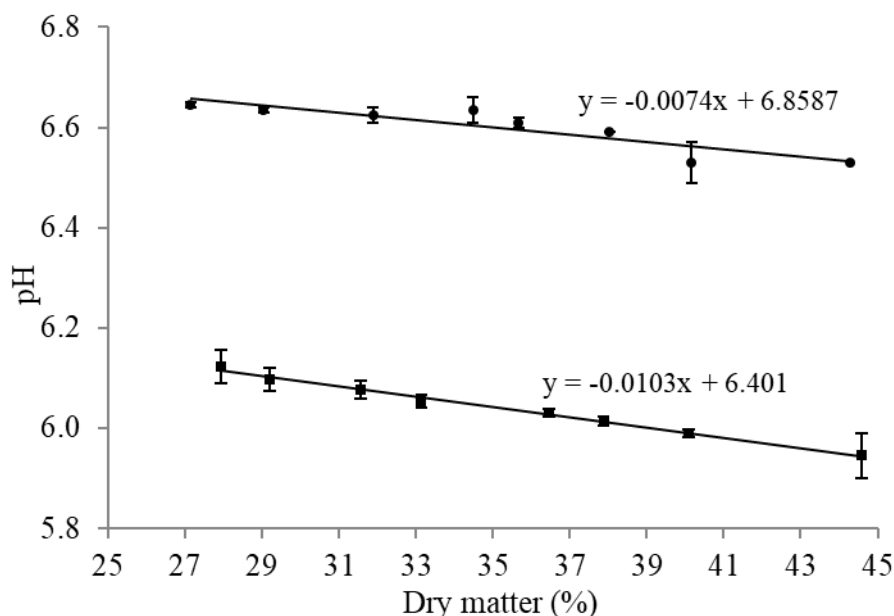


Figure 3.1. pH profile of milk protein concentrates with added lactose (MPC-Lac) (●) or milk permeate (MPC-Perm (■) Samples measured at 50°C, as a function of dry matter (%DM). Values are the means of the data from triplicate analysis.

3.4.3. Viscosity

Analysis of the shear stress versus shear rate flow curves (Figure 3.2 A) for MPC-Lac and MPC-Perm measured before and after evaporation demonstrated that the samples displayed shear thinning behaviour, in agreement with the previous study of Bienvenue, Jiménez-Flores, and Singh, 2003. Prior to evaporation (26% DM), MPC-Lac and MPC-Perm had relatively low apparent viscosity, with values of 3.86 and 5.93 mPa.s, respectively ($P < 0.05$) (Figure 3.2B). However, following evaporation to 45% DM, the apparent viscosity of MPC-Lac reached 30.9 mPa.s, while MPC-Perm had a significantly ($P < 0.05$) higher viscosity at 83.7 mPa.s.

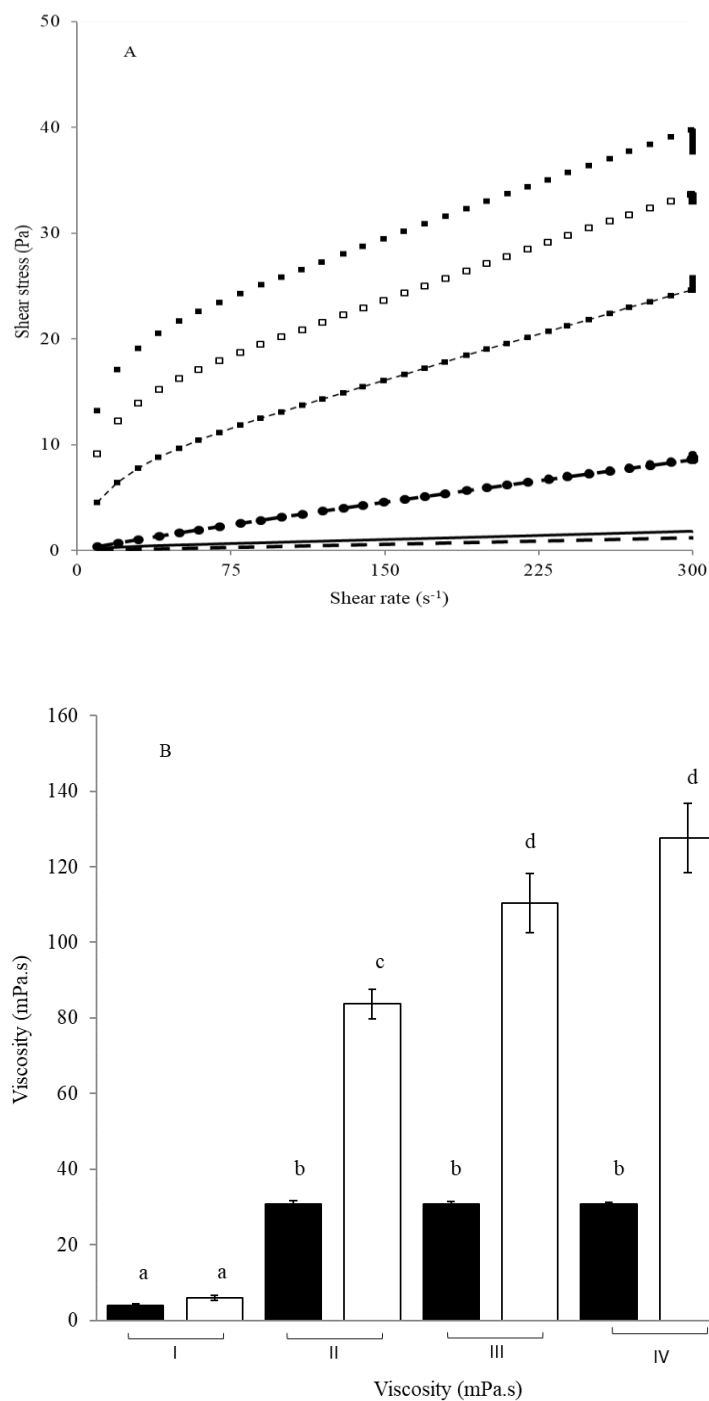


Figure 3.2. Flow curves of milk protein concentrate with added lactose (MPC-Lac) or milk permeate (MPC-Perm) (A); before evaporation at 26% DM (MPC-Lac) (—), (MPC-Perm) (---) and after evaporation at 45% DM; MPC-Lac (—●—), MPC-Perm (—■—). Protein systems stored at 50 C for 30 min after evaporation MPC-Lac (○), MPC-Perm (□) and stored for 90 min MPC-Lac (●) MPC-Perm (■). Apparent viscosity of milk protein concentrate with added milk permeate (MPC-Perm) (□) or lactose (MPC-Lac) (■); (B) Pre (I) and post (II) evaporation and after storage at 50°C for 30 (III) and 90 min (IV). Bars represent standard deviation from triplicate analysis. Values not sharing a common superscript signify significance ($P < 0.05$).

Storing the concentrates for 90 min at 50°C resulted in a significant difference between MPC-Lac and MPC-Perm for age-related viscosity changes. The apparent viscosity of MPC-Lac did not increase after 90 min of storage (30.9 mPa.s), compared to the significant viscosity increase in MPC-Perm (i.e., 127 mPa.s). However, gelation did not occur during the 90 min holding of the MPC-Perm, mainly due to the relatively low solids (45% DM) of the concentrate.

3.4.4. Particle size distribution

Particle size distribution (PSD) profiles of MPC, MPC-Lac and MPC-Perm are shown in Table 3.2 and Figure 3.3. PSD profiles of MPC-Lac and MPC-Perm showed a monomodal size distribution for samples taken before (26% DM) and after evaporation (45% DM). The large volume of particles between 0.01 to 1.0 μm indicated the presence of casein micelles (i.e., 50-200 nm). No significant difference ($P < 0.05$) in particle size values of MPC-Lac solutions were observed before and after evaporation, with $D_{4,3}$ values of 0.28 and 0.29 μm , respectively, with the PSD profile ranging from 0.004 to 0.95 μm . Significantly ($P < 0.05$) higher particle size was observed for MPC-Perm compared to MPC-Lac ($D_{4,3}$ of 1.17 and 0.29 μm , respectively), with no considerable difference before and after evaporation.

Monomodal PSD profiles of MPC-Lac and MPC-Perm powders are shown in Appendix 3A (Figure A3.1) and ranged from 1 to 500 μm . MPC-Perm powders had significantly larger particle size values ($D_{4,3} = 45.36 \mu\text{m}$) compared to MPC-Lac powder ($D_{4,3} = 24.7 \mu\text{m}$).

Table 3.2. Median diameter ($D_{(50)}$), mean diameter ($D_{(4, 3)}$) and percentage ($D_{(10)}$ and $D_{(90)}$) values of milk protein concentrate (MPC) solutions with added lactose (MPC-Lac) or milk permeate (MPC-Perm) measured before and after evaporation and the resultant spray dried powders.

Samples	$D_{(10)}$	$D_{(50)}$	$D_{(90)}$	$D_{(4,3)}$
	(μm)			
MPC	0.09 ± 0.00^a	0.25 ± 0.00^{abc}	0.59 ± 0.01^a	0.47 ± 0.01^{ab}
<i>Before evaporation</i>				
MPC-Lac	0.08 ± 0.00^a	0.23 ± 0.00^a	0.56 ± 0.00^a	0.28 ± 0.00^a
MPC-Perm	0.08 ± 0.00^a	0.30 ± 0.00^{bc}	1.14 ± 0.17^{ab}	1.17 ± 0.35^b
<i>After evaporation</i>				
MPC-Lac	0.08 ± 0.00^a	0.24 ± 0.01^{ab}	0.57 ± 0.01^a	0.29 ± 0.01^a
MPC-Perm	0.09 ± 0.01^a	0.31 ± 0.03^c	1.26 ± 0.33^b	1.00 ± 0.25^{ab}
<i>Spray dried powders</i>				
MPC-Lac	7.58 ± 0.05	20.2 ± 0.35	47.0 ± 0.01	24.7 ± 6.3
MPC-Perm	9.73 ± 1.5	29.5 ± 0.8	103 ± 0.33	45.4 ± 4.85

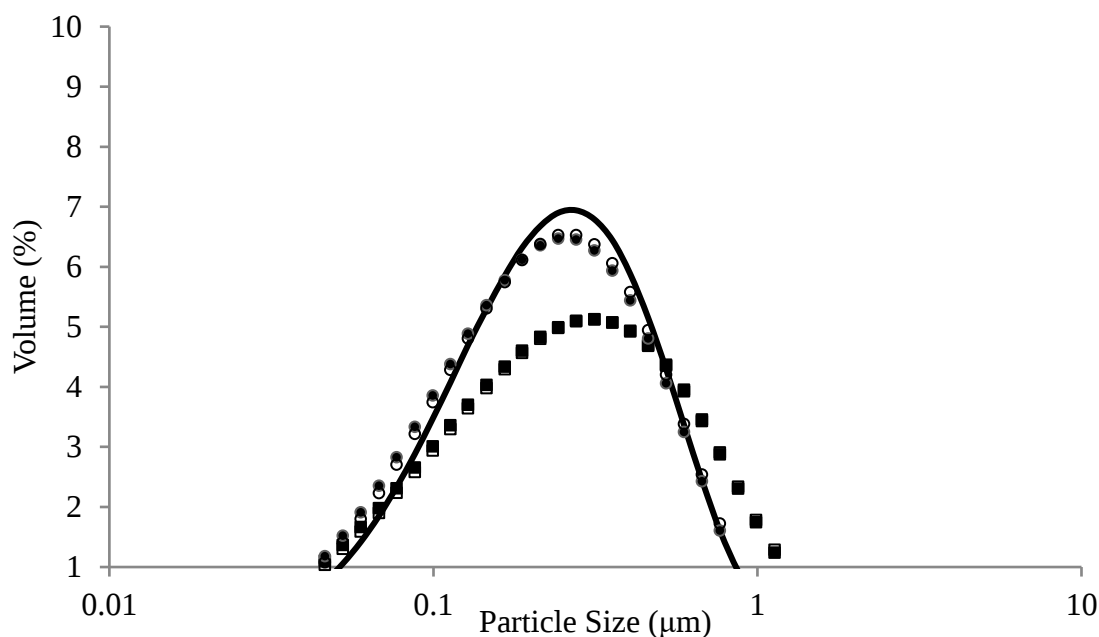


Figure 3.3. Particle size distribution profiles of milk protein concentrate (MPC) (—), milk protein concentrate with added lactose (MPC-Lac) (●) and milk protein concentrate with added permeate (MPC-Perm) (■) before evaporation. MPC-Lac (○), MPC-Perm (□) after evaporation. Values are the means of data from triplicate analysis.

3.4.5. Heat coagulation time, calcium ion concentration and zeta potential

Heat coagulation time (HCT) as a function of pH in the range 6.2 to 7.2 of MPC-Lac and MPC-Perm (26% DM) before evaporation is shown in Figure 3.4. MPC-Lac and MPC-Perm solutions had similar maximum (HCT_{max}) and minimum (HCT_{min}) heat coagulation times at similar pH values. MPC-Lac and MPC-Perm had HCT_{max} and HCT_{min} at ~6 min and <2 min at pH 6.7 and 6.2, respectively. MPC-Perm showed a typical type-A HCT profile, with a decrease in HCT at pH 6.8 to 3 min, followed by an increase in HCT at pH 7.0; however, the HCT decreased again to 3 min at pH 7.2. MPC-Lac had a significantly higher HCT at pH 6.4 compared to MPC-Perm and similar HCT thereafter up to pH 6.8; however, there was a more gradual decrease in HCT at pH >6.8, with HCT_{min} at pH 7.0 (3 min) and a slight increase again at pH 7.2 (4 min).

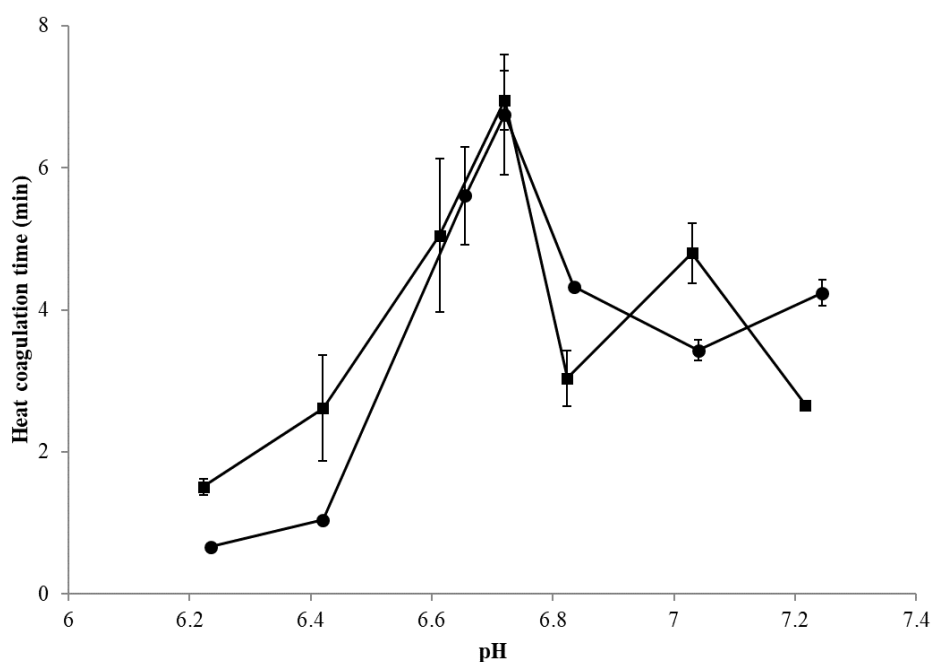


Figure 3.4. Heat coagulation time (HCT) profile of milk protein concentrate with added lactose (MPC-Lac) (—●—) or milk permeate (MPC-Perm) (—■—) prior to evaporation (26% DM) as a function of pH. All values presented are the mean of triplicate analysis. Bars represent the standard deviation of the mean values.

Heat stability and ionic calcium concentration profiles of rehydrated MPC-Lac and MPC-Perm powders are shown in Figure 3.5. The HCT of MPC-Lac remained low (<1 min) from pH 6.2 up to pH 6.8 and eventually began to increase at pH 7.0 up to 6 min and remained constant at pH 7.2. The relatively low heat stability (<1 min) of MPC-Lac was similar to that shown by Crowley et al. (2014) for MPC80 with different concentrations of added lactose at pH values between 6.2 to 6.8. HCT of MPC-Perm increased from 0 min at pH 6.2 up to 13 min at pH 6.7 and decreased thereafter to 5 min at pH 7.2. Overall, MPC-Perm showed higher stability at pH 6.6 to 7.0 compared to MPC-Lac. The calcium ion concentration was significantly higher in MPC-Lac (ranging from 5.42 mM at pH 6.2 to 2.20 mM at pH 7.2) compared to MPC-Perm (2.50 mM at pH 6.2 to 1.10 mM at pH 7.2) across the pH range studied (i.e., pH 6.2 to 7.2) and decreased with increasing pH for both protein solutions (Figure 3.5.).

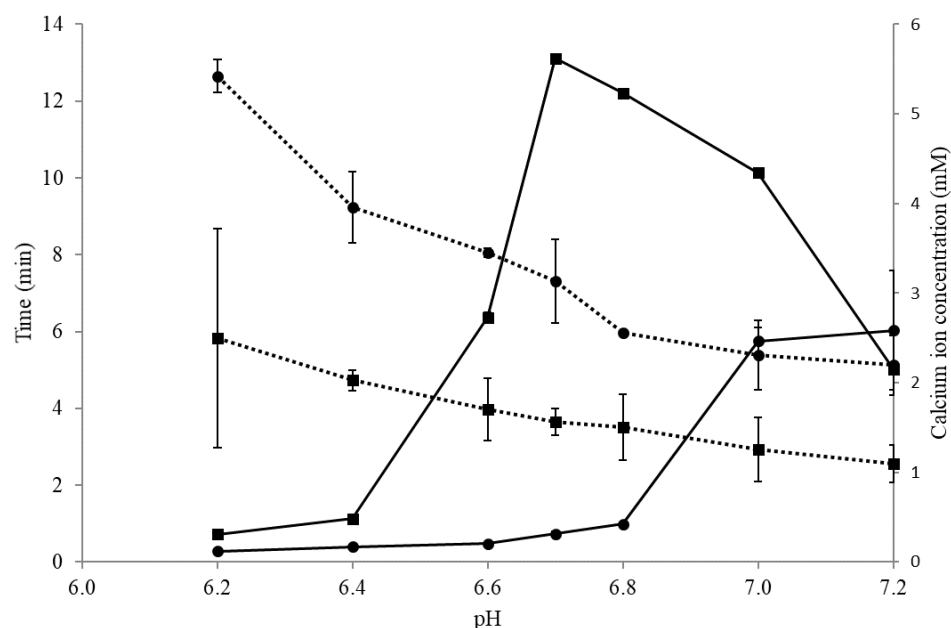


Figure 3.5. Heat coagulation time (HCT) profiles of milk protein concentrate with added lactose (MPC-Lac) (—●—) or milk permeate (MPC-Perm) (—■—) obtained from reconstituted powders (10%, DM) and calcium ion concentration of MPC-Lac (...●...) and MPC-Perm (...■...) solutions measured as a function of pH. All values presented are the means of duplicate analysis. Bars represented the standard deviation of the mean.

The zeta potential (ζ -potential) of reconstituted MPC-Perm and MPC-Lac powders measured as a function of pH is shown in Figure 3.6. The ζ -potential ranged from -18 to -22 mV across the pH range 6.0 to 7.2 for MPC-Lac and MPC-Perm. MPC-Perm and MPC-Lac both showed increasing net negative charge with increasing pH. However, no significant differences ($P > 0.05$) were found between MPC-Perm and MPC-Lac across the pH range 6.0 to 7.2.

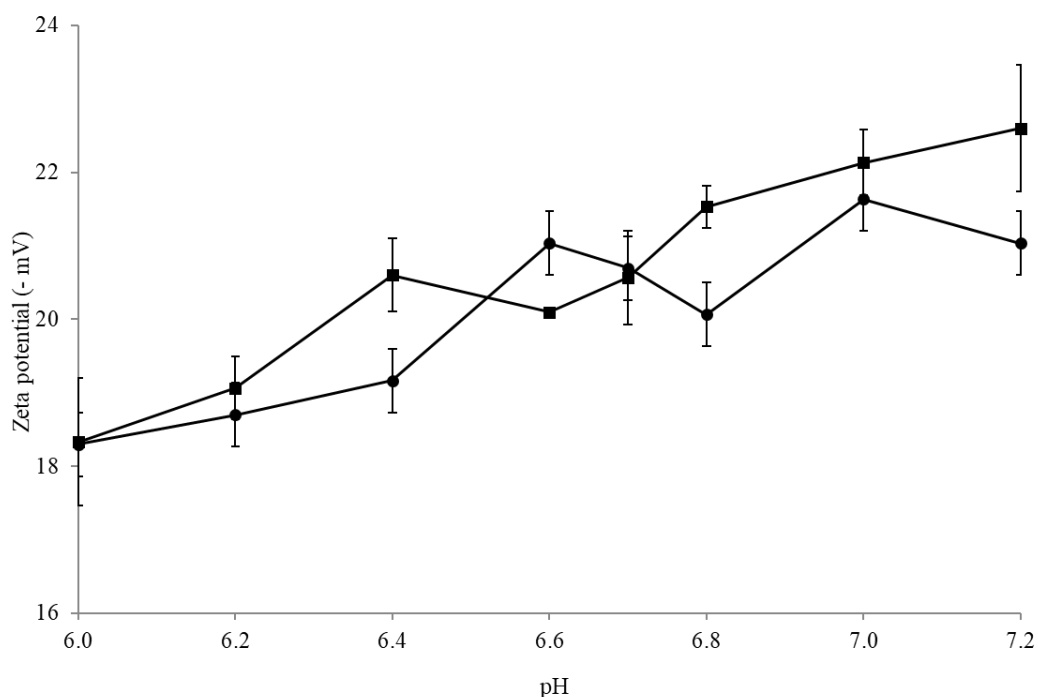


Figure 3.6. Zeta-potential of reconstituted milk protein concentrate with added lactose (MPC-Lac) (—●—) or milk permeate (MPC-Perm) (—■—) as a function of pH at 25°C. All values presented are the means of triplicate analysis.

3.5. Discussion

Mineral-induced changes in viscosity during dairy processing is a well-documented occurrence, with a substantial number of studies on this topic focused on protein denaturation, aggregation, gelation and the complex interactions between proteins and minerals as affected by protein profile, temperature, solids content, pH and time (Anema, 2009; Díaz-Ovalle, González-Alatorre, & Alvarado, 2017; Gandhi, Amamcharla, & Boyle, 2017; Hebishy, Joubran, Murphy, & O'Mahony, 2019; Ho, et al., 2018; Jeurnink & De Kruif, 1993; Liyanaarachchi & Vasiljevic, 2018; Murphy et al., 2018). However, there remain a significant number of unknowns in relating viscosity to protein-mineral complexes, in dairy systems particularly in protein concentrates. The current study has highlighted significant changes to the physicochemical and functional properties of milk protein dispersions with added milk permeate and lactose, as influenced by protein-mineral interactions. Following

evaporation to 45% DM, MPC-Lac and MPC-Perm showed an increased viscosity, with values from 3.9 to 30.9 mPa.s and from 5.9 to 83.7 mPa.s, respectively (Figure 3.2B). The higher viscosity, and associated age-thickening, observed in MPC-Perm samples is similar to that shown in a number of previous studies for skim milk concentrates. Murphy et al. (2018) reported that standardizing the protein content of skim milk with milk permeate, as opposed to lactose, resulted in higher viscosity following evaporation to 45% DM. Karlsson, Ipsen, Schrader, and Erdö (2005) and Sutariya, Huppertz, and Patel (2017) showed that the viscosity of milk depends mainly on the association between whey proteins and casein micelles, but also on heating conditions and the initial composition of the milk (e.g., total solids). In the current study, the measured differences in viscosity were attributed to the differences in mineral content between milk permeate and lactose (Table 3.1.) and their implications for pH and calcium ion concentration, with significantly higher levels of calcium, potassium and magnesium. Interestingly, almost all of the sodium and potassium were present in the serum phase of MPC-Perm and MPC-Lac solutions before evaporation, indicating that they exist mainly as either free ions or soluble salts in milk systems (e.g., NaCl and KCl) (Gaucheron, 2005).

Singh and Newstead (1992) and Carr (1999) suggested that age-thickening in skim milk concentrates is due to precipitation of calcium phosphate on to the surface of casein micelles as the effective concentration of such minerals increases during evaporation. Age-thickening of concentrated milk protein systems has also been linked to hydrophobic interactions, which occur between casein micelles during concentration of skim milk, and may be reversible if the system is subsequently diluted (Snoeren, Klok, Van Hooydonk, & Damman, 1984; Zisu, Schleyer, & Chandrapala, 2013). However, Bienvenue, Jiménez-Flores, and Singh (2003) showed that the

storage of skim milk concentrates for more than 8 h caused irreversible changes to the protein structure due to aggregation of casein micelles, as evidenced by analysis of particle size distribution, even in diluted samples. In the current study, increased particle size was observed in MPC-Perm compared to MPC-Lac (Table 3.2. and Figure 3.3.) and this larger casein micelle size can be correlated with the increase in the apparent viscosity, due in part to the high level of soluble minerals. This is in comparison with the particle size values obtained for MPC-Lac before and after evaporation which were similar to the original MPC80 solution, indicating no significant increase in casein micelle size.

