Manufacture of Casein from Milk Retentates

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by

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Abstract

Casein is the major protein component of cow's milk and is extracted commercially for a wide range of applications. Before casein is precipitated from milk by acid, milk is commonly concentrated by ultrafiltration to increase throughput and partly recover lactose. The degree to which the milk can be concentrated is limited due to higher concentrations producing a tough rubbery curd that causes downstream processing difficulties, particularly when casein is washed to remove calcium, lactose, whey and other impurities.

This thesis examines using milk protein concentrates, MPC70 and MPC85 retentates from ultrafiltration, to manufacture casein. MPC85 was used on a large scale process, and MPC70 was used on lab scale process. Lab scale casein production techniques were developed to produce a similar casein to process scale. Effects of dilution and pH were examined on casein properties and calcium and lactose removal. Diluting the retentates prior to acidification was effective at reducing the residual levels of calcium and lactose in the casein, and no increase in residual whey protein was found compared to conventional casein production. The optimal precipitation pH remained at 4.60, and the resulting casein was not found to have any reduction in its functional performance. Lowering precipitation pH increased casein losses due to fines being formed and difficulty in separating wash water from the curd.

Use of milk retentate for casein manufacture allows greater recovery of lactose and may have positive implications for the economics of the process in reducing washing required to produce high quality casein.

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Nomenclature

a_w	Water activity
CCP	Colloidal calcium phosphate
CMP/GMP	Casein macropeptide / Glycomacropeptide
CN	Casein
Curd	Casein component of milk coagulum
EDTA	Ethylenediaminetetraacetic acid
FTIR	Fourier transform infrared spectroscopy
UF	Ultrafiltration
HTST	High temperature, short time pasteurisation
ICP-AES	Inductively coupled plasma atomic emission spectroscopy
ICP-MS	Inductively coupled plasma mass spectrometry
ISE	Ion selective electrode
LAL	Lysinoalanine
LTLT	Low temperature, long time pasteurisation
MF	Microfiltration
MGFM	Milkfat globule membrane
MIR	Mid infrared spectroscopy
MPC	Milk protein concentrate (often appended with protein content)
MSR	Multi-stage recycle
NIR	Near infrared spectroscopy
RO	Reverse osmosis
RP-HPLC	Reversed phase high performance liquid chromatography
TFA	Trifluoroacetic acid
Tris-HCl	Tris (hydroxymethyl) aminomethane hydrochloride
UHT	Ultra heat treatment
WPC	Whey protein concentrate (often appended with protein content)
WPI	Whey protein isolate
VCF	Volumetric concentration factor
WS1	Wash screen one
WS2	Wash screen two
WS3	Wash screen three
α-La	α-lactalbumin
β-Lg	β-lactoglobulin

Chapter 1. Introduction

1.1 Background

This project was carried out at out Westland Milk Products in Hokitika, on the West Coast of New Zealand's South Island. Westland was first established in 1937 by the amalgamation of several smaller dairy companies. In 2001, during deregulation of New Zealand's dairy industry, Westland's shareholding farmers voted to remain independent. In 2006, Westland constructed a new processing plant which now produces various casein, caseinates and other dairy powders for export. The stated aim of the company is to be the preferred supplier of premium quality dairy and nutritional products, and the investment in protein production is a key part of this.

Casein is the principal protein found in cow's milk. It represents approximately 80% of the mass of milk's total protein component, with the remaining 20% mainly whey protein. Casein consists of α_{S1}, α_{S2}, β and κ casein, as well as subtypes and is present in milk as a stable micelle suspension. It contains minerals such as calcium and phosphate, which also play a role in its stability. Casein is precipitated out of milk on acidification to its isoelectric point of pH 4.6, or when treated with enzymes such as rennet. Casein has been extracted commercially from milk since the early 20th century (Southward 1998). It is now used mainly in nutritional applications and can undergo further processing to produce caseinates. These soluble casein powders are also used in a wide range of nutritional applications. New Zealand is a leading producer and exporter of casein products and primarily produces casein by acid precipitation. In this process, skim milk is acidified either by direct acid addition or the use of lactic acid producing bacteria. This results in the precipitation of the casein curd. The curd is then separated from the whey stream and washed extensively with water to remove impurities such as lactose, calcium and residual whey protein before drying. There are increasingly stringent quality requirements on casein products. The final product should be almost entirely protein, with very low levels of impurities. It should contain none of the calcium that was originally associated with the casein micelles (Walstra et al. 2006) and its mineral content should consist almost entirely of the organically

bound phosphate associated with the casein (Southward 2002). This means that the washing stage of the process is critical to product quality.

1.2 Problem Statement

Ultrafiltration (UF) of skim milk prior to casein manufacture is common practice in the dairy industry. This allows increased throughput due to the reduced volume. Additionally, lactose recovered in the permeate stream at this point can be utilised more easily than the lactose from the whey stream later in the process. Also, as less lactose enters the process, washing efficiency may be improved. UF to a volumetric concentration factor (VCF) of 1.6 – 1.8 prior to casein making is the industry standard, though UF plants are capable of much higher concentration. However, use of high VCF retentates for casein manufacture results in a tough, rubbery curd which is difficult to process, particularly during washing. Studies have shown that as the casein concentration of a milk retentate is increased, greater pH reduction is required to solubilise the minerals present in the casein micelles (Le Graët and Gaucheron 1999). The proportion of calcium that can enter the whey is limited by the amount of water available to take it into solution. Informal communications have also claimed that casein products made in this way have reduced viscosity, a key functional property, than conventionally produced ones.

1.3 Project Aim

The aim of this research is to examine the effects skim milk UF retentate concentration, water addition to UF retentate, and pH have on key casein properties such as toughness, and the residual content of lactose, calcium and whey protein.

1.4 Thesis Structure

An overview of milk composition with a focus on milk proteins and their structure and properties is presented in Chapter Two. The range of commercial milk protein products is then introduced with a discussion of their properties and uses. Current milk processing details are reviewed, before casein manufacture is covered in detail at the end of the chapter.

Materials and methods used for all analyses in the thesis are summarised in Chapter Three.

Before any changes to the process could be investigated, a set of baseline results was required. An assessment of the casein process under normal running conditions is presented in Chapter Four.

A laboratory-based method of casein production that could stand in for a pilot plant and allow small scale trials to be carried out was developed. This is discussed in Chapter Five.

In Chapter Six, retentate is used to make casein under different conditions using the techniques developed in the previous chapter. These results are compared to the initial plant results to assess the effect on the casein produced and help confirm optimal conditions.

Chapter Seven contains conclusions from the research and recommendations for any future work.

Chapter 2. Literature Review

2.1 Introduction

This chapter reviews a range of dairy topics, with particular attention given to aspects relating directly to the work undertaken in the later thesis chapters. The composition of milk is introduced, before a more comprehensive overview of the literature relating to milk proteins, particularly the caseins and the current understanding of their properties. The modern range of milk protein products is discussed, particularly those related to this work. Their commercial uses are also covered where relevant to the properties influenced by their production. The latter half of the chapter focuses on dairy processing, initially on the normal milk and powder processes before moving on to cover the manufacture of casein in detail. Finally, the combination of casein and membrane processing technologies at the centre of the thesis are discussed using what published information is available.

2.2 Milk composition

Milk is the secretion of the mammary gland of mammals and its primary function is for the nutrition of their young. As a result it is one of the most complete food sources known. It is a complex fluid containing fat globules in an emulsion, minerals and some proteins in solution while other proteins are held in colloidal suspension. The main components of milk are summarised in Table 1. The earliest milk of lactation is known as colostrum, and differs in composition from normal milk. A significant difference is the presence of large numbers of antibodies, which function to confer immunity from the mother to her offspring. The composition of milk also varies by species as it is closely linked to the exact requirements of the physiology of the young of that species. The usefulness of milk to the human diet has lead to the domestication of various milk-producing species. The domestic cow *Bos primigenius* in particular is now present across the world and bovine milk is the most well characterised and understood type of milk in the world today. Bovine milk also accounts for the vast majority of milk processed worldwide, though milk from other species such as water buffalo, goat and sheep are significant to various regions of the world. Dairy products represent a significant part of the Western diet, and their consumption in other

cultures is also increasing. Milk derived products are one of the most important sources of calcium in the diet of a large proportion of the world's population.

Table 1: Approximate composition of milk (Walstra and Jenness 1984)

Component	Average Content (% by wt)	Average of Dry Matter (% by wt)
Water	87.3	
Lactose	4.6	36
Fat	3.9	31
Protein	3.25	26
Minerals	0.65	5.1
Organic acids	0.18	1.4
Miscellaneous	0.14	1.1

2.2.1 Lipids

Almost all of the lipid content of milk is in the form of fat globules. These can be easily removed from the parent milk by separation due to gravity. Traditionally this was achieved by 'skimming' the cream layer off the top, leading to the term skim milk for de-fatted milk. Until relatively recently, fat was the most valuable component of milk. Even well into the 20^{th} century, many dairy farms recovered only the cream for sale, while the skim milk was used as stock feed. Changes in dietary patterns and increasing awareness of milk composition meant that the protein fraction of milk is now its most valuable constituent, though milk fat derived products still represent a significant part of the Western diet. The protein component of milk is of major significance to this work and as such, is discussed in greater detail in later sections.

2.2.2 Carbohydrates

Lactose is the major carbohydrate of milk. It is a dissacharide, found in the milk of nearly all mammals and is unique to milk (Walstra et al. 2006). Lactose is hydrolysed by the enzyme β-galactosidase, commonly known as lactase, which is present in the digestive system of young mammals specifically for this purpose. In all mammals except some humans, the amount of lactase produced reduces to a very low level after weaning. The retention of lactase activity occurs in some humans and is thought to be a relatively recent genetic adaptation. Individuals unable to digest lactose are known as lactose mal-digesters or lactose intolerant depending on the severity of the symptoms (Walstra et al. 2006). The prevalence of lactose maldigestion varies by region, with Scandinavia having a rate of around 2% and some Asian countries reaching almost 100%. The rate in New Zealand is around 9% (Vesa et al. 2000). Lactose is utilised commercially in a wide range of applications, which are discussed further in section 2.6.

2.2.3 Salts

The mineral fraction of milk is often expressed as its 'ash' content. This is because standard dairy industry testing uses the reduction of dairy products to ash in a laboratory furnace to give an approximate mineral content. This is not the true mineral content as organic acids such as citrate and acetate are destroyed by ashing. The ashing procedure also transfers organic phosphorus and sulfur to inorganic salts (Walstra et al. 2006). The principal salts of milk are sodium, potassium, calcium, magnesium, chloride, phosphate and citrate (Walstra and Jenness 1984). Some of these are present at levels well below their solubility limit, while others such as calcium and phosphate are present in such high concentrations that they exist in milk only partly in soluble form with the rest associated in a colloidal form with the caseins. These are collectively referred to as micellar or colloidal calcium phosphate (CCP) and play a major role in micellar integrity (Fox 2001). About 67% and 57%, respectively, of the total calcium and phosphate present in milk are in the colloidal phase (Fox and McSweeney 1998).

2.2.4 Proteins

As mentioned, in the modern dairy industry protein is the most valuable dairy component. Bovine milk has a nitrogen content of about 5.3 g per kilogram with around 95% of this is in the form of proteins (Walstra and Jenness 1984). Most of the protein component of milk can be separated broadly into groups depending on their solubility at pH 4.6, as summarised in Table 2 below.

Table 2: Some properties of the main groups of protein in skim milk (Walstra et al. 2006)

Property	Caseins	Globular Proteins	Proteose-Peptone
Present in	Casein micelles	Serum	Both
Soluble at pH 4.6	No	Yes	Yes
Clotted by rennet	Yes	No	Partly
Heat denatured	No	Yes	No

Casein and whey proteins are present in milk in a ratio of around 4:1. Together they represent the vast majority of the total protein fraction of milk, though a large number of other proteins are present at low levels. The principal proteins present in milk have now been well characterised and are summarised in Table 3. This grouping could be substantially subdivided as all of the primary milk proteins exhibit genetic polymorphism (Walstra and Jenness 1984). Multiplication of the nitrogen content of milk and milk products by a Kjeldahl factor of 6.38 is officially accepted to give their protein content (Walstra et al. 2006). Though the Kjeldahl factor differs for individual proteins, this average value provides a relatively accurate approximation and has been agreed upon for use worldwide.

Table 3: Concentration of proteins in milk (Walstra and Jenness 1984)

	Concentration in Milk		Percentage of Total
	g/kg	mmol/m³	Protein (by wt)
Total Protein	33.0	~1490	100.0
Total Casein	26.0	1170	79.5
Whey Proteins	6.3	~320	19.3
MGFM Proteins	0.4		1.2
αS1-casein	10.0	440	30.6
αS2-casein	2.6	110	30.6
β-casein	9.3	400	30.6
γ-casein	0.8	40	2.4
κ-casein	3.3	180	10.1
α-lactalbumin	1.2	90	3.7
β-lactoglobulin	3.2	180	9.8
Blood Serum Albumin	0.4	6	1.2
Immunoglobulins	0.7	~4	2.1
Misc. including Proteose-Peptone	0.8	~40	2.4

The minor proteins of milk are diverse and have only relatively recently become well characterised. Serum albumin is synthesised in the liver and makes its way into the milk through the secretory cells. The milk of all mammalian species that have been examined share this feature (Walstra and Jenness 1984). Immunoglobulins are present in milk to confer immunity to the ingesting calf, though this function is associated more with colostrum than milk due to its much higher immunoglobulin concentration. A very different group of minor proteins is the fat globule membrane proteins. These surround the fat globules in milk and play a role in stabilising it, both by preventing agglomeration of the globules and by preventing the access of lipases present in milk. Additionally, some of these proteins play an important part in various cell processes and defense against bacteria and viruses in the newborn (Fong et al. 2007).

2.2.4.1 Whey Proteins

The whey, or serum, proteins are the protein fraction which is not precipitated from milk at pH 4.6, though aside from this commonality, they share relatively few characteristics. The main whey proteins are α-lactalbumin, β-lactoglobulin, serum albumin and immunoglobulins. β-Lactoglobulin is the major serum protein, and its properties tend to dominate the properties of whey protein preparations, especially the reactions occurring upon heat treatment (Walstra et al. 2006). Proteose peptone is a minor whey fraction which is comprised largely of three different degradation products of β-casein. Another minor whey protein of note is lactoferrin, which as well as being present in whey is found in a range of tissues both in humans and other mammals (Levay and Viljoen 1995). It is a member of the transferrin group, and has an extremely high affinity for Fe^{3+} ions. This gives it anti-bacterial properties, and studies have shown that it has a wide range of other potentially useful properties including anti-cancer and anti-inflammatory effects (Wakabayashi et al. 2006). Lactoferrin is commercially extracted from bovine milk and used as a nutritional ingredient because of these properties.

2.2.4.2 Casein

The ability to separate milk into casein and whey by precipitation at pH 4.6 has been used for centuries, as it forms the basis of many traditional dairy products. Casein was initially thought to be a single protein, until in 1939 its heterogeneity was confirmed using electrophoresis (Mellander 1939). This work found three components of casein, which were named α-casein, β-casein and γ-casein in order of reducing mobility under electrophoresis. It was subsequently discovered by Waugh & von Hippel (1956) that α-casein when treated with $CaCl_2$ could be separated into two further fractions. The calcium sensitive fraction was named α_S-casein and the calcium insensitive fraction, κ-casein. Further work by Annan and Manson (1969) showed that α_S-casein is actually comprised of two proteins, α_{S1} and α_{S2} casein. It was also discovered that γ-casein was actually the C-terminal segment of β-casein after it had undergone proteolysis by plasmin (Groves 1969). As a result, the complete casein protein complement of milk is now known to be α_{S1}, α_{S2}, β and κ caseins. These are

present in the approximate proportions of 4:1:4:1 respectively (Guo et al. 2003). Further diversity can be caused by genetic variation and post-translational modifications such as phosphorylation, glycosylation, disulphide bonding and proteolysis (Ng-Kwai-Hang 2002, Walstra et al. 2006). The 3-dimensional structure of these four casein types has not been measured as they cannot be crystallised for x-ray crystallography, and cannot be dissolved at a high enough concentration for nuclear magnetic resonance (NMR) spectroscopy without causing structural changes (Creamer 2002).

Table 4: Average characteristics of casein micelles (Fox and Brodkorb 2008)

Characteristic	Value
Diameter	120nm (range: 50-500nm)
Surface area	8×10^{-10} cm^2
Volume	2.1×10^{-15} cm^3
Density (hydrated)	1.0632 g cm^{-3}
Mass	2.2×10^{-15} g
Water content	63%
Hydration	3.7 g H_2O g^{-1} protein
Voluminosity	44 cm^3 g^{-1}
Molecular mass (hydrated)	1.3×10^9 Da
Molecular mass (dehydrated)	5×10^8
No. of peptide chains	5×10^3
No. of particles per mL milk	10^{14}-10^{16}
Surace of micelles per mL milk	5×10^4 cm3
Mean free distance	240nm

In milk, the caseins are associated into spherical particles known as casein micelles, properties of which are given in Table 4. As the structure of the micelle has not been directly measured, various techniques have been applied, to allow aspects of its structure to be understood. Caseins have quite different properties from those of most other proteins. They are hydrophobic and have a high charge, which is required to keep them in solution. Structurally, they do not form anything more than short α-helices and have little tertiary structure, which accounts for their stability against heat denaturation (Hallén 2008, Walstra et al. 2006). As milk is generally the sole food source for the developing mammal, the specific biological role of casein within milk

is believed to be mainly a nutritional one. This is evident in a number of its structural features. Casein micelles allow milk to remain a free-flowing, low-viscosity fluid while transporting calcium and phosphate at levels that would otherwise precipitate in the mammary gland (Horne 2002). A high proportion of proline gives the caseins a very loose structure that causes them to be very susceptible to enzymatic hydrolysis, resulting in a well-balanced mixture of amino acids. Casein digestion then results in the release of these large quantities of calcium and phosphorous (Ng-Kwai-Hang 2002).

It has been known since the end of the 19th century that casein particles in milk contain colloidal calcium phosphate (CCP). Of the high calcium content in milk (~1.20 mg/ml), around half is bound to casein as CCP (Hallén 2008). The exact nature of the relationship between colloidal casein particles and calcium phosphate has been extensively researched, but is still not completely understood. The main aspects which have been focused on are the composition of the CCP, the nature of the association between the CCP and casein, and the effect of CCP on casein micelle stability and size (Fox and Brodkorb 2008). The result of this work has been a number of competing theories as to its exact structure. Known for certain is that all of the caseins are distributed evenly throughout the micelle, apart from κ-casein which has been shown to play a key role in the stability of the entire particle (Creamer 2002). Around 12% of total casein is κ-casein, and it is able to stabilise ten times its own mass of the other, calcium insoluble, caseins. This finding, combined with the fact that κ-casein is preferentially hydrolysed when exposed to chymosin are evidence that κ-casein predominates around the surface of the micelle (Farrell 2006, Fox and Brodkorb 2008). The break up of micelles when CCP is removed by acidification or a calcium chelator shows that CCP plays a vital role in the stability of the micelle. The susceptibility of the micelle to dispersal by urea, SDS, high pH and ethanol indicate that hydrogen bonding, hydrophobic and electrostatic interactions must also play a stabilising role (Fox and Brodkorb 2008). Electron microscopy has shown that the casein micelle has an uneven surface, which has been likened to the appearance of a raspberry. This finding was interpreted to mean that the micelle is

composed of sub-micelles as pictured in Figure 1, themselves having the κ-casein clustered around the surface protecting the calcium sensitive caseins (Fox and Brodkorb 2008). This has led to discussions of whether each sub-micelle has this κ-casein layer, or whether their location in the micelle is determined by their κ-casein content, with κ-casein richer sub-micelles clustering around the outside of the micelle, shielding κ-casein deficient ones. The lack of a proposed mechanism for the association of κ-casein with only certain sub-micelles is the main criticism of the sub-micelle model (Horne 2002). The existence of the sub-micelle is still debated by some authors. Another proposed structure is the 'hairy micelle' or Holt model in which calcium phosphate nanoclusters interact with casein molecules through their phosphoserine groups. However, this model does not include a mechanism to limit the growth of the casein gel, there is no role for κ-casein as it lacks a phosphate cluster and it has no explanation for the prevalence of κ-casein at the micelle surface (Horne 2002).

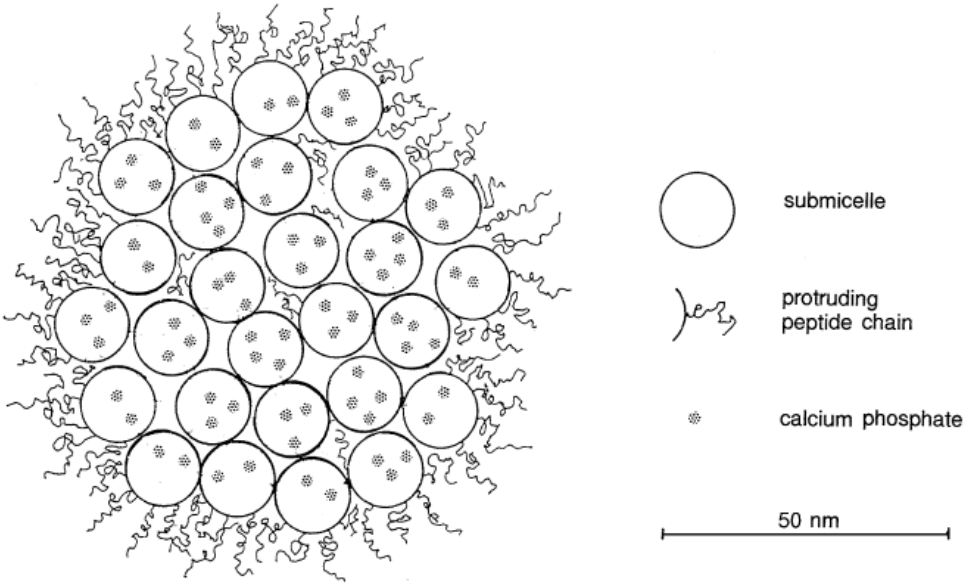

Figure 1: Highly schematic illustration of modified sub-micelle model (Walstra 1999)

Another model has been proposed, in which the assembly and growth of the micelles occurs in two ways. These are bonding between the hydrophobic regions of caseins and bridging across CCP nanoclusters (Horne 1998). This dual-binding model is

depicted in Figure 2. It relies on the previously well-known property of the caseins to self-associate into polymers. Proponents of this model believe it provides satisfactory mechanisms for assembly, growth and termination of growth of the micelle.

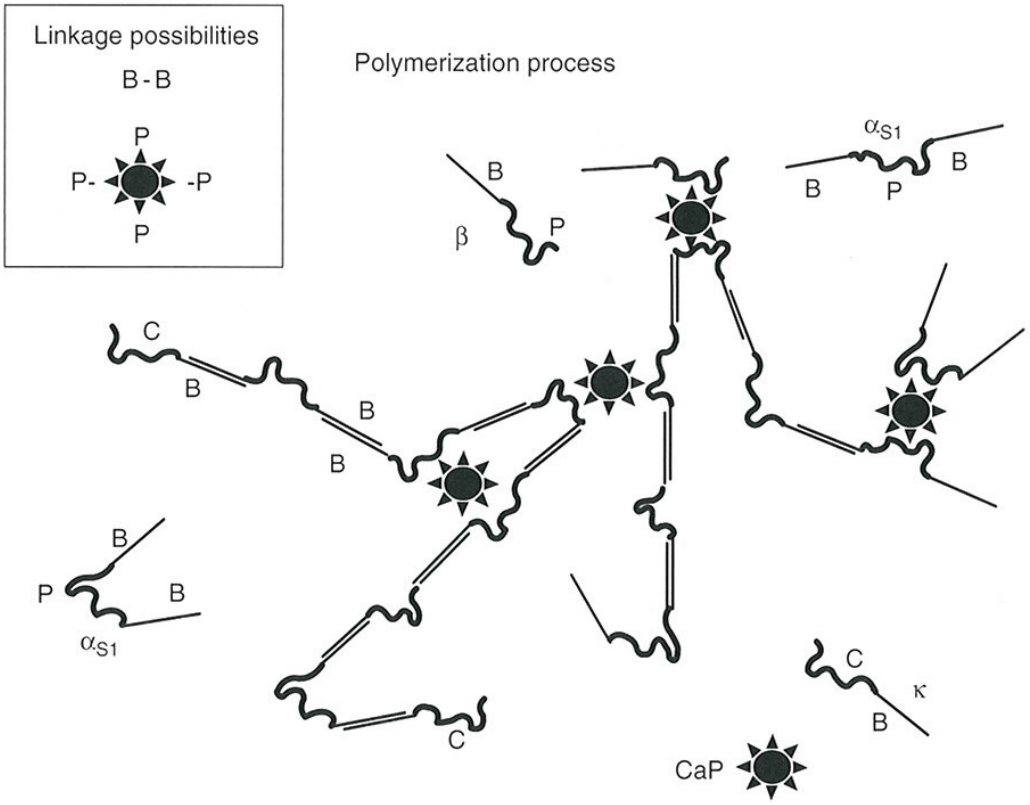

Figure 2: Schematic depiction of casein conformations proposed under the dual-binding model (Horne 2002)

Other models competing with the sub-micelle model also depict the micelle as casein molecules joined by CCP and hydrophobic bonds. Further improvements in electron microscopy may be able to help clarify the situation and allow further refinements to these models (Fox and Brodkorb 2008).

2.3 Coagulation of Milk

The stability of casein micelles in milk is dependant on the negative charge and hydrophilic nature of the C-terminal ends of the κ-casein at the casein micelle surface (Hallén 2008). Coagulation of milk can be induced by broadly two different methods; enzymatic hydrolysis or acidification. These result in quite different casein curd properties, some of which are summarised in Table 5 below.

Table 5: Properties of gels made by rennet or by slow acidification (Walstra et al. 2006)

Property	Rennet Gel	Acid Gel
pH	6.65	4.6
Fractal dimensionality	2.25	2.35
Elastic shear modulus (Pa)	30	100
Fracture stress (Pa)	10	100
Fracture strain	~3	~1
Size of largest pores (μm)	~10	~18
Occurrence of syneresis	Yes	Virtually none

2.3.1 Enzymatic coagulation

Cheese is probably the most well known product of enzymatic coagulation. This process primarily uses rennet, the term for the clotting enzymes originally sourced from the abomasum of calves. The primary enzyme of rennet is chymosin, which has a high specificity for the peptide bond between residues 105 and 106 (Phe-Met) of κ-casein. The hydrolysis of κ-casein results in two segments, and the "hairs" of the micelle being much reduced in length. The segment remaining in the micelles is known as para-κ-casein, while the hydrophilic part released into the whey is known as caseino-macropeptide (CMP) or glycomacropeptide (GMP) and can be isolated for use as a food ingredient (Tek et al. 2005). When approximately 70% of the κ-casein has been hydrolysed the colloidal stability of the micelle is sufficiently reduced for aggregation to begin to occur (Walstra et al. 2006). The micelles form a gel through hydrophobic bonding, which is further solidified by calcium cross-linking between the para-casein micelles. As the cross-links increase the contraction of the gel, whey is expelled from the structure.

2.3.2 Acid coagulation

The other method of milk coagulation is by acidification. This occurs naturally through the effect of lactic acid bacteria, though direct addition of acid achieves the same result. Acid coagulation is used in the production of some cheeses, known as fresh acid cheeses or lactic cheeses. These differ from yoghurts and other fermented dairy products in that some of the moisture is often removed by separation or ultrafiltration prior to inoculation (Lucey 2002).