MPC-Perm and MPC-Lac showed increasing HCT with increasing pH, due to a decrease in calcium ion concentration and an increase in electrostatic repulsion (Figure 3.5. and Figure 3.6.). HCT profiles of protein systems measured at 26% DM were similar to those reported by Lin, Kelly, O'Mahony and Guinee (2018b) for evaporated (i.e., 25% DM) low-heat skim milk. However, the HCT profiles of rehydrated protein powders measured at 3.5% (w/w) protein showed that the heat stability of MPC-Lac across a pH range from 6.2 to 6.8 was significantly lower than MPC-Perm. It might be assumed that the addition of milk permeate to MPC would have resulted in a lower HCT than MPC-Lac, based on the higher levels of minerals in the serum phase of MPC-Perm (Table 3.1.). However, despite the high level of serum calcium in MPC-Perm, calcium ion concentration was significantly lower than MPC-Lac, indicating that the concomitantly higher level of phosphorus in the milk serum was possibly present as phosphate and capable of binding free calcium ions. In contrast, the serum calcium in MPC-Lac was present entirely as ionic calcium (i.e., comparing serum calcium levels (mM) in Table 3.1. and ionic calcium concentration at pH 6.6; Figure 3.5.). This caused a decrease in heat stability at pH values < pH 7.0,

similar to that shown by Crowley et al. (2014) and Lin et al. (2018a). This is supported by the data in Table 3.1. where the calcium to phosphorous ratio was higher for MPC-Lac than MPC-Perm, at 1.26:1 and 0.82:1, respectively. Another reason for the lower HCT in MPC-Lac may be due to heat-induced decomposition of lactose, where the formation of formic acid is produced resulting in acidification (Singh, 2004). However, depending on the casein:whey protein ratio and severity of heating, lactose has been shown to have a stabilisation effect on heating as shown by Murphy et al. (2014), who found that lactose increased the temperature at which β -lactoglobulin denatures, in infant milk formula emulsions. Although it is important to note that at temperatures less than 100°C decomposition of lactose is less likely to occur.

The decrease in ionic calcium concentration with increasing pH (Figure 3.5.) is due to the reduction in soluble calcium phosphate, similar to results presented previously by Augustin and Clarke (1991) and Vaia, Smiddy, Kelly, and Huppertz (2006). Furthermore, the net negative charge of milk proteins decreased with decreasing pH (Figure 3.6.), and because pH values of MPC-Perm measured during evaporation were significantly lower than MPC-Lac solutions (Figure 3.1.), there would be greater propensity for protein-protein interactions to occur. Bienvenue, Jiménez-Flores, and Singh (2003) demonstrated that the decrease in pH and concomitant increase in Ca-ion concentration would lead to a reduction in electrostatic repulsion between casein micelles, facilitating cross-linking and fusion of casein micelles, resulting in higher apparent viscosity. The higher viscosity of the MPC-Perm system has down-stream processing and product quality implications, with one example being increased MPC powder particle size due to higher viscosity of the spray dryer atomizer feed (Power et al., 2020).

Furthermore, Anema, Lowe, Lee, and Klostermeyer (2014) stated that casein micelle size was larger at lower pH values (i.e., pH 6.5) than at pH 7.1 in heated concentrated skim milk due to the association of denatured whey proteins with casein micelles, whereas at higher pH, the dissociation of κ -casein, coupled with the low levels of denatured whey proteins associating with the casein micelles, results in only a small reduction in the size of casein micelles. Previously, Sikand, Tong, & Walker, (2010) showed that enhanced stability of non-fat milk systems (fat content <1.0%), such as low- and medium-heat skim milk was attributed to differences in salt, non-protein nitrogen content (NPN) and buffering capacity between lactose and milk permeate.

3.6. Conclusion

A comprehensive analysis of partitioning of milk minerals between sedimentable and non-sedimentable fractions in milk protein concentrate solutions revealed that increasing the mineral content was primarily responsible for high viscosity and age thickening. However, the addition of permeate to milk protein solutions may not necessarily lead to an increase in the concentration of ionic calcium, which is often considered one of the main factors associated with high viscosity, with the counter ions playing an important role in binding of calcium. As the majority of total calcium was present in the colloidal form, the difference in total calcium content between MPC-Perm and MPC-Lac was relatively low compared to the high concentrations of serum potassium, sodium and magnesium, which appear to be the main contributors to high viscosity and particle size in these MPC-Perm samples. Also of note is that using MPC as a base ingredient offered excellent formulation flexibility

to modify the composition of the serum/aqueous phase of the dispersions by addition of milk permeate or lactose.

3.7. Acknowledgment

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3.9. Appendix

3.9.1. Appendix 3A

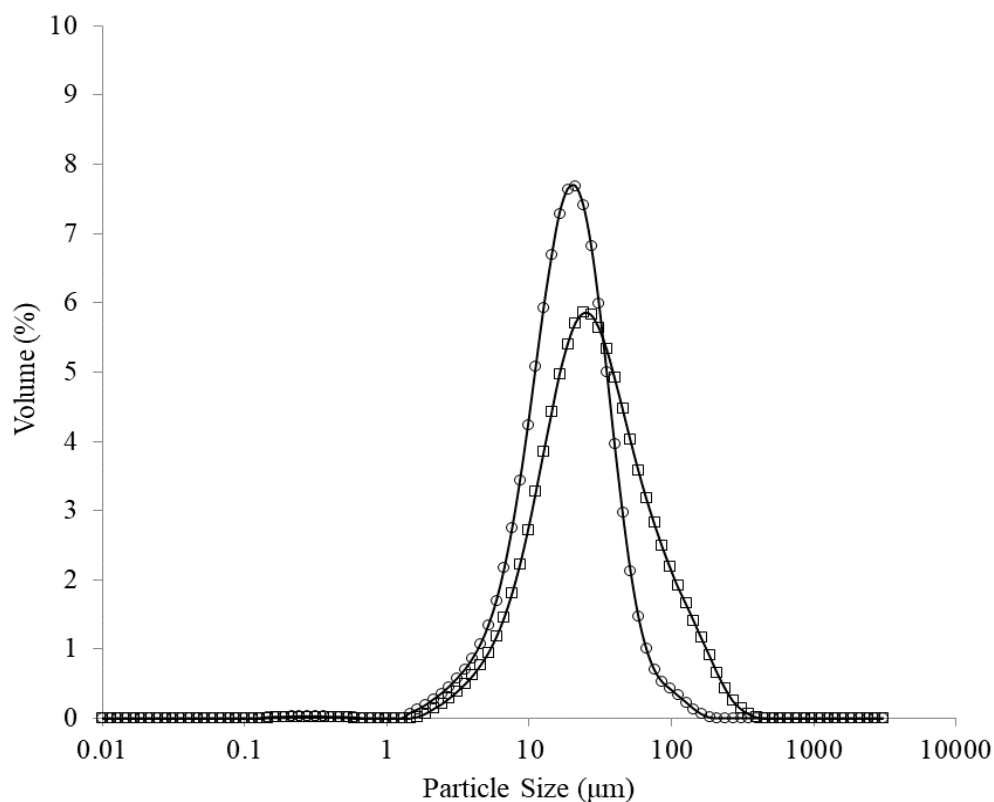


Figure A3.1. Particle size distribution profiles of milk protein concentrate powders with added lactose (-○-) or added milk permeate (-□-). Values presented are the means of data from triplicate analysis.

Chapter 4

Measurement of pH at high temperature in milk protein solutions

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Declaration

This chapter was written by TA and reviewed by all co-authors. TA designed the study with the co-authors and performed all experimental work.

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4.1. Abstract

This study determined the changes in the chemical properties of milk protein ingredients during heat-treatment, with a focus on the *in-situ* measurement of pH. Milk protein concentrate, micellar casein concentrate, sodium caseinate and whey protein isolate powders were rehydrated in either distilled water or lactose-free simulated milk ultra-filtrate (SMUF). Protein dispersions were heated from 25 to 120°C before cooling to 25°C in a custom-engineered hermetically sealed cell, fitted with a pH probe. The direct measurement of pH was successfully performed at temperatures > 100°C, addressing a significant gap in the current literature where pH values are rarely, if ever, carried out at in-process temperatures. This novel approach for measuring pH at high temperature has potential as a laboratory based analytical tool or as a critical control point during industrial high temperature processing.

4.2. Introduction

Thermal treatment is one of the most common unit operations performed in the dairy industry. As such, there have been an extensive number of scientific studies on the impact of thermal treatment on milk stability, casein micelle integrity, whey protein denaturation/aggregation; however, very few have examined the direct effect of temperature on pH (Rose, 1961, 1962; Sweetser & White, 1975). One change occurring during thermal treatment is the heat-induced association of calcium and phosphate in milk and concomitant release of hydrogen ions, resulting in a drop in pH (Fox & McSweeney, 1998; Ma & Barbano, 2003). However, the extent to which pH decreases is effected by the inherent buffering capacity of the milk, and is referred to as the ability of a system to resist changes in pH upon addition of an acid or base. Buffering capacity is affected by milk composition, particularly the protein and mineral profile (Salaün, Mietton, & Gaucheron, 2005).

Although pH plays such a significant role in dairy processing, studies carried out to date have focused on monitoring pH at relatively low temperatures or on samples recovered after high temperature processing (Anema, 2009; Augustin & Clarke, 1991; Chandrapala, McKinnon, Augustin, & Udabage, 2010; Chaplin & Lyster, 1988; Ma & Barbano, 2003; Nieuwenhuijse & van Boekel, 2003; Omoarukhe, On-Nom, Grandison, & Lewis, 2010; Pouliot, Boulet, & Paquin, 1989; Walstra & Jenness, 1984). On-Nom, Grandison, and Lewis (2010) used a rather inventive technique where dialysis of milk was performed at temperatures $>100^{\circ}\text{C}$, and the pH, ionic calcium and total divalent cations of the permeate was then measured at 80°C . Ma and Barbano (2003) measured the pH of milk continuously, *via* the insertion of a pH probe in the holding section of a tubular heat exchanger, at temperatures of 40, 56, 72 and 80°C . Anema (2009) investigated the effect of milk concentration (9.6–38.4%

w/w total solids), temperature (20–80 °C) and time (0–60 min) on the pH and the concentrations of soluble calcium and inorganic phosphate by immersing the pH probe in a holding vessel after milk passed through the heat exchanger. All studies observed a decrease in pH with increasing temperature.

Those that have used indirect pH measurements, such as measuring pH before and after heat treatment, reported this difference to be negligible but not indicating the significant decrease in pH that would have occurred at elevated temperatures. These changes are mainly associated with the shift in dynamic mineral equilibrium between the soluble and colloidal phases caused by temperature (Lucey & Horne, 2009). Omoarukhe et al. (2010) reported that under in-container sterilization conditions (115°C for 15 min), the final pH was lower than initial values by about 0.2 pH units (at 20°C). Also, Nieuwenhuijse and van Boekel (2003) and Tsioulpas, Koliandris, Grandison, and Lewis (2010) suggested there was a slight decrease in pH following UHT sterilization and a decrease of 0.2 to 0.3 pH units during batch sterilization caused by irreversible degradation of lactose. Production of organic acids such as formic acid from lactose degradation at high temperatures known to have an effect on pH decrease.

Given that pH plays such a pivotal role in determining milk protein stability, this study investigated the relationship between temperature and pH during thermal processing of milk protein dispersions suspended in water or lactose-free simulated milk ultrafiltrate (SMUF). The aim of this study was to monitor the pH *in-situ* at temperatures from 25 to 120°C, via a custom-engineered cell fitted with a pH sensor containing an in-built temperature element, in casein- or whey-dominant protein systems.

4.3. Materials and methods

4.3.1. Materials

Sodium caseinate (NaCas), micellar casein concentrate (MCC), milk protein concentrate (MPC80) and whey protein isolate (WPI) powders were supplied by a local dairy company. The MPC, WPI, NaCas and MCC powders contained 80, 90.5, 90.1 and 89.0% protein (w/w), and 7.6%, 8.2%, 4.3% and 8% (w/w, dry matter) ash, respectively.

Table 4.1. Mineral composition of milk protein concentrate (MPC), micellar casein concentrate (MCC), sodium caseinate (NaCas) and whey protein isolate (WPI) reconstituted in distilled water prior to heat treatment.

	MPC	MCC	NaCas	WPI
<i>Minerals</i>	<i>(mg 100 g⁻¹)</i>			
Sodium	134	89.0	1028	699
Magnesium	83.4	99.22	4.31	7.07
Phosphorus	1739	1369	690	106
Sulphur	735	620.3	685	1603
Potassium	631	405.1	12.0	22.7
Calcium	1862	2031	63.3	86.2
Manganese	0.07	0.10	0.10	0.17
Iron	1.02	1.33	1.93	3.36
Cobalt	<0.01	<0.01	<0.01	<0.01
Copper	0.21	0.16	0.19	0.43
Zinc	10.2	7.95	3.26	2.50
Selenium	0.05	0.16	0.05	0.09
Molybdenum	0.05	0.02	0.02	<0.01
Iodine	0.14	0.06	0.14	0.34

Data presented is from a single measurement.

All mineral analysis was carried out using inductively coupled plasma mass spectrometry (Agilent ICPMS 770 Santa Clara, US) for macro (Ca, P, K, Na, and Mg) and trace elements (S, Zn, Fe, I, Mn, Cu, Mo and Se), mineral profile of the powders are shown in Table 4.1. All the chemicals used were analytical grade and supplied by Sigma Aldrich (Arklow, Ireland) unless otherwise stated.

4.3.2. *Preparation of protein dispersions and simulated milk ultra-filtrate (SMUF)*

Whey protein isolate (WPI), milk protein concentrate (MPC), micellar casein concentrates (MCC) and sodium caseinate (NaCas) powders were rehydrated to a final protein content of 1.0, 3.5, 3.5 and 3.5% (w/w, protein), respectively. Dispersion medium was either distilled water (DW) or lactose-free simulated milk ultra-filtrate* (SMUF). SMUF was prepared based on the dry blended method described by Jenness and Koops (1962), with a slight modification as described by Dimpler (2018) where CaCl_2 was added separately. The pH of the SMUF solution was then adjusted to pH 6.60 with 1 N KOH and readjusted if necessary, after 1 h. Protein dispersions were prepared at 40°C, in order to obtain complete solubilisation using an overhead stirrer (IKA EUROSTAR ST D S2, Staufen, Germany) at 600 rpm for 2 h. Dispersions were then kept overnight at 4°C to ensure complete hydration.

4.3.3. *pH measurement and heat treatment*

pH calibration

The pH of protein solutions was measured using a combination pH/temperature sensor EasyFerm Bio HB VP 120 Pt100, supplied by Irish Power and Processing (Co. Kilkenny, Ireland). The pH probe was calibrated at 25°C using standard buffer solutions at pH 4 and 7 (NIST DIN buffer solutions, METTLER TOLEDO, Columbus, USA). The pH measurement method used was based on the NERNST equation, which describes the relation between the potential difference of the electrode and the chemical activity of the ion concentration in the measured medium (4.1).

SMUF was used in this study instead of milk permeate in order to observe the difference between milk minerals and lactose addition. Milk permeate addition would ordinarily be the ideal option in most cases.

$$E = \frac{R \times T}{n \times F} \times \ln \frac{[C]_1}{[C]_2} \quad (4.1)$$

Where, E represents the NERNST's potential, R and F are gas (8.31439 J.mol⁻¹.K⁻¹) and Faraday (96495.7 C.mol⁻¹) constants, respectively, n is the charge number of the measured ion and T is the measured sample temperature (K°). C₁ and C₂ are the active H⁺ ion concentration in solution 1 and solution 2.

Calibration of the pH probe was also performed at 60 and 80°C in order to validate the effect of temperature on calibration and to compare the pH-temperature dependence of the buffer solutions. The accuracy of the buffer solutions used are specified as +/- 0.005 pH units between 0 and 60°C and decreases to +/- 0.008 pH units between 60 and 95°C.

Heat treatment and pH measurement

The heat treatment of protein dispersions from 25 to 120°C was performed under controlled conditions via a Peltier heating system attached to an ARG2 Rheometer (TA Instruments, Crawley, UK). Samples (25 mL) were firstly placed in a custom-engineered stainless-steel cell (SX Engineering Ltd, Cork, Ireland) as shown in Figure 4.1.

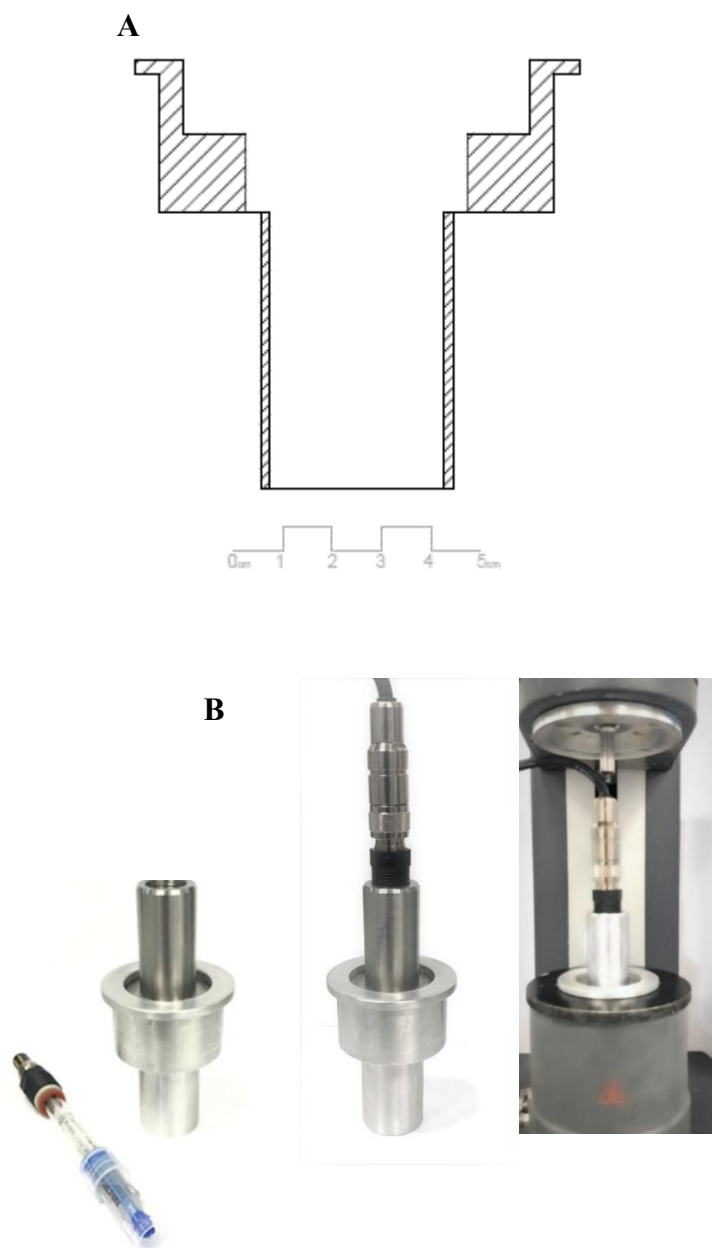


Figure 4.1. Schematic image (A) of the hermitically sealed holding cell and (B) photographic image of the sample-holding cell and pH probe alone, attached together and finally placed in the Peltier heating system of the rheometer.

The pH was measured using a combination pH/temperature sensor EasyFerm Bio HB VP 120 Pt100 (Irish Power & Processing, Kilkenny, Ireland), which was threaded and screwed in to the stainless-steel cell, hermitically sealing the system. The pH/temperature probe combination had an operation range between 0 and 140°C and a maximum operating pressure of 6 bar. The probe has a stable measurement response

time of < 4 s at a pH range between pH 4.0 and 7.0, with a temperature accuracy of $\pm 0.3^{\circ}\text{C}$. Protein dispersions were heated from 25 to 120°C at a rate of $15^{\circ}\text{C min}^{-1}$, held at 120°C for < 2 min before being cooled to 25°C . Temperature and pH were recorded continuously using a pH/ORP converter and data collector (YOKOGAWA EXAxt 450, Amersfoort, The Netherlands). After heat treatment, samples were collected for further analysis. Furthermore, to investigate the effect of time on the accuracy of recording a stable pH reading, separate aliquots of protein dispersions were heated to 40, 60, 80, 100 or 120°C and held for 20 s, with continuous pH data being recorded, before cooling back to 25°C .

4.3.4. *Calcium ion concentration*

The calcium ion concentration of protein dispersions was measured at 25°C before and after heat treatment using a calcium ion meter (Sension+ MM340 benchtop meter, Hach Co., Loveland, Colorado, U.S.A) equipped with calcium selective sensor (sensION+ 9660C, 193 Hach Co., Loveland, Colorado, U.S.A.). Calibration of the sensor, and measurements of calcium ion concentration, was performed as described by Lin, Kelly, O'Mahony & Guinee, (2016). The ion-selective calcium probe was calibrated at 25°C with standard calcium solutions at 0.05, 1.00, 2.50 and 5.00 mM. A 0.1 mL volume of potassium chloride (KCl) stock solution (3 M) was added to 10 mL of all samples, giving an added KCl concentration of 29.7 mM. KCl was added to deliver an electrochemical response that is proportional only to the ion concentration and helps improve the ion selectivity of the probe.

4.3.5. *Buffering capacity*

Buffering capacity was measured as described by Kim, Oh, and Imm (2018) using a Titrand 842 Autotitrator with a TIAMO v.2.2 software package (Metrohm, Ireland Ltd., Carlow, Ireland). A three-point calibration of the pH probe was performed using buffer solutions at pH 4.0, 7.0 and 9.0 prior to performing measurements. Samples (50 ml) were titrated from their initial pH to pH 2.0, through the controlled addition of 0.5 M HCl (20 μ L increments with 30 s equilibration after each addition) followed by alkalization to pH 8 through the addition of 0.5 M NaOH. Titration was performed under continuous stirring at 700 rpm, 25 °C. The buffering index value (dB/dpH) of protein dispersions was determined using the Van Slyke equation (Van Slyke, 1922).

4.3.6. *Statistical analysis*

The results are the average of at least three independent measurements carried out on two replicate trials and are reported as the mean value $\pm$ standard deviation. Statistical analyses were performed using SPSS software (SPSS V.18, IBM, New York, USA) and the level of significance was determined at $P < 0.05$ using analysis of variance and the t-paired test.

4.4. **Results and Discussion**

4.4.1. *Effect of temperature on pH*

The pH of all protein systems decreased linearly with increasing temperature, although there were significant differences in the rate of decrease (Figure 4.2) and the change in pH between protein dispersions when measured before and after heat treatment (Table 4.2).

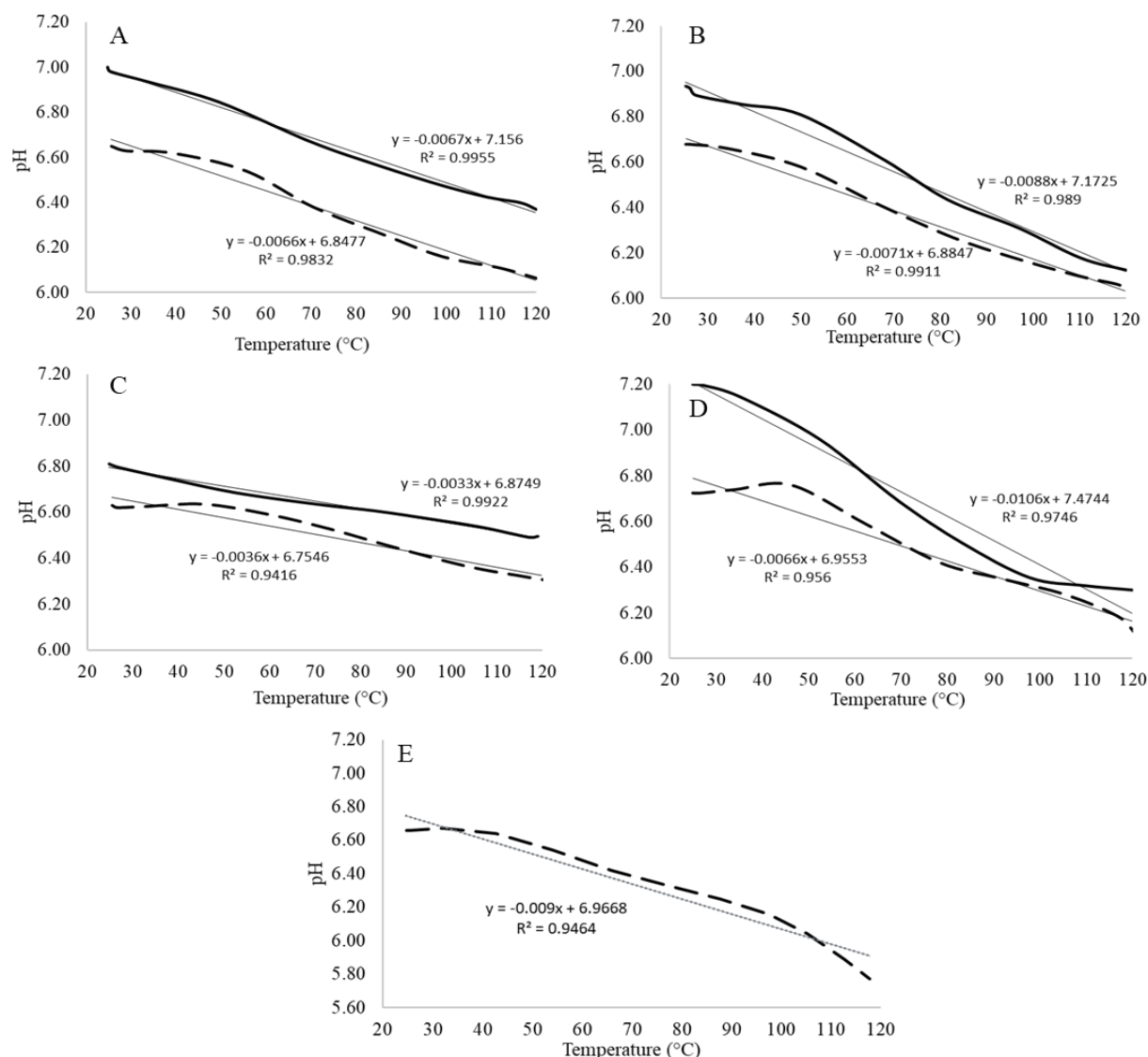


Figure. 4.2. pH profile of milk protein concentrate (A), micellar casein concentrate (B), sodium caseinate (C), whey protein isolate (D) and SMUF alone (E). Protein ingredients were dispersed in either distilled water (—) or in simulated milk ultrafiltrate (---) and measured as a function of temperature from 25 to 120°C.