Various interactions are responsible for the integrity of the casein micelle. The lowering of the pH by acid, either added or produced by bacteria, reduces the negative charge repulsion forces between the casein micelles and as the isoelectric point of the casein micelle (pH 4.6) is approached the micelles begin to aggregate. The lowering of pH by acid causes colloidal calcium phosphate to become soluble and dissociate from the micelle (Walstra and Jenness 1984). This causes the micelle to swell and become more flexible internally (Lucey 2002) before the casein eventually precipitates at pH 4.6.

Depending on the severity of the heat treatment the milk has been subjected to prior to acidification, denatured whey proteins (particularly β-lactoglobulin) may form di-sulphide bonds with κ-casein or each other. The interaction of denatured whey proteins, associated with the micelles, with each other results in increased curd firmness (Lucey and Singh 1998). Although firmer, these gels can also be more susceptible to wheying-off, as the gel has undergone greater rearrangement (Lucey 2002).

2.4 Milk Protein Products

The protein component of milk is utilised to make a vast range of different products. This includes various cheeses, individual protein fractions and modified proteins. Traditionally, the four major commercial casein products were lactic casein, mineral acid casein, rennet casein and caseinates. More recently products with whey included have become established. Of these, total milk protein and milk protein concentrates are the most significant (Munro 2002). This section covers the three most relevant product groups to the project: milk protein concentrates (MPC), whey protein concentrates (WPC) and casein products.

2.4.1 Milk Protein Concentrates

MPCs are milk powders in which the protein content has been increased using membrane processing. These differ from casein and whey products in that the protein ratios within the product should be broadly the same as skim milk. UF retains almost all of the whey and casein while lactose and minerals pass the membrane into the permeate phase. This can also be supplemented with diafiltration, with which the protein content can reach over 85%. An advantage of MPC production is that it provides a source of lactose, which can be further utilised for protein standardisation or processed into a range of products.

MPCs are now widely used ingredients in a range of nutritional applications. They are frequently used in a similar manner to other milk powders, as an ingredient in foods such as desserts, baked goods, low-fat spreads and beverages. They are also an effective way to increase the dairy protein content of foods as their blandness limits the effect on the flavour of the food. The USA is a major export market for MPCs, where they are sometimes a controversial subject due to their perceived displacement of domestic milk products. As more unique functional and nutritional properties of MPCs have been discovered, they have extended the market for high protein dairy products that was once dominated by casein and caseinates (Kelly P. M. 2002).

2.4.2 Whey Protein Products

Whey can be obtained from a variety of different sources. Each of these will produce whey with a different composition. Common types are:

- Whey sourced from cheese-making. As well as the soluble components of milk, it contains GMP from the hydrolysis of κ-casein. Active rennet enzymes and starter bacteria may also be present, as well as lactic acid produced by lactic bacteria. The cheese making process that the whey has come from also influences acidity. Whey with a high salt content can sometimes occur as a result of certain types of cheese making, including cheddar-type cheeses (Walstra et al. 2006).
- Whey from rennet casein manufacture. It shares a number of characteristics with cheese whey and will also contain GMP. However, it will have a lower fat content and contain no starter bacteria or the resulting lactic acid.
- Whey from acid casein manufacture. This contains no rennet or GMP and has a low fat content compared to cheese whey (Walstra et al. 2006). The pH of the whey will be around 4.6 and the shifting of the ionic equilibrium of the casein micelles as a result will cause additional minerals, such as calcium and phosphate, to be present (Foegeding and Luck 2002). The acid source may also have important effects on this whey. For example, lactic casein whey will contain some starter culture and sulfuric acid whey may have increased sulfate levels.

The whey protein products derived from these are primarily whey powders and whey protein concentrates. Whey powders are dried whey, which is often also demineralised. Whey protein concentrates are products made from a whey source in which the protein content has been increased by UF. These are used in specific functional and nutritional applications. The whey protein content of these products ranges from 25% to over 90% (Foegeding and Luck 2002). Generally though, WPCs are produced with protein concentrations from 35 to 90%. If the protein concentration is over 90%, they are known as whey protein isolates, though the exact categorisations can vary. Other compositional properties of whey powders are summarised in Table 6. The processing of whey into whey protein concentrate is

usually achieved by pressure-driven membrane separation techniques such as UF. To get the higher concentrations required for whey protein isolates, diafiltration can also be used. Depending on the whey source, the lactose removed during these steps may be of use. Lactose from cheese or rennet casein making can be utilised for milk protein standardisation of other dairy products. This provides an economical alternative for dairy manufacturers to purchasing dried lactose for the same purpose. Whey from acid casein production is however not as useful for this as it contains additional minerals and salts from the acidification process, and has a low pH.

Table 6: Average composition of whey products (Foegeding and Luck 2002)

Ingredient	Protein (%)	Moisture (%)	Lactose (%)	Fat (%)	Ash (%)
WPC35	34.0 – 35.4	3.5 – 4.0	51.0 – 54.5	3.5 – 5.0	3.1 – 8.0
WPC80	80.0 – 83.0	4.2 – 5.5	4.2 – 10.0	4.2 – 10.0	2.9 – 5.0
WPI	92.0 – 96.1	4.0 – 5.5	0.6 – 2.0	0.4 – 1.0	2.6 – 3.4

Whey protein products are sought after for their functionality, in particular their gel forming properties. Their nutritional benefits are also becoming increasingly recognised. Whey protein is one of the most nutritionally complete proteins known and is rapidly absorbed into the bloodstream after consumption. This has led to it being used in a vast array of nutritional formulations. For many consumers, having a high protein content is the primary concern, even over others such as taste and solubility.

2.4.3 Casein Products

Until the 1960's the major use of casein was in technical, or non-food, applications (Southward 1998). These included adhesives, coatings and plastics. From the 1970's however, the main use of casein products gradually began to transition to food product applications. This was largely as a result of the increased recognition of casein's functional properties in food systems. Primarily these include whipping, foaming, water-binding, thickening, emulsification and textural effects (Southward

1998). Casein is now a commonly used nutritional ingredient and is produced in large quantities, as shown in Table 7 below. Casein must be made from highly skimmed milk, with a heat treatment that denatures as little of the serum protein as possible (Walstra et al. 2006). All methods of casein production involve making it insoluble; the difference in them depends on how this is achieved. Micellar casein is an unusual exception to this as it is processed in a soluble form.

Table 7: Annual production of casein in selected countries (000's tons) (Southward 2002)

Country	1994	1995	1996	1997	1998	1999	Mean
Australia	4.6	6.5	6.2	5.6	9.0	7.5	6.5
Denmark	11.9	12.5	12.7	12.0	-	-	12.3
France	26.4	38.2	35.4	34.4	38.3	42.6	35.9
Germany	8.4	12.4	12.4	10.6	12.9	11.9	11.4
Irish Republic	36.0	42.5	42.7	42.0	42.0	46.1	41.9
Netherlands	-	33.0	-	-	31.5	-	-
New Zealand	79.4	70.0	79.2	92.2	103.7	86.7	85.2
Poland	3.0	3.0	2.2	1.3	6.9	-	3.7

2.4.3.1 Acid Casein

Acid casein is produced by acidifying skim milk to pH 4.6, leading to curd formation. The casein curd must then be separated from the whey, and washed so that as many impurities as possible are removed. It can then be dried as casein powder or continue on through the caseinating process. A variation on this is the use of lactic acid producing bacteria to acidify the milk. In this process the milk is pumped into a silo with the culture added in a frozen or freeze-dried form and held at the optimal temperature for the culture's growth. The culture utilises lactose within the milk to produce lactic acid. Once the casein has coagulated, the silo is pumped out (Southward and Walker 1980). Lactic casein is popular due the perception that it is a

more ‘natural’ process, as well as its flavour. The physical appearance, composition and functional properties of lactic and mineral acid casein are similar (Munro 2002). Disadvantages of this casein process generally relate to the unpredictability that comes with any biological process. Starter culture selection must consider their growth rate, as this directly impacts the throughput of the process. Proteolysis is also a concern as this represents a direct loss of products. Strains such as *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* produce diacetyl during gas-production which can impart a taint into the whey and derived products. Gas production can be a useful property however, as it results in a more open, porous curd. No single bacterial strain is able to satisfy all requirements, so in practice a mixed culture of strains is used. Specific strains can be added or removed to prevent bacteriophage levels becoming too high in the plant and surrounding area. Of the genus *Lactococcus,* only *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* are used as starter cultures in food fermentations (Limsowtin et al. 2002, Southward 2002). Acid casein which is produced by direct acid addition is generally known as mineral acid casein, while casein from the starter culture process is known as lactic casein, not to be confused with the less common production of casein from the direct addition of lactic acid.

2.4.3.2 Rennet Casein

The action of rennet on casein was discussed in section 2.3.1. Commercial rennet casein production may use highly purified rennet preparations from a microbial source or the traditional calf rennet. Although the specifics of the process are different from acid casein, after the coagulation point it undergoes broadly the same process as other casein types. One point of note is that there is a loss of around 4% of protein by weight as the CMP split off from the κ-casein is lost into the whey stream (Walstra et al. 2006).

2.4.3.3 Caseinates

Acid casein products can be further processed by solubilising them in alkali and then drying them. These soluble casein powders are known as caseinates, with a pre-fix normally indicating the alkali used. Common examples are sodium caseinate, calcium caseinate and potassium caseinates. The alkalis used to produce these are NaOH, $Ca(OH)_2$ and KOH respectively. These products have useful functional properties which differ greatly depending on the alkali used. Calcium caseinate in particular is quite different to sodium and potassium caseinate, and requires the use of ammonia in the process to help solubilise the curd. Caseinate is typically a fine white power that is readily soluble in water if properly dispersed (Munro 2002).

2.4.3.4 Micellar Casein

A microfiltration membrane of the correct pore size can be used to remove almost all serum proteins from skim milk. This leaves a retentate with concentrated casein still in its micellar form. The retentate is diafiltered to further remove dissolved substances (Walstra et al. 2006). The resultant product can be dried and then reconstituted with little apparent effect on the micelles. Micellar casein is not a commercial product to the same degree as the others discussed, though its unique properties mean that it may become more common in the future. The development of cross-flow microfiltration technology may facilitate the commercialisation of native micellar casein, as well as its unique whey protein isolate co-product (Daufin et al. 2001).

2.5 Commercial use of milk proteins

Some of the earliest commercial uses for dried casein products were what are now known as the ‘technical’ applications. This was where it was used to make functional objects such as buttons and knitting needles. These types of uses have become far less common due to the ubiquity of plastics. Today, milk protein preparations are far more valuable and are primarily used in foods. Walstra et al. (2006) separates the reasons for the use of milk proteins into the following four main groups:

- Provide foods with a specific nutritive value
- Replace more expensive proteins
- Provide a product with specific physical properties
- Make novel products

2.5.1 Functional Properties

The functional property of a material is its ability to produce a specified property in the environment to which the material is applied (Walstra et al. 2006). The relatively high cost of milk proteins means that they are used often when very specific functional properties are required. Some of these and their associated foods are given in Table 8 below. The properties can often be modified by the processing conditions used to isolate them, meaning that maintaining the consistency of proteins sold into functional applications often represents a significant challenge to manufacturers.

Table 8: Functional properties of milk proteins in food systems (Singh 2002)

Functional Property	Food System
Solubility	Beverages
Emulsification	Coffee whitener, cream liqueurs, salad dressings, desserts
Foaming	Whipped toppings, shakes, mousses, cakes, meringues
Water-binding	Bread, meats, bars, custard, soups, sauces, cultured foods
Heat stability	UHT and retort-processed beverages, soups, sauces, custard
Gelation	Meats, curds, cheese, surimi, yoghurt
Acid stability	Acid beverages, fermented drinks

2.5.2 Functional properties of casein products

The solubility of a dried protein product over a range of pH, temperatures and concentrations can be a good predictor of other functional properties (Singh 2002). Caseins are completely insoluble in water without pH adjustment and so in most commercial applications they are dissolved in alkali before use (Munro 2002). Casein molecules tend to unfold in solution, which can markedly increase their viscosity (Walstra et al. 2006). An essential factor in causing a high viscosity is the association of the molecules, both by hydrophobic and electrostatic interactions (Walstra et al. 2006). Sodium caseinate is becomes exponentially more viscous with increasing protein concentration. At concentrations greater than 15% this can make it very difficult to process, though calcium caseinate does not share this property to anywhere near the same degree (Singh 2002).

Milk proteins have excellent emulsifying properties and are often used to form oil-in-water emulsions and stabilise them against physical changes (Walstra et al. 2006). Caseins and whey proteins are surface active and are rapidly adsorbed (Singh 2002). Caseinates in particular are widely used for this application in foods, where their emulsifying capabilities often see them used over cheaper protein sources. The surface activity of milk proteins also allows them to adsorb to the air-water interface during foam formation (Singh 2002). In the absence of lipid, sodium and potassium caseinates create copious and stable foams. Calcium caseinate is however not so suitable (Walstra et al. 2006). Overrun is a measure of the amount of gas that can be held within a foam. An un-denatured whey protein concentrate at only 3 or 4% concentration can achieve an overrun of 1000% with relatively good stability (Walstra et al. 2006). Caseinates generally give higher overruns than whey protein products but at the expense of foam stability (Singh 2002). Casein micelles are also able to bind large quantities of water in their native state (Singh 2002). This is important in many foods, particularly when used in viscous products such as soups and custards (Singh 2002)

2.6 Commercial lactose utilisation

Increasing use of UF in the dairy industry, and the resultant ability to standardise protein content, has led to greater planning being required around lactose utilisation. The increasing production of WPCs also means that large volumes of whey permeate are available, the major component of which is lactose. Average compositions of some lactose-rich process streams are summarised in Table 9 below. Although New Zealand is a major dairy exporter, some domestic dairy companies import lactose for protein standardisation purposes, while others export lactose and derived products. Depending on the mix of products being made at a factory at a given time, there may be a surplus or deficit of lactose on-site. Dry lactose is a shelf-stable alternative to the requirement for milk permeate to reduce protein content.

Table 9: Average composition (% by wt) of lactose-containing streams (Zadow 1984)

Product	Na	Ca	Mg	K	Ash	NPN	Lactose
Skim milk permeate	1.01	0.43	0.11	2.36	10.0	3.43	84
Cheese whey permeate	1.12	0.70	0.15	2.74	10.2	3.57	86
Whey	0.75	0.70	0.12	2.57	5.0	3.30	77

Early large-scale production of lactose involved removal of proteins from whey, followed by concentration, filtration, crystallisation and centrifugation. This resulted in about a 50% lactose recovery, while the mother liquid was sold as de-lactosed whey powder. This had to be carried out on a large scale to be viable (Zadow 1984). The physical properties of lactose make it a useful additive in the food and pharmaceutical industries. Lactose lacks sweetness compared with other sugars and has a moderate, clean flavour with no aftertaste. This means that lactose is suitable for incorporation into foods or beverages at relatively high concentrations. For example, it can be used at double the concentration of glucose or over triple the concentration of sucrose at equivalent levels of sweetness (Muir 2002). Lactose can also confer ‘body’ or mouthfeel to foods (Muir 2002). However, the nutritional uses

of lactose are limited by the high occurrence of lactose intolerance in many regions (Schaafsma 2002).

Lactose can be used as a substrate for fermentation processes and is used routinely for the production of ethanol (Muir 2002). Pharmaceutical lactose is of a sufficient quality that it conforms to the requirements of national and international pharmacopoeias, rather than the lower standard required for edible lactose (Booij 1985). Quality issues for lactose can include turbidity caused by residual proteins and calcium phosphate. The presence of riboflavin will cause a yellow colour, while minerals will increase the ash content (Booij 1985). The primary pharmaceutical use of lactose is as a tabletting agent or binder. Properties of lactose which make it widely used for this purpose are its neutral taste, low hygroscopicity, low reactivity and good flow properties (Booij 1985). A newer pharmaceutical application is inhalation-grade lactose, in which the lactose crystals are fine enough to be inhaled without causing irritation of the respiratory tract. This product is used in dry-powder inhalers, particularly for asthma and chronic obstructive pulmonary disease. Lactose can also be converted into useful derivatives. Important amongst these are lactulose, lactitol, galacto-oligosaccharides, lactobionic acid and tagatose (Mann 2002, Schaafsma 2002). Lactulose and lactitol have prebiotic effects and are widely used in the treatment of patients with chronic hepatic encephalopathy and chronic constipation as both are not absorbed in the small intestine and are fermented by the intestinal flora (Schaafsma 2002). The main use of Lactulose is as a mild laxative, and it is a growth-promoting factor for *Bifidobacterium* species (Mann 2002, Schaafsma 2002).

The various uses of lactose and derived products mean that lactose is not just a by-product of dairy processing, but can be economically significant if sufficient quantities are available in a useable state. Dairy processors can find themselves in the position of purchasing dry lactose for standardisation at certain times of the dairy season, while having to dispose of excess lactose at others. The price of lactose also varies with other dairy commodity prices so the ability of a dairy manufacturer to remain in a positive lactose balance can be economically important.

2.7 Milk Processing

The perishability and seasonal production of milk meant that traditionally, any surplus was made into more stable products for later use. Butter, ghee, fermented milks and cheese are examples of long-established products that were made for this reason. It is believed that some dried milk was also made by sun-drying (Fox 2002, Pearce 1998). While these products are still widely produced and consumed, the introduction of new technologies over the last 130 years has allowed the development of new products. Liquid products which fit into this category include sweetened condensed milk, UHT milk and ice creams (Fox 2002). These new processing technologies have also allowed the introduction of many dry products such as milk powders and milk protein products. New Zealand's dairy industry is unusual in that the vast majority of the domestic milk supply is exported. This is possible only because of the development of dried dairy products and therefore by necessity, most domestic milk is converted to shelf-stable products. The most common of these is milk powder, which is used as an ingredient in many other foods. Approximate compositions of selected dairy powders are summarised in Table 10 below.

Table 10: Composition (% w/w) of selected powders, adapted from Walstra et al. (2006)

Constituent	Wholemilk Powder	Skimmilk Powder	Whey Powder	Buttermilk Powder	Acid Casein	Sodium Caseinate
Fat	26	1	1	5	1	1
Lactose	38	51	72 - 74	46	0.1	0.1
Casein	19.5	27	0.6	26	88	92
Other Protein	5.3	6.6	8.5	8	-	-
Ash	6.3	8.5	8	8	1.8	3.5
Lactic acid	-	-	0.2 – 2	-	-	-
Water	2.5	3	3	3	10	4

2.7.1 Membrane Separation

Membrane separation is the general term for processes which use semi-permeable membranes to selectively remove solutes from fluids based on their size. The range of applications for these techniques is vast. In the dairy industry alone, membrane separation can be used for pre-concentration, partial demineralisation, protein separation, bacteria removal, brine clarification and wastewater recycling as shown in Figure 3. This represents one of the greatest technological developments in dairy processing during the latter half of the 20th century (Kelly P. M. 2002). The food sector is responsible for 20 - 30% of the total turnover of membrane manufacturers. Of this, the dairy industry represents 40%, of which over 10% is used for protein standardisation (Daufin et al. 2001). Microfiltration (MF), reverse osmosis (RO) and UF are all membrane separation techniques, the only fundamental difference between them being the pore size of the membranes used, which controls the types of materials that may cross them. UF membranes retain macromolecules such as protein, while allowing the passage of lower molecular weight substances such as lactose and minerals.

In a dairy application, membrane elements are generally of the spiral-wound type. These are effectively two sheets of membrane material separated by a supporting sheet and two mesh feed spacers. The assembly is then wrapped around a permeate collecting tube. This design allows the membrane installation to be very compact, thereby helping to minimise capital and installation costs (Kelly P. M. 2002). In an industrial setting, membrane plants are generally configured to operate continuously on a multistage recycle (MSR) system. This allows for the declining volume of retentate due to concentration to be processed in a separate stage, so that diminishing velocity and higher viscosity can be corrected for (Kelly P. M. 2002).

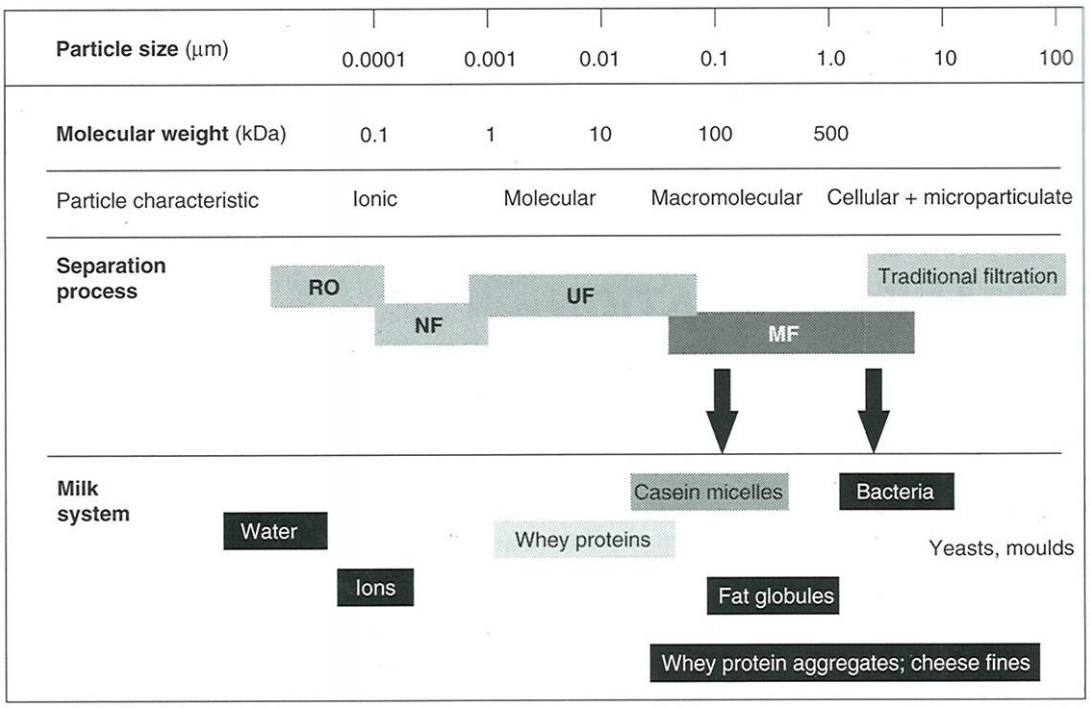

Figure 3: Overview of the membrane separation spectrum as applied to milk processing. RO, reverse osmosis; NF, nanofiltration; UF, ultrafiltration; MF, microfiltration (Kelly P. M. 2002)

2.7.2 Milk reception and liquid processing

After milking, collection and transportation, raw milk is stored on the processing site until entering the plant. The milk first undergoes a heat treatment known as pasteurisation. This is not intense enough to completely sterilise the milk, as a balance must be found between product safety and quality. Instead, pasteurisation is designed to kill all of the non spore-forming pathogenic organisms commonly found in milk. Early pasteurisation systems heated milk to 63 - 65°C for around 30 minutes, before cooling. This process was known as low-temperature long-time pasteurisation (LTLT). This has now been superseded in most cases by plate heat exchanger based systems, which heat milk to 72 - 74°C for at least 15 seconds. This approach has very high-throughput and is known as high-temperature short-time pasteurisation (HTST) (Kelly A. L. and O'Shea 2002).

The next processing step is separation of fat, creating two product streams: cream and skim milk. This is performed on a large scale by centrifugal separators, which operate almost continuously. This processing step is applied in the manufacture of nearly all dairy products, as it allows standardisation of products to a desired fat content (Walstra et al. 2006). The cream can then be further processed into a variety of products including liquid cream, butter or anhydrous milk fat. It may also be added back into the skim milk during further processing, to allow precise standardisation of fat-containing milk products. In this way, the dairy manufacturer can account for the seasonal and geographical variation in fat levels. Fat standardisation has been common practice for many decades, as traditionally fat was the most valuable milk component (Rattray and Jelen 1996).

Most commonly, the next step will be protein standardisation. It was not until 1999 that the Codex Alimentarius allowed the protein standardisation of milk powders by UF and specified a minimum protein value of 34%. The definition of protein standardisation implied by the International Dairy Federation consists of relatively small changes in protein concentration, within the limits of natural variability (Rattray and Jelen 1996). The resulting consistency of the milk or derived products is

beneficial to both milk processors and consumers. The decision to allow protein standardisation was promoted since the 1980s, largely by New Zealand and Denmark, both of whom have a large international trade in milk powders, as well as a natural protein content significantly higher than average (Burgess 1997). During standardisation, the protein content can be increased by UF. Reduction of protein content requires the addition of lactose, which effectively dilutes the protein content by increasing the non-protein solids content. This may be achieved by adding lactose made from dry lactose powder, or by addition of milk permeate. This is commonly done at the start and end of the dairy season, when protein levels elevate beyond their normal range, as shown in Figure 4. From this point any number of dairy products can be produced.

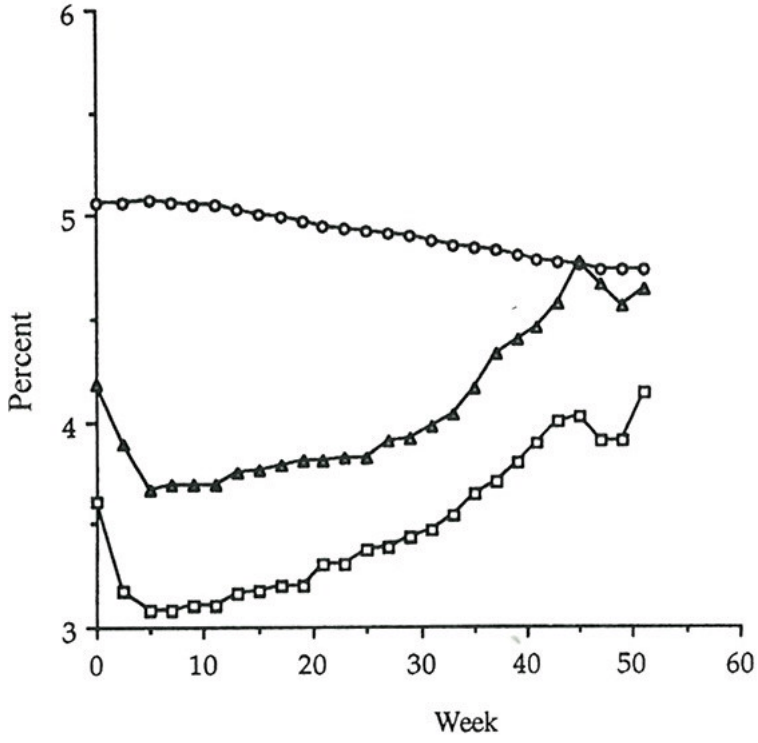

Figure 4: Changes in the concentration of fat (Δ), protein (□) and lactose (○) in milk during lactation (Fox and McSweeney 1998)

2.7.3 Milk Powder Manufacture

As stated, the New Zealand dairy industry primarily produces milk powders for export. Milk powder production is a high-throughput and largely automated process. Economies of scale mean that dairy companies have consolidated and ever-larger drying plants are becoming more common. Liquid milks, protein concentrates and some other products are dried in much the same way, depicted in Figure 5. The initial step after standardisation is a heat treatment known as pre-heating. This is performed using plate heat exchangers at temperatures of 88 - 95°C for 15 - 30 seconds. The objectives of this heat treatment are to destroy pathogenic bacteria and inactivate enzymes. An important consequence of this is the controlled denaturation of whey proteins (Pearce 1998), including the activation of the sulfhydryl groups of β-lactoglobulin, which results in an anti-oxidative effect (Caric 2002).

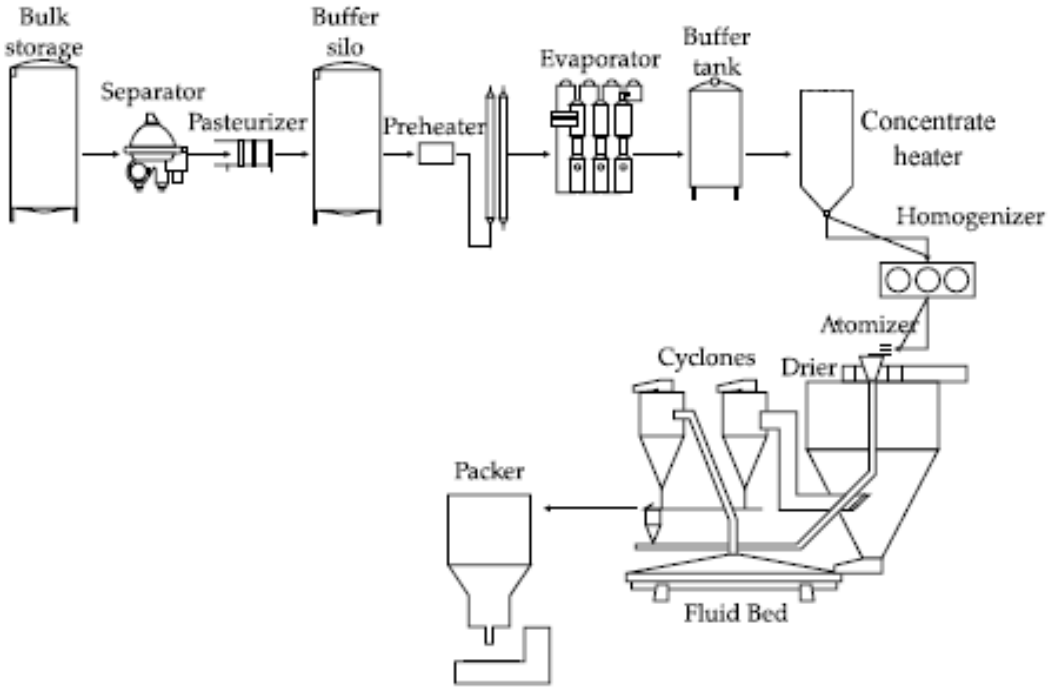

Figure 5: The milk powder manufacturing process. Adapted from Pearce (1998)

The milk next undergoes evaporation in falling film evaporators. These operate under reduced pressure, to allow the evaporation to take place at lower temperatures and reduce heat damage. Modern evaporators utilise multiple effects, with a progressive pressure reduction allowing vapor separated in the first effect to evaporate the water in the second effect and so on. This is quite efficient, using around a tenth the energy of the next drying stage. However, it is only able to concentrate the milk up to approximately 50% total solids (Pearce 1998). Older drying methods such as roller drying have largely been phased out (Caric 2002). Spray drying is now the industry standard. Spray dryers consist of a tall stainless steel cylinder with a conical lower section, though they vary somewhat in design. Heated air enters the drying chamber through the top, and exits lower down the chamber. Milk concentrate enters the top of the drying chamber as an atomised spray. The atomisation of the concentrate is achieved by one of two methods depending on dryer design. Disc dryers contain a disc rotating at 10000 - 20000 RPM into which the concentrate is fed. Nozzle dryers use a series of lances pointing into the drying chamber, tipped with nozzles that atomise the concentrate by pressurising it at 17 – 25 MPa to force it through a specially designed orifice (Caric 2002). Both dryer designs have major impacts on the nature of the milk powder particles produced. Atomising the milk concentrate increases its surface area, so that as it enters the drying chamber concurrently with the heated air there is a rapid and intensive transfer of heat from air to milk, and mass from milk to air (Caric 2002). Evaporative cooling means that during drying the milk powder actually reaches no greater temperature than the outlet air from the dryer (Pearce 1998). Some powder is entrained in the air exiting the dryer and is recovered by cyclonic or bag-house separation. In a two-stage dryer, the powder then enters a fluidised bed for its final drying. Two-stage dryers produce milk powders with better reconstitution properties and have improved heat utilisation compared to single-stage dryers (Caric 2002). The primary advantage of spray drying is the gentle treatment of the milk, by reducing heat exposure and the low residence time. As well, the high level of automation possible with spray drying reduces process costs. Disadvantages include its relatively high energy usage and its large up-front capital cost.

2.7.4 Acid casein manufacture

The commercial production of casein has occurred for most of the 20th century. Until 1960, the majority of this was for non-food applications. This has since however changed to its use now being predominantly in foods, which has resulted in greater requirements for quality and purity (Southward 2002). Many advances have been made during this time, due both to an increased understanding of milk and casein itself, as well as the availability of new processing technologies and equipment. The manufacture of the major types of casein all involve precipitation of casein from skim milk, heating, whey separation, multiple-stage washing, dewatering and drying of the resultant precipitate (Mulvihill 1989). The lactic acid casein process is depicted in Figure 6 below. The focus of this section will be on mineral acid casein, precipitated by sulphuric acid, as this is the primary product of the plant being studied. Sulphuric is the standard acid used for this purpose in New Zealand, while hydrochloric acid precipitation is most commonly used overseas (Southward 2002). Skim milk can be considered the basic material from which all casein products are made (Southward 1998).

2.7.4.1 Precipitation

The initial stages of casein manufacture are known as wet-processing and the post-dewatering stages as dry processing. In mineral acid casein production, acid is added to skim milk as it flows through static mixers welded into the inside of the line. The milk from this point is referred to as the separate components curd and whey, or collectively as the coagulum. This is a key control point for the process, as the rate of acid addition is altered by the operators depending on the pH attained. During steady state running this should not require much adjustment.

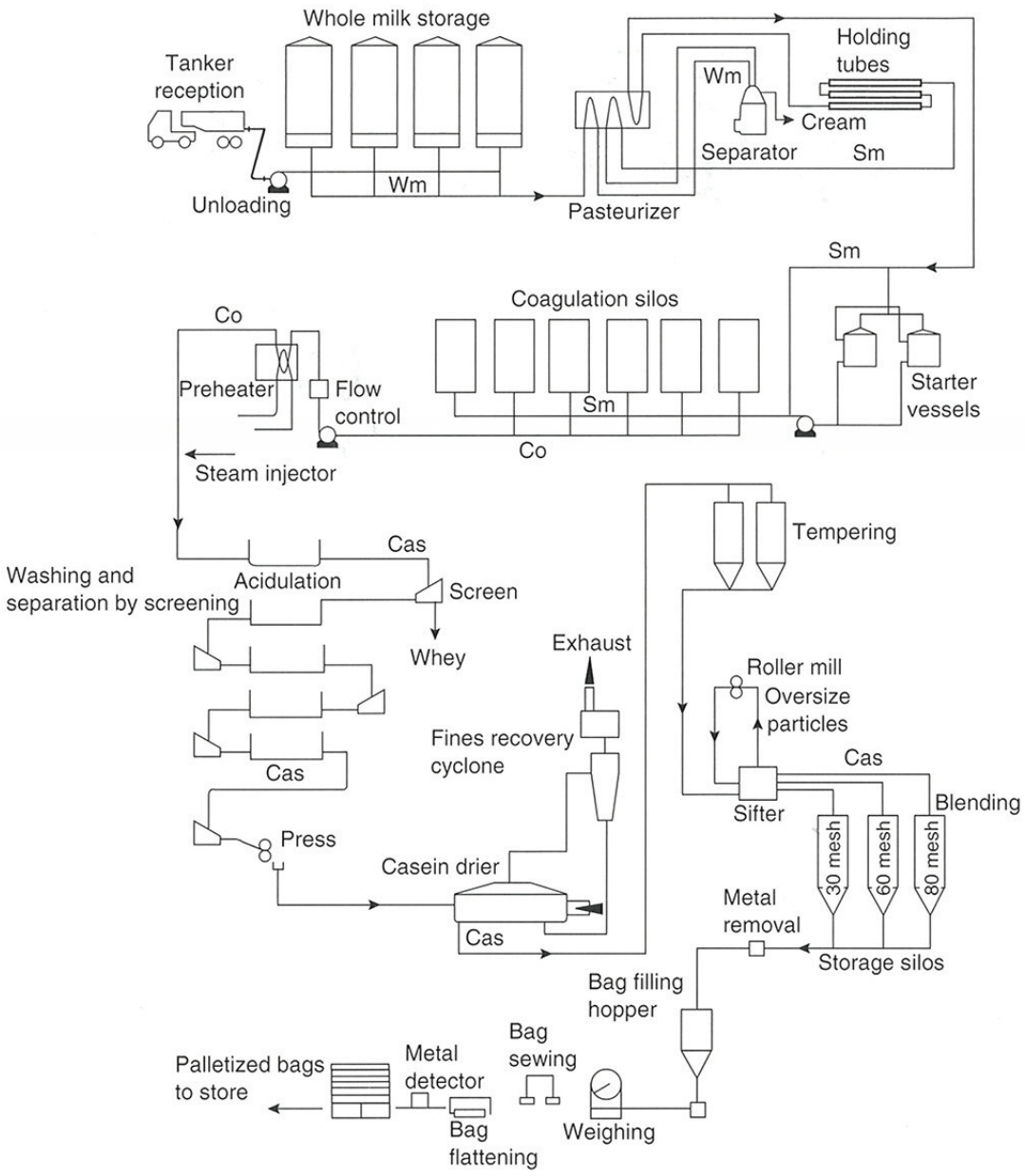

Figure 6: Generalised outline of lactic casein production from skim milk. Annotations: Wm -whole milk, Sm - skim milk, Co - coagulum, Cas - casein (Munro 2002)

2.7.4.2 Cooking

The next stage of the process is known as "cooking" and involves the controlled heating of the curd. Heat causes the curd particles to contract and expel trapped whey, a phenomenon known as syneresis, as well as making it more cohesive and resistant to breakdown during further processing steps. Heating also promotes agglomeration of the casein granules (Southward 2002) which helps prevent the loss of fine particles. The cooking temperature in generally within the range 50 - 57°C, though this may be changed depending on downstream conditions. There are a variety of plant configurations available for cooking of casein. The most common of these are injection of steam directly into the pipeline carrying acidified milk, indirect heating by heat exchanger or some combination of the two (Southward 2002). The plant being studied uses a tubular heat exchanger in conjunction with direct steam injection, a common design. The coagulum then enters the 'low-velocity cooker', a larger diameter pipe with a 10 - 60 second residence time (Southward 2002) that allows the casein to agglomerate due to the less turbulent conditions, resulting in reduced generation of fine particles which may otherwise be lost during subsequent processing steps.

2.7.4.3 Acidulation

After cooking the coagulum begins 'acidulation'. This takes place in a long vat, gently agitated by paddles. The volume of the acidulation vat is sufficient to provide a residence time of at least 10 minutes to all of the coagulum. The purpose of acidulation is to allow time for the dissociation of calcium and phosphate from the casein. Another important aspect of this stage is that it allows continued agglomeration of the casein. Acidulation is especially important to allow equilibrium to be attained between the calcium in the curd and in the whey (Southward 2002). Ideally, the final preparation should contain all of the casein and none of the colloidal calcium phosphate (Walstra et al. 2006). The acidulation vat provides a useful point for assessment of the casein. If curd taken from the vat is hard or rubbery, the pH is probably too high and the acid addition rate may need to be increased. Alternatively, if the curd is overly soft the acid may need to be decreased. The ideal curd at this

stage is soft and spongy but still able to maintain its structure, and should readily expel whey when squeezed.

2.7.4.4 De-wheying

The casein now must be separated from the whey stream in which it is entrained. This is sometimes performed by running the coagulum over screens which retain the curd and allow the whey to drain through. This does not result in a particularly good recovery of whey, as no direct pressure is applied to the curd to expel it. The modern method of de-wheying is a horizontal solid-bowl centrifuge, known as a decanter (Southward 2002). The separated whey stream is then put through a clarifying centrifuge to remove any casein fines before being concentrated by UF for further processing, while the de-wheyed curd continues on to be washed.

2.7.4.5 Washing

The casein stream at this stage consists of casein curd, with some whey trapped between or within curd particles. Impurities contained within the whey, which can affect final casein quality, include lactose, whey proteins, minerals and residual acid. The properties of the curd produced prior to entering the washing system greatly affect the washing stage. With a curd that is too fine, unacceptable amounts of protein will be lost to the wash water and wasted. Conversely, a tough curd will make it more difficult for impurities to diffuse out into the wash water. Washing is a water intensive part of the process and is one of the most heavily studied parts of commercial casein manufacture. Washing in modern casein plants is done by a counter-current system. Under this, the purest wash water encounters curd which has already been mostly washed, while the wash water most concentrated with impurities from previous washing encounters the unwashed curd. This results in the most efficient use of wash water.

2.7.4.6 Dewatering

Residual moisture is removed from the curd prior to drying in a process stage known as dewatering. The washing temperature is important to dewatering as it affects curd texture. With a high wash water temperature, more water is released during dewatering but a tougher, more plastic curd results which is harder to dry. Washing temperature therefore needs to be controlled to optimise the minimum moisture requirement and curd friability (Southward 2002). Efficient dewatering lowers the evaporative load required of the dryer, and results in improved process economics. The plant being studied uses a Conturbex screen centrifuge. This consists of a rotating mesh cone into which the curd is fed. The curd works its way to the wide end of the cone while liquid is removed from the narrow end and through the mesh. This results in a friable curd containing around 50% moisture.

2.7.4.7 Drying

A number of drier types are suitable for casein drying, including pneumatic-conveying ring driers and attrition driers. Most commonly used though are horizontal vibrating fluid-bed driers (Southward 2002). These consist of two levels of perforated trays through which heated air (75 - 115°C) is blown upwards, which in combination with the shaking of the drier, fluidises the casein. The gradually drying casein works its way along the top deck before falling through a rotating grinder, which breaks up any large lumps, then working its way back along the lower deck. Adjustable weirs at the end of the decks set the product depth at each level, and thereby help control the level of fluidisation and residence time. The major control point for the dryer is the outlet temperature. Product off the end of the dryer is closely monitored to be within a specified moisture range. Most moisture is removed during early stages of drying as it evaporates from the particle surface. The later stages require transfer of moisture from the centre to the surface of particles, which is a much slower process (Southward 2002). The robust nature of dried casein results in some potential problems during drying. These include case hardening, in which only the outer layer of the particle is left dried and rubbery, with moisture trapped in the particle.

2.7.4.8 Tempering and Milling

The casein is cooled by air while being conveyed to tempering bins. The purpose of tempering is to provide sufficient time (8 - 24 hours) for moisture equilibration to occur within and between casein particles (Southward 2002). Without this step, moisture remains trapped within the relatively large and robust casein particles. This results in plasticised or rubbery casein and reduces the effectiveness of milling. The casein is then milled and sieved into various particle size fractions. Particle size of casein is generally measured using 'mesh' sizes, which denotes the number of holes per inch in a sieve. Common sizes for casein are 30 and 80 mesh, which correspond to 600 µm and 180 µm apertures respectively. After milling, the casein is blended to ensure uniformity within each batch. It is generally packed into the dairy industry standard 25 kg bags, though much larger bulk bags can also be used. Casein is shelf stable for several years, when stored at temperatures below 20°C and relative humidity below 70%.

2.7.5 Caseinate Manufacture

The caseinate process is the neutralisation of acid casein with alkali (Southward 2002). This can be performed on dried casein, or using dewatered curd directly from the casein process outlined above. The casein or curd is suspended and hydrated in water before being passed through two colloid mills. In the second of these mills, the alkali is injected directly into the hydrated casein. The caseinating reaction then takes place in a heated and agitated reaction vessel. The caseinate solution moves through a series of these vessels until the reaction is complete and the product is spray dried. Caseinates are typically made at concentrations of up to 20% solids (Southward 2002). The viscosity of sodium caseinate in particular makes exceeding this concentration impractical. Exposure of protein to high pH and high temperatures as found in many food processing operations, and particularly during caseinate manufacture, can result in the formation of cross-linked amino acids. These include lysinoalanine (LAL), ornithinoalanine, lanthionine and methyl-lanthionine (Friedman 1999). The presence of LAL, as well as affecting the digestibility and nutritional quality of the protein, has been reported to enlarge the nuclei of kidney cells in rats (Friedman 1999). Because of this, measurement of LAL content is often used as an indicator of the harshness of processing. Avoiding the use of high pH, high temperatures and limiting the proteins exposure time if these treatments are necessary, are essential to minimise LAL content. Customer specifications for commercial protein products often have stated maximum LAL concentrations, generally in the low part per million range.

2.8 Ultrafiltration and Casein Making

This work investigates the effect of using ultrafiltered milk for casein making. The overall aim is the elucidation of conditions under which good quality casein can be made from milk which has been ultrafiltered to remove most of the lactose. UF of milk causes the loss of lactose and soluble salts to the permeate stream, thereby increasing the protein content of the retentate on a solids basis. This means that lactose from the permeate can be recovered in a usable state and the amount of lactose and minerals to be washed out of the casein during washing has also been reduced. In practice, the casein plant being studied has made casein from milk at a volumetric concentration factor (VCF) of up to two, while anecdotal information from other plants is that a maximum VCF of 1.5 – 1.7 is more appropriate. Ideally, the milk would be concentrated several times further, to maximise the recovery of lactose and increase the throughput of the plant, but currently this is not the case. This is because more concentrated retentates result in curd property changes which cause problems in the plant. Primarily, high concentration factors cause the formation of an overly tough curd, which is difficult to wash effectively, and in the worst cases can cause plant blockages. Additionally, the VCF used in the plant already needs to be adjusted over the course of the year to account for the seasonal variation in protein levels. Any change in the size distribution of curd particles is important as efficient solid-liquid separation at the de-wheying, washing and de-watering steps depends on particle size. Secondly, mass transfer from the particles in the washing and drying steps depends on particle size (Teo et al. 1997). The cause of the tough curd phenomenon is believed to be reduced solubility of colloidal calcium phosphate after concentration. Although all of the colloidal calcium phosphate is normally solubilised in milk after the pH is reduced to 4.6, in milk retentate there is much less water present. Figure 7 shows a clear reduction in the proportion of calcium that becomes soluble at decreasing pH as the concentration of casein increases. Of particular interest is the inset, which shows the calcium solubilised at pH 4.6 as a function of casein concentration.

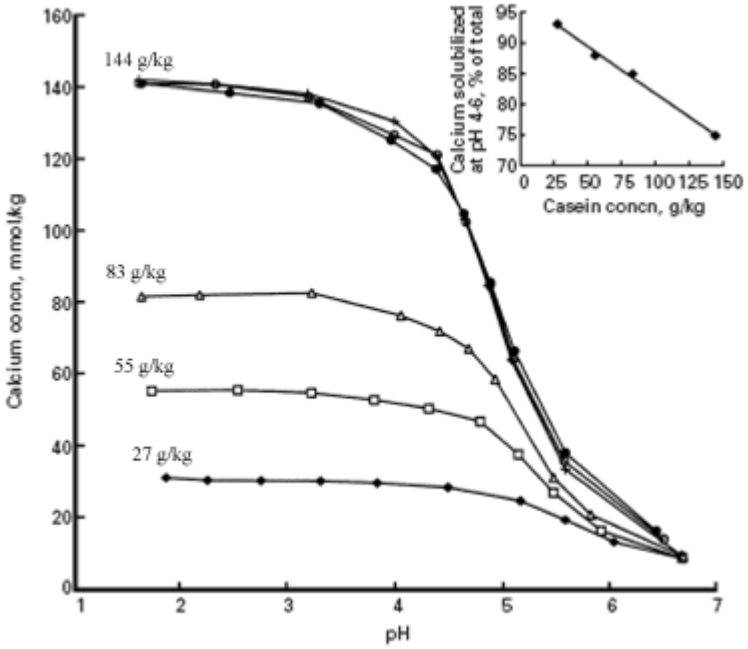

Figure 7: Calcium concentration in the aqueous phase of casein micelle suspensions as a function of pH. Casein concentrations of each data series have been recorded above the first data point of each (Le Graët and Gaucheron 1999)

Richert (1975) specified that casein preferably be precipitated at pH 4.3 to 4.5 as this results in a lower ash content in the final product, due to increased solubilisation of phosphate at these pH levels and preferable curd handling properties at pH 4.3. Presumably, this is because a more 'sloppy' curd performed well in the systems used at the time. Although this is well below the optimal precipitation pH of 4.6, it indicates that lower pH has in some cases been a valid option when retention of minerals in the curd is a problem. The use of UF has been described as "a revolution in cheesemaking" (Mistry and Maubois 1993) and as a result there have been numerous studies of UF retentates in cheese applications published. However, there appears to be almost no published work directly covering its use in commercial casein production available. However, a number of insights can be gained from the work into the effect of UF on cheese making and then applied to elements of the casein process.

When milk is ultrafiltered at its normal pH (6.7), mineral salts (Ca, Mg, P) bound to casein micelles are concentrated in the same proportion as the proteins. This increases the buffering capacity of the retentates and consequently modifies basic parameters of the cheesemaking process such as acidification kinetics and final pH value (Mistry and Maubois 1993). This point is equally true of casein making, which as a continuous process is potentially even more at risk from deviations from normal conditions than carefully monitored batch cheesemaking. Some of these effects could be mitigated by pH adjustment prior to UF. Reduction of milk pH from 6.6 to 6.0 and 5.6 increases the calcium content of UF permeate from 0.38 g/kg to 0.50 g/kg and 0.80 g/kg respectively. Consequently, a UF retentate at a VCF of five obtained at pH 5.6 has a Ca content 2.6 times that in milk instead of 3.8 times for the UF retentate obtained at pH 6.6 (Le Graët and Gaucheron 1999). Other options include the addition of NaCl (0.5 - 0.9%) to UF retentate during or after UF to increase the ionic strength, reducing the ionisation of casein phosphoseryl groups and consequently leading to solubilisation of colloidal calcium phosphate in the permeate (up to 15 - 18% depending on the pH and amount of NaCl added) (Mistry and Maubois 1993). Although these approaches may not be practical for the purposes of this work, it is clear that there are various means of avoiding the problem of mineral retention in milk retentate sourced products.

2.9 Conclusion

This chapter introduced a range of milk topics, with the aim of giving context to the more detailed discussion of the function, structure and properties of casein. This also applied to casein processing, with the more common milk powder process being covered due to its cross-over with the preparation of the skim milk required for casein manufacture.

The ongoing work to uncover the precise structure of the casein micelle and the nature of the interaction between the individual casein proteins was also reviewed. For the purpose of this work, the acid precipitation of casein is already understood well enough as the departure of CCP from the micelle structure is known to be key to the phenomenon. There is little information available in the scientific literature on the effect of UF prior to casein manufacture. Some crossover however exists with cheese-making, so some of the information available on that topic is of use. The work of Le Graët et al. (1999) provides analytical data which supports in-plant observations. Overall, the practical effect on casein manufacture of UF retentate use has not been published though may have been studied by private companies. This means that initially the effect of the problem has to be examined, as most of the information on the effects comes from observations or informal discussions. This thesis aims to clarify these points and find a solution whereby the desire for increased process efficiency and lactose recovery can be achieved without compromising product quality.

Chapter 3. Materials and Methods

3.1 Introduction

This chapter presents the analytical methods used in this research. These include standard dairy industry methods, some modified, which were used to characterise the products used and produced in both the commercial process and laboratory experiments. Other methods were also used to provide additional information to that normally required during the production of casein products.

3.2 Calcium by complexometric titration

The method determines calcium content using a complexometric back-titration. A known quantity of EDTA solution is added to the dissolved sample, which is then titrated with a calcium chloride solution in the presence of indicator. This test has been widely applied to calcium measurement of milk and milk powder but is also suitable for protein products with some changes to the sample preparation. The test method is based on an EDTA titration described by Vogel (1989).

Testing procedure

Casein was weighed accurately into a 150 ml Erlenmeyer flask and mixed with 30 ml deionised water. 0.1 M hydrochloric acid was then added to solubilise the sample. For casein testing the particles must be finely milled and may require heating up to 60°C under agitation before the sample is completely dissolved. A volume of 0.010 M EDTA solution 10 ml in excess of that sufficient to complex all of the calcium present was added. Magnesium sulfate solution was then added before the pH was adjusted to exceed 10 by adding 8 M sodium hydroxide. Under these conditions, all of the magnesium is present as $Mg(OH)_2$ instead of an EDTA complex since calcium forms a more stable complex with EDTA than magnesium does (Kaur 2007). After sufficient mixing, 0.1 g of Patton and Reeders indicator (2-hydroxy-1-(2-hydroxy-4-sulpho-1-naphthylazo)-3-napthoic acid) was added and the sample was titrated with 0.01 M $CaCl_2$. A subtle colour change from purple to pink indicates the end-point. Calcium concentration was calculated by:

Where:

V = Volume, in millilitres, of standard EDTA solution added to sample

T_1 = Volume, in millilitres, of calcium chloride solution used in the back titration

W = Sample weight in grams

3.3 Calcium by ion selective electrode

An ion selective electrode (ISE) generates an electrical potential from the activity of a specific ion in solution. This can then be measured by a device which gives a numerical output.

Use with titration

The electrode was used in combination with the EDTA titration in an attempt to improve end-point detection. This was attempted to see if the two methods could provide higher sensitivity and greater accuracy, particularly for the relatively dilute whey samples. The titration was carried out as detailed earlier, aside from the presence of the calcium electrode in the sample. The volume of titrant added was recorded with the corresponding electrical potential in mV given by the electrode across a range of values from before to after the endpoint. For measurement, an Orion ionplus® calcium combination electrode was used. This is a single junction combination electrode. A Metrohm 744 pH meter operating in mV mode was used as the output device.

3.4 Lactose by Phenol Sulphuric method

Lactose content is an important property for casein products as residual lactose can cause quality issues in the casein, the main one being a noticeable brown colour. Lactose is one of the key impurities removed during curd washing and as such, residual lactose is a good indicator of washing efficiency. This test utilises the reaction of carbohydrates with phenol in sulfuric acid. Casein samples were dissolved in sodium bicarbonate solution to release lactose, then removed by acid precipitation. Although the test measures all soluble carbohydrates, in this case that is effectively only lactose. This test method is from the International Dairy Federation Standard 106:1982 *Casein and Caseinates. Determination of Lactose content. Photometric method.*

Testing Procedure

1 g dry casein samples were weighed into 75 ml stoppered glass tubes to which 25 ml 0.4% $NaHCO_3$ was added. The tubes were placed in a 65°C waterbath until the sample was dissolved. After cooling, the pH was adjusted down to 4.4 – 4.6 with 0.05 M H_2SO_4. The sample volume was made up to 50 ml with water and the precipitated casein was filtered off. The filtrate at this point was checked for clarity to ensure that no casein remained in solution. In a test tube 1 ml of the filtrate was mixed with 1 ml phenol before 5 ml 98% H_2SO_4 was rapidly added. The reaction gives a pink-orange colour which was measured in a spectrophotometer at 490 nm. A standard curve was constructed using lactose standards at 20, 40 and 60 µg/ml, from which the lactose concentration of the original casein could be calculated using:

Where:

c = The lactose content as obtained from the calibration graph, expressed in grams.

W = The weight, in grams, of the sample.

50 = Factor to account for the 50 ml sample size.

3.5 Moisture

The reference method for moisture is the gravimetric method and is suitable for use on all milk protein products. Moisture is calculated from the weight loss of the sample during drying. The method is based on the International Dairy Federation Provisional Standard 78C:1991.