The solvent media also had a major effect, with the pH of protein ingredients dissolved in SMUF lower than in water, but the rate of pH decrease (pH units °C⁻¹) was similar (Figure 4.2). The rate of pH decrease as a function of temperature and WPI-DW were -0.0088, -0.0067, -0.0033 and -0.0106 pH units °C⁻¹, respectively. The pH change as a function of temperature was also linear for protein systems in the presence of SMUF, with the rate of pH decrease measured as -0.0071, -0.0066, -

0.0036, -0.0066 and -0.0090 pH units °C⁻¹ for MCC-SMUF, MPC-SMUF, NaCas-SMUF, WPI-SMUF and SMUF respectively.

Using the rate of pH decrease is a good means of comparing the effect of temperature on pH across samples and against previously published data. A study by Chaplin and Lyster (1988) measured the change in pH of skim milk as a function of temperature between 0 and 80°C. These authors reported a linear coefficient of -0.0073 pH units °C⁻¹ for skim milk, which is similar to MCC-SMUF and MPC-SMUF (-0.0071 and -0.0066 pH units °C⁻¹) in the present study measured over a temperature range of 25 to 120°C. Similarly, Ma and Barbano (2003), who measured the pH *in-situ* of milk at set temperatures of 40, 52, 72 and 80°C in a tubular heat exchanger, reported a gradient of -0.0078 pH units °C⁻¹. While these studies all measured pH directly at set temperatures, On-Nom, Grandison, and Lewis (2010) heated milk at 120°C for 60 min in a retort sterilization system and took dialysis bags *in-situ* before measuring pH of the dialysate at 20°C. These authors highlighted the issue of being unable to measure pH *in-situ* at sterilization temperatures (120°C); however, they reported that the pH of the resulting permeate was pH 5.92 when measured at 20°C. Similarly, in the present study, a pH of 5.79 was obtained for SMUF at 120°C and pH 5.70 when cooled back to 25°C, indicating that the pH change was irreversible (Δ pH 0.95; Table 4.2).

Interestingly, NaCas-DW and NaCas-SMUF had similar linear coefficients (0.0033 pH units °C⁻¹) indicating that it is the calcium phosphate content in milk based systems that determine the pH-temperature dependence. While, On-Nom et al. (2010) directly measured the pH in milk and in milk permeate at temperatures up to 80°C, reporting a pH gradient of ~ -0.010 pH units °C⁻¹ for across a temperature range of 20 to 80°C. They also showed that the

Table 4.2. pH of protein dispersions before (25°C) and after (120°C) heat treatment.

	Initial pH at 25°C	pH at 120°C	pH at 25°C after heat treatment	Δ pH
<i>Protein dispersions reconstituted in water</i>				
MPC	6.98 $\pm$ 0.03	6.37 $\pm$ 0.02	6.82 $\pm$ 0.01	0.16
MCC	6.94 $\pm$ 0.01	6.13 $\pm$ 0.01	6.63 $\pm$ 0.01	0.31
NaCas	6.81 $\pm$ 0.01	6.49 $\pm$ 0.01	6.73 $\pm$ 0.01	0.08
WPI	7.20 $\pm$ 0.03	6.30 $\pm$ 0.03	7.00 $\pm$ 0.01	0.20
<i>Protein dispersions reconstituted in SMUF</i>				
SMUF	6.65 $\pm$ 0.02	5.79 $\pm$ 0.01	5.70 $\pm$ 0.02	0.95
MPC	6.65 $\pm$ 0.02	6.06 $\pm$ 0.02	6.37 $\pm$ 0.01	0.28
MCC	6.68 $\pm$ 0.01	6.03 $\pm$ 0.01	6.37 $\pm$ 0.03	0.31
NaCas	6.63 $\pm$ 0.05	6.31 $\pm$ 0.01	6.44 $\pm$ 0.01	0.19
WPI	6.73 $\pm$ 0.03	6.11 $\pm$ 0.02	6.06 $\pm$ 0.01	0.67

SMUF – simulated milk ultra-filtrate

Δ pH – Difference in pH before and after heat treatment, measured at 25°C

pH of milk and their corresponding ultrafiltration permeates (i.e., protein-free) were closely aligned during heat treatment up to 80°C, indicating that although protein may contribute to buffering, the pH of milk is mainly defined by the mineral composition. Heat treatment of milk induces a re-equilibration between HPO_4^{2-} , H_2PO_4^- and $\text{H}_3\text{PO}_4^{3-}$ in the serum phase, which can result in the release of H^+ ions and concomitant decrease in pH (Chandrapala, McKinnon, Augustin, & Udabage, 2010). The observed decrease in pH is mainly associated with the precipitation of primary and secondary calcium phosphate, with simultaneous release of H^+ ions. Therefore, and as expected, the larger decrease in pH was observed for MCC and MPC ingredients compared to NaCas (Figure 4.2), due to the higher level of calcium and phosphorous in the micellar protein systems (Table 4.1). However, what is interesting in the micellar casein ingredients dispersed in SMUF is that while the overall pH was lower than in water, the rate of decrease was similar.

In the present study, the pH measured before and after heat treatment of MPC, MCC and NaCas in water or SMUF was $\sim$ 0.1 to 0.3 units lower than the initial value (Table 4.2; Supplementary Table 4.1). This is in agreement with Nieuwenhuijse and

van Boekel (2003) who reported a 0.2 to 0.3 pH unit decrease after batch sterilization at 120°C. However, it is important to note that for short-time heat treatment processes such as pasteurization and UHT, the pH usually returns to its initial value after cooling (On-Nom et al., 2010), indicating that large irreversible changes to milk pH are both temperature and time dependent. Tsioulpas et al. (2010) reported that the level of ionic calcium in skim milk decreased from 1.87 to 1.78 mM after in-container sterilization (121°C × 15 min) and that the pH decreased from pH 6.86 to 6.62 due to H⁺ ion release and the degradation of lactose. The calcium ion concentration of protein dispersions are shown in Table 4.3. MPC-DW and MCC-DW showed the highest levels of ionic calcium before heat treatment at 2.82 and 2.24 mM, respectively, while calcium-depleted NaCas-DW and WPI-DW were too low to measure. The presence of SMUF dramatically increased the ionic calcium level of WPI-SMUF and NaCas-SMUF to 2.0 and 1.00 mM, respectively, due to the presence of calcium ions in the SMUF. However, the addition of SMUF resulted in a reduction in calcium ion concentration in MPC-SMUF and MCC-SMUF (2.36 and 2.01 mM, respectively), due to the addition of counter ions in the form of citrate and phosphate. All protein dispersions reconstituted in SMUF displayed a decrease in the level of ionic calcium after heat treatment.

Table 4.3. Calcium ion concentration (mM) of protein dispersions before and after heat treatment.

	Calcium ion concentration (mmol L ⁻¹)	
	Before heat treatment	After heat treatment
<i>Protein dispersions prepared in water</i>		
MPC	2.82 ± 0.0	2.70 ± 0.0
MCC	2.24 ± 0.0	2.43 ± 0.0
NaCas	< LOD	< LOD
WPI	< LOD	< LOD
<i>Protein dispersions prepared in SMUF</i>		
SMUF	1.59±0.0	0.43 ± 0.2
MPC	2.36±0.0*	1.47± 0.0*
MCC	2.01±0.0*	1.63±0.3*
NaCas	1.04±0.0*	0.88±0.0*
WPI	2.04±0.0*	0.42±0.0*

All measurements were performed at 25 °C.

LOD = limit of detection

* Indicates calcium ion concentration values that differed significantly ($P < 0.05$) before and after heat treatment.

It is known that pH is strongly temperature and mineral/ion dependent, with Kaombe, Du and Lewis (2011) previously suggesting that dialysis and UF of milk at high temperature provides the best means yet for estimating the pH and ionic calcium of milk at that temperature. However, with the exception of ionic calcium measurements, this study has shown actual measured pH data at high temperature.

4.4.2. Buffering capacity of milk protein dispersions

The buffering curves obtained from the acid-base titrations of milk protein dispersions are shown in Figure 4.3 and 4.4, presented as the buffering index versus the change in pH. MPC-DW and MCC-DW had a net buffering peak area between pH 6 and pH 4 on titration from their initial pH to pH 2 (Figure 4.3. A and B). This buffering peak is attributed to the solubilisation of colloidal calcium phosphate and

the concomitant release of phosphate ions. Free phosphate ions associate with free H^+ ions causing an increase in the buffering capacity between pH 6 and 4. Note: this study uses the term free H^+ ions but in fact acid species are more accurately represented as hydronium ions (H_3O^+) due to the complex nature of hydrogen in aqueous solutions.

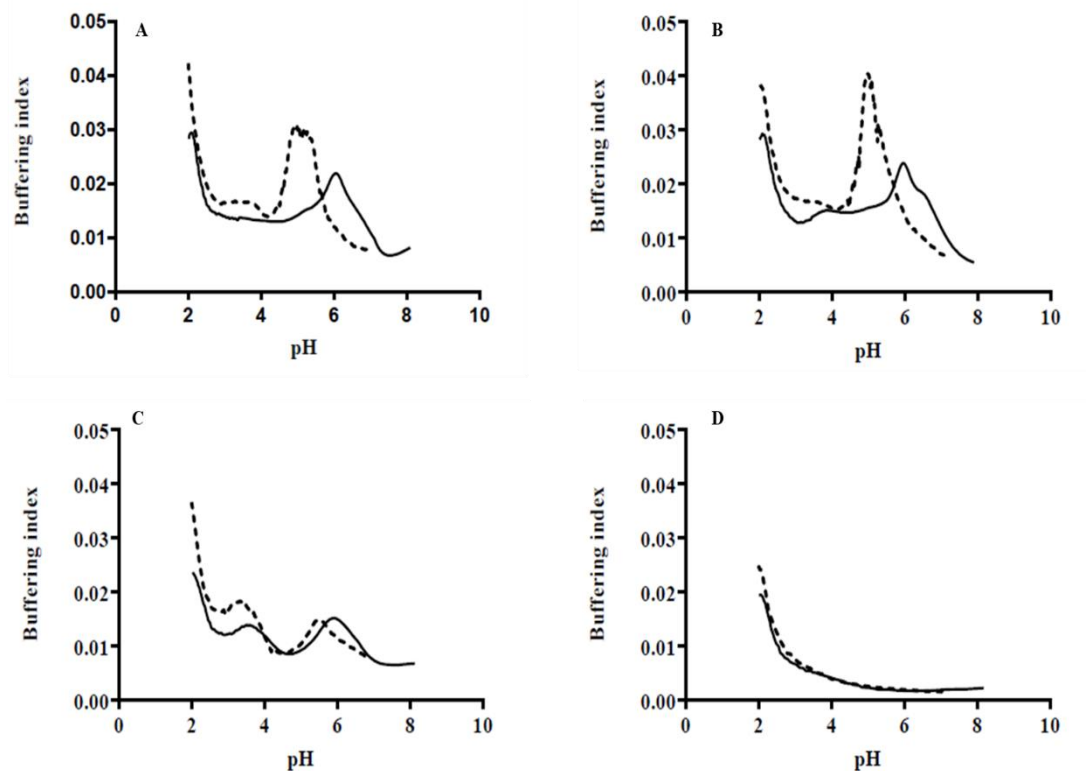


Figure.4.3. Acid-base buffering curves of milk protein concentrate (A), micellar casein concentrate (B), sodium caseinate (C) and whey protein isolate (D) dispersed in distilled water. Dotted and black lines represent acidification and alkalization, respectively.

A number of previous studies (Lucey & Fox, 1993; Lucey, Gorry, & Fox, 1993; Upreti, Bühlmann, & Metzger, 2006) have reported that, at approximately pH 6.0, the solubilisation of hydroxyapatite ($Ca_5(PO_4)_3(OH)$) commences, with complete solubilisation achieved at pH 5.0. Likewise, MPC-SMUF and MCC-SMUF (Figure 4.4. B and C) showed a similar buffering profile to MPC-DW and MCC-DW on acidification. This is due to the fact that colloidal calcium phosphate associated with

micellar casein affects buffering capacity to a greater extent than the minerals and ions added to the aqueous phase in the form of SMUF. However, on the back titration from pH 2.0 to pH 8.0, two net buffering peak regions were observed for MPC-SMUF and MCC-SMUF as shown at pH 4.0 and 6.0 (Figure 4.4), unlike for MPC-DW and MCC-DW which only showed one buffering peak at pH 6 (Figure 4. 3). SMUF alone showed much lower BI values (~ 0.01) at all pH values. The maximum buffering observed at pH 4.0-5.0 is in agreement with Wolfschoon, Spiegel, and Hernandez-Zenil (2017) who showed a maximum buffering capacity in the pH region of 4.0–4.8 in milk permeate. It is important to note that the relationship between buffering capacity (performed at 25°C) and changes in pH as a result of temperature are not entirely correlated, as the former method includes the addition of H^+ ions in the form of HCl, compared to the innate release of H^+ ions from milk minerals in the latter. In Section 3.1, the pH decrease as a result of heating was caused by the release of H^+ ions from HPO_4^{2-} , $H_2PO_4^-$ and H_3PO_4 during the formation of calcium phosphate, as opposed to the dissociation of calcium phosphate during acidification in the buffering capacity method. Therefore, milk systems with the same measured pH value does not always mean that the same chemical interactions occurred to achieve it. For instance, taking SMUF with a pH of 5.7 obtained as a consequence of heat treatment is not the same as pH 5.7 attained through the addition of H^+ ions in the form of HCl. Both methods ultimately have the same pH value but the ratio of total:free H^+ ions is very different, as the system with HCl addition contains a higher total hydrogen ion concentration.

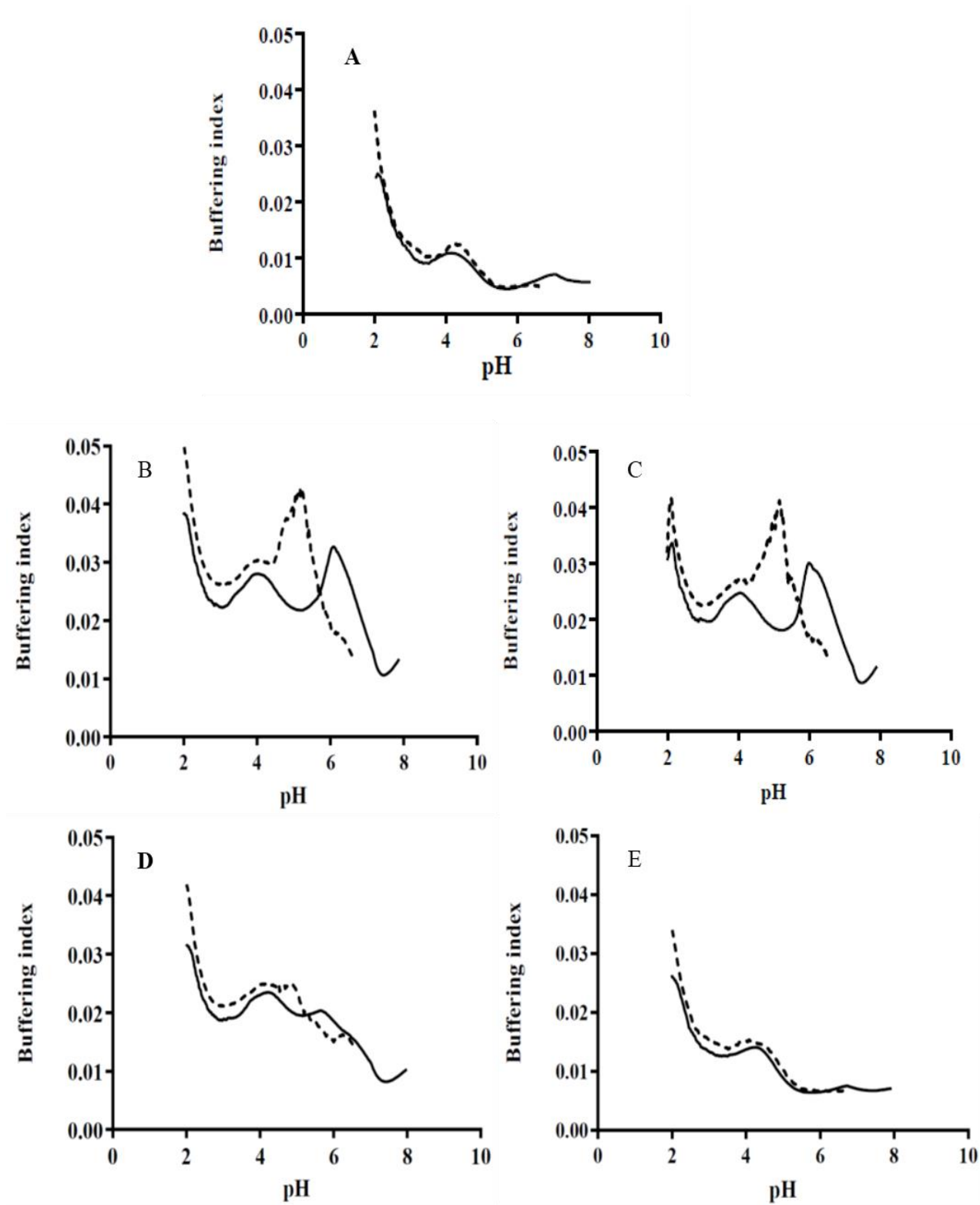


Figure. 4.4. Acid-base buffering curves of simulated milk ultrafiltrate (SMUF) (A), milk protein concentrate (B), micellar casein concentrate (C), sodium caseinate (D) and whey protein isolate (E) dispersed in SMUF. Dotted and black lines represent acidification and alkalization, respectively

While the former involves a release of H^+ ions from the phosphate group during calcium phosphate formation, the latter dissociates calcium phosphate and, as HCL is added, the H^+ ions are associated to the free phosphate groups, causing an increase in buffering index.

During the addition of 0.5 N NaOH to casein dominated protein solutions, H^+ ions are released from the side chains of carboxyl groups ($COOH$) on amino acids, resulting in the formation of COO^- ions (Bylund, 1995). The buffering maxima at pH 6.0 is associated with calcium phosphate ($Ca_5(OH)(PO_4)_3$, $Ca_3(PO_4)_2$, $Ca_4H(PO_4)_3$ or $CaHPO_4$) precipitation and the concomitant release of H^+ ions from HPO_4^{2-} and $H_2PO_4^-$ which combines with the alkali titrant (OH^-), resulting in increased buffering capacity (Salaün et al., 2005; Upreti et al., 2006). Previously, Upreti et al. (2006) suggested that increased buffering at approximately pH 6.0 was mainly associated with the formation of $CaHPO_4$ or $Ca_4H(PO_4)_3$, rather than $Ca_{10}(PO_4)_6(OH)_2$ hydroxyapatite precipitate or $Ca_3(PO_4)_2$ based on theoretically modelled pH buffering curves. The buffering maxima between pH 3 to 5 observed in MPC-SMUF and MCC-SMUF during alkalization was due to the presence of citrate salts, present as part of the SMUF addition.

For the non-micellar casein system, NaCas-DW had buffering capacity peaks at pH 7.0-5.0 and at pH 4.0-3.0 during acidification (Figure 4.3.C). The buffering index values in the pH range 7.0-5.0 were less than that observed in MPC and MCC systems, mainly due to the absence of colloidal calcium phosphate. WPI-DW did not have any distinctive buffering peak, although the formation of a shoulder at $\sim$ pH 3 is related to the association of H^+ ions to carboxylic groups on amino acids (Salaün et al., 2005), while, WPI-SMUF (Figure 4.4C) showed a relatively similar profile to the SMUF solution alone.

4.5. Conclusion

Although the effects of heat treatment on various milk systems has been extensively studied over the years, knowledge of thermally induced pH changes in dairy protein systems is limited, with measurements taken before and after heat treatment the most common approach. The unique feature of this study allowed for the measurement of pH directly *in-situ* as dispersions were heated to 120 °C under controlled conditions. The impact of temperature on pH showed that the rate of decrease was linear and largely reversible upon cooling for casein-dominated systems but not for whey protein dispersions. While the addition of soluble minerals in the form of SMUF caused a greater decrease in pH, it followed a similar trend to protein ingredients dispersed in water. Overall, unlike for one-off individual measurements, the continuous recording of pH has significant implications for dairy processing, facilitating new insights in to heat stability of milk and milk protein products.

The *in-situ* high-temperature pH measurement technique, developed and proven to work effectively in this study, can facilitate rapid and continuous measurements, which help understand critical process attributes during high temperature processing. This technique is a useful tool for the dairy industry, particularly for early identification of pH fluctuations in certain products.

4.6. Acknowledgment

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Chapter 5

Measuring pH of skim milk and milk permeate at ultra-high temperatures at laboratory and pilot scale

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Declaration

This chapter was written by TA and reviewed by all co-authors. TA co-designed the study and performed all of the experimental work.

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5.1. Abstract

The aim of this study was to examine changes in the pH of skim milk and skim milk ultrafiltration permeate on heating from 25 to 140°C. Results show that the decrease in pH with increase in temperature up to 140°C was not linear. Hydrogen ion release due to changes in the milk mineral balance are responsible for the reduction in pH with increase in temperature. The presence of milk proteins offered little buffering against the drop in pH. The precipitation of calcium phosphate resulted in sediment in milk permeate heated above ~70°C, but this did not occur in skim milk, with the pH remaining lower in milk permeate after heat treatment when measured at 25°C. This study has shown that *in-line* pH measurements of milk at ultra-high temperatures is feasible, and could prove useful at laboratory and pilot-scale for studying interactions within, and stability of, more complex formulations with added minerals.

5.2. Introduction

One of the most significant physicochemical properties of skim milk, affecting its processability and quality of many finished products (e.g., evaporated milk, infant formula) is the heat stability. During dairy processing, milk can be exposed to temperatures anywhere between 4 and 145°C, designed in most cases to retard/inactivate microbial (pathogenic and spoilage microorganisms, all spore-forming and non-spore-forming bacteria) and enzyme activity (enzyme inactivation for indigenous, exogenous and endogenous enzymes) or to induce desired physical attributes (e.g., improved emulsification, gelation, heat stability) (Deeth, 2021; Lindsay, Robertson, Fraser, Engstrom, & Jordan, 2021; McSweeney, Aydogdu, Hailu, O'Mahony, & McCarthy, 2022). To this effect, the behaviour and stability of milk at high temperatures is critical for maintaining efficient processing and producing products of acceptable and consistent quality. The most commonly used laboratory technique for determining heat stability of milk is the subjective *oil bath method*, developed by Davies and White (1966), involving the pH adjustment of milk solutions, prior to submerging the samples in tubes into oil at 120 or 140°C and recording the elapsed time for coagulation or the appearance of flecks, known as the heat coagulation time (HCT). The heat stability of milk at 140°C varies widely and is affected by a number of factors, such as microbial quality, protein content and profile, mineral composition, total solids and, in particular, the concentration of hydrogen ions (pH) which has a direct correlation with heat stability (Dumpler, Huppertz, & Kulozik, 2020). Bulk bovine milk and milk samples from most individual cows show as a type A HCT profile, which has a local minimum at ~pH 6.9-7.1 and maximum stability at pH 6.6-6.8. The local minimum observed at ~pH 6.9-7.1 has been reported to be a consequence of dissociation of κ -casein from the micelle with associated whey

proteins (Anema & Klostermeyer, 1997; Dimpler et al., 2020; Kudo, 1980; Rose, 1961). Ultimately, there are four main mechanisms which effect heat-induced changes in milk and cause a decrease in the stability of micelles; association of calcium and phosphate with the micelles, association of denatured/aggregated whey proteins to micellar casein, dissociation of κ -casein from the micelles, and/or formation of organic acids through lactose degradation (Anema & Li, 2000; Singh, 1995).

There have been numerous studies and review articles describing the heat stability of milk, concentrated milk and factors influencing heat stability, as well as the heat-induced changes in milk prior to coagulation. Previously, Fox (1981) reviewed the heat-induced pH changes in milk, in particular discussing the heat treatment of milk from a study performed by Sweetsur and White (1975). This study used extrapolation of pH data obtained at ≤ 90 °C to generate pH values at 140 °C, as this was the only feasible method at the time. However, a recent study by Aydogdu, O'Mahony, and McCarthy (2022) the pH changes in milk protein systems when heated to 120°C and found a linear regression between pH and temperature due to the release of hydrogen ions during the formation of calcium phosphate. This study was performed under static conditions using a Peltier heating system, and provided new insights to the actual pH decrease of protein solutions and how the presence of milk salts affect these changes.

Developing this previous work further, the aim of this study was to focus on obtaining pH data *in-situ* at temperatures up to 140 °C in both a laboratory-designed system for rapid, high throughput analysis and also in-line within a customised pilot-scale tubular heat exchanger.

5.3. Materials and methods

5.3.1. Materials

Low-heat skim milk powder was produced in Moorepark Technology Limited, as described by Magan et al. (2019). Total nitrogen (TN) and non-protein-nitrogen (NPN) levels were determined using the Kjeldahl method according to ISO 8968-1 (2001). The total protein content of the skim milk powder was 37.2% (w/w), using a nitrogen-to-milk protein conversion factor of 6.38. The NPN level was 0.042%, w/w. All the chemicals used were analytical grade and obtained from Sigma-Aldrich (Arklow, Ireland), unless otherwise stated.

5.3.2. Rehydration of skim milk, pH adjustment and ultrafiltration

Skim milk powder was reconstituted in water to a final total solids (TS) content of 9%, w/w. The rehydrated skim milk was stored overnight at 4°C in order to ensure full hydration. Following overnight agitation, aliquots of skim milk (~1 L; tempered at 25°C) were pH adjusted from pH 5.6 to 7.2, at 0.2 unit increments using 0.5 M HCl or NaOH under constant stirring. The pH adjusted samples were stirred for 2 h and pH was re-adjusted if necessary. In order to generate milk permeate from the skim milk samples, ultrafiltration (UF) of the pH-adjusted skim milks was performed at 25°C using a 10 kDa molecular weight cut-off Vivaflow 200 cross flow cassette membrane (Sartorius AG, Göttingen, Germany) connected to a peristaltic pump. The pH of the milk permeate solutions were not re-adjusted after ultrafiltration in order to avoid introducing additional ions. The temperature of the skim milk during UF was controlled using a water bath throughout the filtration process in order to minimise compositional variations in the permeate. Skim milk permeates were collected after UF for further analysis.