Testing Procedure

To ensure the sample was representative, the bulk sample was thoroughly mixed. A 50 g sub-sample was passed through a test sieve with a nominal aperture size of 500 µm to check the particle size was small enough for moisture to diffuse out during drying. A sample unable to pass the sieve would require grinding. An empty metal drying dish was dried for at least an hour in an oven (102±2°C) then the weight recorded after cooling in a dessicator. 5±0.0001 g casein was placed in the dish before it was put back in the oven for three hours. The dish was then allowed to cool in the dessicator before being weighed and placed back in the oven for one hour before being re-weighed. Once constant weight was achieved the moisture was calculated as follows:

Where:

W_0 = The weight, in grams, of the empty dish

W_1 = The weight, in grams, of the dish and un-dried sample

W_2 = The weight, in grams, of the dish and dried sample

3.6 Spectroscopic rapid analysis

Near-infrared (NIR) and mid-infrared (MIR) spectroscopy are analytical methods that utilise the absorption of light in the infra-red area of the spectrum, used here for measuring total solids, protein and lactose. These are the wavelengths from approximately 0.8 – 2.5 µm for NIR and 2.5 - 20.0 µm for MIR. If a robust calibration is available, results can be obtained almost instantly. This has made NIR analysis widely used as a cost and time saving method for industry.

Testing Procedure

Milk and milk retentate samples were analysed for total solids, protein and, for MPC85, lactose on a Milkoscan FT2 (Foss Analytical, Denmark). This instrument uses a Fourier transform infrared (FTIR) interferometer which scans the MIR spectrum using a diode laser. No sample preparation was required as the instrument is designed for the analysis of viscous fluids. The instrument was controlled by an external PC running Foss Integrator software. The software package was capable of partial least squares (PLS), modified PLS calibrations and principal component analysis. Instrument calibrations were created and maintained using the Foss WinISI software package in conjunction with reference results obtained by IDF standard methods from an accredited dairy testing laboratory.

3.7 Protein by Kjeldahl method

Reference protein content for all sample types was calculated from total nitrogen content determined by the Kjeldahl method. This consists of digestion of the sample using 98% sulfuric acid, with copper (II) sulfate present as a catalyst, to convert organic nitrogen into ammonium sulfate. After digestion the ammonium sulfate is converted to ammonia by heating with sodium hydroxide. The ammonia is steam distilled into an excess of boric acid solution to form borate, the quantity of which is then determined by titration with hydrochloric acid. The method does not give a true protein value as the non-protein nitrogen (NPN) component of the product is included. The NPN value can be tested separately and subtracted if required. The total nitrogen value obtained by this method is converted to protein content by multiplication by 6.38. This is the agreed conversion factor for bulk dairy protein, though individual proteins may require different values. This method is based on International Dairy Federation Standard 20-2B:2001.

Testing Procedure

The sample was initially weighed and placed into a glass digestion tube with potassium sulfate to raise the boiling point of the acid and copper sulfate catalyst. Retentates required 1 ml of sample, while powders expected to be over 80% protein (all of those tested in this work) required 0.2 g of sample. 15 ml concentrated sulfuric acid was added to the tubes before being placed in a Foss Tecator digestion block (Foss Analytical, Denmark) where they were progressively heated up to 425°C and held for at least 135 minutes. The conversion of ammonium sulfate and the acid titration were performed automatically in a Kjeltech 8400 analyser unit with a Kjeltech 8420 sampler unit (Foss Analytical, Denmark). The instrument also calculates protein values automatically but the calculation used is as follows:

Where:

Vs = The volume, in millilitres, of acid titrant used in the determination

Vb = The volume, in millilitres, of acid titrant used in the blank sample test

M = The exact molarity of the standard volumetric solution of acid

W = The sample mass, in grams

For dairy products, the total nitrogen value must be multiplied by 6.38 to give the protein content.

3.8 Protein Profile by RP-HPLC

The protein profiles of skim milk, UF retentate and casein samples were analysed by reversed phase high performance liquid chromatography (RP-HPLC). RP-HPLC separates components based on their hydrophobicities and can be applied well to milk proteins.

Buffer preparation

The loading buffer (buffer A) used was an aqueous solution of 0.1% TFA. This was prepared by adding 1 ml of TFA to 999 ml water. Elution buffer (buffer B) was 0.1% TFA in acetonitrile, prepared by adding 0.5 ml TFA to 499.5 ml acetonitrile. Sample buffer was prepared by adding 0.78 g Tris-HCl, 0.65 g tri-sodium citrate and 30 mg DDT in 42 ml water. Once these were dissolved, 24 g urea was added. Dilution buffer was prepared by dissolving 18 g urea in 45 ml 0.1% TFA (buffer A).

Sample preparation

Preparation of casein samples for analysis used a method based on that of Alim et al. (2005). 50 mg of dried casein sample was weighed accurately into a 35 ml sample container and 2 ml of sample buffer was added. These were mixed by inversion for an hour in an automatic horizontal mixer. These samples were then centrifuged at 14,000 g for 10 min before being diluted by adding 200 µl of sample into 800 µl of dilution buffer and mixed thoroughly. These were then centrifuged at 14,000 g for 10 min before being transferred to 1.5 ml glass vials with rubber septum closures for HPLC analysis.

Analytical Method

The HPLC used was a Shimadzu modular system consisting of an SCL-10AVP system controller, LC-10ADVP binary pump unit, FCV-10ALVP gradient valve, SIL-10ADVP auto-injector, CTO-10AVP columns oven and SPD-M10AVP diode array detector. HPLC system control was by external PC running Class VP 7.0 software (Shimadzu Corporation. Kyoto, Japan). The separation itself used a Zorbax 300SB C8 analytical column with a length of 150 mm and internal diameter of 4.6 mm. (3.5 µm, 300 Å. Agilent Technologies, USA) which was maintained at 45°C during the analysis. The gradient conditions used were an in-house method developed from published methods (Bonfatti et al. 2008, Bordin et al. 2001). The method used a flow rate of 0.48 ml/min and the sample injection volume was 10 µl. Peaks were identified by comparison to standard profiles obtained from previous work using protein standards.

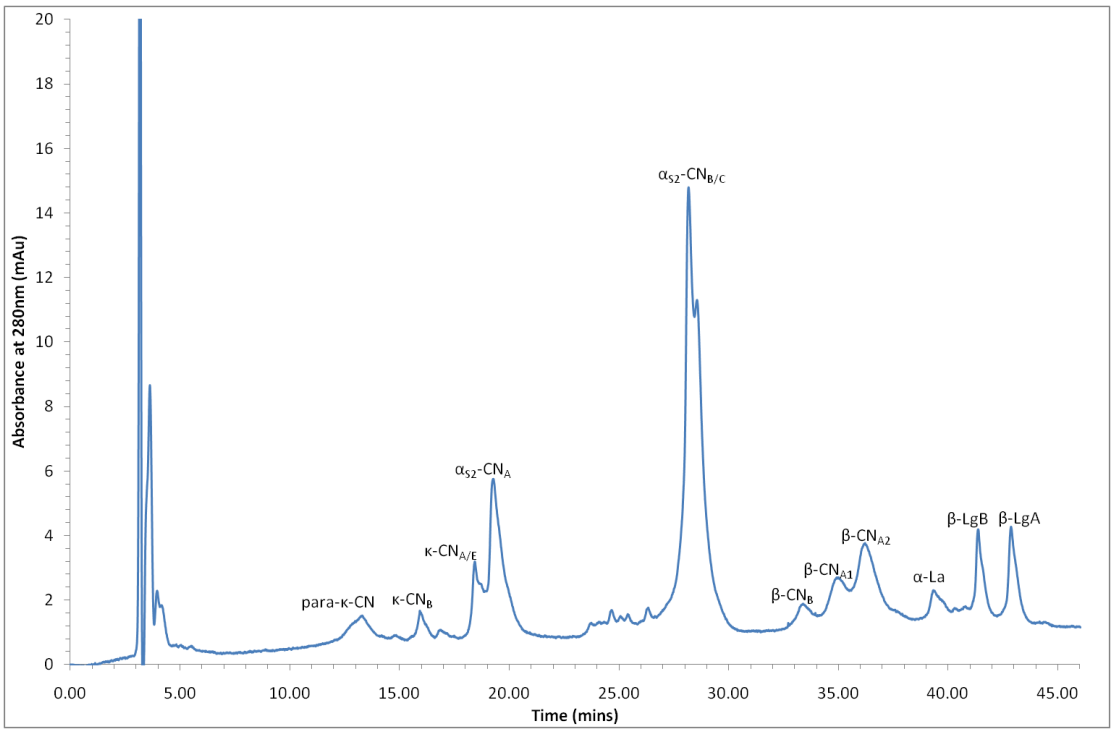

Figure 8: Example chromatogram showing milk protein peaks in skim milk

3.9 Total solids

The solids content of liquid milk and retentate samples was measured by the gravimetric method. Moisture is initially removed from the sample by evaporation on a steam bath before oven drying. Total solids are the percentage of residue remaining as a percentage of the initial sample weight. The method is based on the International Dairy Federation Standard 21B:1987.

Testing procedure

A dry, empty metal drying dish was accurately weighed. Approximately 4 ml of milk of 1 g of concentrated milk was then placed in the dish and the weight accurately recorded. The dish was placed on a boiling waterbath for 30 minutes before being transferred to a drying oven (102 ±2°C) for two hours before being weighed. The dish was then placed back in the oven for an hour before re-weighing. This was repeated until successive weighing steps differed by less than 0.1 mg. The result was calculated as follows:

$$\frac{\qquad}{\qquad}$$

Where:

W_0 = The weight, in grams, of the empty dish

W_1 = The weight, in grams, of the dish and un-dried sample

W_2 = The weight, in grams, of the dish and dried sample

This procedure is commonly used in conjunction with fat testing using the Roese-Gottlieb method to report the non-fat solid (SNF) content of dairy products.

3.10 Water activity

Water activity (a_w) is a measure of the energy status of the water in a system, and is therefore more useful than water content as a measure of perishability in foods. It represents only the 'unbound' water in a system, or that which is free to react. Water activity measurements were used as a convenient alternative to full moisture analysis by drying due to the speed and non-destructive nature of the test. These measurements were carried out using an Aqualab 3TE (Decagon Devices, Inc. Washington, USA.).

Testing procedure

The instrument measures the water activity of a sample by the chilled-mirror dew point technique. In this, the sample is equilibrated with the headspace of a sealed chamber containing a mirror. The chamber is then chilled until condensation is detected on the mirror and the temperature at which this takes place is recorded. The sample is cycled through this a number of times to give a more accurate reading. The temperature of the sample was controlled to 25°C and repeatability was to within $a_w \pm 0.003$.

3.11 Particle size analysis by laser diffraction

Particle size of dried samples was measured by a Mastersizer MS2000 (Malvern Instruments, UK). This instrument passes the sample through a laser beam and then utilises Mie theory to calculate the particle size distribution from the scattering pattern produced.

Procedure

The wet dispersion cell of the Mastersizer was used to disperse the sample and pass it through the measurement cell. The dispersant used was de-aerated isopropanol. Samples were well mixed and representatively sampled before sufficient mass was added to dispersant flowing through the wet cell to achieve between 10% and 20% obscuration of the laser, as this is the optimal measurement level. The scattering pattern was then measured for 20 seconds before conversion to a numerical particle size distribution by Mastersizer 2000 software, version 5.30 (Malvern Instruments, UK).

3.12 Viscosity by glass capillary viscometer

The viscosity of liquids can be measured by a range of methods. Glass capillary viscometers, sometimes known as Ostwald viscometers, are able to measure relatively small sample volumes to a high degree of accuracy.

Procedure

Temperature control is critically important in accurate viscosity measurement. This is difficult using the capillary method as a line of sight must be maintained with the viscometer. The apparatus used consisted of a wide-mouthed two litre conical flask, half submerged in a 25°C water bath. This meant that the viscometer was clearly visible while a small pump was used to circulate water between the flask and water bath at a rate of 10 litres per minute.

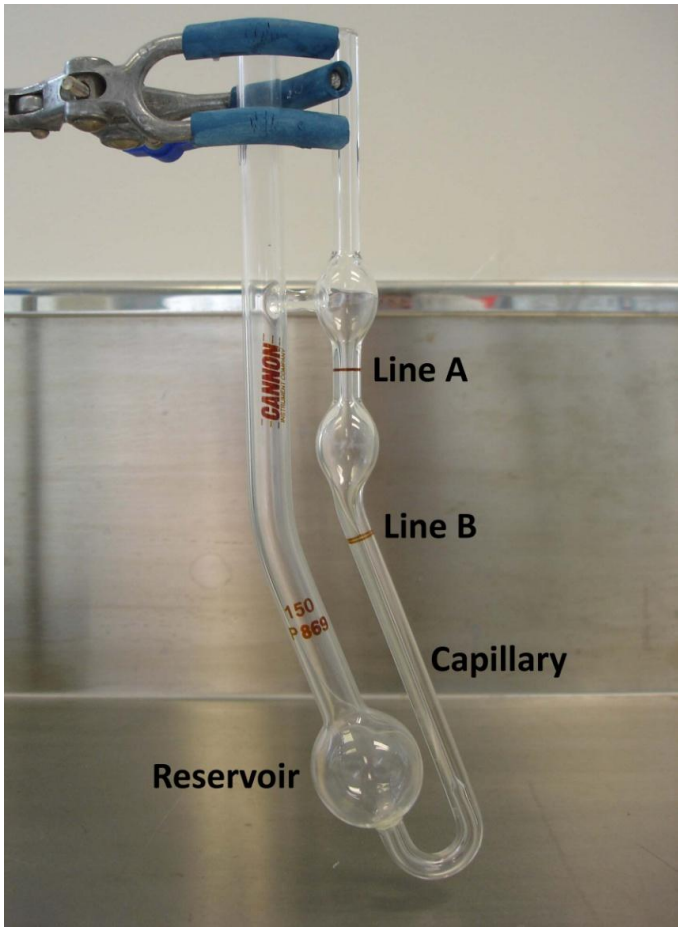

Figure 9: Glass capillary viscometer

Chapter 4. Assessment of Current Casein Process

4.1 Introduction

The primary aim of this work was to determine the effect of high pre-concentration of skim milk retentates on the casein products subsequently made from them. If possible, this would lead to a set of operating conditions under which the maximum amount of lactose could be recovered from the milk without degrading the quality of the resultant products. The initial stage was to characterise the effect of processing on the properties of the current casein so that any changes made later could be assessed and compared. Another aspect was that it would provide more data from which a lab-scale procedure to mimic the processing plant could be developed and tested. Although casein is thoroughly tested, the testing is focused mainly on the quality of the final product, and does not look at changes during processing in detail. This meant that useful methods of establishing the effects of milk composition on the casein process had to be found.

The casein plant studied in this work was broadly outlined in the literature review. The limitations imposed by commercial production meant that at this stage there was no ability to alter the running conditions, so in-process product sampling followed by laboratory testing of manufacturing runs was the most practical way to characterise the current product.

Although the casein process involves multiple unit operations all of which had the potential to be of interest, key points of the process to be monitored had to be selected. As the pre-concentration conditions and the resultant effect on calcium solubility were expected to change the firmness of the precipitated curd, only sample points which would reveal the effect of these conditions on the final casein product were used.

As stated earlier, this specific area does not appear to have been covered greatly in the scientific literature. Studies on the effect of parameters such as temperature, time and concentration on acid milk gels have been performed (Anema 2008, Gastaldi et al. 1997). The effect of precipitation temperature and pH on casein curd particle size and calcium content has been studied. This has a small degree of crossover with this work, though even the authors of that work acknowledged that the literature contained little fundamental information on many casein processing steps (Jablonka and Munro 1985).

4.2 Results

To gather information on normal plant running conditions, a set of standard sample points was defined.

Retentate: Sample of UF retentate prior to acid addition, taken either from end of UF membranes for a spot sample, or a storage silo.

Acidulation: Taken from a fixed point at the start of the acidulation vat. Sample consists of curd and whey. Curd samples were tested either directly as collected or rinsed to remove whey before analysis.

WS1: Wash screen 1, the first screen separating wash water from curd.

WS2: Wash screen 2, the second screen separating wash water from curd.

WS3: Wash screen 3, the third screen separating wash water from curd.

Conturbex: Sample point at base of screen bowl centrifuge used for dewatering. These samples were curd containing ~50% residual moisture.

Drier: Un-milled casein sample from end of Pillet drier.

Wet curd samples taken through the process from acidulation to dewatering were dried to $a_w < 0.400$ and manually crushed. They were then tested for residual lactose and calcium content.

4.2.1 Pre-concentration conditions

A standard casein production run was selected for monitoring. Samples of the low VCF UF retentate being used were taken from the pre-acidification balance tank.

Table 11: RP-HPLC peak areas for skim milk and low concentration UF retentate

Peak	Peak Area		Area %	
	Skim milk	UF Retentate	Skim	UF Retentate
para-κ-casein	63203	78299	3.88	2.93
κ-casein B	29034	45664	1.78	1.71
κ-casein A/E	76607	145032	4.70	5.44
αS2-casein A	217473	352476	13.34	13.21
αS1-casein B/C	750883	1302654	46.05	48.83
β-caseinB	38141	59308	2.34	2.22
β-casein A1	83743	149366	5.14	5.60
β-casein A2	174253	259842	10.69	9.74
α-lactalbumin	37621	43010	2.31	1.61
β-lactoglobulin B	75371	98195	4.62	3.68
β-lactoglobulin A	84321	134146	5.17	5.03

Table 11 shows the peak areas obtained for this retentate as well as skim milk from the same time period. It should be noted that the skim milk sample was not the actual parent milk of the retentate but was taken at approximately the same time.

Figure 10 below shows clearly the increase in protein concentration with no apparent change to the overall proportions of individual proteins.

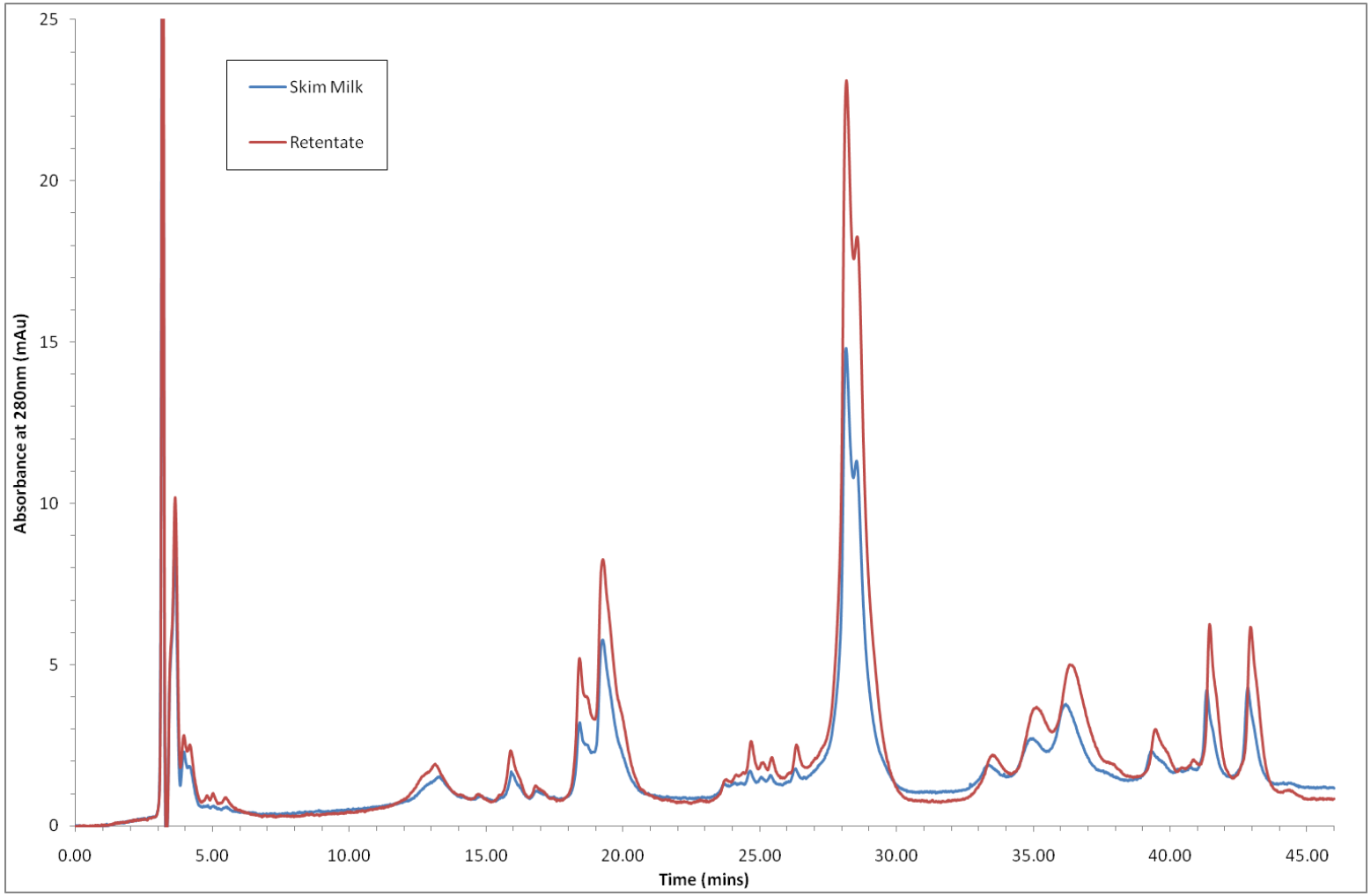

Figure 10: RP-HPLC chromatogram with overlaid traces of retentate used for casein making and skim milk.

To calculate the concentrations of the individual proteins the following extinction coefficients were used: κ-casein 10.5, α_{S1}-casein 10.05, α_{S2}-casein 11.5, β-casein 4.7, α-lactalbumin 20.06 and β-lactoglobulin 9.41. The calculated results are given in Table 12. The value obtained for total protein of 3.8% is plausible for the milk sample and time of season during which it was taken.

Table 12: Casein and whey protein concentrations in low concentration retentate and skim milk

Peak	Retention time (minutes)	Proteins (%)	
		Skim milk	UF Retentate
para-κ-casein	13.1	0.12	0.15
κ-casein B	15.9	0.06	0.09
κ-casein A/E	18.4	0.15	0.28
α_{S2}-casein A	19.3	0.37	0.59
α_{S1}-casein B/C	28.2	1.49	2.59
β-casein B	33.6	0.16	0.25
β-casein A1	35.1	0.36	0.64
β-casein A2	36.4	0.74	1.11
α-lactalbumin	39.5	0.04	0.04
β-lactoglobulin B	41.5	0.16	0.21
β-lactoglobulin A	43.0	0.18	0.29
Total		3.82	6.23

The samples were also tested in the plant by FTIR and later by reference methods, as shown in Table 13 below.

Table 13: Composition of retentate sample

	Retentate (FTIR)	**Retentate (Reference)**
Protein	8.81	6.70
Total Solids	14.50	12.68

4.2.2 Curd washing

Table 14: Lactose results for washed and dried casein samples from normal casein manufacture

Sample	Weight (grams)	Absorbance	Lactose content of test (µg)	Lactose monohydrate (% by wt)
Acidulation	0.5005	3.11	354.94	3.55
WS1	1.0005	1.88	214.43	1.07
WS2	1.0002	0.61	69.80	0.35
WS3	1.0026	0.20	21.45	0.11
Conturbex	1.0018	0.05	4.98	0.02

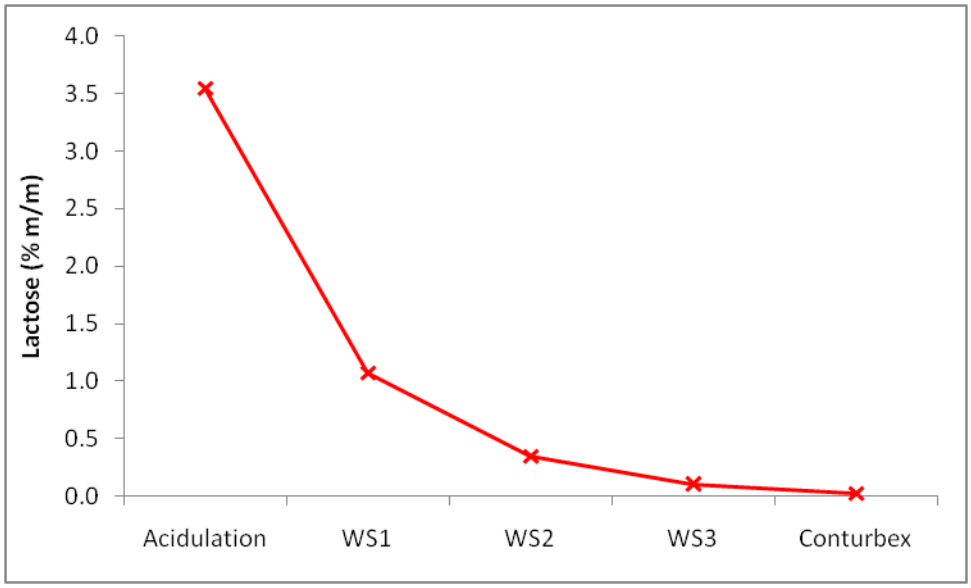

Figure 11: Reduction in lactose through casein washing process

Lactose removal is the key measure of washing effectiveness. The reduction through the four washing stages is clearly identifiable in Table 14 and Figure 1. An acidulation sample was included for completeness but as the absorbance result was

above the optimal linear range for the spectrophotometer the result should not be considered as accurate at the other sample points.

Since a large number of calcium measurements were required, it was planned to avoid having to perform the full EDTA back-titration for all samples. Initially the calcium electrode was used for direct measurement of calcium with liquid, or dissolved solid samples. However, attempts at this showed a large amount of drift in the results. The calcium electrode was then used in conjunction with the titration as outlined in section 3.3. The intention was that this would allow the titration to be performed more quickly as fixed amounts of titrant could be added to the sample and the potential measured at each point. The endpoint could then be interpolated from the change in the potential recorded from the electrode, using the Gran plotting method to find it precisely. This worked relatively well, though the problems with electrode drift, combined with the relatively small titration steps required meant that it was more time-consuming than expected. In the end the conventional titration using a burette was the most effective option for all calcium measurements.

Table 15: Calcium results by EDTA titration for washed and dried casein samples from normal casein manufacture.

Sample	Sample (grams)	EDTA added (ml)	$CaCl_2$ standard used (ml)	% Ca (m/m)
Acidulation	0.4010	10	3.15	0.69
WS1	0.4010	10	7.90	0.21
WS2	0.4010	10	9.00	0.10
WS3	0.4007	10	9.70	0.03
Conturbex	0.4014	10	10.00	0.00

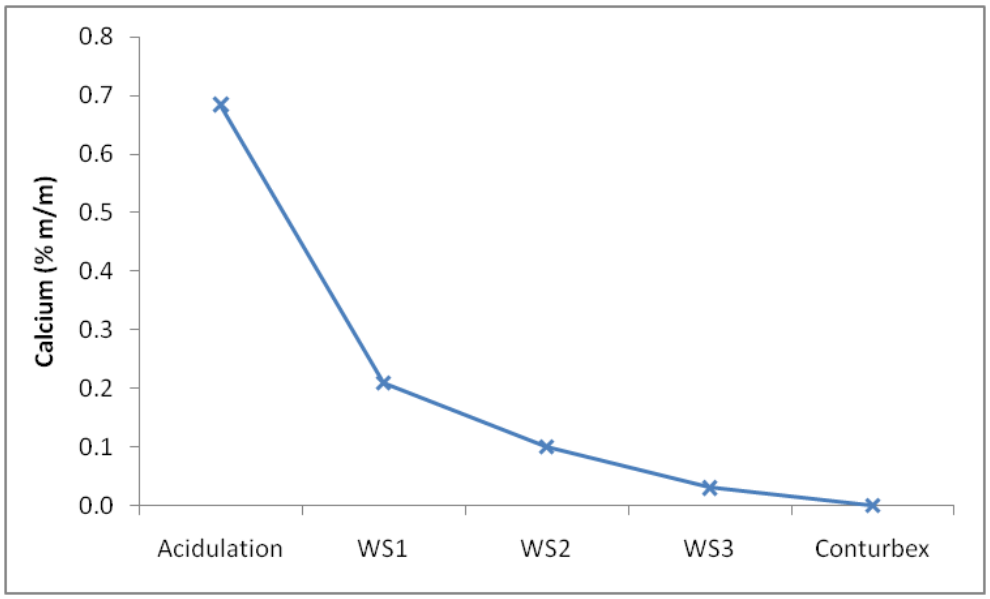

Figure 12: Reduction in residual calcium during casein washing process

The calcium results are given in Table 15 and plotted in Figure 12. The values obtained for this run were comparable to those measured in earlier runs, but at the lower end of the range. This indicates that a reasonably high level of variation exists due to changes in processing conditions.