5.3.3. *In-situ pH measurement and heat treatment at 140°C*

Aliquots of the pH adjusted skim milks and their corresponding milk permeates were subjected to heat treatment using an oil bath equipped with a custom engineered stainless steel holding cup. The holding cup was fitted with a combination pH probe (Hamilton EasyFerm Bio HB, Station Road, Birmingham, UK) with a VP 120 type connection and built in temperature sensor Pt1000, supplied by Irish Power and Processing (Stoneyford, Co. Kilkenny, Ireland). The processing specifications for the sensor are as follows; temperature 0-140°C, pH 0-14 and a maximum pressure of 6 bar. The calibration of the pH probe was performed as described previously by Aydogdu et al. (2022). The oil bath (Buchi heating bath B-305, BÜCHI Labortechnik, Flawil, Switzerland) temperature was set to 140°C prior to analysis, individual samples were placed in a stainless steel holding cup and immersed in the oil and pH and temperature recorded until a sample temperature of 140°C was reached. After a 5 s holding period, the stainless steel sample holding cell was immediately placed into an ice bath and allowed to cool. Heated samples were subsequently collected and further analysis was carried out within 24 h.

5.3.4. *Heat coagulation time of skim milk*

Heat coagulation time (HCT) of skim milk (9% TS) samples were measured at 140°C as a function of pH (5.6 to 7.2) as described by Davies and White (1966). pH adjusted samples (Section 2.2) were equilibrated for 1 h at 25°C prior to measurement and re-adjusted if necessary. Samples were placed in glass tubes and immersed in an oil bath at 140°C. HCT was monitored and recorded as the time elapsed between immersing the sample in the oil bath and protein aggregation visibly detected.

5.3.5. *Mineral composition*

Skim milk permeate samples were collected before and after heat treatment at 140°C. UF generated milk permeates collected after heat treatment were carefully sampled in order to collect the serum phase without disturbing any sedimented mineral deposits (centrifugation was not required). Macro elements (calcium, phosphorous, potassium, magnesium and sodium) were determined via inductively coupled plasma-optical emission spectrometry (Agilent ICPMS 7700, Santa Clara, CA, USA) as described by Cruijsen, Poitevin, and Brunelle (2019). Samples were digested in a microwave digestion system (CEM, MARS 6, Dublin, Ireland). Liquid sample (4 mL) was mixed with nitric acid and incubated for 15 min. Samples were then heated to 200 °C and held for 15 min at a pressure of 55 bar.

5.3.6. *Calcium ion concentration*

The ionic calcium concentration of skim milk and all milk permeate samples within the pH range 5.6-7.2 were measured before and after heat treatment, and analysed as described by Aydogdu, Ho, Ahrné, O'Mahony, and McCarthy (2021), with a calcium ion selective electrode (sensION+ 9660C, Hach Co., Loveland, CO, USA). The ion selective calcium probe was calibrated with freshly prepared standard calcium solutions at 0.05, 1.00, 2.5 and 5.00 mM at 25°C. Prior to probe calibration or sample measurements, a 3 M potassium chloride stock solution was prepared, from which 0.1 mL was added to 10 mL of all standard and sample solutions in order to enhance the ion selectivity of the measurement. All measurements were performed at 25°C under constant stirring.

5.3.7. *Heat treatment of skim milk using a tubular heat exchanger with in-line pH measurement*

Pasteurized liquid skim milk and milk permeate, prepared by ultrafiltration using a 10 kDa molecular weight cut-off polymeric membrane, were obtained from Moorepark Technology Ltd. (Moorepark, Fermoy, Co. Cork). The total solids and protein content of the liquid skim milk was 8.97% and 3.29% (w/w), respectively, with a NPN content of 0.036%, w/w. Milk permeate had a total solids content of 5.85%, w/w, and a NPN content of 0.030%, w/w. The macro element composition of the skim milk was as follows: calcium (35.5 and 6.4 mM), magnesium (5.1 and 2.8 mM), phosphorus (37.4 and 12.2 mM), potassium (44.0 and 39.1 mM) and sodium (18.5 and 14.6 mM), respectively.

The skim milk and milk permeate were thermally treated using a pilot-scale tubular heat exchanger (OMVE Netherlands B.V. Gessel 61 3454 MZ De Meern, the Netherlands), equipped with preheater, final heater, holding tubes and two cooling sections. The final heater temperature was incrementally increased from 25 to 65, 75, 85, 90, 100, 120 and finally to 140 °C at a flow rate of 10 L h⁻¹ and holding time of 23 s. The holding duration of 23 s was designed to ensure sufficient time and volume of product in the holding tube in order to obtain a stable pH reading. A custom-built stainless steel T-piece pipe fitting, with pH probe incorporated, was attached to a flexible stainless steel holding tube as shown in Figure 5.1. The temperature of the product was concomitantly recorded from the temperature sensor within the pH probe (as described in Section 2.3), which correlated to $\pm 1.5^{\circ}\text{C}$ with the reading from the temperature sensor on the holding tube section of the heat exchanger. The backpressure valve was adjusted from 2.3 bar at temperatures < 100 °C to 3.8 bar at temperatures $\geq 100^{\circ}\text{C}$.

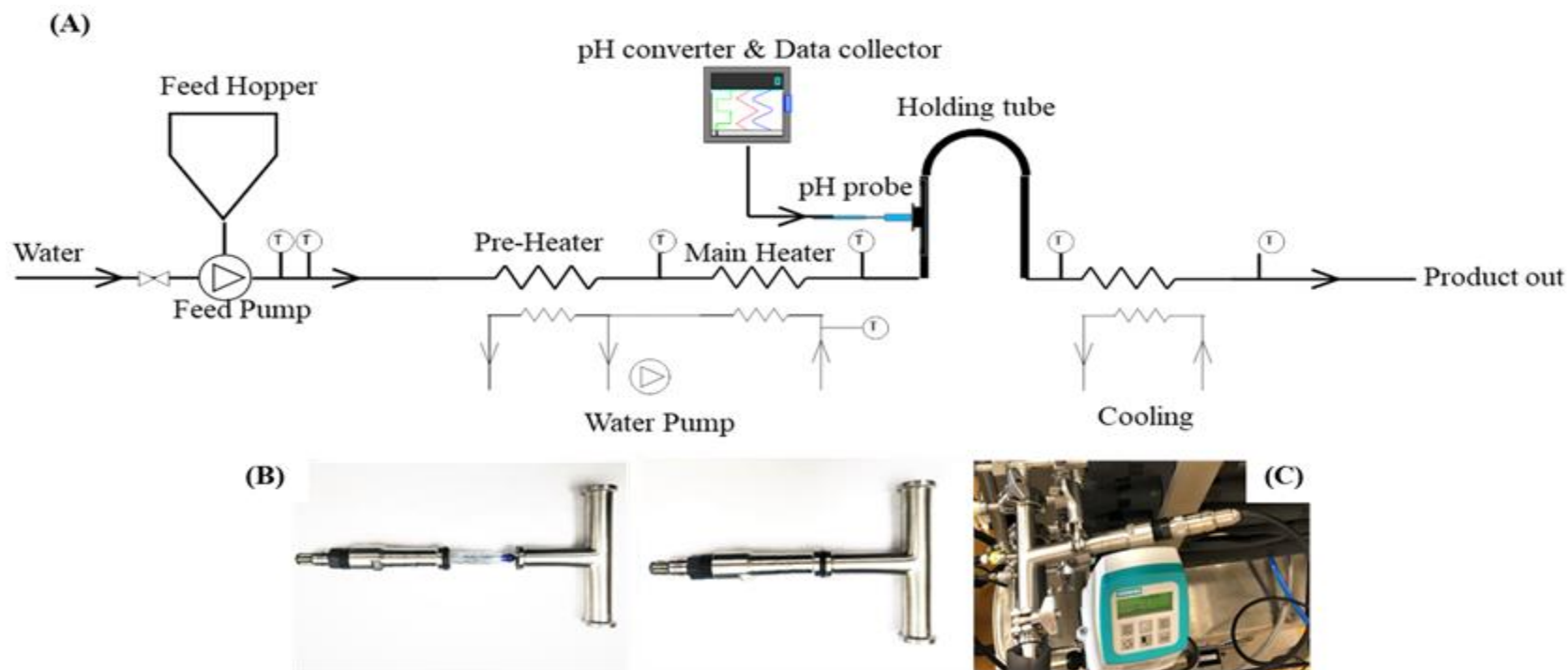


Figure 5.1. Line diagram of the pilot-scale heat treatment system with pre-heater, final heater, cooling sections, and modified holding tube with pH probe insert (A) and photographic images of pH probe in a stainless steel Tee-fitting and (B) and photographic image of the pH probe inserted in the holding tube (C).

5.4. Results

5.4.1. Laboratory-scale heat treatment (oil bath method)

Mineral and calcium ion concentration of skim milk and milk permeate samples

The mineral profile of the original skim milk and of the corresponding milk permeate solutions obtained at each pH are shown in Table. 5.1. The mineral profile of the original skim milk was typical of that shown before with potassium present at the highest concentration (45.5 mM) followed by phosphorus (33.4 mM) and calcium (32.0 mM). The pH of the original skim milk was 6.7 but its corresponding milk permeate was slightly lower at pH 6.6, with the level of potassium in the milk permeate similar to that in the original skim milk. However, there was a significantly lower level of calcium (7.34 mM) and phosphorous (11.0 mM) in the milk permeate at pH 6.6, as the majority of calcium and phosphorus is bound in the casein micelle. Adjusting the pH of skim milk from 6.7 to 7.2 resulted in a reduction in the level of calcium and phosphorus in the resultant milk permeate, alternatively adjusting the pH of skim milk to more acidic values (i.e., < pH 6.6) resulted in an increase in calcium and phosphorous. Similarly, in Figure 5.2., the calcium ion concentration increased with decreasing pH for both milk permeate and skim milk, due mainly to the solubilisation of calcium phosphate in the skim milk.

Table 5.1. Mineral profile (in mmol) of skim milk powder and corresponding ultra-filtration permeates obtained from pH-manipulated skim milk, measured before and after heat treatment.

pH	Skim milk	Milk permeates								
	6.7	7.2	7.0	6.8	6.6	6.4	6.2	6.0	5.8	5.6
Sodium	14.7 ± 1.1	16.0	17.5	16.9	14.5	14.6	14.6	14.4	11.2	11.7
Magnesium	4.60 ± 0.8	2.2	2.5	2.6	2.7	2.8	2.8	3.0	2.5	3.0
Phosphorus	33.4 ± 1.4	9.4	10.1	11.3	11.0	12.1	12.9	14.5	14.7	15.8
Potassium	42.5 ± 1.0	36.6	39.0	41.9	41.5	42.5	39.9	39.8	37.4	34.7
Calcium	31.4 ± 2.7	5.9	6.4	7.8	7.4	8.4	8.5	10.6	11.3	14.3

Data presented is from a single measurement.

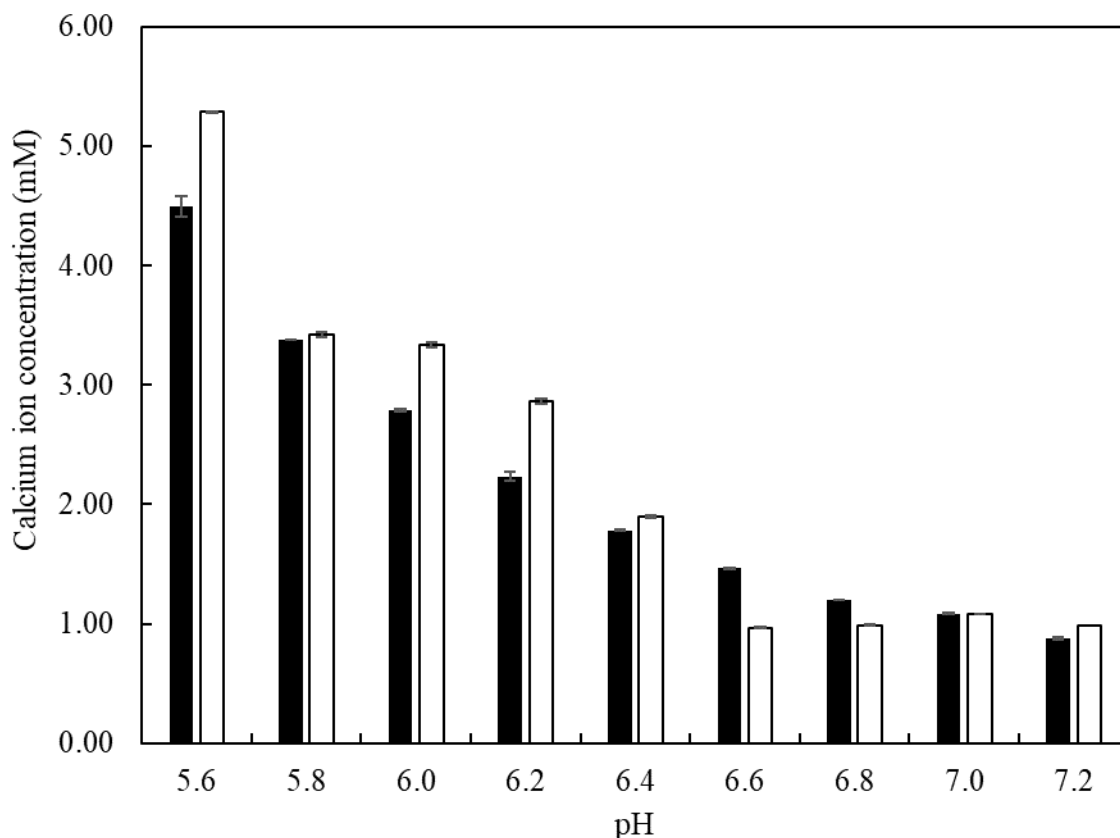


Figure 5.2. Ionic calcium concentration of skim milk (■) and corresponding milk permeates (□) as a function of pH from 5.6 to 7.2 at 25°C.

pH and heat coagulation time of skim milk and milk permeate solutions

The pH profile of skim milk and milk permeate solutions measured as a function of temperature is shown in Figure 5.3, with the changes in pH inversely related to temperature. The measurement of pH for skim milk was not possible when the pH of the skim milk was pre-adjusted to below pH 6.4, due to significant protein aggregation, with fouling on the probe preventing accurate measurement of pH in this pH range (Figure 5.3F-I). Due to the inability to measure pH in skim milk samples at pH <6.4, milk permeate solutions offered insights in to the decrease in pH at 140°C without the issue of protein aggregation. Plotting the pH of milk permeate solutions as measured at 25°C versus their corresponding pH values at 140°C is shown in Figure 5.4. For milk permeate at a pH of 7.0 (25°C), the actual pH at 140°C was 6.07, while

a pH of 5.4 at 25°C for milk permeate decreased to pH 4.65 at 140°C. The heat coagulation time (HCT) of skim milk as a function of pH is shown in Figure 5.5, with a typical type A profile observed. The HCT of the skim milk was less than 1 min at $\text{pH} \leq 6.2$ with a maximum HCT at pH 6.8 of 13.3 min. HCT decreased at pH 7.0 to 5.3 min before a subsequent increase at pH 7.2 to 10.3 min. Interestingly, the pH of milk permeate solutions measured at 140°C (Figure 5.4) appear to follow a similar profile with a reduction in pH of milk permeate obtained from skim milk at pH 7.0. The low heat stability at pH values below 6.4 is mainly due to the high concentration of calcium ions in acidified skim milk as shown in in Figure 5.2.

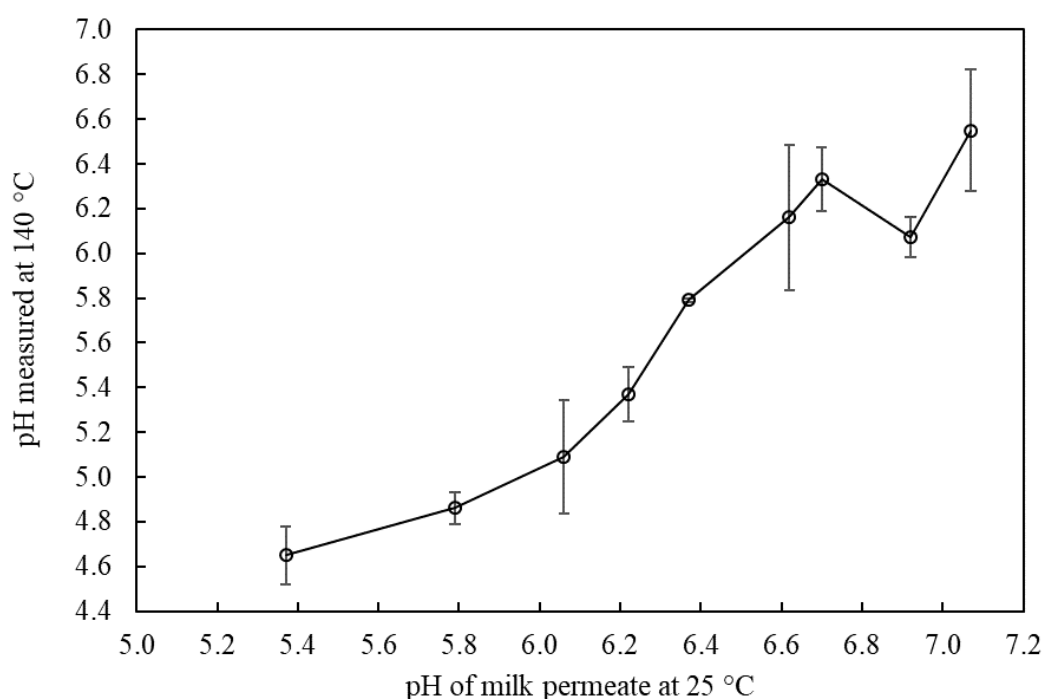


Figure 5.4. The pH of milk permeate measured at 140°C versus its corresponding pH prior to heat treatment.

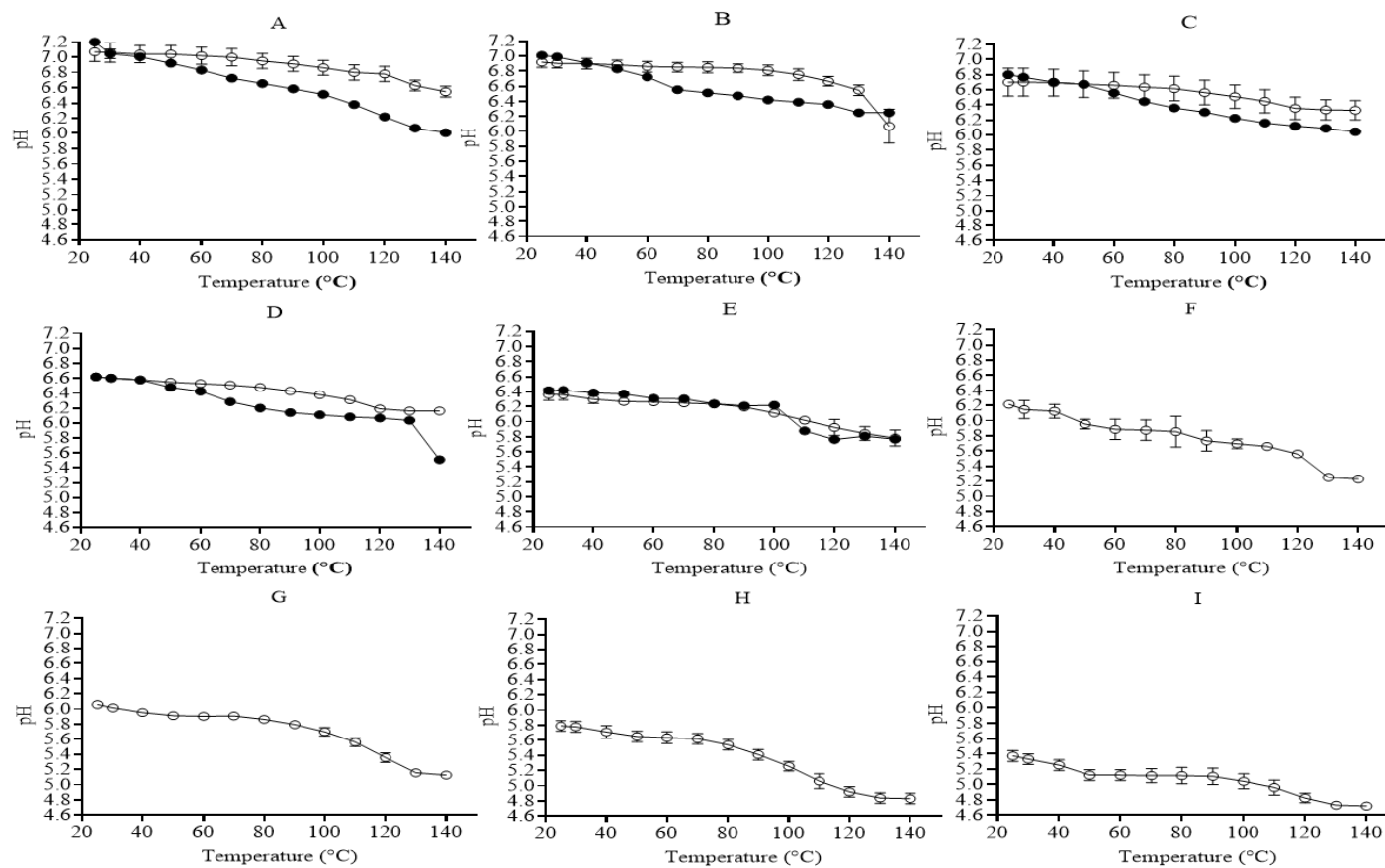


Figure 5.3. pH profile of skim milk (●) and corresponding permeate solutions (○) as a function of temperature between 25 and 140°C, with the initial pH of the skim milk adjusted to 7.2 (A), 7.0 (B), 6.8 (C), and 6.6 (D), 6.4 (E), 6.2 (F), 6.0 (G), 5.8 (H) and 5.6 (I).

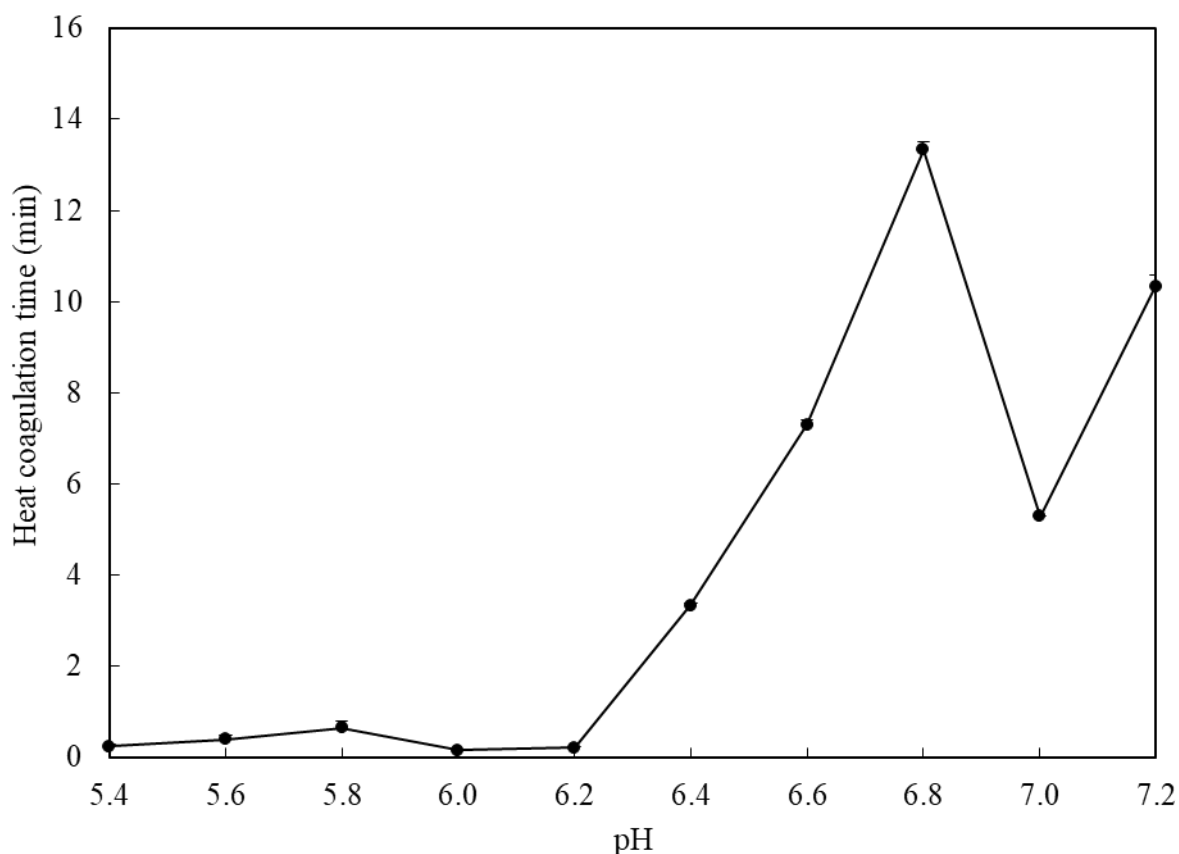


Figure 5.5. Heat coagulation time of skim milk measured across a pH range of 5.4 to 7.2 at 140 °C.

5.4.2. Pilot-scale heat treatment in a tubular heat exchanger

pH data of skim milk and milk permeate during heating

The pH profile of the skim milk measured *in-line* during continuous heat treatment is shown in Figure 5.6A. The pH of the skim milk at pH 6.7 (25°C) gradually decreased to ~ pH 6.2 at ~ 90°C, where little to no decrease in pH was observed thereafter on heating up to 140°C. The pH-temperature profile allowed for a second-order polynomial regression with a good fit ($R^2 = 0.981$). The pH-temperature profile for milk permeate (Figure 5.6B) was different from that of the skim milk, with a slight increase in pH from 6.68 at 25°C, to ~ pH 6.75 at 65°C before the pH decreased to 6.26 at 125°C and remained relatively constant thereafter. The pH-temperature profile of milk permeate could be fitted with a polynomial regression (R^2 value ~0.93).