4.2.3 Casein properties

The dried final product was not of direct interest to this study, as the pre-dewatering part of the process was the main focus. However, to help ensure that the run sampled was representative of normal production, the quality of the final product was checked. This product met the relatively broad codex standard, as well as the more rigorous standard generally required for acid caseins and appeared in all respects to be typical of high grade New Zealand manufactured casein, summarised in Table 16.

Table 16: Casein final product quality results

Attribute	Codex Alimentarius Standard	Standard Specification	Result
Minimum protein (dry)	90	95	97.37
Maximum moisture	12	10	9.63
Maximum milk fat	2.0	1.5	0.71
Maximum ash	2.5	2.2	
Maximum lactose	1.0	0.2	0.05
Maximum free acid	0.27	6.2	5.3
Casein colour (max)		3	2
Scorched particles		A	A
Foreign matter (max)		1	1
Insolubles (max)		3	1

4.3 Discussion

The purpose of this initial work was to gain a better understanding of the casein process during normal production. This is because, although the product is well characterised for sale, the testing regime normally performed on the dried product is designed to show the quality of the product to customers and does not provide detailed enough information to adequately assess the processing itself. The retentate was also analysed by FTIR spectroscopy, which gave a protein result of 8.81% and total solids of 14.5%. This protein result is higher than the 6.23% calculated from the HPLC peak areas. This may be due to a problem with the HPLC analysis, such as protein denaturation during sample preparation. Alternatively, the FTIR analysis may be at fault as the sample was outside of the calibration's normal range at that particular concentration. The HPLC analysis was performed several days after the sample was taken, so it was not available for further testing. The quality of the HPLC results is further supported by subsequent testing of the protein and total solids by reference methods, which gave results of 6.7% and 12.68% respectively. Whatever the reason for the discrepancy, the initial HPLC results were still able to confirm that no major changes in the proportions of proteins took place during the UF process. The α-lactalbumin peaks in the HPLC analysis were lower than expected, though the reason for this was not clear.

The major consequence of using concentrated retentate for casein production is its effect on the resultant casein curd. This is why the washing section of the process was such a focus. The lactose results obtained showed clearly the effectiveness of the washing system at removing lactose entrained within the casein curd. Approximately 70% of the lactose present at each stage sampled had been removed by the next stage. These results are comparable to values obtained in separate testing and are consistent with the final product values routinely obtained. Lactose is required to be under 0.1% and around 0.2% is where browning of the product can occur. These results show that the washing system functions well and may therefore be able to continue to remove lactose effectively even with non-ideal curd.

Calcium had special significance in this work because the supposed reduction in its ability to move from the casein micelles into the whey during acidification was proposed as a key factor to overcome before casein of acceptable quality could be made using retentate. Initial testing for baseline calcium solubility data was intended to be performed on whey, in a similar procedure to that employed by Le Graët and Gaucheron (1999). However, although this would allow direct comparisons to their work, it would not yield as much information about the direct effect of changing concentrations and pH on the casein micelles as direct measurement of retained calcium in the casein fraction itself would. The same samples were therefore used for the calcium testing as the earlier lactose testing.

Calcium testing was initially expected to be far more convenient than the lengthy lactose test through using the calcium ISE. However, despite multiple attempts, the ISE never achieved the level of accuracy or reliability required. Calibration using standards showed that it was able to perform adequately, but when used in protein solutions either directly or in conjunction with a titration was much less accurate. The measured potential drifted over time to such a degree that the recording of results was almost impossible. This may have been due to the relatively impure samples in which it was being used. A possible reason could be a reaction between protein and silver ions in the plug junction of the electrode. The next attempted method was the use of the ISE to help detect the endpoint of an EDTA titration. This did work to some degree, though the endpoint was not found to be any sharper than could be seen using the conventional method with indicator, as shown in Appendix A. This was because fixed volumes of titrant had to be added and the result recorded after each, whereas the normal titration proceeds using a burette until the change is seen. The high pH used during the titration was also likely to damage the ISE over extended use. Although somewhat time consuming, the conventional EDTA titration proved to be the most accurate and reliable method of calcium measurement that was readily available.

Chapter 5. Laboratory scale processing

5.1 Introduction

Performing trials in the plant to assess the effect of the UF retentates was not practical due to it being in constant use for commercial production through the dairy season. There was no access to a pilot scale plant and even subtle changes in the type of casein process could have major effects on the casein produced, making the replication of the process difficult. With all of these factors taken into account, the most suitable way to begin the investigation was the development of a laboratory procedure which could reproduce the commercial process on a lab scale. The intention was to be able to trial different retentates and produce casein which could then be analysed and compared to that from the full-scale process.

Producing acid casein from retentates in the laboratory presented some additional challenges compared to the relatively straightforward precipitation of normal milk. Additionally, the intention was to mimic the commercial process as closely as possible rather than just separating a pure casein fraction. Without the ideal equipment available, some experimentation was required to find a suitable method. This section outlines the steps used in this procedure and some of the findings during its development.

5.2 Results

Each stage of the process is presented as separate sub-sections for direct comparison to the commercial process.

5.2.1 Precipitation

The initial challenge was adding the acid in an effective way. In the plant, excellent mixing of the acid into the milk is achieved as the acid is injected directly before it encounters the static mixers. In the laboratory, adding the acid directly into a container of milk results in localised precipitation of casein at the point of addition while other parts of the milk may still not have been exposed to acid. This effect was even more pronounced in the retentates used in this study because of their high viscosity. Good mixing is then very difficult as the precipitated casein may still contain pockets of acid and un-reacted milk. Extreme low pH causes a very fine-grained curd, as shown in Figure 13, with pockets of low pH due to inadequate acid mixing. To avoid this, the milk was kept cold while the acid was added, to slow the precipitation enough that the acid could be well mixed into the milk. When warmed, the casein precipitation occurred more evenly through the sample. This is similar to the approach used by Teo et al. (1997). Other recommendations for laboratory preparation of caseins include performing the acidification at 2°C using dilute acid and holding for 30 minutes before warming to 30-35°C. This procedure allows the CCP to dissolve. The dilute acid is recommended to avoid localised precipitation (Fox and McSweeney 1998).

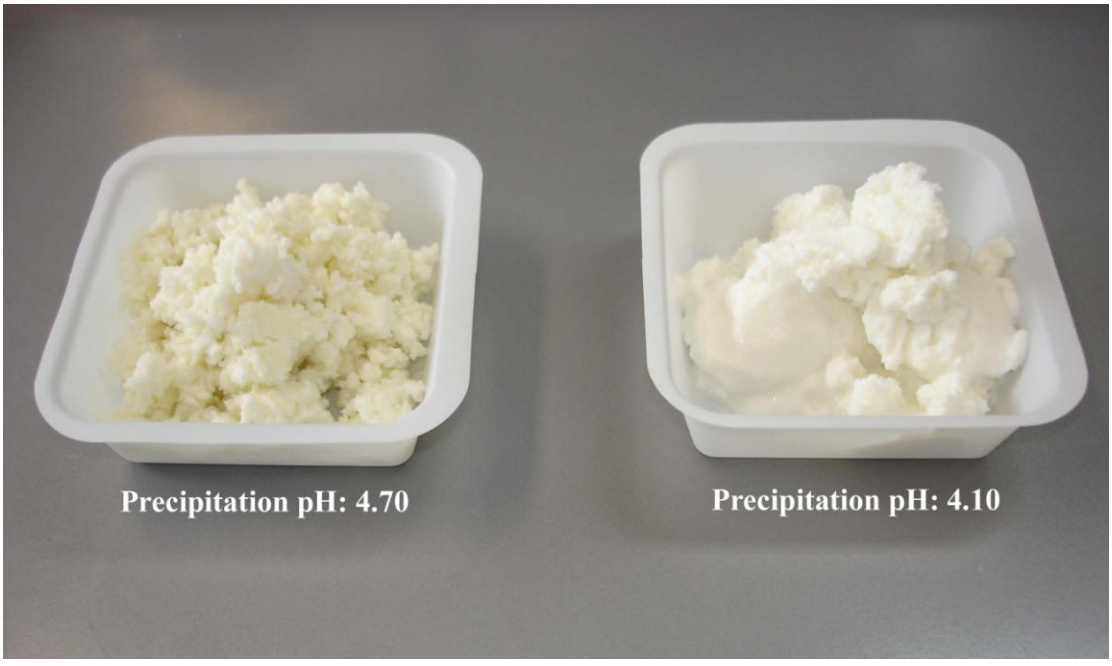

Figure 13: Effect of low pH on casein curd properties.

5.2.2 Cooking

Casein cooking in the plant utilises direct steam injection, which is then followed by a large diameter cooking vessel in which the curd particles agglomerate, helping to reduce the number of fines. This is difficult to replicate in the laboratory. Heating in a waterbath is not fast enough, and requires vigorous stirring to achieve an even temperature through the sample. The different concentrations of UF retentate samples also heat at differing rates and hotplates and other types of direct heat tended to cause only localised cooking. The best compromise was use of a microwave. This provided an even, rapid heat with good temperature control and resulted in a curd similar to that from the commercial process as shown in Figure 14. The lack of stirring also helped minimise the generation of casein fines. To mimic the gentle agitation and long holding time of the acidulation vat, each sample was placed in a horizontal centrifuge device at a speed of 20 RPM after being cooked.

Figure 14: Acidified milk before (left) and after (right) being 'cooked' in the laboratory

5.2.3 Washing

Curd washing was arguably the most difficult part of the commercial process to accurately replicate. Trying to match the efficiency of the washing system would be impossible, so the main effort was placed into finding a very reproducible procedure. In this way all the samples could be compared directly to each other, but could only be compared to the process samples in a relative sense. To do this, the curd was mixed with ultrapure water and allowed to stand for five minutes before being centrifuged. The separated wash water was then decanted off through cheese cloth. This was repeated three times to keep some commonality with the commercial process. As can be seen in Figure 15 this stage did result in some breakdown of the curd and generation of more fines which increases the loss of casein into the wash water.

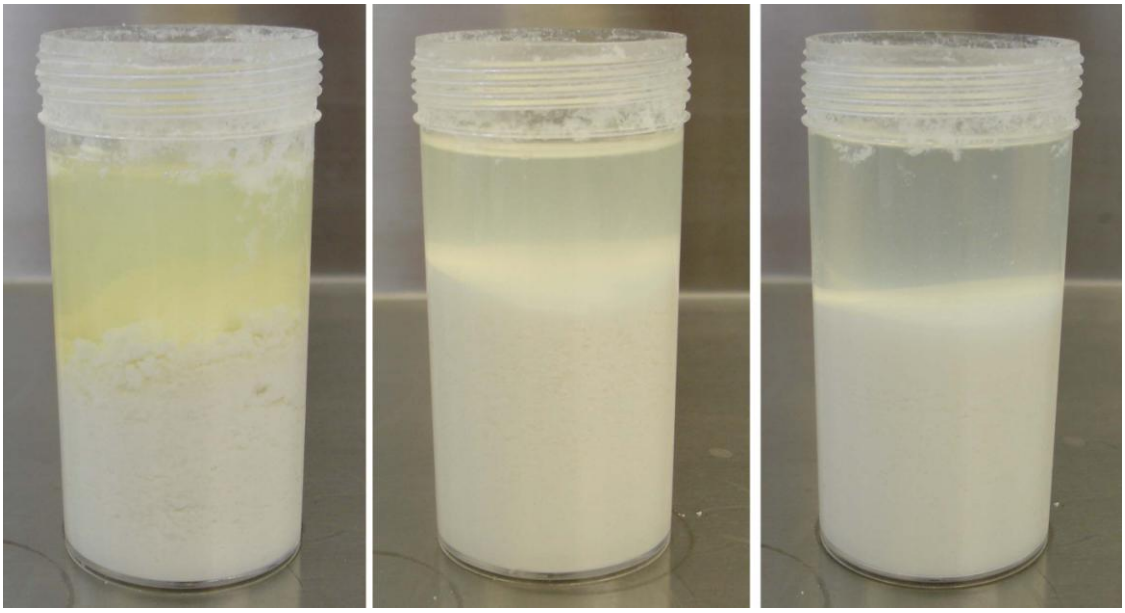

Figure 15: Casein samples with wash water during laboratory washing. Progressing from left to right.

The differences between this method and the plant washing tube and screen system are significant. As washing is a key element of this study, the conditions were kept as consistent as possible. A similar washing technique was used by Jablonka and Munro (1985), who also looked at residual calcium as part of their study. Figure 16 below shows samples obtained from the plant and can be compared to the previous Figure 15. Aside from the finer particles of the laboratory samples and increased whey during initial washing, the two sets are similar in appearance.

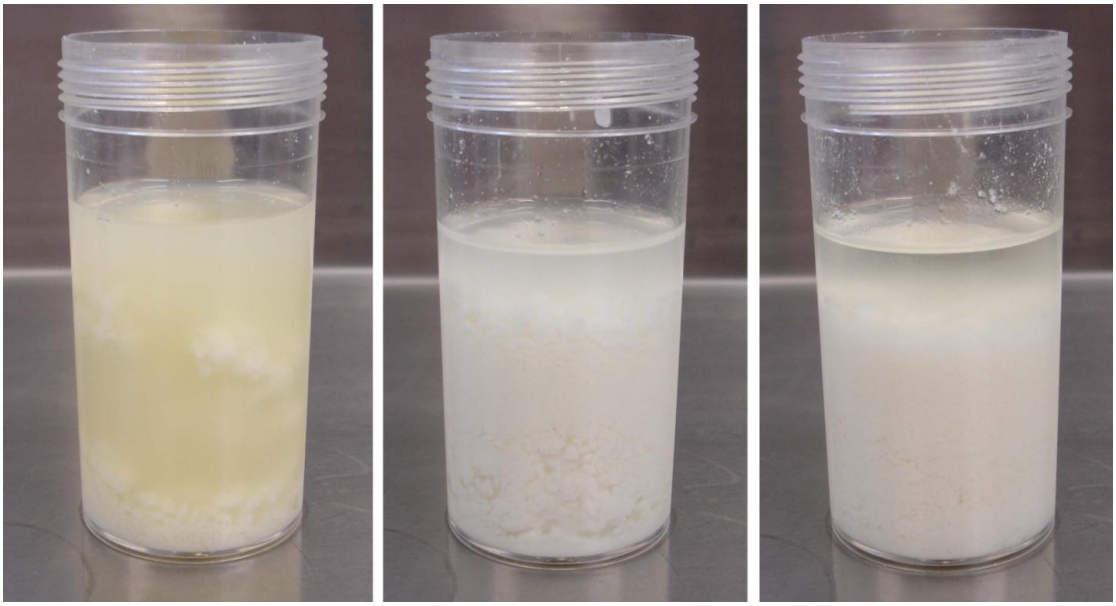

Figure 16: Samples of casein with retained liquid from commercial process. Left to right are: Acidulation, WS1 and WS2.

5.2.4 Drying

Drying the curd is another step which was difficult to fully replicate. The casein plant utilises a shaking bed to convey the casein, as well as a "mincer" which helps to break up large lumps. Simply placing curd in an oven results in an extremely plasticised sample which is impossible to further process, or in some cases even analyse. It was found that the first few hours of drying were the most important. If the curd was repeatedly cut with a spatula during this time, the resulting small particles did not tend to re-agglomerate as they dried. Although manually intensive, this results in far less work during subsequent analysis and results in finer particles which more closely resemble commercial casein directly after drying. High pH curd was very difficult to dry due to its extremely rubbery texture. Medium to low pH curds all dried well as shown in Figure 17, where a conventional and very low pH curd have a similar appearance after drying.

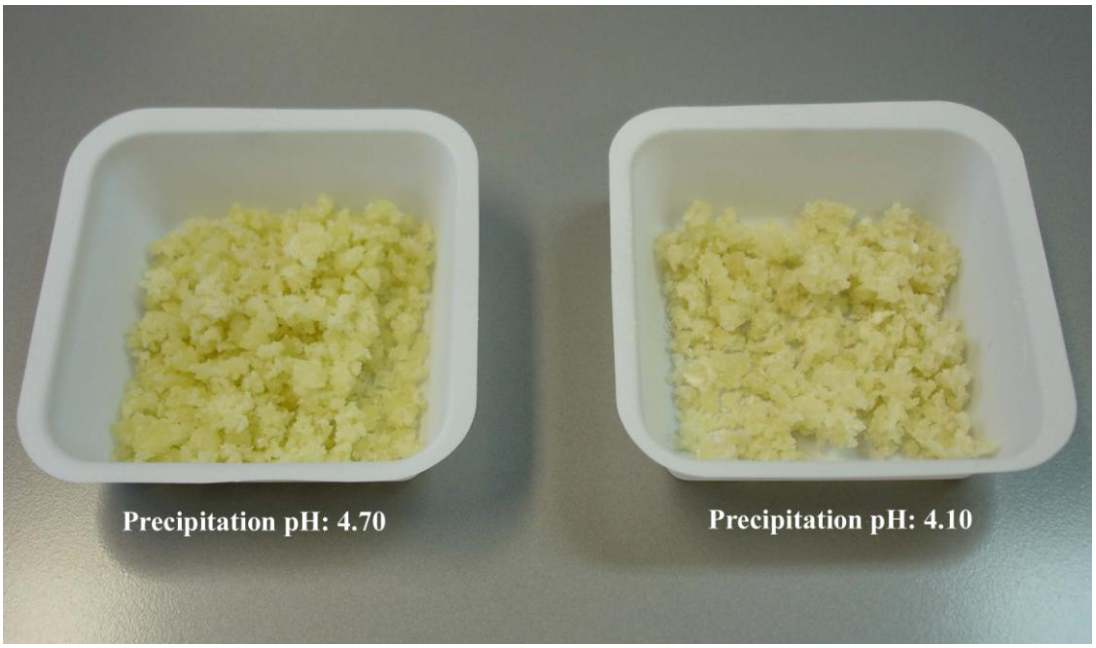

Figure 17: Casein samples as shown in Figure 14, after oven drying.

5.2.5 Milling

A number of approaches were trialled to mill the particles. Although laboratory scale mills are available, these were prohibitively expensive for this work. To allow small-scale milling of the samples, a section of stainless steel pipe had a cap welded onto one end. A plunger section that fitted into it was then machined from a solid steel section. This allowed the casein to be placed in the pipe section while a hammer was used to drive the plunger into it. Although this method produced a wide particle size distribution, enough suitable particles were produced that they could be sieved out and retained. As shown in Figure 18, the difference between the dried casein produced this way and that from the plant is actually quite small. If the particles were not sufficiently dried, or only case-hardened, there was no way to mill them effectively. Samples taken directly from the plant presented fewer difficulties. Casein from throughout the washing stages was already in the form of a cohesive curd, and could simply be dried in the oven before milling. Samples that had not been de-wheyed, such as the acidulation vat, could be rinsed or dried as they were depending on the testing they were intended for.

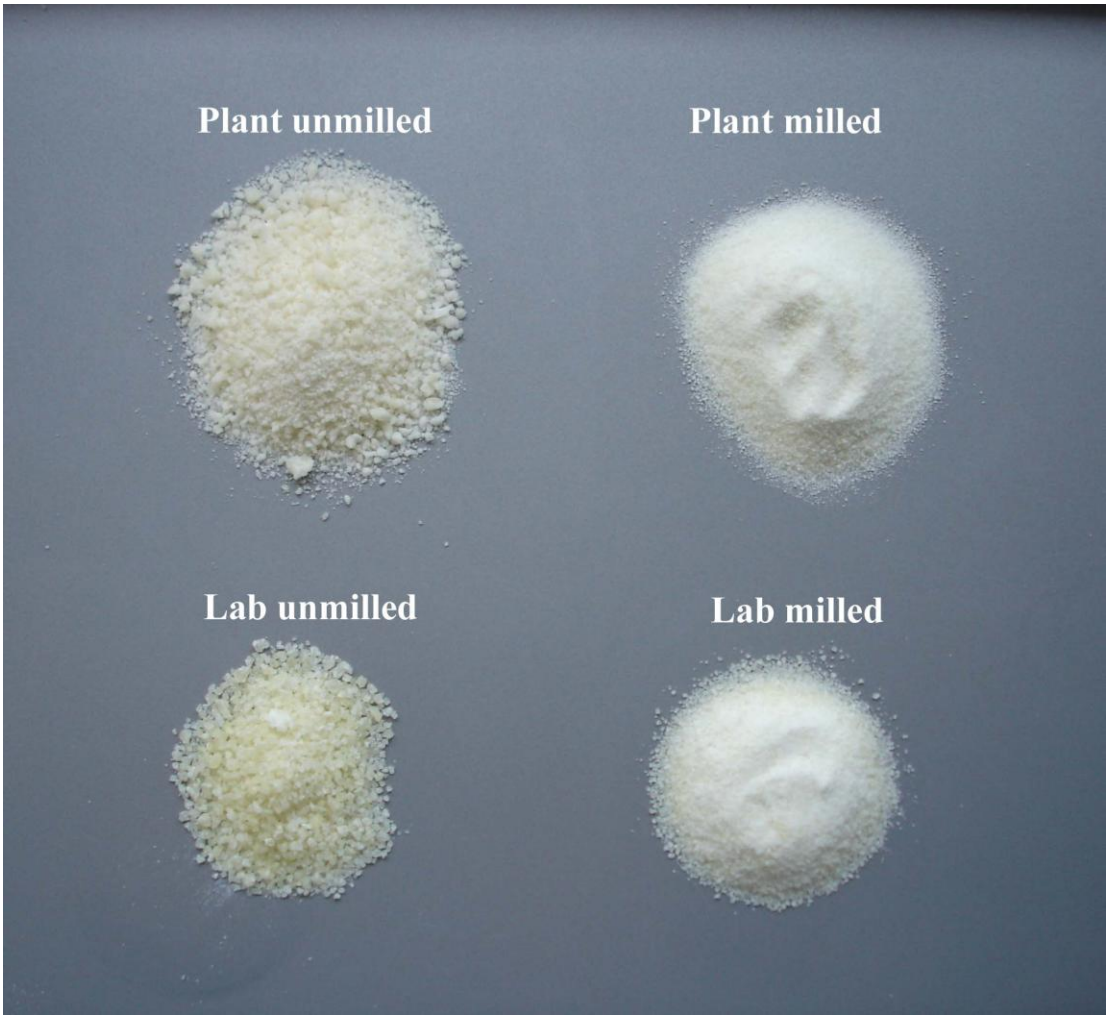

Figure 18: Comparison of casein samples from the plant to those produced in the laboratory.

The particle size distribution of the hand-milled samples was determined by laser diffraction on the MS2000. Some commercial casein samples were also tested to compare the milling. These showed that on average the hand-milled samples were much coarser than the commercial samples, but the spread of sizes was similar. Table 17 summarises some particle size parameters for the samples. The values for d(0.1), d(0.5) and d(0.9) are the size in μm which 10%, 50% and 90% of the particles are below, respectively. The particle size distributions of these samples are shown in Figure 19. All subsequent testing of the prepared samples only required that they be fine enough to solubilise, so the slight size difference was not a concern. However, if this milling technique was to be used to test casein properties in which particle size was important, another method would likely have to be used.

Table 17: Particle size parameters for standard casein and lab-milled casein samples

	Plant Sample	**Lab - High conc, high pH**	**Lab - Low conc, high pH**	**Lab - Low conc, low pH**	**Lab - High conc, low pH**
d (0.1)	177.9	145.4	101.0	148.5	107.5
d (0.5)	394.1	473.2	346.8	539.3	417.5
d (0.9)	703.2	1114.4	934.6	1207.2	1075.0

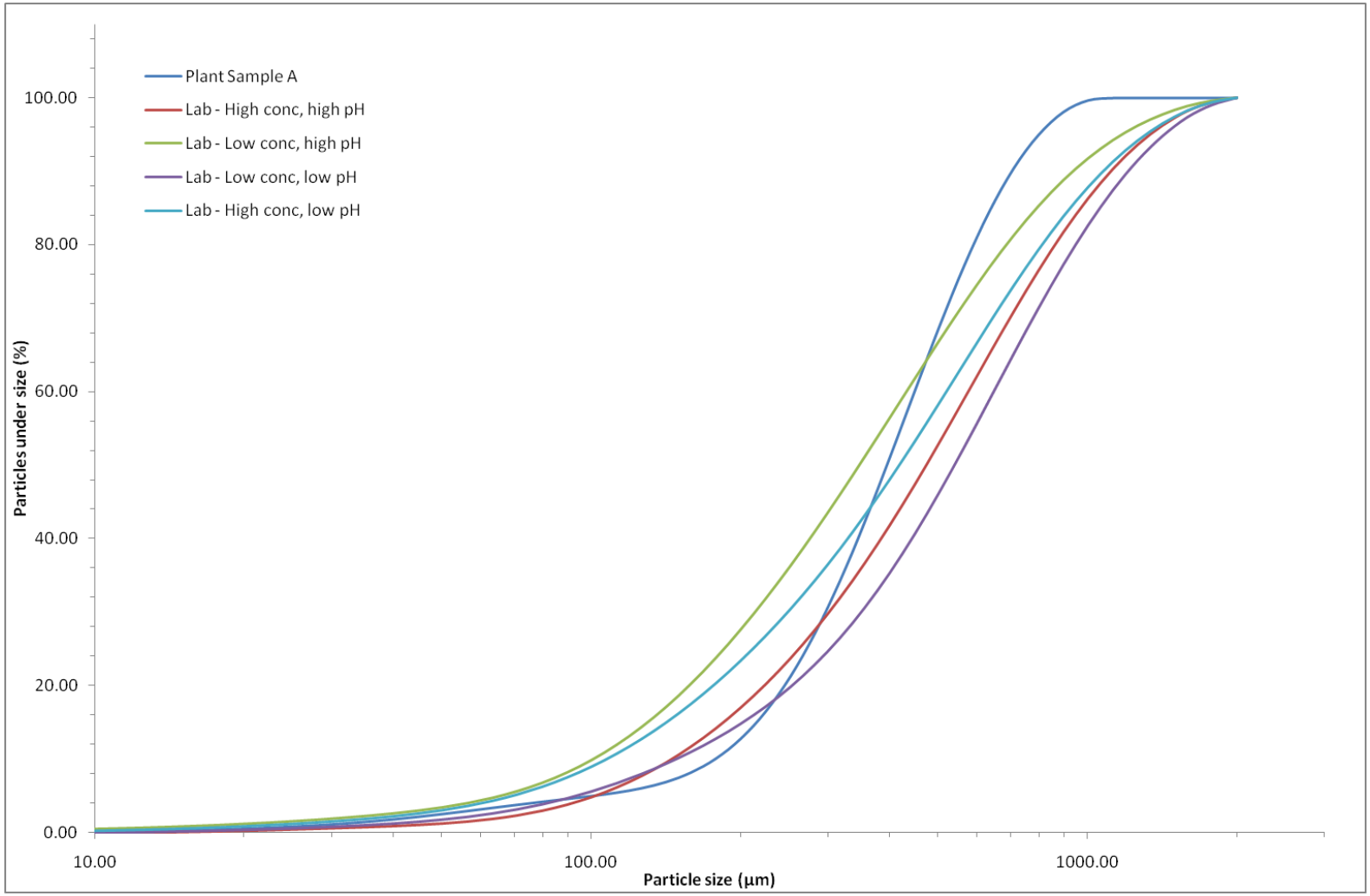

Figure 19: Particle size distribution of standard casein product and lab-milled casein samples

5.3 Discussion

Although precipitating casein from milk is a relatively straightforward task, making a casein preparation which adequately matched the commercial plant was more challenging. The precipitation of highly concentrated retentates was the first issue, as these formed an extremely viscous gel as soon as acid was added. The cold acid approach seemed to fix this problem, and after acidulation a very even curd structure was obtained. Adjusting the acidification temperature from that used in the plant is not without risk of changing the curd properties. Acidification temperature, as well as acidification time, has been shown to affect the rheological properties of acid milk gels (Anema 2008). However, as this work was looking at gross curd properties rather than the properties of a slow set gel, this was not expected to have a major effect. The limitations of the laboratory procedure excluded any alternative means of acidification without risking localised precipitation. Another factor at the precipitation stage was the increased buffering capacity of the UF retentates. This meant a replicate of each sample needed to be titrated with acid to establish the correct addition rate before adding this fixed volume of acid to the actual sample. The stirring of the samples is also likely to have resulted in additional fines compared to the plant process. The temperature control available with microwave heating of the curd was not as good as cooking by heat exchanger. However, this is unlikely to have had any substantial effect on the experiment as the treatment still caused the required changes in the properties of the curd and all samples received the same heat treatment. Curd washing was the experimental step which differed most from the commercial process. There was no practical way to replicate wash tubes and screens with the laboratory equipment available. The batch washing technique used however did allow a very consistent treatment of all samples, which in this case was more useful than directly replicating the plant. Comparison of Figure 15 and Figure 16 shows that laboratory washing samples did have finer curd particles than samples from the plant. The de-wheying stage is also more effective in the plant, as the first laboratory wash sample appears to contain a large amount of whey. The effectiveness of the washing stage was later tested; this is detailed in Chapter Six. As the procedure was designed to compare the effect of process changes, the absolute values of the

retained impurities compared to those from the commercial process was less important than the change in impurity levels between different sample treatments.