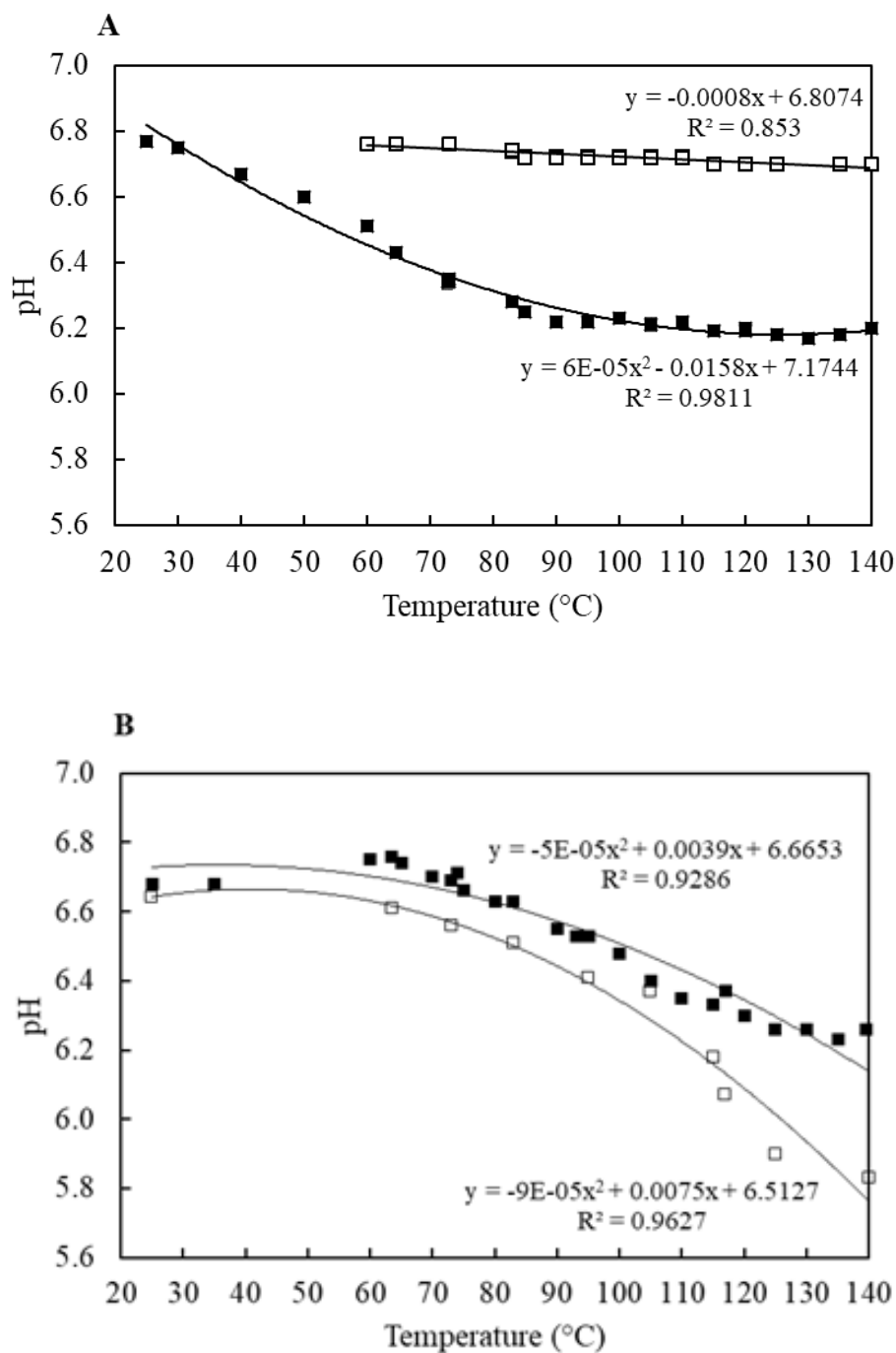


Figure 5.6. pH data of skim milk (A) and pH data of milk permeate (B) measured in-line as a function of temperature within a tubular heat exchanger (■) and pH of skim milk and milk permeate measured at 25 °C (□) after each heat treatment temperature.

The pH values of skim milk and milk permeate samples collected after heat treatment and measured at 25°C (Figure 5.6A and B) were significantly different from one another. The pH reduction observed in skim milk at 140°C was reversible upon

cooling compared to milk permeate which resulted in a further reduction in pH upon cooling (Figure 5.6B). Although, the changes in pH of milk permeate were largely reversible up to $\sim 70^{\circ}\text{C}$, at heat treatment temperatures above this the pH decreased even further upon cooling. The reversibility of pH in skim milk may be due to casein binding the newly formed calcium phosphate, as described by Nieuwenhuijse and Huppertz (2021), whereas casein-free systems such as milk permeate or whey have little ability to stabilise calcium phosphate, as previously shown by Aydogdu et al. (2021) for whey protein isolate dispersed in simulated milk ultrafiltrate. Tercinier, Ye, Anema, Singh, and Singh (2017) showed that α_s - and β -casein had a high affinity for binding hydroxyapatite, through the presence of phosphoserine residues in their primary structure. Pouliot, Boulet, and Paquin (1989a) also showed that formation of calcium phosphate during heat treatment of milk at 85°C was largely reversible and re-solubilised upon cooling of the milk to 4°C .

Mineral composition of milk permeate before and after heat treatment

The irreversible changes in pH upon heating were reflected in the soluble calcium and phosphorous levels in the milk permeate, as shown in Figure 5.7. Above 110°C , a sharp drop in the concentration of calcium and phosphorus was observed with 1.87 and 8.86 mM soluble calcium and phosphorous remaining in solution after heat treatment at 140°C . This equates to a 70% and 27% reduction in calcium and phosphorus, respectively. The sedimentable calcium: phosphorus ratio after heating at 140°C was 1.76.

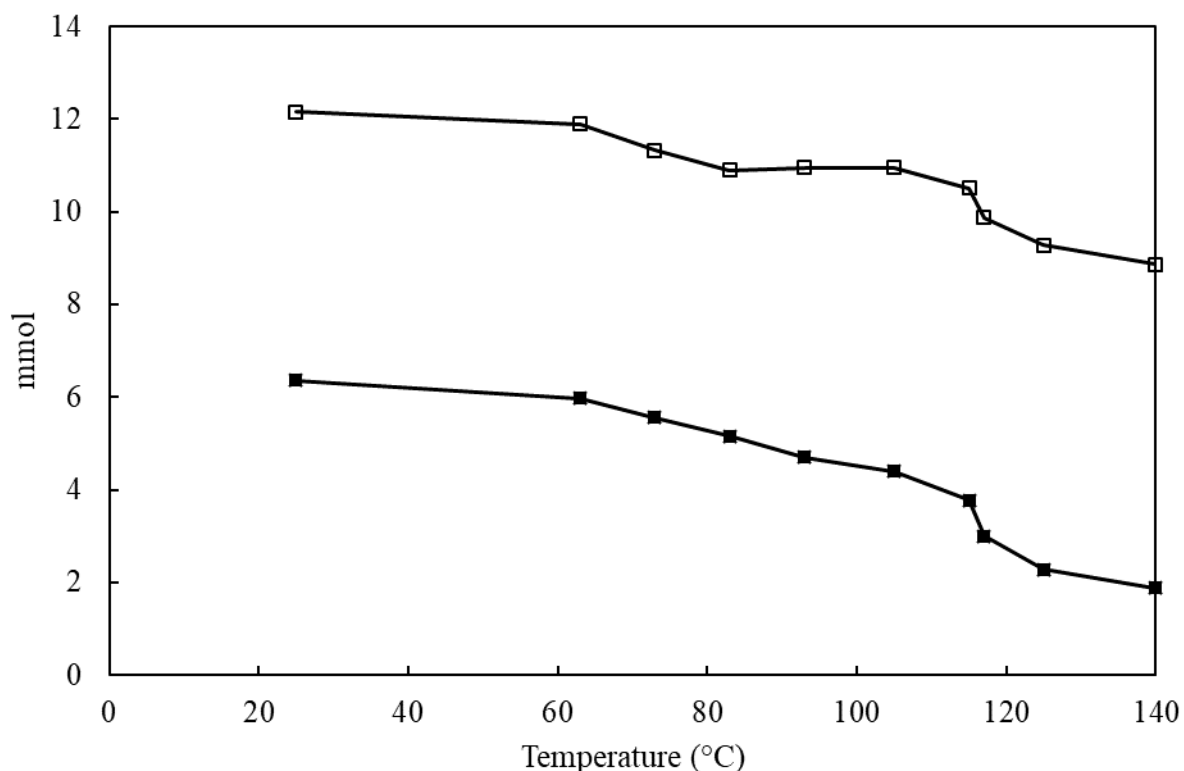


Figure 5.7. Level of soluble calcium (■) and phosphorous (□) in milk permeate (initial pH 6.8) after heat treatment at different temperatures in the OMVE tubular heat exchanger. Data presented is from a single measurement.

5.5. Discussion

Measurement of heat stability by either the subjective or objective assay are the most commonly used laboratory techniques, as described by O'Connell and Fox (2000), and as heat stability of milk is affected by pH, the most widely used and relevant means of assessing heat stability is over a pH range. However, a poorly understood dimension of milk heat stability is the extent to which pH changes during heat treatment, particularly at temperatures over 100 °C, and how this influences heat stability. The current study has shown that the decrease in pH with temperature is non-linear across the temperature range of 25 to 140°C for both skim milk and milk permeate (Figure 5.6A and B). In fact, the pH of milk permeate slightly increased when heating from 25 to ~65 °C, before decreasing thereafter as shown in Figure 5.6B. This non-linear response of pH to increasing temperature has not been reported to-

date, with the vast majority of analyses performed by measuring pH before and subsequently after heat treatment. Of the studies that measured pH at elevated temperatures, Chandrapala, McKinnon, Augustin, and Udabage (2010) found the decrease in pH with temperature to be linear when measured in reconstituted skim milk from 25 to 90 °C. Similarly, Aydogdu et al. (2022) reported that the pH-temperature profile could be fitted as a linear model in milk protein solutions when heated from 25 to 120°C. Aside from the work of Aydogdu et al. (2022), most studies have focused on measuring pH at temperatures <100°C or on samples recovered after high temperature processing. On-Nom, Grandison, and Lewis (2010) investigated the dialysis and ultrafiltration as methods for monitoring mineral partitioning and pH change at high temperatures. They reported a linear decrease in the pH and ionic calcium level as temperature increased from 20 to 110°C. However, as shown in Figure 5.6 of the present study the non-linear decrease in pH up to 140°C is reasonable given that a linear response does not take in to account the innate buffering capacity of milk.

The current study also showed using both the oil bath and tubular heat exchanger that because the pH decrease is not linear, the pH of milk remains significantly higher (Figure 5.2 and 5.6) than previously assumed. A review by Fox (1981) of the early work on heat coagulation by Rose and Tessier (1959) stated that the pH of milk serum was pH 5.9 at 110°C and that by extrapolating the data the pH at 140°C could potentially be as low as pH 5.5. Similarly, Sweetsur and White (1975) were unable to determine that there is a levelling off period in pH in both skim milk and milk permeate at temperatures above 110°C and therefore unlike when these authors extrapolated their data to give a potential pH reading of 4.9 for milk at 140°C, the pH is actually significantly higher as shown in Figure 5.6A. Indeed, a number of

previous studies have debated how skim milk can remain stable for several minutes at 140°C if the pH is actually as low as previously thought (Fox, 1981; Rose and Tessier, 1959). However, by combining data from Figure 5.2 and 3C in the present study we can assume that the skim milk was stable against heat-induced coagulation for ~ 13 min at 140 °C at an actual pH of 6.2. Adjusting skim milk to pH values below 6.2 offers little information in terms of heat stability as the pH at 140°C is potentially pH 5.0 or even lower. A study by Kaombe, Du, and Lewis (2011) proposed a question as to whether UF permeate collected at high temperature and subsequently measured at 20°C, is representative of the pH of the milk at the higher temperatures during thermal processing. From the data shown in Figure 5.6B the pH of milk permeate does not begin to decrease until temperatures above 70°C, but that once heated and cooled there is a further decrease in pH. Conversely, the increase in pH for skim milk upon cooling, which did not occur for milk permeate, indicates that the calcium phosphate remains insoluble in milk permeate after cooling but either reverses in skim milk or as previous studies have suggested the newly formed calcium phosphate is adsorbed within the casein micelle or to casein peptides (Cross, Huq, Palamara, Perich, & Reynolds, 2005; Huppertz & Nieuwenhuijse, 2022; Pouliot, Boulet, & Paquin, 1989b). One aspect arising from the present study that requires future consideration is the low pH value of milk permeate at 140°C when adjusted to pH 7.0 prior to heat treatment (Figure 5.4). The fact that this decrease correlates with the decrease in the HCT of skim milk (Figure 5.5) is not clearly understood. The decrease in HCT of skim milk in the range of pH 6.9 to 7.1 is most commonly linked with the dissociation of κ -casein from the micelle, leading to protein aggregation (Anema & Li, 2000). Whether the decrease in pH of the milk permeate is related to the type of calcium phosphate formed when the milk permeate is at pH 7.0 (Figure 5.3B) is currently unknown. It should be noted that

not only the extent of calcium phosphate precipitation, but also the type of calcium phosphate that precipitates is strongly pH dependent. This will determine the amount of liberated hydrogen ions, the amount of residual buffering salts, and hence the pH of the final solution.

Overall, though this study should provide a foundation for similar work across a multitude of applications, such as in high solid concentrates complex nutritional formulas and ingredient manufacture.

5.6. Conclusion

This study successfully designed, developed and implemented a real-time, in-line pH measurement system capable of providing data at temperatures up to 140 °C. The measurement of pH during high temperature treatment of skim milk and protein-free milk permeate has shown that the decrease in pH with increase in temperature up to 140°C is not linear. Therefore, applying a linear pH-temperature coefficient for milk systems is not sufficient to describe the pH changes at ultra-high temperatures. Heat treatment caused a release of hydrogen ions mainly due to the formation of calcium phosphate. The presence of milk proteins offered little in terms of buffering against the drop in pH; however, the presence of casein micelles meant that the heat-induced pH changes were reversible in skim milk upon cooling, compared to milk permeate where the pH was lower after cooling than that measured at 140°C. The data from this study can act as a benchmark for heat stability measurements particularly for UHT products, enabling a better understanding on the safety and quality parameters for industrial applications such as UHT processing, sterilization and cleaning-in-place procedures.

This study brings further opportunities to the area of dairy research by providing a novel tool/approach for investigating highly complex pH-mineral, protein-mineral interactions. Further studies on complex product matrices including infant formulas, follow-on formulas, and nutritional/medical feeds would benefit from the *in-line* pH measurement technique presented in this work, where the addition of minerals, vitamins and carbohydrates are common practice

5.7. Acknowledgement

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Chapter 6

The relationship between composition and heat coagulation in type A and type B skim milks

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Declaration

This chapter was co-designed, written by TA and reviewed by all co-authors. Yonas Hailu (YH) assisted with the protein quantification analysis of the skim milk. Citric acid analysis was performed by Eurofins Food Testing Ireland Ltd. TA performed all other laboratory experiments and pilot plant process.

6.1. Abstract

This study aimed to examine the differences in skim milk composition over the early lactation period in Ireland and determine its influence on heat stability. Overall, the macro-composition of the skim milks did not significantly change from early February to late April, with only a minor decrease observed in both protein and solids content. A heat coagulation Type B profile was observed for skim milk samples collected in February and March, compared to skim milk from April that showed a Type-A profile with two local maxima at pH 6.6 and 7.2. The casein to whey protein ratio did not differ significantly between any of the skim milks, maintaining a ratio of 85:15, respectively. Heat-induced disassociation of κ -casein from the micelle was similar for all milk samples with levels of dissociation increasing at higher pH values. There was a decrease in citric acid levels in skim milk samples collected over time, while milk permeate samples produced by ultrafiltration showed a decrease in calcium, phosphorous, sodium and magnesium. Overall, this study showed for the first time that there was a shift in HCT profiles from Type B to Type A in bulk skim milk samples, however, it was not possible to decisively determine the compositional factors governing these HCT profiles.

6.2. Introduction

The composition of bovine milk changes due to a multitude of factors, mainly, genetics, animal health, stage of lactation, diet, and climate (Auldist, Walsh, & Thomson, 1998). Global bovine milk production may be divided into two distinct groups; indoor feeding systems where milk production continues throughout the year, and outdoor systems, where the majority of milk production is based on the seasonal growth rate of pasture. Milk produced from indoor feeding systems is less subject to climate and external factors, while calving is spread throughout the year and therefore the effect of stage of lactation is nullified. The outdoor pasture based feeding systems, located mainly in Ireland and New Zealand, main milk production follows the pattern of pasture growth from early Spring to late Autumn/early winter (O'Brien, Mehra, Connolly, & Harrington, 1999; O'Brien, Moran, & Shalloo, 2018). The pasture based dairy system is often perceived as more environmentally sustainable with additional benefits in terms of animal welfare. The Irish dairy system is primarily based on spring-calving herds (January to April), grazed on pasture which results in a pattern of seasonal variation in milk composition that is defined by the stage of lactation (O'Brien & Guinee, 2022; Timlin et al., 2021).

Typically, milk production volumes are higher during the spring and summer months when there is an abundance of fresh grass and other forage for cows to graze. This period is often referred to as the "flush" or "peak" season. The large volumes of milk in spring and early summer means that dairy processors must produce most of their commodities and finished products over a relatively short period. The transition period from spring to summer is considered critical, as the milk composition changes sharply and this brings challenges during milk processing (Grimley, Grandison, & Lewis, 2009). Studies showed that stage of lactation is one of the main factors

controlling raw milk composition (protein, fat, lactose and mineral contents) and further nutritional value, physicochemical properties, processing efficiency and functionality of milk and milk products in seasonal systems (Hayes et al., 2023; Li, 2020; O’Callaghan et al., 2016; Timlin et al., 2021).

Considering the significance of seasonal variations on dairy processing, there is surprisingly a limited number of studies published on the effect of seasonal variations on milk processing and product functionality. The major focus on this area is based around cheese making, particularly cheese yield and physical properties (Gulati et al., 2018; Kefford, Christian, Sutherland, Mayes, & Grainger, 1995; Lucey, 1996), while a few studies have focused on butter production and quality as well as production challenges in certain dairy ingredients. Auldist, Walsh, and Thomson (1998); Chen, Lewis, and Grandison (2014) suggested that milk supply may be more suited to the manufacture of certain products at different times of the year or even on a day to day basis to utilize optimum composition for end-product functionality. Similarly, Kelly (1982) investigated the addition or removal of urea to obtain heat stable skim milk powder over a 12-month period. Urea addition improved the heat stability during mid-lactation, which initially showed poorer stability behaviour compared to other milk obtained from other months. Grandison (1988) studied the seasonal variation in deposit formation within a heat exchanger at temperatures between 110-140°C and reported that there was a complex relationship between milk composition and fouling over the season.

A possible reason for the low number of studies on the topic of seasonal variation in milk processing, is that there are only a few countries worldwide producing milk on a pasture based system, and secondly the high cost involved in examining seasonal variation on milk processing and end-product functionality might

prove prohibitive. However, one of the most common and rapid means of discriminating milk samples based on heat stability is the heat coagulation time (HCT)-temperature method, established to examine heat-induced coagulation over a pH range, usually between pH 5.8 to 7.4. Milks can either be defined as having a HCT profile of either Type A or Type B, where the former is a curved profile with a maximum HCT at approximately pH 6.7 before a decrease at pH 7.0-7.1 before a HCT increases again. This is in comparison to a Type B profile where the HCT increases as a function of increasing pH. Therefore, this study was designed to investigate the effect of milk composition and physiochemical properties on heat stability over a relatively narrow window in the early season milk pool.

6.3. Materials and methods

6.3.1. Milk collection, evaporation and spray drying

Pasteurised (73 °C for 15 s) liquid skim milk was supplied by a local dairy company over a two month period in 2022, commencing on the 17th February (sample point; SP1), 1st March (SP2), 22nd March (SP3) and 22nd April (SP4); approximately, 1800 kg of skim milk was obtained for each SP. Ultrafiltration was performed using two 10 kDa molecular weight cut-off (MWCO) spiral-wound polymeric membranes consisting of a 31 mil spacer, to generate milk permeate streams from each SP. UF was performed using a pilot scale membrane filtration plant (GEA Process Technologies, Dublin, Ireland), under continuous mode at ~8 °C, with the permeate stream collected in a separate tank, as described in detail in Chapter 5.

A sub-sample of the skim milks was evaporated at 65°C using a single-effect falling film evaporator operating in recirculation mode (Anhydro F1 Lab; Copenhagen, Denmark). Concentrates were spray dried using a single-stage spray

dryer (Anhydro Laboratory Spray Dryer, SPX Flow Technology, Anhydro F1 Lab) equipped with a two-fluid nozzle atomisation system operating in counter-flow mode at inlet and outlet temperatures of 185 and 85°C, respectively.

6.3.2. *Compositional analysis*

Nitrogen composition and ash content

Total nitrogen (TN), non-protein nitrogen (NPN) and non-casein nitrogen (NCN) content) were determined by the Kjeldahl (IDF 20–4:2016). Crude protein (CP), true protein (Guinee & O’Callaghan), casein (CN) and serum protein (SP) contents were determined from N fraction analysis using a conversion factor of 6.38.

Mineral analysis

Mineral analysis was performed using inductively coupled plasma optical emission spectroscopy (Agilent ICPOES 770 Santa Clara, US) for macro and trace elements as described by Cruijssen, Poitevin, and Brunelle (2019). As the preliminary step, samples were digested in a microwave digestion system (CEM, MARS 6, Dublin, Ireland). Liquid sample (4 mL) was mixed with HNO₃ and equilibrated for 15 min after which samples were heated to 200°C and held for 15 min at 55 bar. The calcium ion concentration of skim milks was measured at 25°C before and after heat treatment using a calcium ion meter (Sension+ MM340 benchtop meter, Hach Co., Loveland, Colorado, U.S.A) equipped with calcium selective sensor (sensION+ 9660C, 193 Hach Co., Loveland, Colorado, U.S.A.). Calibration of the sensor, and measurement of calcium ion concentration, was performed as described by Lin, Kelly, O’Mahony, and Guinee (2016). The ion-selective calcium probe was calibrated at 25°C with standard calcium solutions at 0.05, 1.00, 2.50 and 5.00 mM. A 0.1 mL volume of

potassium chloride (KCl) stock solution (3 M) was added to 10 mL of all samples which deliver an electrochemical response that is proportional only to the ion concentration and helps improve the ion selectivity of the probe. The pH of all samples was recorded using a bench top pH meter with built-in temperature sensor (Mettler Toledo) at 20°C. Citrate analysis was performed by Eurofins Food Testing Ireland Ltd (North City Business Park, Dublin, Ireland).

6.3.3. *Buffering Capacity*

Buffering capacity was measured as described by Kim, Oh, and Imm (2018) using a Titrand 842 Autotitrator with a TIAMO v.2.2 software package (Metrohm, Ireland Ltd., Carlow, Ireland). A three-point calibration of the pH probe was performed using buffer solutions at pH 4.0, 7.0 and 9.0 prior to performing measurements. Samples (50 ml) were titrated through the controlled addition of 0.5 M HCl from their initial pH to pH 2.0, (20 μ L increments with 30 s equilibration after each addition) followed by alkalization to pH 8 through the addition of 0.5 M NaOH under continuous stirring (700 rpm, 25 °C). Temperature of the sample was maintained at 25°C by water bath with an attached holding cup. The buffering index value (dB/dpH) of protein dispersions was determined using the Van Slyke equation (Van Slyke, 1922).

6.3.4. *Reverse-phase high performance liquid chromatography*

Reversed phase-high performance liquid chromatography (RP-HPLC) analysis was completed for protein quantification using a Poroshell 300SB-C18 (Size: 2.1 mm diameter x 7.5 mm, 5 mm; Agilent Technologies, Ireland) column equipped with a Zorbax poroshell guard column (Size: 2.1x12.5x5 mm; Agilent Technologies).

Skim milk (200 ml) was diluted in 7 M urea buffer containing 20 mM Bis-Tris propane and 71.5 mM 2-mercaptoethanol (pH 7.5) at a 1:20 ratio (v/v). Diluted samples were left for 1 h at 23 °C prior to filtration using a 0.2 µm syringe (Agilent Technologies, Econofiltr, PES 25µm). The HPLC gradient system detailed in McCarthy et al. (2017) was used for separating proteins and data was processed using Waters Empower® software. Protein analysis was carried out in triplicate.

6.3.5. *Heat coagulation time*

Heat coagulation time (HCT) of skim milk (9% TS) were measured at 140°C as a function of pH (6.0 to 7.2) as previously described by Davies and White (1966). Samples were pH adjusted with addition of 0.5 M NaOH or HCl solutions under constant stirring and equilibrated for 1h at room temperature prior to measurement and re-adjusted if necessary. Samples were placed in glass tubes and immersed in a pre-heated oil bath at 140 °C. HCT was monitored and recorded as the time elapsed between immersing the sample in the oil bath and visibly detectable protein aggregation (aggregates/flecks) observed.

6.3.6. *Dissociation of κ-Casein*

Assessment of the Dissociation of κ-casein from the casein micelle was performed as described previously by Anema and Klostermeyer (1997), with some modifications. Rehydrated skim milk powders (9%, w/w) were pH adjusted to 6.6, 6.9 and 7.2 using 0.5 M NaOH at 23 °C. Sub-samples of skim milk (25 mL) were then heated at 90 °C for 15 min in a temperature controlled water bath, before being cooled in ice water. Unheated and heated skim milk samples were centrifuged at 100,000 g for 60 min at 25 °C using a Sorvall Discovery 90SE ultracentrifuge (Kendro

Laboratory Products, Asheville, NC, USA). The clear serum phase was then carefully removed and frozen until further analysis. The proportion of κ -casein present in the serum phase was determined by HPLC as described in Section 2.3.