None of the laboratory milling techniques available were aggressive enough to reduce the size of the dried casein particles, which initially presented a problem. Almost all of the test methods required that the casein be dissolved, and this was impractical without some sort of particle size reduction. The manual milling device finally used gave a wide particle size distribution compared to the commercial process. However, the particle size itself should not have affected any of the subsequent testing. The values given in Table 17 and Figure 19 show that the final size was similar to that of the commercial casein sample. The samples obtained by this procedure were small, less than 6 g of dried casein was recovered in all samples. This allowed most intended testing to be performed but prevented some other types of test such as viscosity using a Brookfield viscometer from being practical. Although the procedure outlined was time consuming and relatively labour intensive, it produced good quality casein samples which could be measured using the standard analytical methods without any additional preparation.

Chapter 6. High concentration retentate casein-making

6.1 Introduction

Using UF retentates for casein making on a commercial basis does not appear to have been covered in detail in the literature. One of the most relevant papers in the area is the work of Le Graët and Gaucheron (1999), which determined the levels of minerals upon acidification of milk ultrafiltrates. However, that work focused on the aqueous phase of the milk, and did not examine the effect on casein precipitation. The work described in this chapter instead placed the focus on the residual calcium content of the casein itself. This meant that other factors, such as the structure of the casein curd itself, became important because of their effect on the ability of minerals to be washed out of the casein. The techniques developed in the previous two chapters were employed to assist in this. In the work of Le Graët and Gaucheron, rennet was added to the samples to help whey recovery. This technique was also employed here, to allow casein to be recovered from samples where the pH was well above the isoelectric point of casein. The intention of the trial and experimental work described was to establish whether UF retentates caused the expected processing problems during casein precipitation and whether adjusting concentration or pH could mitigate these effects. Finally, from the experiments some optimal conditions for the production of casein from UF retentates could be identified.

6.2 Results

As outlined previously, milk is routinely concentrated to a VCF slightly below 2.0 before being used for casein manufacture, the precise level depending on the protein concentration of the milk. Membrane concentration equipment of a suitable scale for laboratory trials was not available on-site. This meant that this experimental stage was reliant on the full-scale UF plant, and was timed around the preparation of retentate for MPC production at a suitable concentration. Choosing an appropriate retentate concentration for the laboratory trials had to be made on the basis of availability as well as suitability. A range of samples of MPC70 and 85 samples were either tested or compositional data retrieved from recent analyses to show the normal variation in the product and ensure that only samples representative of normal production would be used in experiments. These are summarised in Table 18 and Table 19 below. Note that only the MPC85 calibration set included lactose results, so this is not available for other retentates. This was due to only MPC85 having sufficient lactose results available when the calibrations were last modified. MPC85 initially seemed to be the most advantageous product for this work as its production recovers more lactose. However, as permeate is perishable, an excess can result in disposal costs. MPC70 contains more lactose, but is still highly concentrated. It was also produced more frequently during the course of the project so was a more practical source material.

Table 18: Variation in composition of MPC85 retentate (% by weight)

	Sample A Reference	Sample B Reference	Sample B FTIR	Sample C FTIR	Sample D FTIR
Protein	14.63	13.14	13.35	13.74	12.98
Lactose	-	-	0.78	0.79	0.62
Total Solids	16.47	15.84	15.58	16.08	15.35

Table 19: Variation in composition of MPC70 retentate (% by weight)

	Sample A FTIR	Sample B FTIR	Sample C FTIR	Sample D FTIR
Protein	12.08	12.41	12.56	12.25
Total Solids	16.52	17.16	17.21	16.88

6.2.1 Plant Trial

Soon after the initial work on this project began a quantity of MPC85 retentate, unable to be dried due to the dryer being off-line, was available. This presented the opportunity for an impromptu full-scale trial of its use in the casein plant. Approximately 8 m^3 of retentate was diluted with 12 m^3 of RO water in the retentate storage silo before running it through the casein plant on the end of a standard mineral acid casein production run. Exact volume measurements could not be obtained, so silo level indicators and operator judgement were used. Samples of the diluted retentate were analysed by FTIR at the start of the trial. Samples were also taken from the retentate silo before and after dilution for subsequent reference testing. These results are summarised in Table 20, with a comparison to a sample of the low VCF retentate normally used.

Table 20: Composition of pre and post dilution retentate used in trial

	Retentate (Reference)	**Diluted (Reference)**	**Diluted (FTIR)**	**Normal casein feed**
Protein	14.63	4.69	4.36	12.68
Total Solids	16.47	5.35	4.80	6.70
Lactose			0.81	

These results show that the retentate was diluted to a final protein concentration only slightly above that of un-concentrated milk, i.e. less concentrated than the retentate normally used in the casein plant. While this was not ideal, the trial still gave insights into the practicality of using diluted retentates and any effects on the final casein. It also provided a comparison to previous attempts at casein production from more concentrated retentate.

Initially, during the precipitation stage, pH control was difficult. Larger variations in pH than were expected occurred, which was likely due to changing feedstock from normal conditions to diluted retentate while the plant was running. As RO water was used to dilute the retentate, it had a lower buffering capacity; hence the greater change in pH during acid addition. Numerous adjustments to the acid addition rate were required before the plant began to run steadily, at which point the pH of the acidulation vat was found to be 4.68. With only approximately 20 m^3 of diluted retentate available and a throughput of 14 m^3/hr there was limited time to ensure steady state, though this was achieved after 30 minutes. Some key process variables are summarised in Table 21. No major differences were noted during the run, though the operators observed that while passing over the washing screens, the curd appeared whiter than normal. This is consistent with the whiter appearance of milk retentates, particularly those at high concentrations, due to the lower lactose and mineral content compared with normal milk.

Table 21: Processing conditions used during plant trial

Cooking rate (m3/hr)	Indirect cooker temperature (°C)	Cooking temperature (°C)	3rd Wash temperature (°C)	4th Wash temperature (°C)
14.0	35.0	51.8	72.0	42.1

Samples were collected through the wet processing part of the process and refrigerated overnight. Dried samples were also collected but could not be confirmed as being completely free of residual casein from the earlier part of the run so were not analysed further. There was no visible difference in the appearance of the final casein produced from the retentate and the conventional casein produced earlier on the same plant. In the laboratory, the acidulation vat sample was gently rinsed to remove trapped whey, then all samples were dried at 60°C in a ventilated oven until reaching a water activity of a_w <0.400 (approximately 20 hours) and milled by hand using the method outlined in section 5.2.5. These were then analysed for residual calcium

content using the EDTA titration method. The results are shown in Figure 20, plotted against the conventional casein samples from Chapter Four. The retained calcium in the curd through the early part of the process was significantly lower than during normal processing. The samples were also tested for lactose. Again these are plotted against casein samples from Chapter Four in Figure 21.

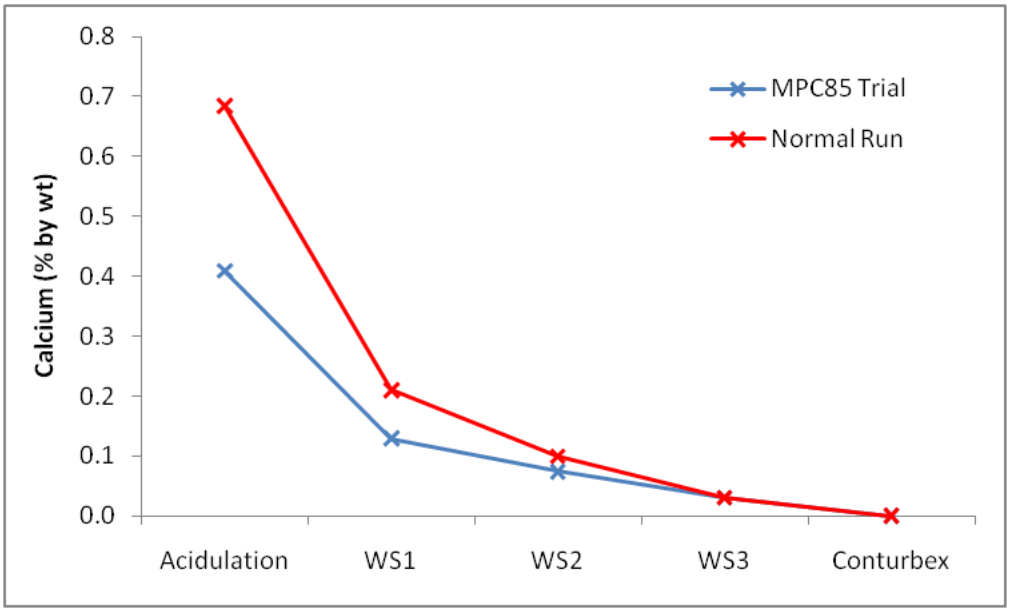

Figure 20: Calcium results for dried samples collected through process

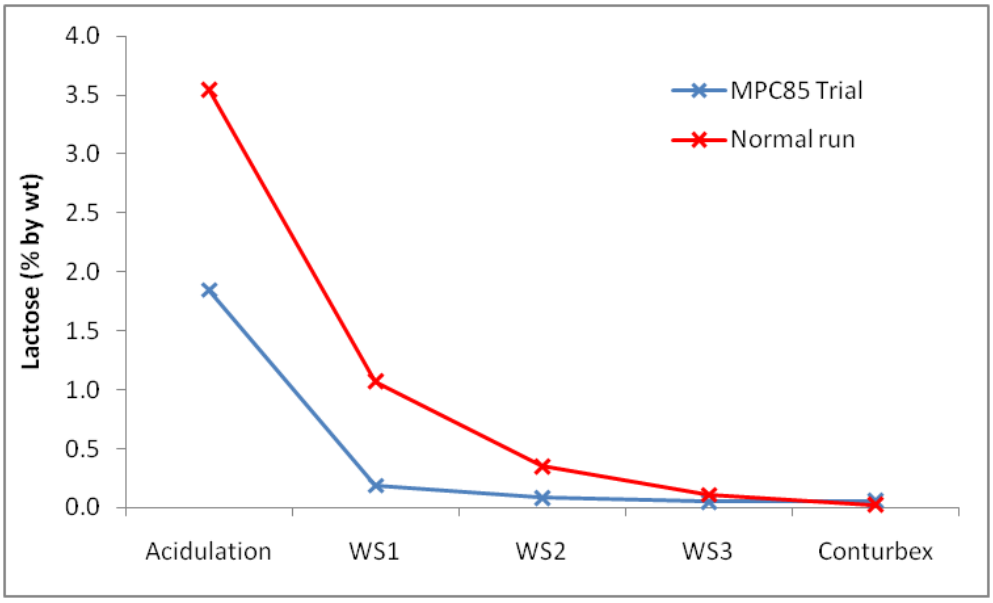

Figure 21: Lactose results for dried samples collected through process

6.2.1.1 RP-HPLC comparison of samples

The trial samples were also analysed by RP-HPLC. This was to determine whether the residual whey protein content differed from normal casein production and to confirm that the unusual treatment of the milk prior to casein-making, or differences in the way this product dealt with processing, may have caused some protein damage. For comparison, the samples obtained during normal processing described in Chapter Four were also analysed. Figure 22 shows the traces obtained for these samples. The main observable difference is that the β-casein peaks are less well resolved in the samples obtained from the washing system compared to those of the de-watered casein. Possible reasons for this are not clear. However, this difference occurs in the WS3 samples from both the conventional casein and the trial product. Overall, the results clearly show that there is little difference between the two sets of samples, with any variations small enough to be a result of normal sample and testing variation. Around the 40 minute mark of the run, where the whey proteins elute, there is a small increase in absorbance. However, this was undetectable as a peak and is similar in size with both sample sets. Overall these results showed the absence of any detectable residual whey protein in the trial product.

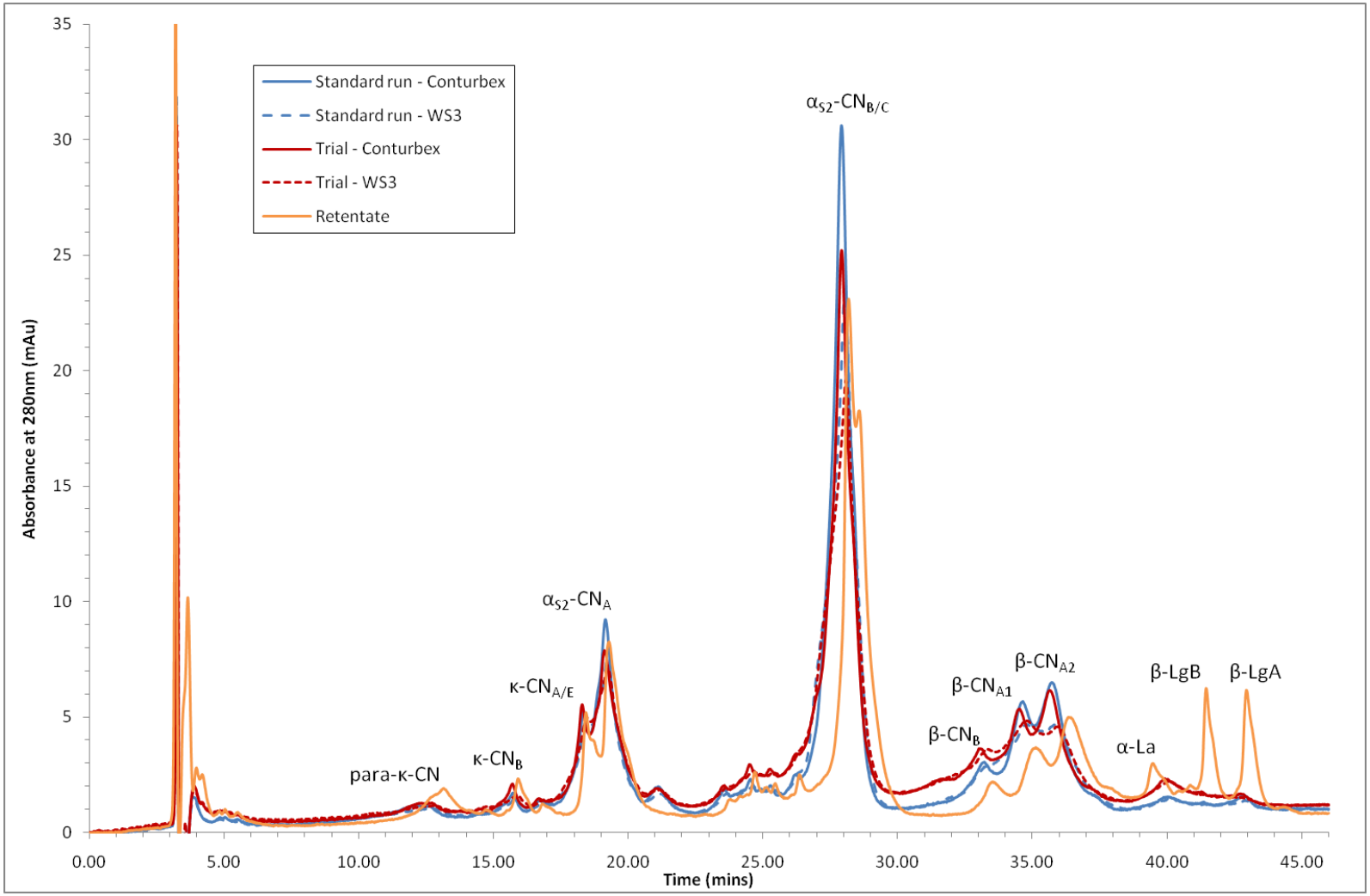

Figure 22: RP-HPLC chromatograms showing trial and standard product from process against starting retentate.

6.2.1.2 Viscosity results

Informal communications with production staff had indicated a concern that increased retentate concentration resulted in lower viscosity in the final product. Although no mechanism for this was proposed, to ensure that the supposed viscosity changes were not an issue, viscosity measurement was carried out using the procedure outlined in section 3.12. This also provided an opportunity to check for unnoticed product changes as viscosity is a key functional property. Excellent temperature control was accomplished using the circulation pump, which is reflected in the repeatability of the tests as shown in Table 22. No reduction in caseinate viscosity through the use of the diluted MPC85 retentate was observed. In fact the trial sample had a higher viscosity, though at less than 10% this was within the range of up to 20% sometimes seen during routine testing of caseinates. The increase may be due to this testing error or the different composition of the trial sample. Previous research has shown that the addition of salts including $CaCl_2$, NaCl and NaH_2PO_4 can change the viscous properties of sodium caseinate solutions, and that calcium addition in particular appears to act as a viscosity limiting factor (Konstance and Strange 1991). These effects are likely to relate to their influence on solubility of the proteins, just as the use of monovalent or divalent salts such as Na^+ and Ca^{2+} in caseinate manufacture dramatically changes their viscous properties. It has been suggested that this is caused by the salts effectively increasing protein concentration by competing with the casein for water (Loveday et al. 2007).

Table 22: Comparison of sodium caseinate viscosities

Normal Conditions - Conturbex

Efflux time (Sec)	Viscometer constant (mm2/s2)	Kinematic viscosity (cSt)
158.01	0.035	5.53
159.17	0.035	5.57
156.86	0.035	5.49
157.75	0.035	5.52
157.73	0.035	5.52
157.77	0.035	5.52
157.61	0.035	5.52
157.54	0.035	5.51
157.51	0.035	5.51
157.45	0.035	5.51
157.29	0.035	5.51

Plant Trial - Conturbex

Efflux time (Sec)	Viscometer constant (mm2/s2)	Kinematic viscosity (cSt)
169.20	0.035	5.92
169.11	0.035	5.92
169.01	0.035	5.92
169.01	0.035	5.92
169.10	0.035	5.92
168.70	0.035	5.90
168.48	0.035	5.90
168.36	0.035	5.89
168.58	0.035	5.90
168.20	0.035	5.89
168.26	0.035	5.89

6.2.1.3 Trial Summary

The run itself, and the casein produced, yielded useful data about the effect of diluted retentates on the process. The washing system coped well with the changed curd properties, though the over-dilution would have helped with this. This was demonstrated by the fact that washing was much more effective during this run than under normal conditions. The extra water used in the dilution may even result in a reduction in total water use if the wash water volumes could be decreased. Overall, the trial showed clearly that casein can be successfully made in this plant from high VCF retentates by the use of dilution prior to precipitation.

6.2.2 Initial Laboratory Trial

The plant trial successfully proved some elements of the concept, but to build upon those findings casein-making experiments had to be carried out in the laboratory. The work consisted of two main trials following the procedure developed earlier in Chapter Five. The initial trial was designed to establish the effect of a very wide range of pH and concentration treatments on the casein.

A four litre sample of freshly concentrated MPC70 retentate was obtained directly from the UF plant and refrigerated overnight. Milk retentates have different buffering capacities due to the differences in the composition and distribution of minerals and proteins between the aqueous and micellar phases as reviewed by Salaün et al (2005). To account for this, before starting casein preparation samples were titrated with 10% sulphuric acid to determine the total volume required to achieve a specified pH. Figure 23 displays the curves obtained for the MPC70 sample prior to the initial laboratory trial.

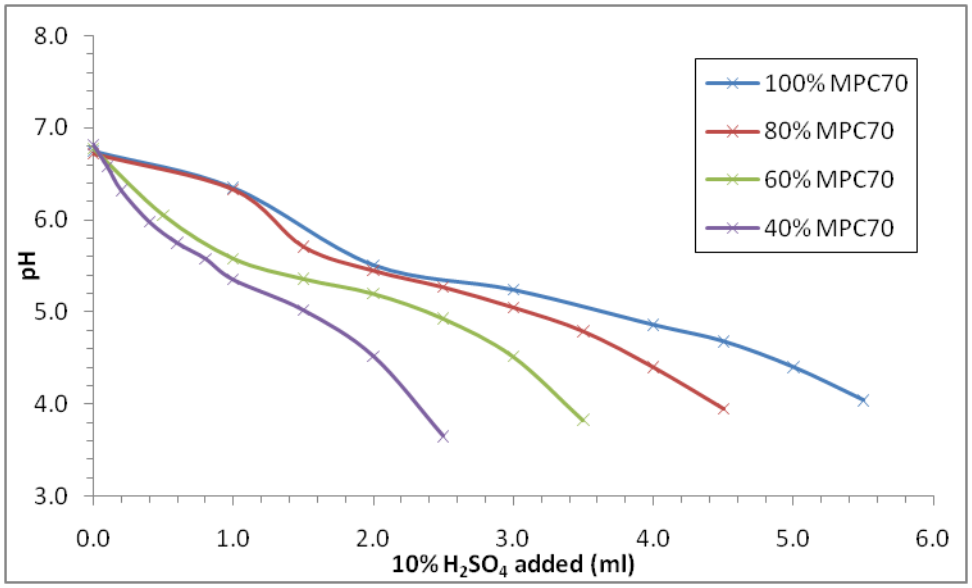

Figure 23: Acid addition to MPC70 retentate at different dilutions showing buffering effects.

The design of this experiment is summarised in Table 23. In all, 24 samples of approximately 100 g each were made, which were separated into three sets (100%, 80% and 60% MPC70) with eight subsamples in each. Each of the 24 had a specific quantity of acid added, based on the results shown in Figure 23, to give a range of final pH values as shown in Table 23. 70 µl of commercial rennet was also added to each sample, as the samples at high pH would not have precipitated. In this way the residual calcium across an extremely wide range of pH values could be measured in the casein.

The three sets of eight samples described were all treated as individual samples with the same procedure followed for each, other than the differing dilution and acid additions. The target pH were: natural, ≈5.5, ≈5.2, ≈4.9, ≈4.7, ≈4.5, ≈4.3 and ≈4.1. As the table illustrates, these were difficult to achieve in some cases. Accurate pH measurement was only possible on separated whey after cooking, so no further pH adjustments were able to be made. The yield of dried casein has not been reported as the drying and hand-milling procedures used resulted in inconsistent losses between samples.

Table 23: Experimental summary of laboratory MPC70 casein-making

Sample	Retentate (g)	Water (g)	H2SO4 (ml)	Final pH	Wet Curd (g)	Whey Calcium (%)	Casein Calcium (%)
1	100.25	0.00	0.00	6.69	43.05	0.040	2.46
2	100.17	0.00	1.50	5.57	34.48	0.101	1.95
3	100.01	0.00	2.00	5.34	35.78	0.138	1.64
4	100.09	0.00	3.50	4.94	42.08	0.242	1.15
5	100.02	0.00	4.25	4.76	46.20	0.269	1.03
6	100.08	0.00	4.85	4.49	50.96	0.253	1.19
7	100.04	0.00	5.25	4.31	56.17	0.274	1.12
8	100.03	0.00	5.50	4.19	57.57	0.264	1.18
9	80.04	20.53	0.00	6.68	30.56	0.034	2.60
10	80.02	21.80	1.25	5.58	24.55	0.111	1.78
11	80.10	20.05	2.00	5.25	26.00	0.169	1.31
12	80.10	20.10	3.00	4.93	33.41	0.248	0.56
13	80.03	20.04	3.50	4.74	33.89	0.272	0.36
14	80.07	20.42	4.00	4.44	38.96	0.275	0.58
15	80.28	20.27	4.25	4.22	43.62	0.256	0.63
16	80.08	20.29	4.50	4.09	46.69	0.256	0.64
17	60.16	40.28	0.00	6.74	24.00	0.027	2.51
18	60.04	40.02	0.50	6.04	20.90	0.058	2.12
19	60.23	40.72	1.00	5.62	19.50	0.099	1.58
20	60.11	40.41	2.50	4.89	25.57	0.218	0.16
21	60.05	40.28	2.75	4.70	30.40	0.228	0.16
22	60.25	40.18	3.00	4.51	34.54	0.234	0.06
23	60.25	40.36	3.25	4.33	35.61	0.236	0.06
24	60.64	40.22	3.50	4.11	33.91	0.239	0.04

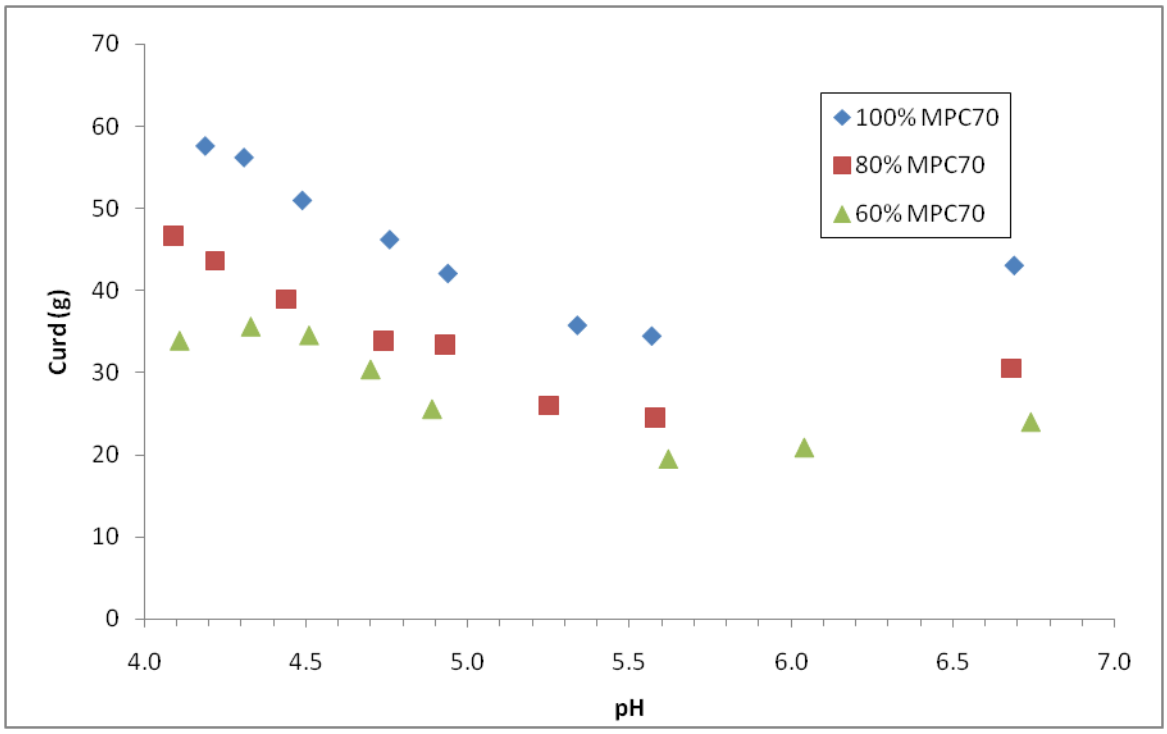

Figure 24: Wet curd mass recovered

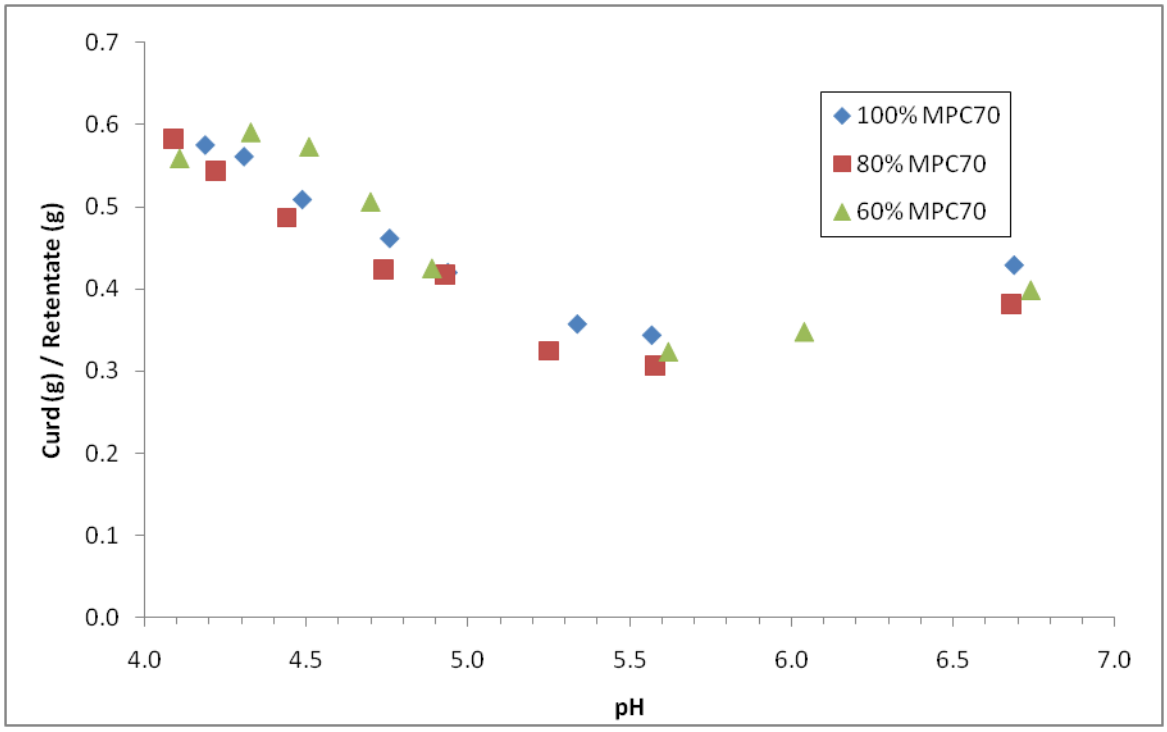

Figure 25: Recovered curd normalised for original retentate mass

The mass of wet curd recovered varied in line with expectations from the solubility of casein at different pH. The use of rennet will have influenced these results so they cannot be compared directly to a normal mineral acid casein precipitation. The raw results are plotted in Figure 24, while Figure 25 shows them normalised for the initial quantity of retentate present in the sample. These show that the highest recovery occurs at pH below 4.5 and in the lowest concentration samples.

Calcium was measured for all whey and casein samples, also summarised in Table 23. Whey analyses were first performed in duplicate and in some cases triplicate; these results can be found in Appendix B. The variation in results was very small, less than 0.01% in all whey samples and 0.2% for the caseins. The majority of the variation is likely to come from the indistinctness of the end-point when determining it visually. The release of calcium into the whey upon acidification is clearly illustrated in the whey results, plotted in Figure 26. By pH 4.7, the calcium content for all three sample sets has begun to level off. The pH 4.49 result in the 100% set is slightly lower than would be expected by the general trend; this can be attributed to the difficulty of getting a clean separation of whey at low pH due to the softness of the curd as discussed previously. These calcium results are presented in Figure 27 normalised for the initial retentate concentration. The increased solubility of the calcium as the retentate concentration is decreased is readily apparent below pH 5. Above this point, where the casein has been predominantly coagulated by rennet, there is little difference between the sample sets.

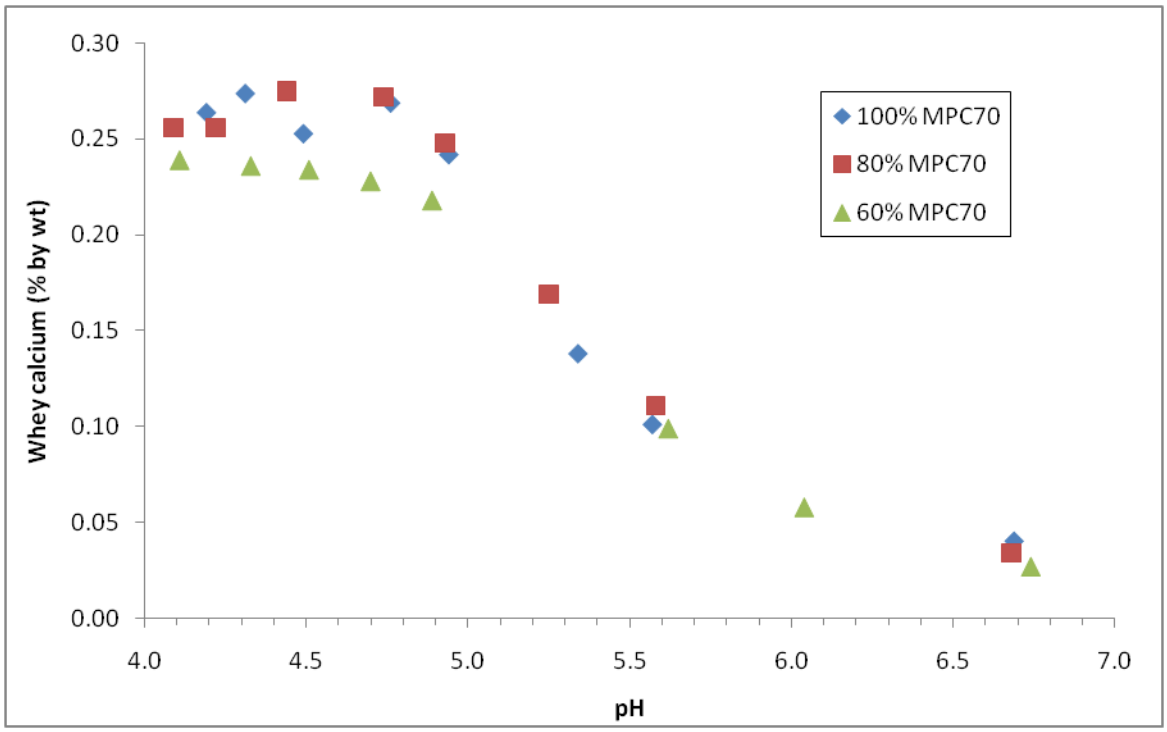

Figure 26: Calcium results for whey samples across a wide pH range

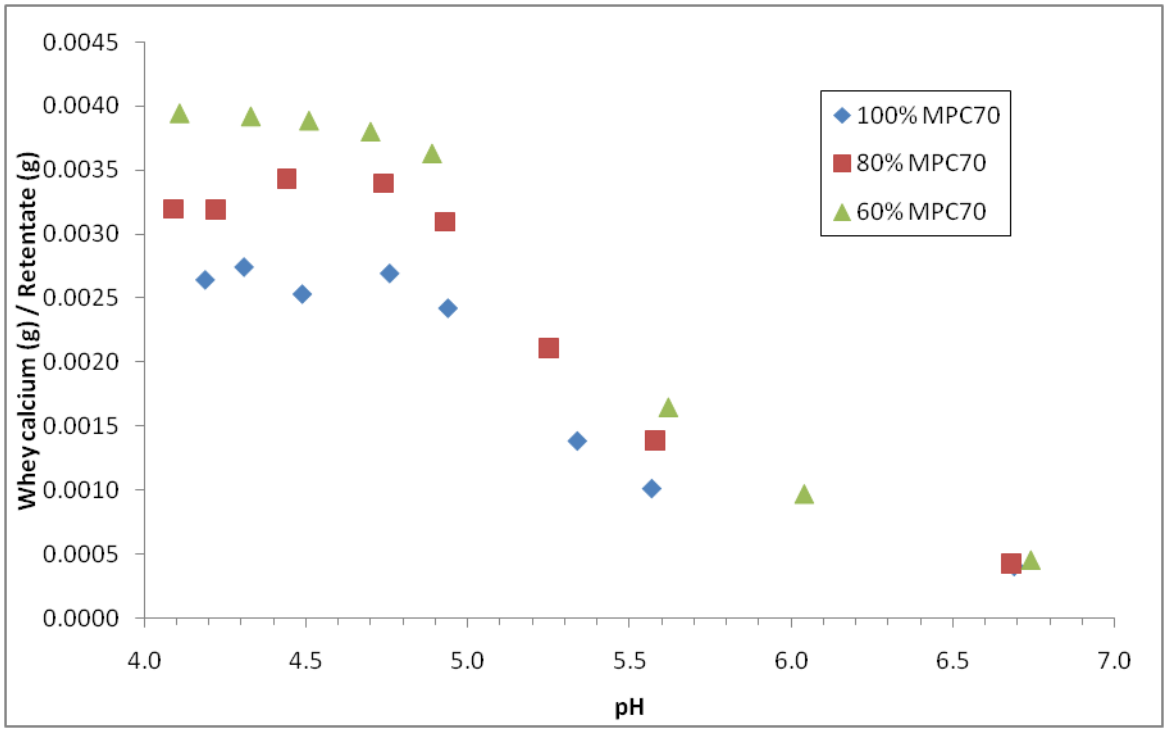

Figure 27: Whey calcium results normalised for retentate concentration

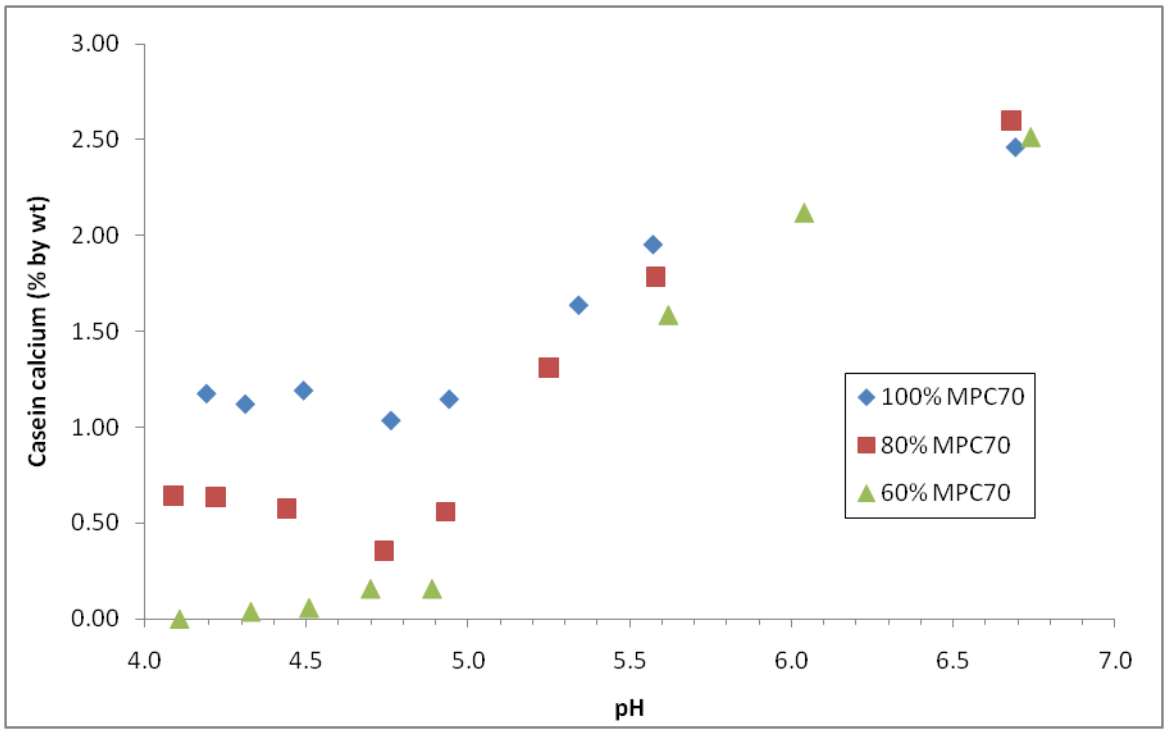

Figure 28: Calcium results for casein samples across a wide pH range

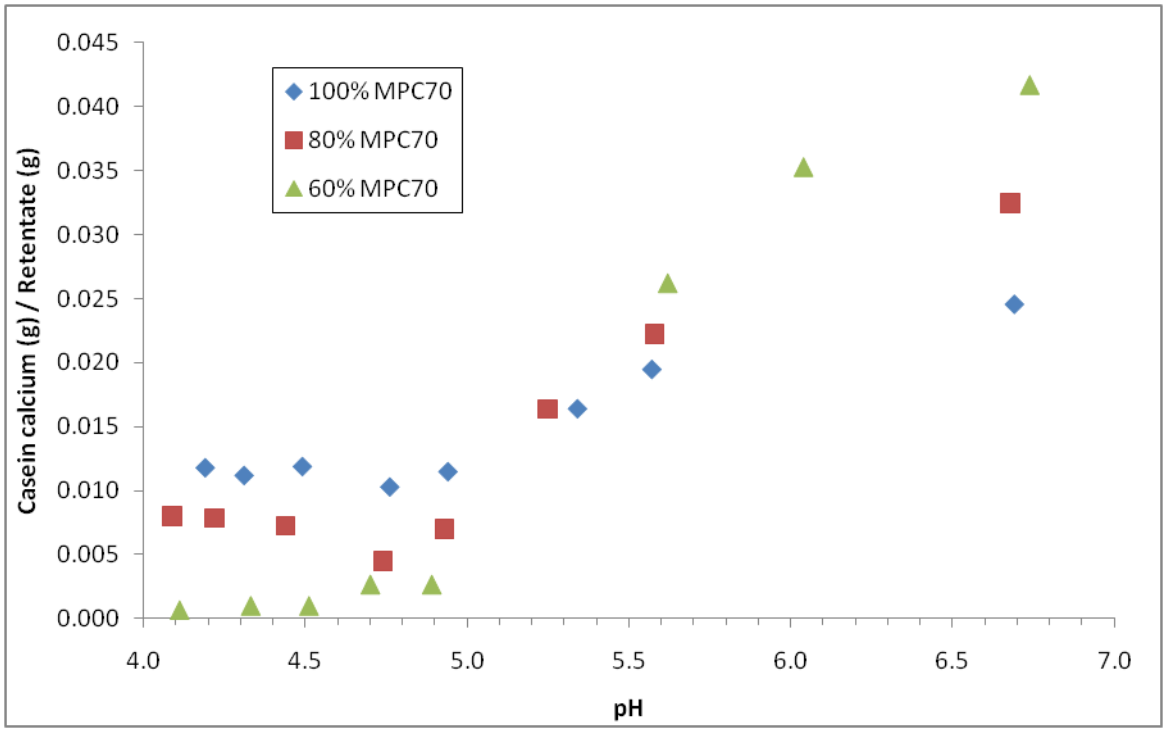

Figure 29: Casein calcium results normalised for retentate concentration

The casein calcium results were of more direct relevance than those from the whey as the intention of the experiment was to determine the effect on casein. These are summarised in Figure 28. Higher pH samples in particular appeared to share more physical properties with rennet casein than acid casein during their preparation such as a very dense curd which was difficult to wash. This was due to the acid precipitating a much lower proportion of the total casein. This made the measurement of these samples difficult, as can be seen by the difference between the calcium results for the un-acidified samples of the three dilutions in both the whey and casein analyses. Figure 29 shows these same results normalised for the original retentate concentration. The key finding from this was that by around pH 4.7, the calcium levels for all calcium samples had reached steady-state. That is, they either did not reduce or reduced by only a small amount as the precipitation pH was further lowered. In this set of samples, only the 60% MPC70 at the correct pH produced casein with calcium results comparable to those found during the assessment of standard processing from Chapter Three. As stated earlier, the differences between the laboratory and commercial procedures mean that the absolute values obtained in these experiments would not exactly match those obtained when scaled up to a commercial process. However, the conditions resulting in good curd properties will still apply to a similar process at any scale.

This initial experiment clearly showed the solubilisation of calcium upon acidification of the retentate. The addition of rennet was useful to be able to show the effect of acidification across a full range of pH conditions, but the differences that it caused in the precipitation conditions meant that the findings could not be directly applied to a commercial process. The findings from this experiment were therefore used to help plan a second laboratory trial in which likely plant conditions could be more closely matched.

6.2.3 Second Laboratory Trial

The second full laboratory experiment again made use of MPC70 directly from the UF plant as it was the most concentrated MPC product available. The sample obtained was analysed by reference methods from Chapter Three to give a total solids result of 18.25% and protein of 13.44%. Table 24 outlines the sample preparation used for this experiment. Unlike the previous experiment, the four sets of diluted retentates were prepared as batches before being split into six sub-samples for acidification. This was to minimise any variation in concentration between subsamples of the same set. The acid addition information from the first experiment was used to try and give a range of final pH values between 5.2 and 4.2, though the final values were not able to be measured until the whey had been separated.

The mass of the separated whey phase after cooking was recorded. These results are given in Table 24. Recovered wash water masses are shown in Table 25, with the asterisked results indicating samples where more water was present, but containing a large number of casein fines. All of these are the one or two samples with the lowest pH in each sample set, showing clearly the degree to which the low pH caused a very soft curd resulting in large losses of fine casein particles. As this was making the recording of yields difficult, only two washes were used for samples in this experiment. This also provided an opportunity to investigate the sensitivity of the final casein to the washing procedure itself.

Table 24: Experimental design and recoveries from second laboratory trial

Sample	Retentate (g)	Water (g)	Acid (g)	Final pH	Whey (g)	Curd (g)	Loss (g)
1	70.15	30.07	2.30	5.09	77.55	24.89	0.1
2	70.13	30.05	2.60	5.00	69.96	32.48	0.3
3	70.01	30.01	3.00	4.83	59.11	43.25	0.7
4	70.11	30.05	3.20	4.72	57.48	45.74	0.1
5	70.09	30.04	3.40	4.56	37.07	66.04	0.4
6	70.25	30.11	3.80	4.26	56.63	43.08	4.4
7	60.05	40.03	2.10	5.05	79.61	22.45	0.1
8	60.02	40.02	2.70	4.75	64.92	37.95	-0.1
9	60.03	40.02	3.00	4.53	61.60	41.61	-0.2
10	60.02	40.01	3.20	4.35	56.67	46.72	-0.2
11	60.00	40.00	3.30	4.26	52.33	51.12	-0.2
12	60.09	40.06	3.40	4.16	65.65	34.99	2.9
13	50.02	50.02	1.70	5.16	85.82	15.80	0.1
14	50.01	50.01	2.00	5.00	81.93	20.09	0.0
15	50.02	50.02	2.25	4.77	71.71	30.58	0.0
16	50.01	50.01	2.50	4.53	68.68	33.78	0.1
17	50.01	50.01	2.70	4.33	72.21	30.61	-0.1
18	50.01	50.01	2.70	4.24	73.03	26.33	3.4
19	40.00	60.00	1.45	5.15	88.87	12.34	0.2
20	40.04	60.07	1.65	4.96	86.10	15.62	0.0
21	40.02	60.04	1.85	4.75	79.96	21.75	0.2
22	40.05	60.08	2.00	4.58	78.61	22.87	0.7
23	40.02	60.03	2.20	4.29	78.30	23.69	0.3
24	40.00	60.00	2.25	4.16	76.59	22.49	3.2

Table 25: Experimental results from second laboratory trial

Sample	Retentate Concentration (%)	Final pH	Washwater #1 (g)	Casein Calcium (%)	Whey Calcium (%)
1	70	5.09	43.74	0.781	0.190
2	70	5.00	50.96	0.526	0.219
3	70	4.83	50.58	0.341	0.242
4	70	4.72	52.42	0.321	0.257
5	70	4.56	48.06	0.411	0.261
6	70	4.26	42.69*	0.354	0.271
7	60	5.05	46.53	0.680	0.180
8	60	4.75	45.61	0.290	0.223
9	60	4.53	39.04	0.370	0.232
10	60	4.35	37.75	0.391	0.236
11	60	4.26	37.94*	0.371	0.236
12	60	4.16	37.74*	0.175	0.239
13	50	5.16	45.69	0.685	0.146
14	50	5.00	46.99	0.361	0.171
15	50	4.77	46.89	0.220	0.184
16	50	4.53	40.02*	0.240	0.189
17	50	4.33	35.65*	0.185	0.193
18	50	4.24	38.02*	0.180	0.195
19	40	5.15	46.55	0.515	0.117
20	40	4.96	46.43	0.270	0.137
21	40	4.75	44.62	0.150	0.146
22	40	4.58	40.17	0.080	0.150
23	40	4.29	35.46*	0.055	0.152
24	40	4.16	37.72*	0.050	0.154

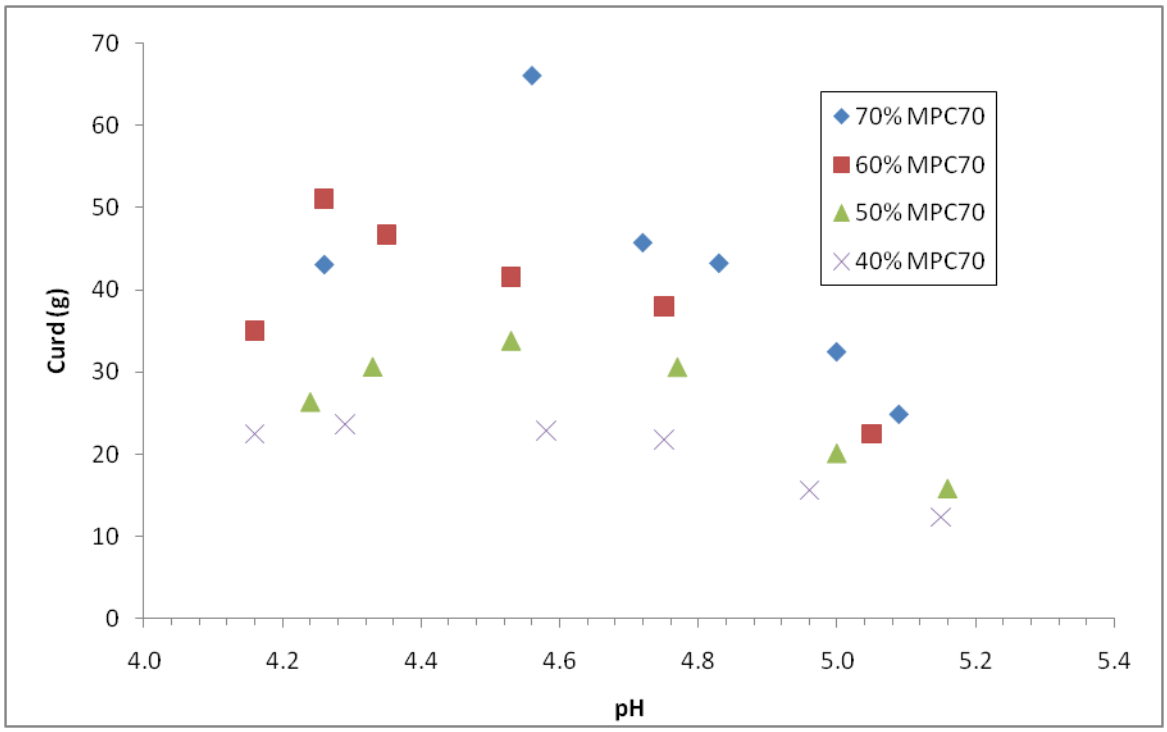

Figure 30: Wet curd mass recovered

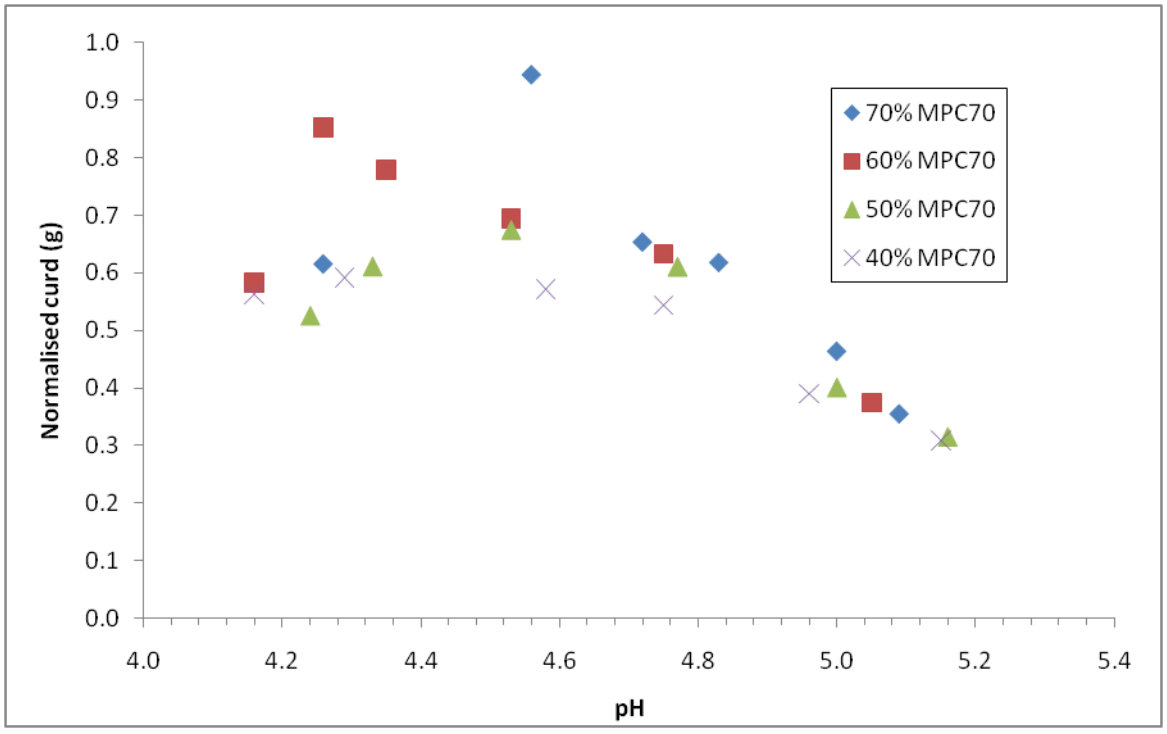

Figure 31: Curd mass normalised for retentate concentration

Visual assessment of the samples from this experiment during their preparation, particularly the precipitation stage, showed that they more closely matched the physical properties of those from normal production. As the maximum target pH values were only slightly over five, there was no need for rennet addition. This trial therefore was a much closer representation of plant conditions than the initial one. The recovered mass of casein is plotted in Figure 30. These results are also shown normalised for initial retentate concentration in Figure 31. These are comparable to the results from the first laboratory trial though do show more scatter. This resulted mainly from the softness of low pH curd resulting in losses into the whey and wash water.