6.3.7. Casein micelle size and ζ -potential

Zeta potential measurement of liquid skim milk and retentate streams were measured using a Zetasizer-nano (Malvern Instruments, Worcestershire, UK). Skim milk samples were diluted (1:20) in their own corresponding milk permeate. Measurements were performed using a dispersant refractive index of 1.33, sample refractive index of 1.38 and viscosity of 0.89 mPa s at 25 °C.

6.4. Results

6.4.1. Composition of skim milks and milk permeates

The mean compositional data of skim milks sampled (SP1-SP4) over the early lactation period (February to April) are presented in Table 6.1. The total solid (TS) content of skim milk from SP1 was the highest at 9.36% TS, with skim milks obtained in March and April all less than 9.0% TS. O'Brien et al. (1999) and Chen et al. (2014) previously reported a reduction in solids content from winter to summer milk. Similarly, the total crude protein content of the skim milks was lower across the SP from 3.54 % in February (SP1) to 3.27% in April (SP4); however, NPN content remained constant at 0.03% (w/w) for all skim milks.

Milk permeate obtained by ultrafiltration of the skim milks (SP1-SP4) all had similar total solids content ranging from 5.21-5.51% (w/w). Interestingly, there was crude protein in the milk permeate samples, indicating that there was permeation of protein through the 10 kDa polymeric membranes, although it was present at very low

levels 0.16-0.21% (w/w). No significant difference in pH was observed between skim milks and their corresponding milk permeates and with no obvious trend found as a function of SP.

The majority of the lactation/seasonality studies carried out on pasture based milk production have reported an increase in protein content across the entire year that includes early, mid and late lactation (Auldist, Coats, Rogers, & McDowell, 1995; O'Connell, Kelly, Tobin, Ruegg, & Gleeson, 2017; Phelan, O'keeffe, Keogh, & Kelly, 1982). Li, Ye, and Singh (2019) reported that-protein content of skim milk increases from early to late lactation from 3.44 to 4.43% (w/w) for a seasonal calving herd in New Zealand.

Table 6.1. Compositional and physical properties of skim milk samples obtained from a commercial supplier on the 17th of February (SP1), 1st of March (SP2), 22nd of March (SP3) and 22nd of April (SP4) 2022, and their corresponding milk permeates.

	SP1		SP2		SP3		SP4	
	Skim milk	Milk permeate	Skim milk	Milk permeate	Skim milk	Milk permeate	Skim milk	Milk permeate
Total Solids (% (w/w))	9.36	5.48	8.97	5.28	8.50	5.21	8.91	5.51
Total protein (% (w/w))	3.54	0.21	3.35	0.19	3.29	0.18	3.27	0.16
NPN (% (w/w))	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Ash (% (w/w, dry matter basis))	8.47	9.47	8.64	9.62	8.41	9.41	8.18	9.0
Ionic Calcium (mmol)	2.89	3.22	2.90	2.87	3.66	4.37	2.91	3.13
pH	6.80	6.83	6.85	6.90	6.73	6.78	6.83	6.84
Size	206.63		183.89		186.6		200.13	
ζ -Potential (mV)	11.7		12.47		12.8		12.37	

6.4.2. Mineral analysis and buffering capacity

The mineral profile of the skim milks, displayed in Table 6.2, showed that there was no obvious trend between the skim milks across the SPs. All six major minerals were in-line with levels typical of skim milk. Milk permeate samples however did display a downward trend in calcium (864.9 down to 653.9 mg/100 g), phosphorous (908.1 down to 814.6 mg/100 g), magnesium (147.4 down to 124.9 mg/100 g) and sodium (643.7 down to 522.9 mg/100 g) over the sampling periods of SP1 to SP4. Similarly, there was a significant ($P < 0.05$) decrease in citrate levels (Figure 6.1) from skim milk taken at SP1 (11.9 mM) and SP2 (11.2 mM), compared to SP3 (9.8 mM) and SP4 (9.9 mM). The average citrate content of skim milk has previously been reported as ~10 mM, of which more than 85% is found as soluble citrate and the remaining portion is bound to micellar casein (Akkerman, Larsen, Sørensen, & Poulsen, 2019; Holt & Jenness, 1984). Ionic calcium levels in skim milk from SP1, SP2 and SP4 were all similar at ~2.9 mM with SP3 having an ionic calcium level of 3.66 mM. The higher level of ionic calcium in SP3 may be related to the slightly lower pH value in this skim milk sample.

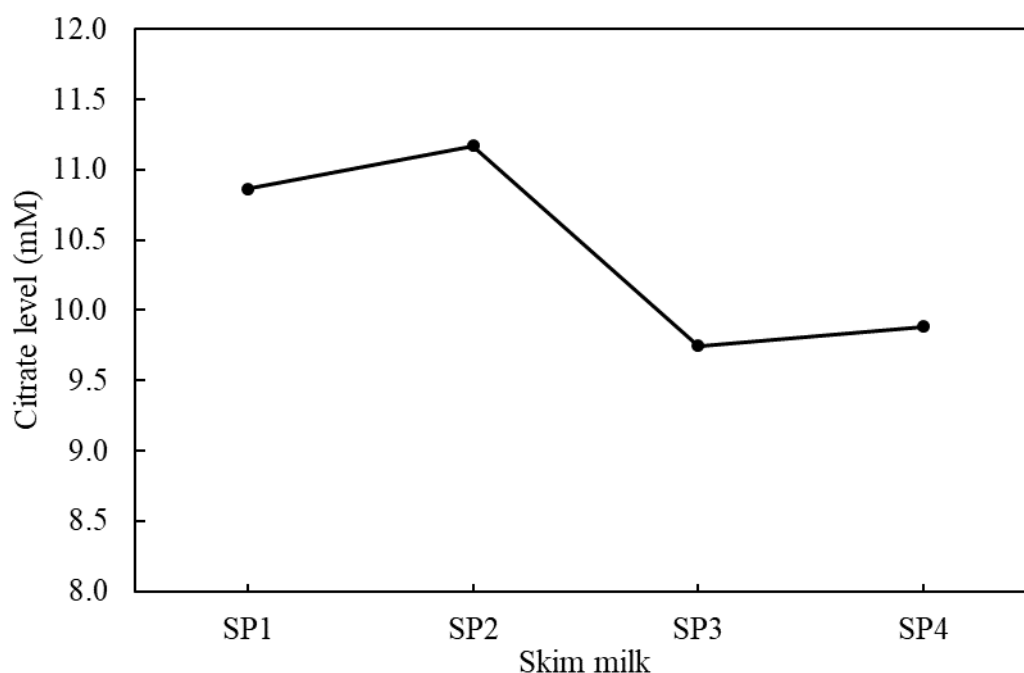


Figure 6.1. Citrate concentration of rehydrated skim milk (9.0%, w/w), where the original liquid skim milk was obtained from a commercial supplier on the 17th of February (SP1), 1st of March (SP2), 22nd of March (SP3) and 22nd of April (SP4) 2022.

The buffering capacity (BC) profile of the skim milks are shown in Figure 6.2. All skim milks followed a relatively similar trend during both the acidification and alkalisation steps, with a major buffering peak observed from pH 6 to 4 during acidification (buffering index ~ 0.03). When skim milks were titrated with an alkali solution, the main peak region was observed at pH 6 to 8 for all skim milks. It is known that BC of milk is highly dependent on the inherent levels of phosphate, citrate, lactate, carbonate, acetate cations i.e., calcium, magnesium as well as casein and whey proteins (Salaün, Mietton, & Gaucheron, 2005), milk salts being the predominant constituent contributing the BC.

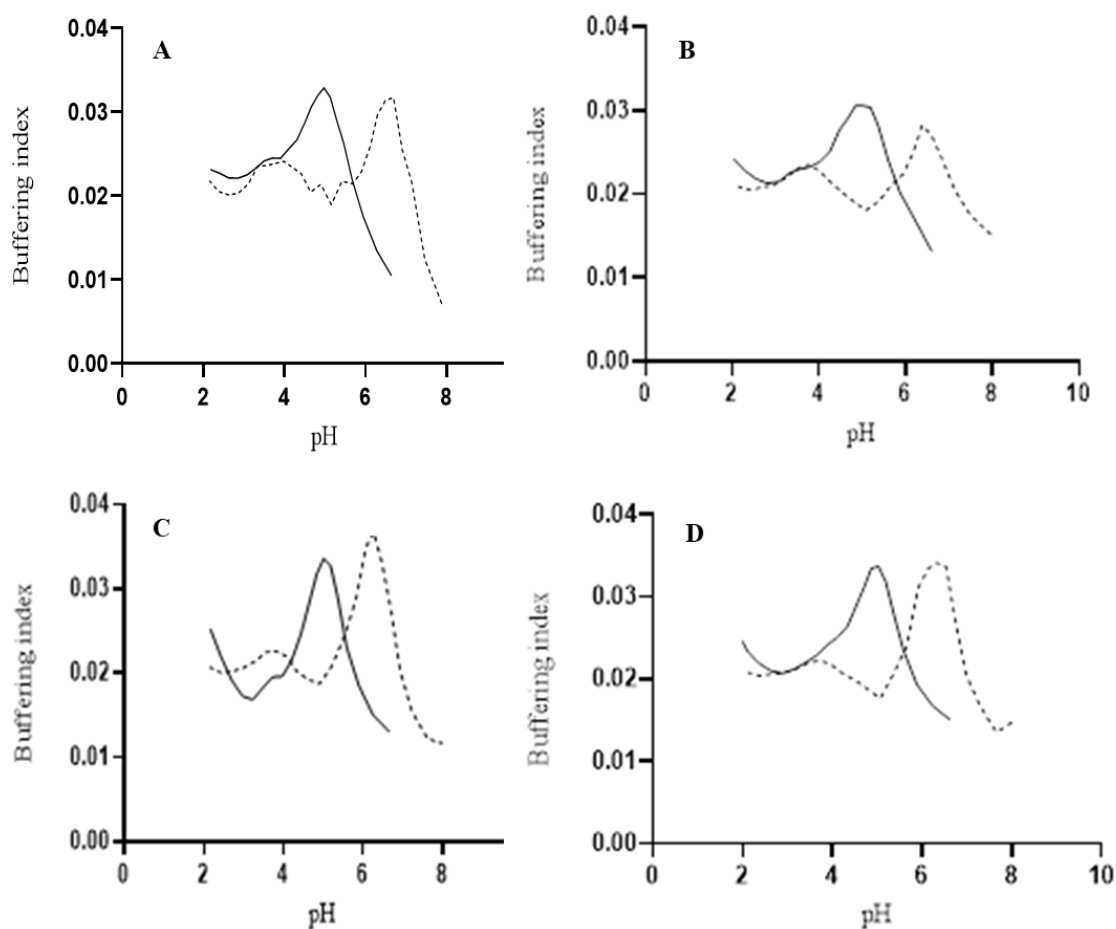


Figure 6.2. Acid-base buffering curves (measured at 25°C) of rehydrated skim milk powders (3.5% protein, w/w), where the original liquid skim milk was obtained from a commercial supplier on the 17th of February (SP1; **A**), 1st of March (SP2; **B**), 22nd of March (SP3; **C**) and 22nd of April (SP4; **D**) 2022. Solid and dotted lines represent acidification and alkalislation, respectively.

Table 6.2. Mineral analysis of skim milk samples obtained from a commercial supplier on the 17th of February (SP1), 1st of March (SP2), 22nd of March (SP3) and 22nd of April (SP4) 2022, and their corresponding milk permeates

		Ca	K	Mg	Na	P	Zn
Sample point		<i>mg/100g, dry matter</i>					
Skim milk	SP1	1538	1811	132.1	434.5	1205	5.72
	SP2	1616	1983	140.2	509.1	1280	5.9
	SP3	1518	1846	133.3	406.5	1202	5.25
	SP4	1486	1793	128.6	444.9	1208	5.16
Permeate	SP1	864.9	2631	147.4	643.7	908.1	0.35
	SP2	767.0	2513	137.1	553.8	825.4	0.25
	SP3	720.9	2619	134.7	550.0	852.9	0.17
	SP4	653.9	2512	124.0	522.9	814.6	0.18

6.4.3. Heat coagulation time, casein micelle size and ζ -potential

Heat coagulation time (HCT) profiles of skim milk samples were measured as a function of pH at 140°C (Figure 6.3). Aliquots of skim milk taken from SP1, SP2 and SP3 showed that the HCT increased in conjunction with increasing pH from pH 6.2 to 7.2, characteristic of a type B HCT profile (Dumpler, Huppertz, & Kulozik, 2020). This is in contrast to SP4 where the HCT increased from pH 6.2 to 6.6 (~ 13 min), then decreased at pH 6.8 (5.43 min) remained low at pH 7.0 before increasing again at pH 7.2 (21 min). The HCT profile of SP4 skim milk was typical of a type A profile. The type B profile does not display a local minimum or maximum heat stability; it increased with increasing pH, reaching a maximum at pH 7.2. Interestingly, there was no significant difference in the HCT profiles among the skim milks taken at SP1, SP2 and SP3. These values were relatively similar to that reported by Loveday, Weeks, Luo, and Cakebread (2021) for early season skim milk obtained from individual cows. One of the major points of interest is that at approximately the natural pH of the skim milks (~pH 6.8; Table 6.1), the early lactation milks had greater heat stability at 140 °C (9.25 to 11.2 min), than the milk taken in April at SP4 (5.43 min). Chen, Grandison, and Lewis (2014) also reported higher heat stability in autumn and winter milk compared to spring and summer milk.

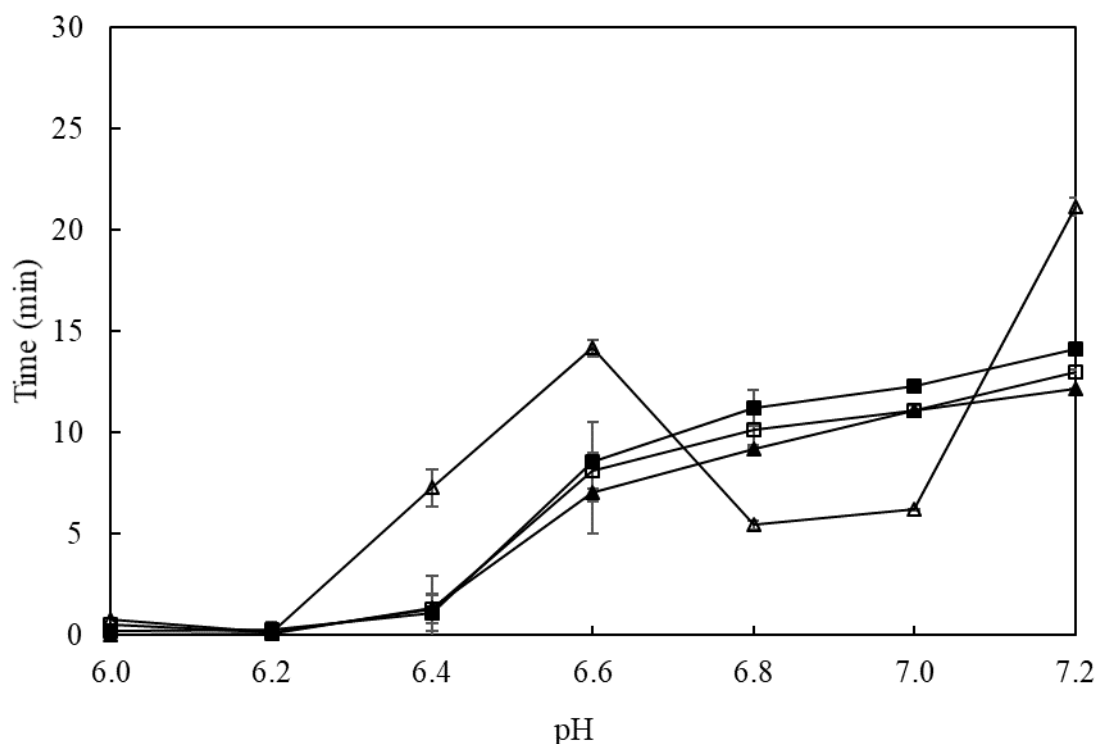


Figure 6.3. Heat coagulation time profiles of rehydrated skim milk (9.0%, w/w), where the original liquid skim milk was obtained from a commercial supplier on the 17th of February (SP1; ●), 1st of March (SP2; ■), 22nd of March (SP3; ▲) and 22nd of April (SP4; ◆) 2022, measured as a function of pH at 140 °C.

The casein micelle size did not vary significantly from SP2 to SP4 (183.9, 186.6 and 200.1 nm, respectively), however it was slightly higher for the milk collected in SP1 (206.6 nm). This was in agreement with the previous work by (de Kruif & Huppertz, 2012). Similarly, no significant difference was found between the ζ -potential of skim milks, with values ranging from -11.7 to -12.8 mV.

6.4.4. Protein profile and κ -casein dissociation

The protein profile of the skim milk samples is shown in Table 6.3, with the relative milk protein fractions all present at levels in-line with literature. However, κ -casein and α_{s2} -casein levels were slightly elevated in SP4 skim milk compared to milk

from all other SPs. The α -lactalbunin: β -lactoglobulin ratio was also slightly higher in SP4 milk compared to milks from SP1, 2 and 3. Figure 6.4 shows the level of κ -casein present in the serum phase before and after heat treatment at 90 °C. There was no major correlation between pH and κ -casein dissociation prior to heat treatment. However, after heat treatment there was a significant increase in levels of κ -casein dissociation with level of serum κ -casein present in the skim milk samples increasing drastically at pH 6.9 and 7.2. The relation between the pH and κ -casein dissociation observed in the current study is similar to the previous work by Anema and Klostermeyer (1997).

Table 6.3. Protein profile of skim milk samples obtained from a commercial supplier on the 17th of February (SP1), 1st of March (SP2), 22nd of March (SP3) and 22nd of April (SP4) 2022

Sample	κ -	α_{S2} -	α_{S1} -	β -	α -la	β -lg b	β -lg a
g / 100 g protein							
SP1	14.6	6.5	33.6	30.4	3.4	6.1	5.4
SP2	13.6	6.5	33.3	31.0	3.3	6.8	5.5
SP3	14.1	6.5	32.8	31.8	3.1	6.5	5.1
SP4	15.9	7.0	31.6	30.8	3.4	6.4	4.8

6.5. Discussion

In regions where bovine milk production is based on the availability of fresh pasture the effect of stage of lactation is a significant factor on bulk milk composition and processability. However, aside from the known seasonal fluctuations in the content of milk protein, fat and lactose throughout the year, there is very little in-depth knowledge on the topic. The continuous changes in bulk milk properties in countries of seasonal milk production can lead to issues with fouling and unpredictable end-product functionality. This study has shown that the HCT profiles of bulk skim milk obtained from a commercial supplier changes significantly over time from a type B profile in milks collected in February and March to the more common type A profile

in April. Many previous studies have shown milks with a type B profile, but this has always been observed in milks taken from individual cows. An early study by Tessier and Rose (1964) examined the difference between HCT type A and B profiles, with two thirds of the 33 individual milk samples classified as type A and the remaining as type B, while all bulk milk samples collected were of a type A profile. Furthermore, it is not unusual for individual cows to switch between a type A and type B profile. A comprehensive study on HCT profiles of individual cows by Loveday et al. (2021) showed that type A was the most dominant profile observed across the sampled cows and that a number of cows changed from one type of HCT profile to another over the yearly lactation period. However, as the majority of cows are producing type A milk, the variation in HCT profile is often diluted out in commercial bulk milk. The significance of the HCT data shown in the current study (Figure 6.3) is that a representative sample (1800 kg) was taken from a commercial supplier, indicating that a type B HCT profile dominated the milk pool over the early lactation period.

It is also important to note that the data shown in this study was based on one season and may not be representative of every year, but highlights that bulk commercial skim milk can indeed change from one HCT type to another. Milks taken from the same supplier in spring 2023 all showed type A HCT profiles (data shown in Appendix A). This data offers new insights, in that, HCT profiles may not be specific to the stage of lactation but are affected by a combination of diet, which is often dictated by weather conditions and the effect on grass growth. From a compositional viewpoint, there was a number of changes and trends that occurred over the sampling period (Tables 6.1 and 6.2). However, one obvious trend was the decreasing levels of calcium and phosphorus in the milk permeate samples, although this was not the case

for the skim milk samples themselves. This might indicate that it is the composition of the serum phase of the milks that is pivotal in determining the HCT profile.

Citrate levels in the skim milks also decreased over time (Figure 6.1) and this trend continued in to the summer months (data not shown). A study by Garnsworthy, Masson, Lock, and Mottram (2006) previously reported a significant reduction in citrate level in milks from early- to mid-lactation, decreasing from 11.3 to 9.7 mmol/L. One issue with correlating lower citrate levels in skim milk from SP4 to a type A HCT profile, is that the citrate level was also similar in skim milk from SP3 which had a type B profile. Casein micelle size and ζ -potential did not change over the early period which might have been originally thought to affect HCTs but this was also found by Li et al. (2019). The HCT minimum at pH 6.8 – 7.0 is attributed to the dissociation of κ -casein from the casein micelle, causing protein destabilization by the combined effect of high temperature and calcium induced-aggregation. The slightly higher level of κ -casein in skim milk at SP4 (Table 6.3), compared to the other skim milks might be thought to have some influence on HCT profiles. However a previous study by Tessier and Rose (1964) showed that the addition of κ -casein to skim milk, changed the HCT profile from a type A to type B and correspondingly the addition of β -lactoglobulin to skim milks changed type B profiles to type A. However, an interesting finding in the protein profile specifically is the level of κ -casein dissociation (Figure 6.4), with no difference between any of the skim milk samples after heat treatment at 90 °C. This suggests that although type B milks have similar levels of κ -casein dissociation it is not causing the same destabilization effect as it is in type A milks. Thus, there must be some other factor overriding the heat-induced protein destabilization effect and providing greater heat stability in the pH range of 6.8 – 7.0 (as shown in Figure 6.3). It is well known that the increased electrostatic repulsion at

pH 7.2 is the reason behind the high heat stability in type A and type B milks, even though there is significant κ -casein dissociation at this pH. Greater electrostatic repulsion at pH 6.8-7.0 in type B milks compared to type A might be an area to examine in future work.

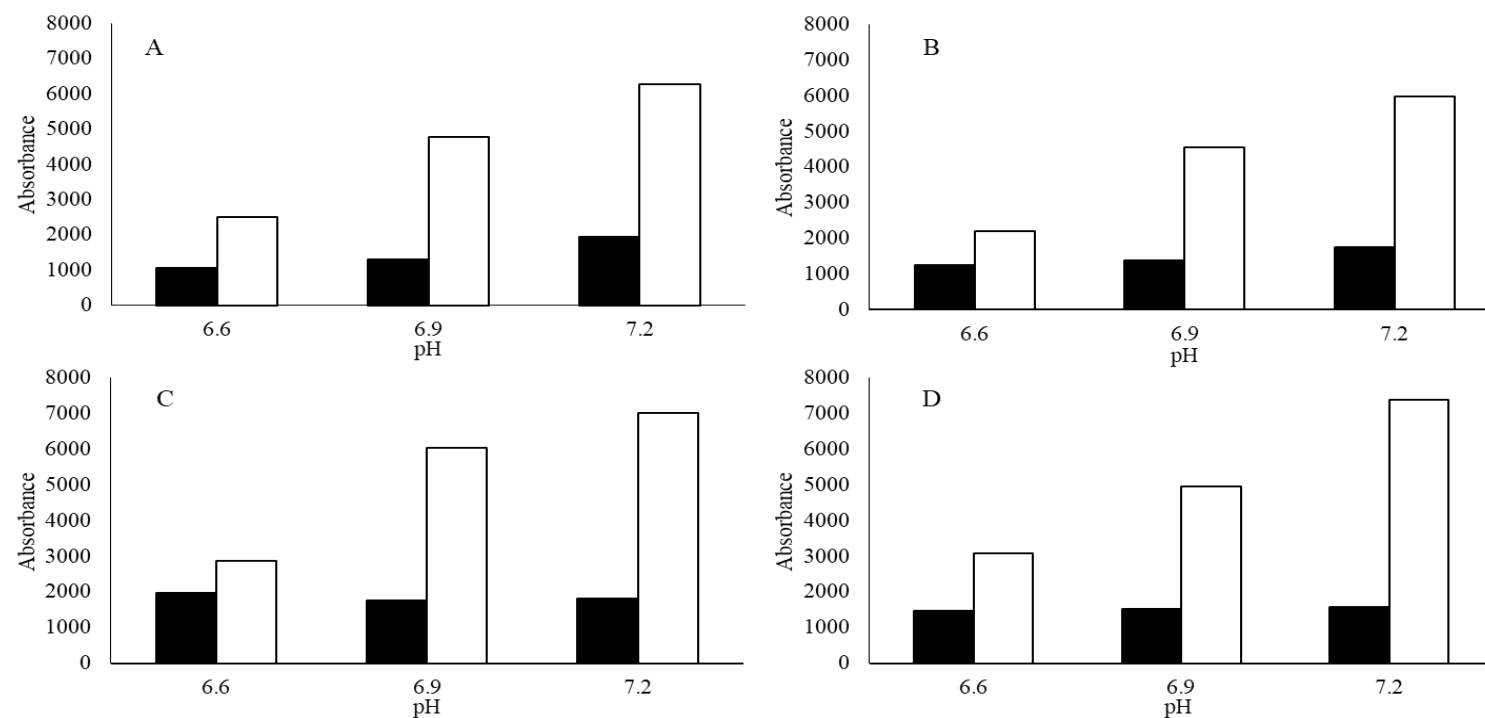


Figure 6.4. HPLC data showing levels of serum κ -casein in pH adjusted rehydrated skim milk (9.0%, w/w) before (filled bar) and after (open bar) heat treatment ($90\text{ }^{\circ}\text{C} \times 15\text{ min}$). Original liquid skim milk was obtained on the 17th of February (SP1; **A**), 1st of March (SP2; **B**), 22nd of March (SP3; **C**) and 22nd of April (SP4; **D**) 2022.

6.6. Conclusion

This study has shown for the first time that the HCT profile of commercial skim milk can change between Type B and Type A. However, the specific factor governing HCT profile was difficult to determine and it may be that there is a multitude of compositional factors defining heat stability. A reduction in citrate level, an increase in κ -casein content or a reduction in some of the major serum minerals may all play a combined role in defining HCT profiles. It is also important to remember that it is not just the HCT profile of a type A or type B curve that is of interest, but that there can be a major shift in the heat stability of skim milk at its natural pH. It is at this region on a HCT profile that is of most relevant to dairy processors and one that the industry must be cognisant of.

6.7. Acknowledgement

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Chapter 7

Membrane filtration performance of early season skim milk and subsequent heat stability properties of milk protein concentrates

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Declaration

This chapter was written by TA and reviewed by all co-authors. TA co-designed the study and performed all of the experiments. Surabhi Subhir (SS) lend assistance during the pilot scale membrane filtration. Yonas Hailu (YH) assisted with the protein quantification analysis of the skim milk.

7.1. Abstract

This study aimed to examine the differences in skim milk composition collected over the spring period (February, March and April) in Ireland and determine its influence on membrane performance and the subsequent heat stability of high protein retentates. Under steady state conditions, there was no differences in permeate flux between any of the skim milk samples obtained throughout spring period. The average permeate flux varied between 6.1 to 7.2 L/m²/h for 36 mil spacers and 6.6 to 8.5 L/m²/h for 46 mil spacer membranes. Ultrafiltration retentate samples contained ~9.0% total solids and 3.5% protein. There were no major differences in mineral profile between retentates generated from skim milk across the spring period. This was also the case for milk permeate samples. The HCT data for all retentate samples was of a type B profile, with heat stability increasing with increasing pH. However, addition of milk permeate generated from skim milk in April combined with the retentate stream produced in February converted the HCT profile from a type B to a type A profile, indicating the importance of the milk serum phase in defining the HCT profile.

7.2. Introduction

In this, the concluding experimental chapter of the thesis, the relationship between milk protein and milk mineral chemistry and its influence on heat stability was assessed, taking in to consideration the learnings from Chapters 3, 4, 5 and 6. The membrane filtration of skim milk to produce milk protein concentrate and milk permeate offers two benefits, one the opportunity to assess how early season skim milk composition might affect membrane performance and secondly allowing for the manipulation of serum minerals by swapping milk permeate from April with milk protein concentrate from February. Previously, chapter 6 has shown that the heat coagulation time (HCT) profile of skim milks obtained in February and March were of a type B profile compared to milk obtained in April displaying a type A profile. Aside from the milk composition effect on membrane performance it was decided to also assess how spiral-wound polymeric membranes with the same molecular weight cut-off but with different spacer sizes would influence permeation flux. Spacers, situated between the porous membrane allow for the feed material to pass along the length of the membrane (Abdul Latif, Lau, Low, & Azeem, 2021). For viscous feeds, a larger spacer size is often used to accommodate the flow of liquid in to the membrane but this concomitantly reduces the membrane surface area, and so may negatively affect flux performance. Although, there are a number of critical factors governing ultrafiltration performance, mainly membrane type, pore size, feed composition, temperature of filtration, volume concentration factor and transmembrane pressure, all of which affect permeation rate and particle diffusivity (Ng, Haribabu, Harvie, Dunstan, & Martin, 2017). It is the composition of the skim milk during milk protein concentrate (MPC) production that is most variable, effecting fouling (particles caught

in the membrane pores) and concentration polarization (accumulation of retained particles on the membrane surface).

Therefore, as skim milk composition is affected by stage of lactation, and with Irish milk production predominantly based on spring-calving herds (Timlin et al., 2021), the transition period across January to April is considered critical for milk processing, particularly around heat treatment and ultrafiltration performance (Grimley, Grandison, & Lewis, 2009). Fouling can negatively affect membrane performance due to the accumulation of material such as protein, inorganic salts and colloidal particles which reduce the permeate flow rate, can change the retention characteristics of the membrane and can reduce its lifespan (Grandison, Youravong, & Lewis, 2000). However, membrane fouling is usually associated as a negative factor that hinders efficiency, but it can be used to alter the selectivity of the module by aiding in the retention of desired components. To get this information it is vital to carry out membrane filtration under continuous steady state conditions to identify relevant flux performance.

The objectives of the study were to investigate if seasonal variations in skim milk might influence ultrafiltration performance and secondly to investigate the effect of the milk serum phase on HCT profiles of milk protein concentrates.

7.3. Materials and methods

7.3.1. Materials

A local dairy company supplied pasteurised liquid skim milk ($73\text{ }^{\circ}\text{C} \times 15\text{ s}$) over a three-month period from February to April 2022, representing early season milk in the Irish production system. Four 10 kDa molecular weight cut off (MWCO) spiral-wound polymeric membranes were supplied by Synder Filtration (SynderFiltration,

Vacaville, CA, USA), two membranes were configured with 31 mil (~ 0.79 mm) spacer and the other two consisted of 46 mil (~ 1.17 mm) spacer. All the chemicals used were analytical grade and supplied by Sigma Aldrich (Arklow, Ireland) unless otherwise stated.

7.3.2. Ultrafiltration of skim milk

A total of four independent membrane trials were performed, commencing on the 17 and 18th of February (sample point; SP1), 1st and 2nd of March (SP2), 22nd and 23rd of March (SP3) and on the 22nd and 23rd of April (SP4). Approximately, 2000 kg of pasteurized skim milk was obtained for each SP, with each SP divided into two 1000 kg batches. The first batch was processed using two polymeric membranes consisting of a 31 mil spacer (~ 0.79 mm) and the second batch processed using two membranes consisting of a 46 mil spacer (~ 1.17 mm), as shown in Appendix 7A (Figure A7.1). The total surface area for two 31 and two 46 mil spacer membranes was 13.4 and 10.8 m², respectively. Filtration was performed using a pilot-scale membrane plant (GEA Process Technologies, Dublin, Ireland). Prior to filtration, the plant was cleaned according to the standard cleaning-in-place procedure (CIP), as previously described by Subhir, McSweeney, Fenelon, Magan, and Tobin (2022). Filtration was performed under continuous feed-and-bleed mode at ~ 8 °C, with the permeate and retentate streams collected in separate tanks. A volume concentration factor (VCF) of 3 was maintained throughout filtration, with a transmembrane pressure (TMP) of 2 bar applied. The mass of all streams was recorded including the dead/residual volume of the membrane plant, which was collected as a final flush post processing to minimise solids loss, ensuring an accurate mass balance. All processing data was recorded from the start-up of the plant to final shutdown (i.e., flow rates,

cross-flow velocities, pressures, temperature and energy consumption; Table 7.1), using a digital data logger (Endress + Hauser AG, Reinach, Switzerland) and collated for assessing membrane filtration performance. The skim milk feed was sampled prior to ultrafiltration, while retentate and permeate streams were sampled hourly for further compositional analysis.

The total mass of the starting skim milk, permeate and retentate streams were recorded. The recovery (%) of total solids (TS) and total nitrogen (TN) of each stream were calculated (equation 7.1 and 7.2) to determine the partitioning dynamics and efficiency of ultrafiltration and results are shown in Appendix 7B; Table A7.1.

$$\%TS \text{ recovery} = \frac{(\text{kg of TS in permeate}) + (\text{kg of TS in retentate})}{(\text{kg of TS in skim milk})} \times 100 \quad (7.1)$$

$$\%TN \text{ recovery} = \frac{(\text{kg of TP in permeate}) + (\text{kg of TP in retentate})}{(\text{kg of TP in skim milk})} \times 100 \quad (7.2)$$

7.3.3. *Evaporation and spray drying*

A sub-sample of the starting skim milk and retentate samples were evaporated at 65°C using a single-effect falling film evaporator operating in recirculation mode (Anhydro F1 Lab; Copenhagen, Denmark). Concentrates were spray dried using a single-stage spray drier (Anhydro Laboratory Spray Dryer, SPX Flow Technology, Anhydro F1 Lab) equipped with a two-fluid nozzle atomisation system operating in counter-flow mode at inlet and outlet temperatures of 185 and 85 °C, respectively.

7.3.4. *Compositional analysis*

Total nitrogen (TN) and non-protein nitrogen (NPN) were determined by the Kjeldahl method (IDF 20-4: 2016). Calculation of crude protein (CP), true protein

(Guinee & O'Callaghan), casein (CN) and serum protein (SP) contents were determined from N fraction analysis using a nitrogen to protein conversion factor of 6.38. Total crude protein (CP) calculated by multiplying the protein conversion factor (6.38) by TN content.

Mineral analysis was carried out using inductively coupled plasma optical emission spectroscopy (Agilent ICPOES 770 Santa Clara, US) for macro and trace elements as described by Cruijssen, Poitevin, and Brunelle (2019). As the preliminary step, samples were digested in a microwave digestion system (CEM, MARS 6, Dublin, Ireland). Liquid sample (4 mL) was mixed with HNO₃ and equilibrated for 15 min after which samples were heated to 200°C, and held for 15 min at 55 bar. The calcium ion concentration of protein dispersions was measured at 25°C before and after heat treatment using a calcium ion meter (Sension+ MM340 benchtop meter, Hach Co., Loveland, Colorado, U.S.A) equipped with calcium selective sensor (sensION+ 9660C, 193 Hach Co., Loveland, Colorado, U.S.A.). Calibration of the sensor, and measurements of calcium ion concentration, was performed as described by Lin, Kelly, O'Mahony, and Guinee (2016). The calcium ion-selective probe was calibrated at 25°C with standard calcium solutions at 0.05, 1.00, 2.50 and 5.00 mM. A 0.1 mL volume of potassium chloride (KCl) stock solution (3 M) was added to 10 mL of all samples, giving an added KCl concentration of 29.7 mM. KCl was added to deliver an electrochemical response that is proportional only to the ion concentration and helps improve the ion selectivity of the probe. The pH of all samples was recorded using a bench top pH meter with built-in temperature sensor (Mettler Toledo SevenCompact S220, Hamilton, New Zealand) at 20°C.

7.3.5. Viscosity measurement

Viscosity of the liquid skim milk and composite retentate samples were measured using an AR-G2 controlled-stress rheometer (TA Instruments, Crawley, UK), equipped with a parallel plate geometry. Measurements were conducted at 8°C to investigate the rheological behaviour of skim and retentate stream at ultrafiltration process temperature. Samples were pre-sheared at a shear rate of 200 s⁻¹ for 30 s, followed by a shear rate ramp from 0.1 to 300 s⁻¹ over 5 min with the temperature controlled using a Peltier system (±0.1 °C). All analysis were performed in duplicates.

7.3.6. Heat coagulation time

The heat coagulation time (HCT) of skim milks (same as chapter 6) and milk protein concentrates (3.5% protein, w/w) was measured at 140°C as a function of pH ranging from 6.0 to 7.2, as previously described by Davies and White (1966). Samples were pH adjusted with addition of 0.5 M NaOH or HCl solutions under constant stirring and equilibrated for 1 h at room temperature prior to measurement and re-adjusted if necessary. Samples were placed in glass tubes and immersed in a pre-heated oil bath at 140 °C. HCT was monitored and recorded as the time elapsed between immersing the sample in the oil bath and visibly detectable protein aggregation (aggregates/flecks).

To evaluate the effect of the serum phase on the HCT profile, a recombined milk was formed using ultrafiltration retentate from SP1 and milk permeate from either SP1 or SP4. The milk permeate was added to the retentate to reduce the solids and protein concentration back to levels similar to skim milk (i.e., 9.4% TS and 3.5% protein; as shown in Table 7.2). The recombined systems were allowed to stir for 2 h and left overnight to equilibrate before HCT anal was performed as described above.

7.3.7. Statistical analysis

The results are the average of at least duplicate measurements and are reported as the mean value $\pm$ standard deviation.

7.4. Results and Discussion

7.4.1. Membrane performance

Membrane filtration performance was monitored by measuring the changes in permeate flux over a 5 h period at $\sim 8-9^{\circ}\text{C}$ (Figure 7.1). During the initial change from water to skim milk, the flux was $\sim 35 \text{ L/m}^2/\text{h}$ and then decreased over the first 100 min of filtration before eventually reaching steady state (Figure 7.1). The initial decrease in permeate flux is caused by the initial viscosity increase in relation to water and subsequent fouling due to milk components, mainly that caused by the presence of micellar casein, whey proteins and residual fat within the skim milk (Ng et al., 2017). The effect of mineral precipitation on the membrane was most probably negligible due to the relatively low temperature of filtration (Puri, Singh, & A. O'Mahony, 2020).

Table 7.1. Operational parameters during ultra-filtration of skim milk.

Parameters	31 mil spacer				46 mil spacer			
	SP1	SP2	SP3	SP4	SP1	SP2	SP3	SP4
Recirculation flow rate (L/h)	4242	4472	4677	4580	4712	5159	5296	5079
Retentate flow rate (L/h)	42.1	45.7	48.9	45.5	41.0	48.1	48.8	44.6
Permeate flow rate (L/h)	81.9	88.3	96.5	89.1	79.1	94.5	94.3	87.7
Permeate flux (L/m ² /h)	6.1	6.6	7.2	6.7	7.3	8.8	8.8	8.1
Processing temperature (°C)	8.4	8.9	9.3	9.1	7.9	8.8	9.1	9.1
Energy parameters								
Feed Pump (kW h)	0.2	0.2	0.1	0.2	0.1	0.1	0.1	0.1
Recirculation Pump (kW h)	1.0	1.0	1.1	1.1	1.0	1.0	1.1	1.1
Energy consumption* (kW h/L)	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01

*per unit of permeate

The average permeate flux, under steady state continuous conditions, over a time period of 200-300 min were 6.1, 6.6, 7.2 and 6.7 L/m²/h for skim milk obtained from SP1, 2, 3, and 4, respectively, when 31 mil spacer membranes were used (Figure 7.1A). The average permeate flux for the 46 mil spacer membranes between 200-300 min run-time were slightly higher than the 36 mil spacer membranes, with 6.6, 8.5, 7.8 and 7.0 L/m²/h for SP1, 2, 3 and 4, respectively (Figure 7.1B). This was given that the permeate flow rates (Table 7.1) were similar for 31 mil (surface area 13.4 m²) and 46 mil spacer membranes but that 46 mil membranes have significantly lower membrane surface area (10.8 m²). The larger spacer size also led to a significantly ($P < 0.05$) higher cross-flow velocity, which may benefit a reduction in foulant build-up, or reduce the effect of concentration polarization on the surface of the membrane

(Table 7.1). Over time, there is a build-up of particles retained at the membrane surface, which influences the selectivity of the membrane. This means that at the membrane surface the solute concentration is actually greater than the feed solution, but under steady state conditions, as shown in Figure 7.1, the flow of solutes to the membrane surface is equal to the solute that permeates through the membrane. Interestingly, there was no significant ($P > 0.05$) difference in permeate flux in relation to the skim milks obtained across the spring period. Although, this might have been noticed had the protein content been increased further, but this would have required the incorporation of diafiltration water, changing the composition of the feed and the subsequent flux performance.

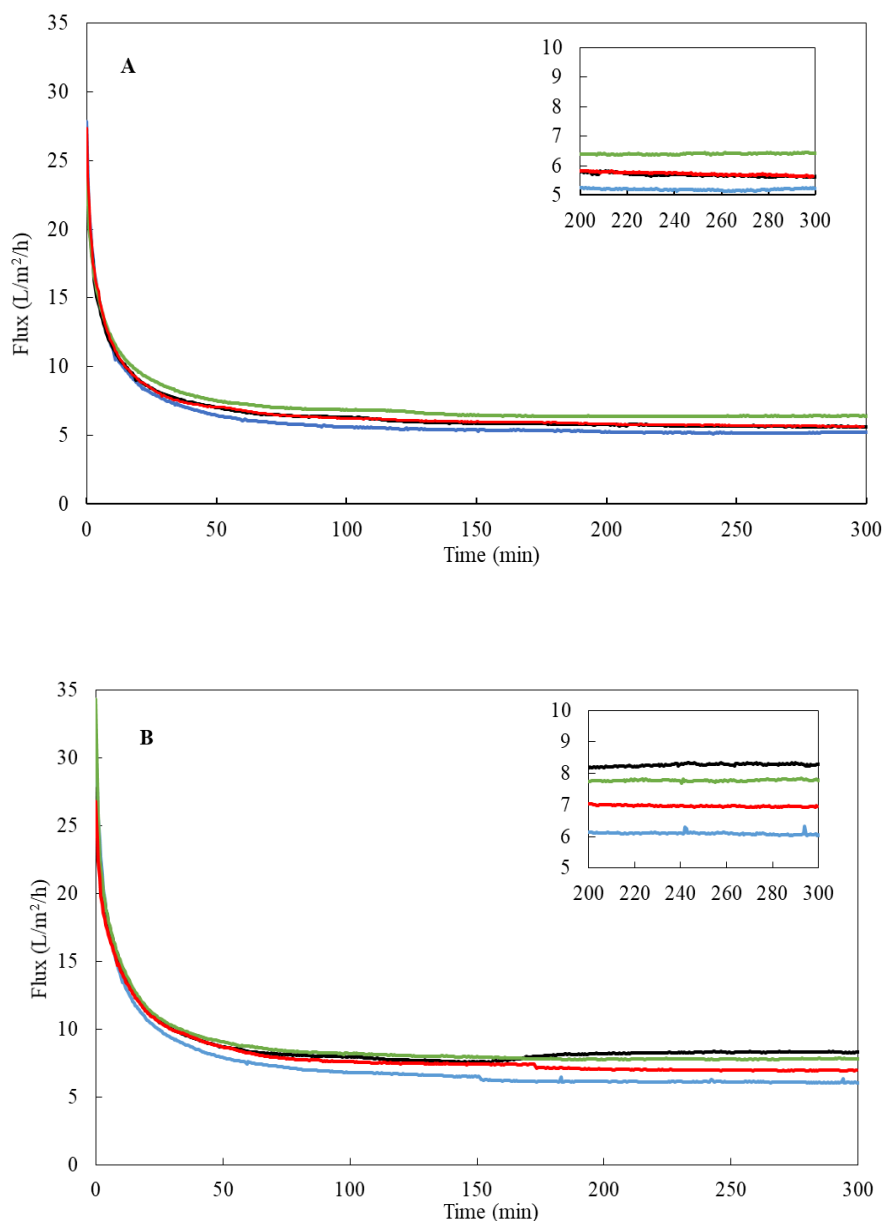


Figure 7.1. Permeate flux (L/m²/h) measured as a function of time (min) during ultrafiltration of skim milk from SP1 (—), SP2 (—), SP3 (—) and SP4 (—) using membranes with a 31 mil spacer (A) and 46 mil spacer (B).

7.4.2. Composition

Compositional data of the skim milks and their corresponding permeate and retentate fractions are shown Table 7.2. The total solids content (TS) of skim milk from SP1 was the highest at 9.36% which then decreased to <9.0% TS in SP2, 3 and 4. For the most part, previous data has shown the trend in solids content for whole

milk across a seasonal period. This is often not reflective of skim milk as a depression in fat content can occur between early spring and early summer period, while in general the fat content increases throughout the year (as discussed in chapter 1). Previously, O'Brien, Mehra, Connolly, and Harrington (1999) showed that the TS of whole milk increased from January to April. The solids content of the permeate and retentate streams for all sample points were $\sim 5.5\% \pm 0.5$ and $14\% \pm 1.0$, respectively. The protein content shown in Table 7.2 was slightly higher in milk from SP1 and 2 at 3.5% (w/w) compared to SP3 and 4 at 3.3% (w/w), while the protein content in the ultrafiltration retentate samples were $\sim 8.5\% \pm 0.5$ (w/w). Interestingly, all milk permeate streams contained nitrogen but once divided by the nitrogen to protein conversion factor of 6.38 this equate to similar values for NPN (0.03%, w/w), indicating that during filtration there was no significant loss of protein from the retentate side of the membrane to the permeate side but only the permeation of NPN. The ash content of the milks at 0.7-0.8% were similar to those previously reported by O'Brien et al. (1999), while no major differences in the pH values were observed for all skim milks, retentate streams and permeates (pH 6.7 to 6.9). Previous studies have reported that the pH of autumn milk to have a lower pH compared to milk in spring and summer (Chen, Lewis, & Grandison, 2014; Timlin et al., 2021). The ionic calcium content of skim milk did not significantly change from SP1, 2 and 4 SP4 (2.91 mmol), although skim milk from SP3 was higher at 3.66 mmol. Higher levels of ionic calcium shift the mineral equilibrium towards calcium phosphate production, which increases the level of H^+ and concomitantly reduces the pH. Interestingly, skim milk collected during SP3 had the highest ionic calcium level with 3.66 mmol and lowest pH value of 6.73, contrary to skim milk collected during trial-1 with lowest ionic calcium level of 2.69 mmol and highest pH value of 6.80.

The mineral content of the skim milk, ultrafiltration retentate and permeate samples are shown in Table 7.3, with no major differences between the retentate samples and likewise amongst the permeate samples when compared across the sampling points. The high level of calcium and phosphorous associated with the retentate streams is in-line with its association with the casein micelle, compared to monovalent ions such as sodium and potassium which readily permeated the membrane. Aydogdu, Ho, Ahrné, O'Mahony, and McCarthy (2021) showed the distribution of these minerals between micellar casein bound minerals and those in the serum phase (Chapter 3). Had diafiltration water been added in the current study, then the level of these salts would be even lower in the retentate streams.

Table 7.2. Compositional data of skim milk obtained at sample points (SP) 1, 2, 3 and 4 and subsequent ultrafiltration permeate and retentate samples generated using membranes with either 31 or 46 mil spacer configuration.

	SP1					SP2					SP3					SP4				
	Skim Milk	Permeate		Retentate		Skim Milk	Permeate		Retentate		Skim Milk	Permeate		Retentate		Skim Milk	Permeate		Retentate	
		36	41	36	41		36	41	36	41		36	41	36			36	41	36	41
		mil	mil	mil	mil		mil	mil	mil	mil		mil	mil	mil	41 mil		mil	mil	mil	mil
TS (% (w/w))	9.4	5.5	5.5	14.9	13.7	8.97	5.3	5.5	13.9	13.7	8.5	5.2	5.4	13.8	13.3	8.5	5.5	5.2	14.5	13.6
TP (% (w/w))	3.5	0.2	0.2	8.7	8.3	3.5	0.2	0.2	8.2	8.2	3.3	0.2	0.2	8.1	8.9	3.3	0.2	0.2	8.6	8.6
NPN (% (w/w))	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Ash (% (w/w))	0.8	0.3	0.6	1.2	1.1	0.8	0.2	0.5	1.2	1.1	0.7	0.4	0.5	1.1	1.1	0.8	0.4	0.4	1.1	1.1
Ionic calcium (mmol)	2.9	3.2	3.1	3.5	3.1	2.9	2.9	2.8	3.0	2.9	3.7	4.4	3.6	4.4	3.4	2.9	3.1	3.0	2.4	2.1
pH	6.8	6.8	6.9	6.9	6.8	6.9	6.9	6.9	6.8	6.8	6.7	6.9	6.8	6.8	6.7	6.8	6.8	6.8	6.8	6.8

TS – total solids

TP – total protein (nitrogen to protein conversion factor used was 6.38)

NPN – non-protein nitrogen

Table 7.3. Mineral composition of skim milk and ultrafiltration retentate and permeate samples from SP1 to SP4.

	<u>Ca</u>	<u>Cu</u>	<u>Fe</u>	<u>K</u>	<u>Mg</u>	<u>Mn</u>	<u>Na</u>	<u>P</u>	<u>Zn</u>
	mg/100g, dry matter								
Skim milk									
SP1	1537.5	0.1	0.2	1810.7	132.1	0.03	434.5	1204.9	5.7
SP2	1615.9	0.1	0.3	1983.3	140.2	0.03	509.1	1280.3	5.9
SP3	1517.8	0.1	0.3	1846.0	133.3	0.04	406.5	1202.4	5.3
SP4	1485.5	0.1	0.3	1793.4	128.6	0.04	444.9	1208.5	5.2
Retentate									
R1-31 mil	1853.9	0.1	0.4	1174.8	119.7	0.04	275.0	1305.7	8.6
R2-31 mil	1740.9	0.2	0.4	1190.3	113.6	0.04	263.8	1235.7	7.6
R3-31 mil	1718.5	0.1	1.1	1056.3	112.8	0.05	250.1	1191.5	7.4
R4-31 mil	1920.9	0.1	0.5	1174.7	123.8	0.05	264.6	1369.7	7.9
R1-46 mil	1812.4	0.2	0.5	1064.8	114.0	0.04	257.6	1260.9	8.9
R2-46 mil	1798.8	0.1	0.4	1105.9	113.3	0.05	248.9	1260.2	8.0
R3-46 mil	1769.7	0.1	0.4	1105.5	116.3	0.06	263.0	1229.0	7.5
R4-46 mil	2208.2	0.2	1.2	1334.4	141.9	0.07	291.6	1567.3	9.4
Permeate									
P1-31 mil	864.9	LOD	0.5	2631.2	147.4	0.02	643.7	908.1	0.4
P2-31 mil	766.9	LOD	0.6	2512.9	137.1	0.02	553.8	825.4	0.3
P3-31 mil	720.9	LOD	0.04	2618.9	134.7	0.02	550.0	852.9	0.2
P4-31 mil	653.9	LOD	0.1	2512.2	124.0	LOD	522.9	814.6	0.2
P1-46 mil	792.9	0.02	0.3	2434.8	136.7	LOD	580.6	838.6	0.6
P2-46 mil	702.9	LOD	0.1	2365.2	126.5	LOD	534.6	795.4	0.5
P3-46 mil	656.7	LOD	0.2	2417.4	122.6	LOD	538.7	773.6	0.4
P4-46 mil	709.6	LOD	0.04	2721.7	133.9	LOD	563.0	884.6	0.5
LOD, limit of detection									

7.4.4. Viscosity of skim milks and ultrafiltration retentates

Viscosity profiles measured as a function of shear rate at 8°C are shown in Figure 7.2. There were no differences in the average skim milk viscosity across the shear rate ramp from 0.1 to 300 s⁻¹, ranging from 2.7 to 3.1 mPa.s at a shear rate of 300 s⁻¹. Similarly there were no significant differences between the retentate samples with viscosity ranging from 6.0 to 7.5 mPa.s. The viscosity of the retentate samples at 8°C was relatively low and well below a critical viscosity that might cause membrane blocking. This is given that the protein content of the samples (Table 7.2) was ~8.5% protein and ~14% solids. Puri et al. (2020) previously reported significantly higher viscosity values for ultrafiltration retentate samples measured at 5°C, than those reported in the current study. Although the protein content was also higher at ~12.1% (w/w) and with the measurement temperature slightly lower at 5°C. Shu-Sen (1988) studied the effect of viscosity on ultrafiltration flux and showed that the relationship between flux is inversely proportional to feed viscosity, although it is not exactly a linear relationship.

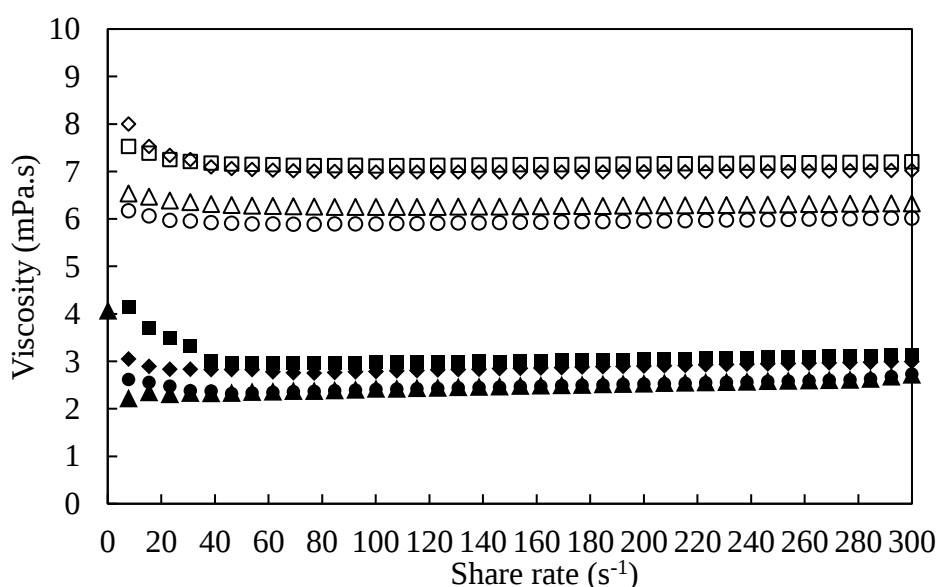


Figure 7.2. Viscosity measured as a function of shear rate at 8 °C for skim milk samples obtained at sample point (SP) 1 (●), SP2 (▲), SP3 (◆) and SP4 (■) and their corresponding retentate streams from SP1 (○), SP2 (△), SP3 (◇) and SP4 (□).

7.4.5. Heat coagulation time of skim milks and ultrafiltration retentates

Heat coagulation time (HCT) profiles of reconstituted skim milk and milk protein concentrate (MPC) powders (rehydrated at 3.5% protein, w/w) are shown in Figure 7.3. As shown in chapter 6 and discussed therein, skim milk obtained from SP1, 2 and 3 showed a continuous increase in heat stability with increasing pH, typical of a type B profile. However, skim milk from SP4 showed a typical type-A profile, with two local maxima at pH 6.6 and 7.2 with a HCT of 13 and 21 min, respectively (Note: HCT profiles of skim milk are the same data shown in chapter 6 and discussed in detail therein).

For all retentate samples (i.e., MPC), the heat stability values increased with increasing pH, typical of a type B profile. Previously, a study by Crowley et al. (2014) showed that for rehydrated MPC powders containing 35 and 50% protein (dry matter basis) the HCT profile was type A; however, for powders containing $\geq 60\%$ protein (w/w) the heat stability was of a type B profile. In general, Crowley et al. (2014) showed that the higher the protein content of MPC powders the lower their heat stability in the pH region of 6.6 to 7.0. When considering the heat stability of milk and related protein systems, a number of factors are known to be influential, particularly in relation to their serum phase. Most notably the destabilising influence of ionic calcium causing heat-induced calcium bridging, and the stabilising role of urea, which can buffer against the significant pH decrease during HCT. Therefore, the heat stability of milk is usually associated with the proportion of κ -casein, total calcium, citrate, phosphate, urea and lactose content (Huppertz, 2016; Singh, 2004).

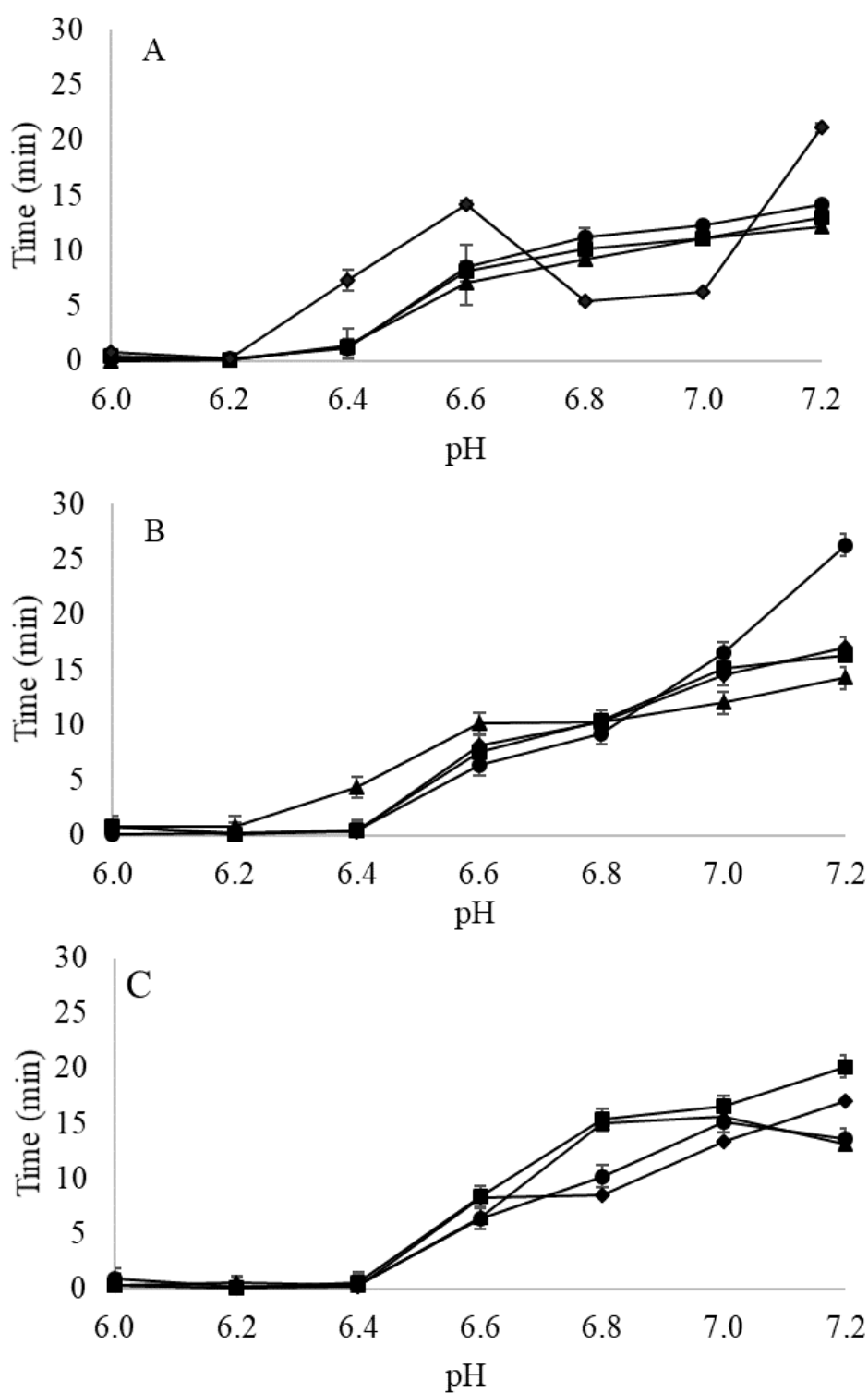


Figure 7.3. Heat coagulation time-pH profiles (140°C) of skim milks (A), and milk protein concentrates obtained from membrane filtration using 31 mil (B) or 46 mil (C) spacer membranes. Symbols represent milk obtained in February (●), early March (■), late March (▲) and April (◆).

7.4.6. *Heat coagulation time of recombined skim milk*

Given the significant role the milk serum phase plays in defining the HCT profile, further exploration was necessary. Performed by recombining ultrafiltration retentate obtained from skim milk at SP1 with its own milk permeate, and secondly recombining the same retentate with milk permeate generated from SP4. The hypothesis being that the serum phase is to a large extent defining the HCT profile, despite the fact that was no notable difference in mineral composition between milk permeates, as shown in Table 7.3. Figure 7.4 shows that recombining the retentate and milk permeate from SP1 resulted in a HCT type B profile, similar to the original skim milk for SP1 (Figure 7.3A). However, combining milk permeate from SP4 with retentate from SP1 resulted in a change to a type A profile with a maximum heat stability at pH 6.6 and 7.1, and a decrease in heat stability in the pH region 6.8-7.0. Confirming that by maintaining the same retentate and changing only the milk permeate the HCT profile can be altered from a type B to type A profile. Crowley ... (2014) showed that the addition of urea to MPC80 solutions increased the heat stability in the pH region above pH 6.8, but did not convert the HCT profile from a type B to a type A. Conversely, the addition of lactose decreased the heat stability of MPC80 suspensions, but again only in the region above pH 6.8 and did not effect the HCT profile type. To the authors knowledge, the data presented in Figure 7.4 is the first time that the change in milk HCT profile has been shown by altering the serum phase.

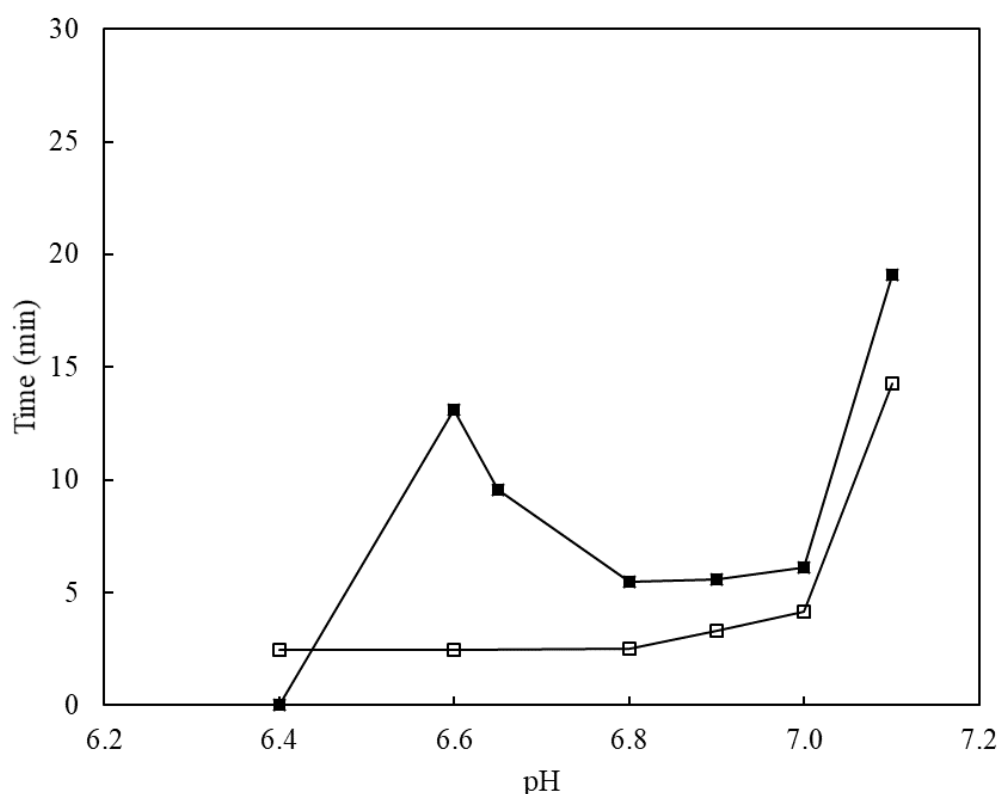


Figure 7.4. Heat coagulation time-pH profiles of milk protein systems. The recombination of milk permeate and MPC from SP1 (□) and secondly milk permeate from SP4 with MPC from SP1 (■).

7.5. Conclusion

This study demonstrated that the serum phase of milk is the predominant factor effecting the actual type of HCT profile, i.e., either type A or B. Moreover, while the study did not identify a specific compositional factor responsible for this influence, it is most likely minor differences across a number of constituents that interlink and effect HCT. The manufacture of MPC from early lactation skim milk from February to April did not significantly affect the processing efficiency; although had diafiltration water been added and a higher protein content achieved then differences in permeate flux across skim milks may have become more apparent. This concluding chapter of the thesis has highlighted scope for exciting future work on which to build. The exchange of milk sera with milk protein concentrates, produced from skim milks

with different HCT profiles, is an area of research that could really elucidate an important aspect of milk chemistry.

7.6. Acknowledgement

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7.8. Appendix

7.8.1. Appendix 7A

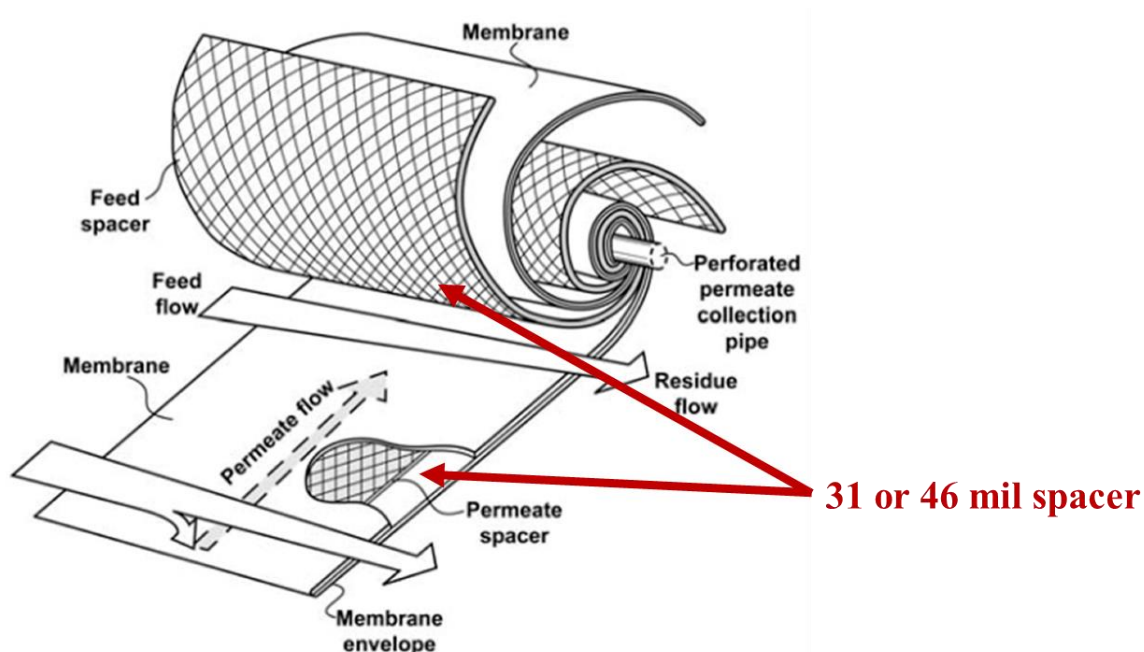


Figure A7.1. Schematic of a spiral wound polymeric membrane highlighting the spacer component, adapted from Abdul Latif, Lau, Low and Azeem, 2021.

7.8.1. Appendix 7B

Table A7.1. Percentage recovery of total solids and total nitrogen, as described in Section 7.2.

	31 mil spacer				31 mil spacer			
	SP1	SP2	SP3	SP4	SP1	SP2	SP3	SP4
Total solids (%)	98.0	97.1	96.4	97.2	97.0	97.5	96.8	98.8
Total nitrogen (%)	97.0	97.7	98.3	99.9	97.2	96.2	95.9	>100*

* Result of >100% due to $\pm$ standard deviation in the results of the Kjeldahl analysis.

Chapter 8

General discussion and suggestions for future research

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Declaration

This chapter was written by Tugce Aydogdu (TA) and reviewed by all co-authors.

8.1. Summary of key findings

The multitude of factors affecting the heat stability of dairy-based systems can be broadly grouped into either (1) protein, or (2) mineral-based. The protein- and mineral-based factors can be further sub-divided into sedimentable and serum fractions, with the environmental factors of temperature and duration of heat treatment effecting this equilibrium. However, it is the almost infinite number of processing combinations (not just thermal processing) practiced by the dairy industry that makes this area of dairy chemistry so incredibly complex. Hydrogen ion concentration, an element that transcends all aspects of dairy chemistry, is well established but often poorly understood. There is an ongoing objective of increasing heat stability of dairy protein systems in an effort to minimise viscosity development during thermal treatment, often associated with protein denaturation and aggregation, with undesirable consequences ranging from heat exchanger fouling to gelation, all of which make such thermal processes less efficient and sustainable due to energy, water and cleaning chemical utilisation (Ho et al., 2018). Optimization of thermal processing operations, *via* real-time monitoring, offers significant potential in terms of flexibility to minimise protein aggregation and gelation. **Chapter 2** discussed the interrelationship between dry matter content, minerals, temperature, and pH, and provided important insights into some of the new developments in *in-line* pH measurement and data collection under challenging processing conditions (i.e., high temperature and pressure). The chapter also highlighted where the dairy industry could potentially benefit from the incorporation of such technology, particularly in ultra-high temperature processing and for products such as read-to-drink beverages in the infant, adult and medical formulation sector.

Continuing from the suggestions in Chapter 2 for a need to examine pH *in-situ*, **Chapters 4 and 5** enabled these new insights into the heat stability of milk and milk protein products through the design, development and implementation of a novel *in-situ* approach to pH measurement under ultra-high temperature treatments. Unlike *off-line* individual measurements, the continuous measurement and recording of pH at high temperatures have significant implications for dairy processors to make informed decisions rapidly. More specifically, the work completed in **Chapter 4** involved the design and development of a laboratory-scale high-temperature pH measurement system, which was further developed into the format of a retrofit plug-and-play *in-situ* pH measurement system at pilot scale in **Chapter 5**. These novel studies showed for the first time the *in-situ* pH measurement at temperatures exceeding 100°C for a number of different targeted dairy systems with varying total solids, protein and mineral profiles. The measurement of pH during high-temperature treatment of skim milk and protein-free milk permeate showed that the decrease in pH with increasing temperature up to 140 °C is not linear, which was first observed in **Chapter 4** at lower measurement temperatures (25-120°C). This proved that the predictions made in previously published studies were incorrect, where simply applying a linear pH-temperature coefficient were poor at predicting pH changes at ultra-high temperatures (Chandrapala, McKinnon, Augustin, & Udabage, 2010; On-Nom, Grandison, & Lewis, 2010). As shown in **Chapter 4**, each milk system displays different temperature-pH profiles due to their inherent chemical compositions, which are found to be mainly controlled by the calcium phosphate content and protein profile. Also, the presence of soluble minerals in the form of simulated milk ultra-filtrate resulted in a greater decrease in pH compared to lactose (**Chapter 3**), which has significant

implications for the protein standardization practices during skim milk powder production.

While protein standardization is widely adopted in the dairy industry, the studies published have largely focused on calcium phosphate precipitation and fouling (Rosmaninho & Melo, 2006; Ojaniemi, Pättikangas, Jäsberg, Puhakka, & Koponen, 2020). There is a gap in our comprehensive understanding of the influence of milk serum minerals on heat stability, protein concentrate viscosity and age thickening.. Therefore, it was important to investigate thoroughly these changes, the role of protein-mineral interactions, and the influence of milk serum minerals on protein concentrate viscosity, age thickening, and heat stability. The work of **Chapter 3** demonstrated that the mineral content was primarily responsible for the high viscosity and age thickening of milk concentrates. Furthermore, the work suggested that the formation of calcium complexes (such as precipitation of calcium phosphate on to the surface of casein micelles, association of ionic calcium to the negatively charged casein micelle surface or association of whey proteins with positively-charged free calcium ions) might not be the only factor influencing the viscosity and age gelation properties of concentrates, but rather a multitude of factors are at play. The time-dependent viscosity increase and eventual age thickening, might be associated with rearrangements in the micellar structure and/or changes in the number/strength of chemical bonds between the casein micelles (Bienvenue, Jiménez-Flores, & Singh, 2003).

Learnings relating to the dominating role of minerals on protein-protein interactions, viscosity, and heat coagulation time (**Chapters 3 and 4**) were further assessed in **Chapters 6 and 7**, with commercial bulk skim milk obtained across the Spring period in Ireland to determine if the changes in milk composition from early

lactation influence the heat stability and subsequent membrane filtration performance. The change in heat coagulation time profile was reported for, the first time (**Chapter 6**) for commercial bulk milk samples from February to April (early lactation) without any significant difference in milk composition between any of the samples. Previous studies reported a change in the heat coagulation time (HCT) profile of the milk from individual cows and associated this with a significant change in mineral profile (i.e., total calcium, ionic calcium, and phosphorus content). It is presumed that the variation in HCT profile and mineral composition might be more apparent when milk from individual cows is investigated compared to bulk milk, as the individual variations might be eliminated. However, from an industrial dairy processing perspective, learnings obtained using commercial bulk milk would be more relevant and informative for further investigations and actions. One question to be considered is the importance of HCT-pH profile type, when for the most part, milk is not heat treated at pH values as low or as high as those performed in the oil bath method. However, what the HCT profile shows is that milk at pH values in the range of 6.6 to 6.8 can have significant differences on heat stability, dependent on whether they follow a type A or type B profile (**Chapter 6**). Aside from the heat stability at the natural pH of milk, Chapters 4 and 5 show the extent to which pH can decrease at temperatures up to 120 and 140°C, respectively. Therefore, milk heat treated at pH 6.6 can display reductions in pH to values as low as ~ pH 6.2.

In **Chapter 7**, commercial bulk skim milk obtained across the Spring period was further assessed for membrane filtration performance during milk protein concentrate production via ultra-filtration. **However**, skim milk from February to April did not significantly affect the permeate flux of the milks during ultrafiltration. However, one caveat with the trials performed in **Chapter 7** was that filtration was

performed under continuous feed-and-bleed conditions to obtain industrially relevant data, but it was decided not to incorporate diafiltration water to increase the protein content further, as this would influence membrane performance data. Had the protein content being increased by washing out more serum lactose and minerals then there may have been a difference in heat stability of the MPCs obtained from the different skim milks.

This thesis has generated a considerable body of new research that provides a novel approach to *in-line* measurement of pH in laboratory and industrial settings and new insights into the complex relationships between milk composition (particularly mineral composition) and thermal stability during processing. These findings deliver a greater understanding on some of the unknowns in fundamental dairy chemistry and provides insights that can be extrapolated to the thermal processing of complex nutritional formulations (e.g., infant formulas) where mineral addition is common practice.

Overall, the work carried out in this thesis not only contributes to the field of protein and mineral chemistry but has real impact and applications for industry. The information provided on *in-line* high temperature-pH measurement in conjunction with the work focused on protein-mineral interactions and its effect on heat stability is both useful for milk processors and nutritional companies producing infant and medical formulations. Next steps: Ideally for the promotion and uptake of the knowledge generated from this research, continued communication to the wider scientific and industry community is needed, particularly if its adoption into laboratory and pilot plant settings are to be fulfilled.

8.2. Suggestions for future research

Some suggestions for further investigation and follow-up studies that would complement the work presented in this thesis are as follows:

- Further work on high temperature pH measurements is certainly worth continuing (**Chapter 4**), particularly for complex dairy systems such as infant formulas, follow-on formulas and medical nutritional products, where UHT and retort thermal treatments are commonly used. The methodology shown in Chapter 4 is laboratory based, low cost, with high throughput, making it ideal for benchmarking the heat stability-pH relationship of numerous dairy products.
- The ability of in-line/*in-situ* pH measurements may also be worth pursuing for pilot or industrial scale applications (**Chapter 5**). Commercially available probes and housings, constructed from titanium or ceramic, may be an option to replace the glass probe used in **Chapters 4 and 5**. These probes are known to be used in the pharmaceutical industry and can withstand ultra-high temperatures and pressures with good durability (**Chapter 2**). Validation of the titanium probes for in-line pH measurement at ultra-high temperatures would be of benefit to the dairy industry.
- The study reported in **Chapter 6**, based on the HCT profile of skim milk collected within the early part of the lactation season, could be continued and expanded to include milk from a number of seasons. Factors such as weather conditions and fodder quality may affect milk profile and quality and subsequent HCT. However, individual factors such as animal age and health may not be as influential, when sampling bulk milk from commercial suppliers, as opposed to individual cow milk

sampling, which has previously been examined. Therefore, it is worth investigating the HCT of commercial skim milks from several dairy plants across a number of seasons. This might provide a better understanding of the observed changes in the HCT profile seen in **Chapter 6**. However, the work presented in **Chapter 6** explains that macro compositional factors alone may not elucidate the reasons for type A or type B profile and that further in-depth analysis is required. This type of work is costly, time consuming, and might be better suited to a larger multi-partner funded project.

- Continuing the same theme, lactational effects on membrane filtration performance and high protein heat stability is worth examining further (**Chapter 7**), mainly to produce MPC ingredients with higher protein content (i.e., >80%, w/w) where processing differences between early season milks may be more pronounced (i.e., permeate flux, membrane fouling and overall process efficiency).

8.3. References

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