The calcium content of the wash water from the first washing step was also measured. These samples all contained less than half the calcium of their corresponding whey samples. Further washing samples were not measured as their contribution to the calcium mass balance would have been negligible due to the relatively low concentrations found. Whey and casein samples were measured using the same procedure used in the initial laboratory trial and are shown in Table 25. Figure 32 depicts the calcium results in the whey, where there is a constant increase in the calcium content as the pH is lowered, particularly in the 70% and 60% sample sets. For the 50% and 40% samples the increase in calcium has almost stopped by pH 4.6. The normalised results from Figure 33 are interesting in that they appear to show little variation with retentate concentration. However, when compared to results from the first laboratory trial, they agree closely. The 100% and 80% retentate sample sets from the first trial have lower calcium concentrations, while the other five sets match very closely. This shows that for the samples at 70% and lower and once the pH is below five, little additional calcium enters the whey.

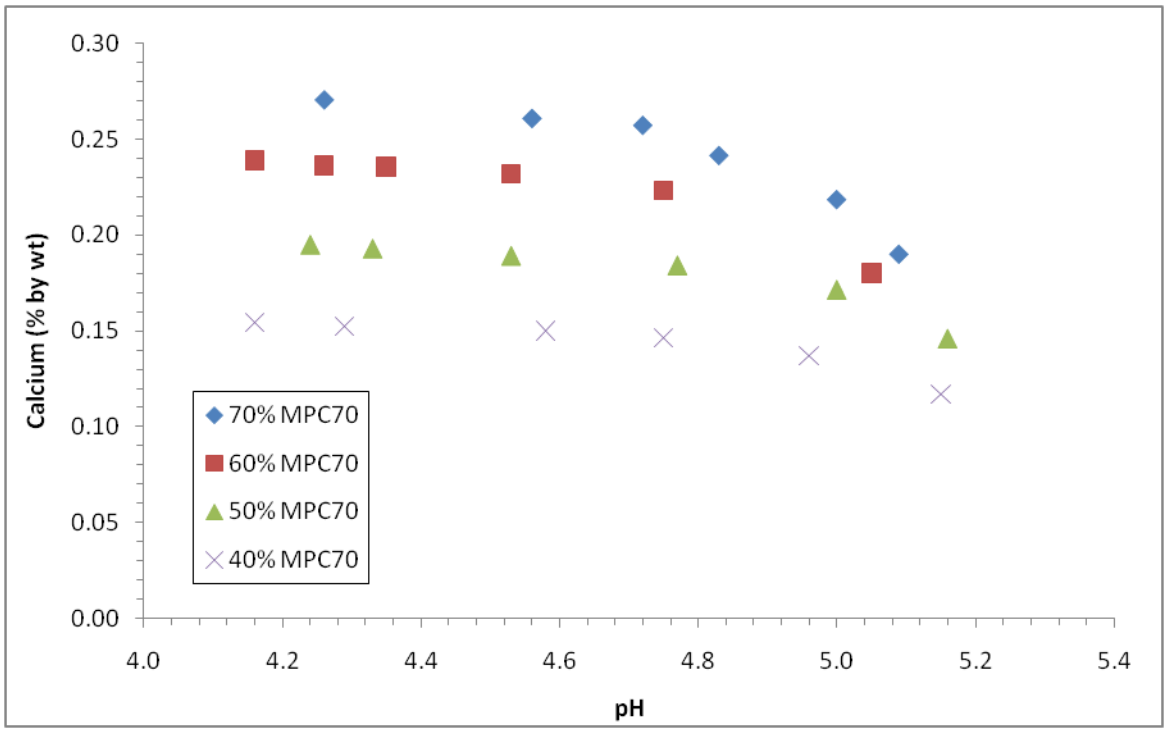

Figure 32: Calcium results for whey from MPC70 casein from second laboratory trial

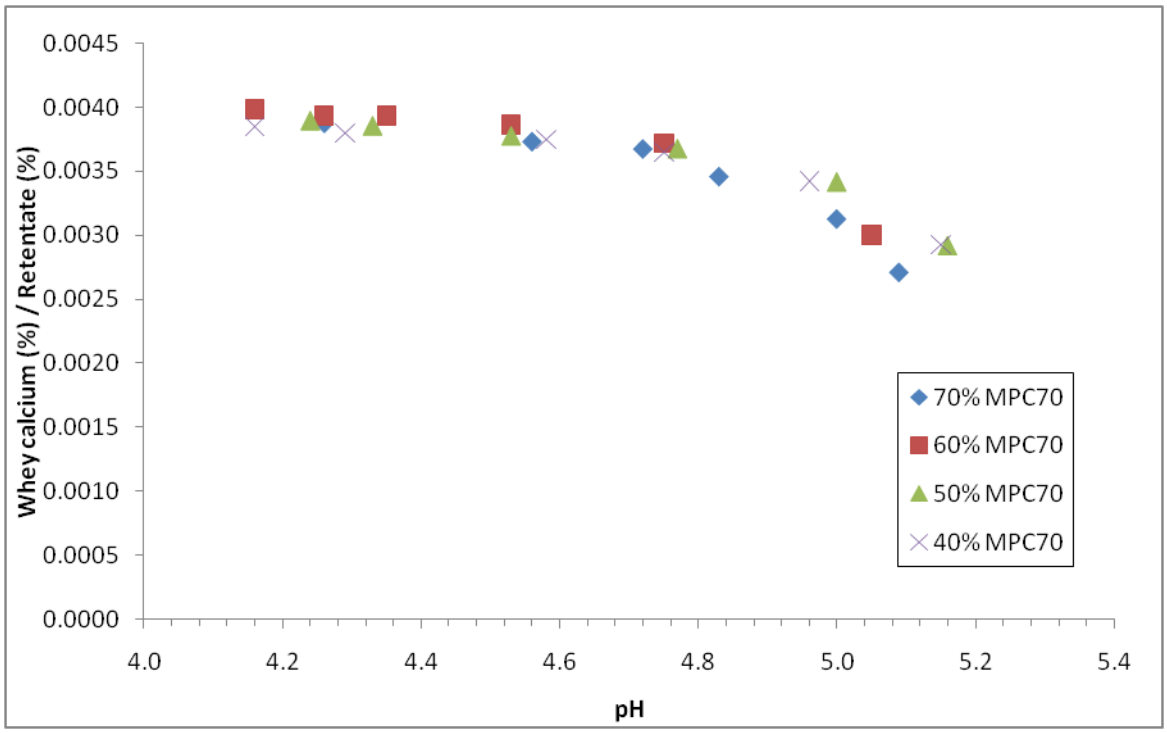

Figure 33: Whey calcium results normalised for retentate concentration

In the casein sample calcium results shown in Figure 34, the results are higher than those obtained in the first experimental trial. This is likely due to the omission of the final wash during this experiment as outlined. The effect of washing is therefore critically important to the calcium values obtained when precipitating casein in the laboratory and means that the scope for reduction of wash water usage may be limited. However, this would vary greatly in a commercial plant from the laboratory procedure used here. The normalised results from Figure 35 show clearly once again that calcium removal is better at lower retentate dilutions and pH values between 4.2 and 4.6. From these results the optimal precipitation conditions are to continue precipitating at pH 4.6, with retentate concentrations at 50% and below. These results are well within the range achieved in casein using reconstituted skim milk at 9% total solids concentration by Jablonka and Munro (1985).

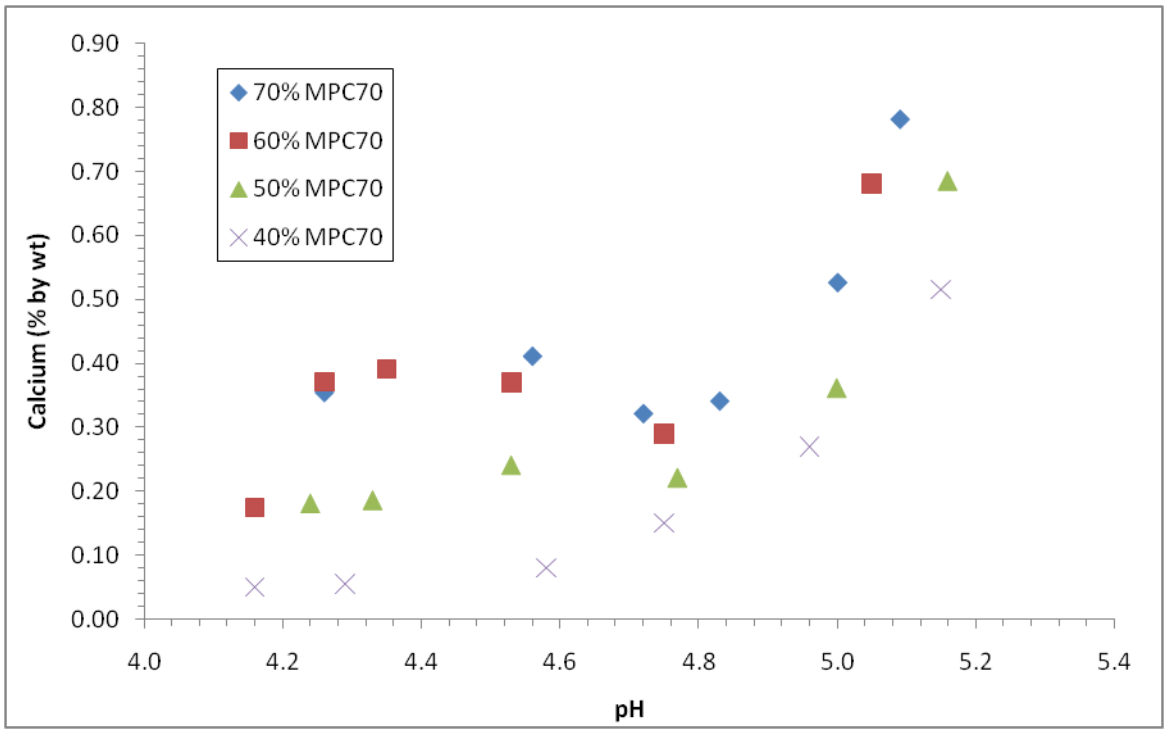

Figure 34: Calcium results for MPC70 sourced casein from second laboratory trial

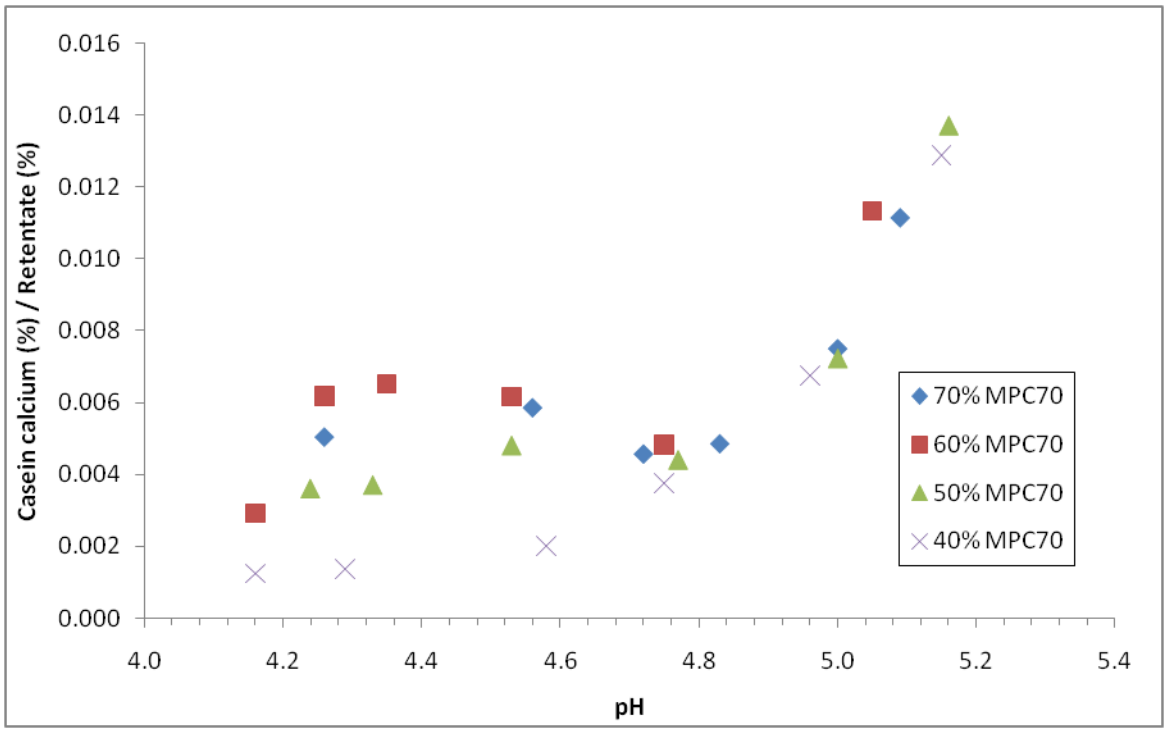

Figure 35: Casein calcium results normalised for retentate concentration

6.3 Discussion

The plant trial using MPC85 retentate was a useful opportunity to see the practical effect of using a retentate in commercial scale casein production. The total solids reference result of 4.8% for the diluted retentate showed that the over-dilution had brought the concentration to less than half that normally used in the plant. The dilution itself was carried out in a very impromptu manner, using silo level indicators as a measurement system. This aspect would need to be improved if this trial was to be repeated in a more meaningful way. Comparing the casein produced from the normal UF retentate at the start of the run, to the trial production at the end, there is little obvious difference. It should be noted though that some of the conventional casein will have been included with the dry trial product, and the extensive blending used before casein packing helps average out any deviations. The whiteness of the curd noticed during washing may have been due to the presence of far fewer impurities, mainly lactose, than would normally be expected. Since this was not visible in the dried casein, this means that the washing system is effective at removing these during normal casein production anyway. Viscosity testing of the casein was as a result of discussions with manufacturing staff which indicated there may have been a correlation between increased UF and reduced solution viscosity in the resulting product. It presented a good opportunity to investigate changes in a key functional property of casein products. The standard test method for sodium caseinate uses a Brookfield spindle-type viscometer to measure a 15% solution at 25°C. The lengthy sample preparation time and the casein quantities yielded made this impractical, so capillary viscometry was used instead. The temperature was carefully controlled and good repeatability was observed using this technique. No other functional properties of the trial product were tested as viscosity is the primary one for casein products.

Overall, although limited in the specific areas outlined, the trial effectively proved the concept that milk can undergo extensive UF to recover usable permeate, be diluted with water, and then be used to successfully make casein. However the excessive dilution meant that the ability of the plant to process less dilute retentates remained unknown. This is why the lab trials were required to study the properties of casein produced in this manner in more detail.

This first experiment using high VCF retentate for casein production in the laboratory was not designed to mimic the plant exactly, but to produce data similar to the graphs published by Le Graët and Gaucheron (1999). This was the rationale for the very wide pH range used. Using samples with pH of well over five meant that rennet addition was required to recover usable casein samples. The different properties of acid and rennet caseins meant that the samples obtained could not be compared directly to the plant samples. Samples with greater acid addition were visually much more similar to acid casein than those at higher pH, due to the reduced influence of the rennet. Calcium testing of the dried casein samples showed clearly the expected trend of reduced calcium upon pH reduction; however the data was much noisier than intended. Samples were tested in duplicate and in some cases triplicate, with the same result. This means that the differences must be as a result of the preparation procedure itself, particularly the batch washing system used. Although not ideal, they compare very well with results published by Jablonka and Munro (1985), who used a similar test method. Their work also shows an increase in residual calcium at high precipitation pH, though it was most dramatically increased by high precipitation temperature. Reducing precipitation temperature may therefore be another potential area of investigation. It is likely that a large part of the variation in results is due to inconsistencies in the de-wheying and washing steps as performed in the laboratory. The lack of access to small-scale UF and analysis equipment did limit the scope of this work as different retentate compositions could not also be trialled.

The only method of reliable calcium measurement available, as discussed previously, was the EDTA titration. Off-site access was potentially available to inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma atomic emission spectroscopy (ICP-AES). ICP-MS can handle no more than around 0.1% total dissolved solids as the sample must be nebulised, and levels of more than 100 ppm require dilution. ICP-AES was more robust in terms of sample preparation, but would have a depressed response if protein was present. As no small-scale UF was available on-site, the samples were unable to be tested by these methods. Additionally, the addition of preservatives or use of freezing had been shown to affect results in the past. The initial intention of the work had been to use more sophisticated methods of mineral analysis to obtain precise results that could then be used to model the concentration of various species under different concentration and pH conditions. However, it seems likely that even if those techniques had been used the variation in the prepared casein samples themselves would have prevented the development of a useful model from being achievable. Although the EDTA titration lacks the precision of these instruments, the level of accuracy achieved has shown that it was suitable for this work.

The second experiment focused on the pH range from 5.2 to 4.0 and was intended to be more applicable to the commercial process as the addition of rennet was not required. A comparison of the two experiments shows that any slight changes to the washing procedure used results in quite different residual calcium levels in the casein. For this reason the shape of the curves obtained are more useful than the absolute values when trying to compare to a full-scale washing system. The 80% and 70% sample sets continued to show variation in the calcium levels even once the pH 4.6 point had been passed. This was probably due to the variability in washing as the curds remained very dense even as the pH was reduced past this point. The other two sets, 60% and 50%, reached a steady state by around pH 4.6. This indicates both that the use of a pH below the normal precipitation value is unjustified, and that the 50% set in particular was at a level of dilution practicable for processing. Therefore the

dilution factor used in the plant trial was approximately twice what is required for adequate curd properties during wet curd processing by this method.

Chapter 7. Conclusions and Recommendations

The Westland Milk Products casein process was characterised under normal running conditions, analysing the effectiveness of the tube/screen washing system at reducing key impurities in the precipitated curd. This was followed by laboratory experiments examining the effect of producing casein from skim milk ultrafiltration retentate (MPC70). With adequate dilution, and careful control of pH, MPC70 retentate (initial solids and protein content of 18.3% and 13.4% respectively) can be acidified and washed to produce high quality casein. The use of dilution is simple, requires no capital expenditure and should not adversely affect plant throughput or operating costs. The effect of dilution and acidification pH on colloidal calcium phosphate (CCP) solubility during casein precipitation from MPC70 retentate was explored. This showed that CCP solubility increased as retentate concentration was reduced. MPC70 retentate at between 50% and 40% of original concentration was found to produce curd with good washability. Reducing acidification pH below the normal range of 4.60 – 4.65 was not found to be a practical means of reducing the residual calcium levels in casein made from these retentates. Lowering pH increased casein losses due to an increase in the formation of fines and difficulty in separating wash water from the curd. This effect was very pronounced in the laboratory and would have a detrimental effect on yields in the commercial process.

A plant trial was conducted where MPC85 retentate with a total solids content of 16.5% and protein of 14.6% was diluted and used to make casein. Analysis of this product showed it to be of excellent quality. Low levels of calcium and lactose indicate the curd was very well washed and RP-HPLC testing was not able to detect any residual whey protein. Previous observations indicated that the use of high concentration UF retentates may result in caseinates with lower than normal viscosities. This was investigated by making a 5% total solids sodium caseinate solution from the trial casein and comparing it to conventionally-produced casein. The trial sample in fact had a slightly higher viscosity, though the change was not thought to be significant.

7.2 Further work

Based on the findings of the literature review, plant analysis, plant trial and experiments the following recommendations for further work can be made:

- Repeating the experimental conditions used in this work, but with more detailed analysis of the mineral compositions of the feed material and all of the resultant streams. Ideally this would be done with ready access to laboratory-scale membrane separation and more sophisticated analytical techniques such as ICP-AES or mass spectrometry. The data obtained could then be used in conjunction with chemical speciation software to optimise the concentration and pH conditions.
- More investigation into the effect of UF retentate use on the whey stream is required. WPC is sold as a highly functional ingredient so even subtle changes to its composition may be commercially important. The addition of dilution water which must be subsequently removed to concentrate WPC may have an impact on process economics.
- Repeated trials, either on a pilot or full-scale plant are needed to confirm these findings. The use of plants with different washing systems would be important to this, as these can vary widely in design and possibly their ability to wash the more cohesive curds obtained.

Chapter 8. References

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Chapter 9. Appendices

Appendix A Calcium ISE with EDTA titration

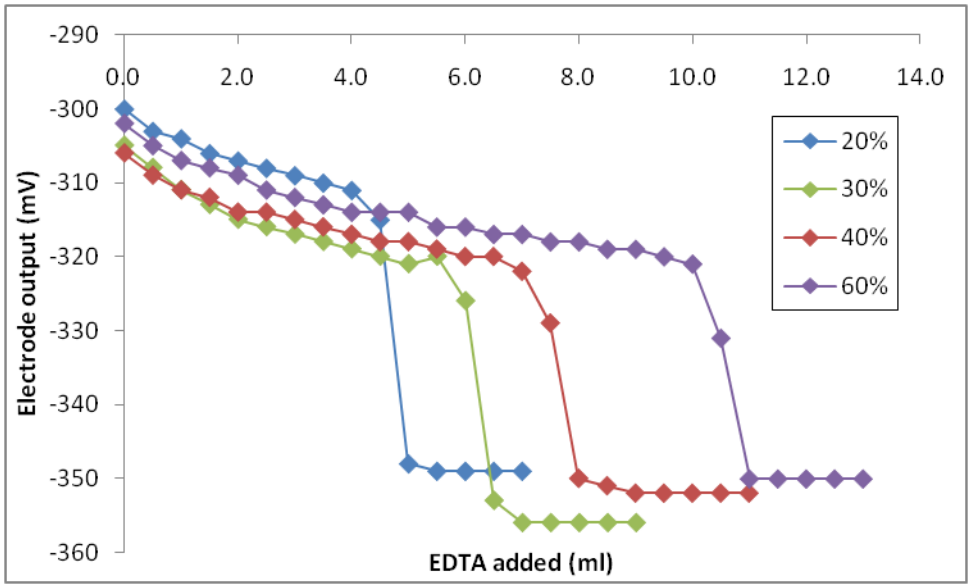

Figure 36: Example output of calcium ISE during EDTA titration. EDTA added by auto-pipette to diluted whey samples.

Appendix B Calcium data from first Laboratory Trial

Whey results

Table 26: Raw calcium results – whey from laboratory trial one

Sample	Concentration	pH	W	V	T1	% Ca
1	100%	6.69	4.0324	10	6.00	0.040
1	100%	6.69	4.0260	20	16.00	0.040
1	100%	6.69	4.0141	10	6.00	0.040
1	100%	6.69	4.0265	10	5.90	0.041
2	100%	5.57	4.0241	20	9.90	0.101
2	100%	5.57	4.0167	25	14.80	0.102
3	100%	5.34	4.0358	20	5.90	0.140
3	100%	5.34	4.0170	50	36.40	0.136
4	100%	4.94	4.0153	50	26.10	0.239
4	100%	4.94	4.0358	35	10.20	0.246
5	100%	4.76	4.0204	50	22.90	0.270
5	100%	4.76	4.0030	50	23.30	0.267
5	100%	4.76	4.0043	50	23.20	0.268
6	100%	4.49	4.0265	35	9.50	0.254
6	100%	4.49	4.0044	50	24.60	0.254
6	100%	4.49	4.0031	50	24.80	0.252
7	100%	4.31	4.0294	50	22.40	0.275
7	100%	4.31	4.0042	50	22.65	0.274
8	100%	4.19	4.0029	50	23.50	0.265
8	100%	4.19	4.0110	50	23.80	0.262
9	80%	6.68	4.0039	10	6.65	0.034
9	80%	6.68	4.0036	10	6.55	0.035
10	80%	5.58	4.0228	20	8.90	0.111
10	80%	5.58	4.0177	25	13.75	0.112
11	80%	5.25	4.0221	25	8.10	0.168
11	80%	5.25	4.0188	35	18.00	0.170
12	80%	4.93	4.0115	35	10.20	0.248
13	80%	4.74	4.0175	35	7.70	0.272
13	80%	4.74	4.0293	35	7.75	0.271
14	80%	4.44	4.0159	35	7.65	0.273
14	80%	4.44	4.0193	35	7.20	0.277
15	80%	4.22	4.0033	35	9.30	0.257
15	80%	4.22	3.9970	35	9.60	0.255
16	80%	4.09	4.0124	35	9.40	0.256
16	80%	4.09	4.0206	35	9.20	0.257
17	60%	6.74	4.0136	10	7.40	0.026
17	60%	6.74	4.0203	10	7.10	0.029
18	60%	6.04	4.0096	20	14.15	0.058
18	60%	6.04	3.9978	25	19.20	0.058

19	60%	5.62	3.9995	25	15.10	0.099
19	60%	5.62	4.0015	25	15.10	0.099
20	60%	4.89	4.0090	25	3.40	0.216
20	60%	4.89	4.0242	35	13.00	0.219
21	60%	4.70	4.0155	25	2.20	0.228
21	60%	4.70	4.0151	35	12.10	0.229
22	60%	4.51	4.0045	25	1.70	0.233
22	60%	4.51	4.0046	35	11.60	0.234
23	60%	4.33	4.0039	35	11.50	0.235
23	60%	4.33	4.0071	35	11.40	0.236
24	60%	4.11	4.0259	35	11.00	0.239
24	60%	4.11	4.0060	35	11.10	0.239

Casein Results

Table 27: Raw calcium results – casein from laboratory trial one

Sample	Concentration	pH	W	V	T1	% Ca
1	100%	6.69	0.1022	25	18.40	2.59
1	100%	6.69	0.1058	10	3.50	2.46
1	100%	6.69	0.1027	10	3.75	2.44
2	100%	5.57	0.1005	10	5.10	1.95
2	100%	5.57	0.1012	10	5.10	1.94
3	100%	5.34	0.1020	10	5.90	1.61
3	100%	5.34	0.1077	10	5.60	1.64
4	100%	4.94	0.1014	10	7.10	1.15
4	100%	4.94	0.1022	10	7.10	1.14
5	100%	4.76	0.1007	10	7.40	1.03
5	100%	4.76	0.1032	10	7.60	0.93
6	100%	4.49	0.1027	10	7.10	1.13
6	100%	4.49	0.1084	10	6.70	1.22
6	100%	4.49	0.1009	10	7.00	1.19
7	100%	4.31	0.1216	10	6.60	1.12
7	100%	4.31	0.1007	10	6.80	1.27
8	100%	4.19	0.1125	10	6.70	1.18
8	100%	4.19	0.1023	10	7.00	1.18
8	100%	4.19	0.1036	10	7.00	1.16
9	80%	6.68	0.1018	10	3.40	2.60
9	80%	6.68	0.1001	10	3.50	2.60
10	80%	5.58	0.1000	10	5.50	1.80
10	80%	5.58	0.1023	10	5.45	1.78
11	80%	5.25	0.1009	10	6.70	1.31
12	80%	4.93	0.1006	10	8.60	0.56
12	80%	4.93	0.1030	10	8.60	0.54
13	80%	4.74	0.1015	10	9.10	0.36
13	80%	4.74	0.1018	10	9.10	0.35
14	80%	4.44	0.1323	10	8.10	0.58
15	80%	4.22	0.1012	10	8.40	0.63
16	80%	4.09	0.1029	10	8.35	0.64
17	60%	6.74	0.1037	10	3.50	2.51
18	60%	6.04	0.1050	10	4.45	2.12
19	60%	5.62	0.1037	10	5.90	1.58
20	60%	4.89	0.1020	10	9.60	0.16
20	60%	4.89	0.1015	10	9.70	0.12
21	60%	4.70	0.1105	10	9.60	0.15
21	60%	4.70	0.1016	10	9.60	0.16
22	60%	4.51	0.1033	10	9.85	0.06
22	60%	4.51	0.1035	10	9.85	0.06
23	60%	4.33	0.1056	10	9.90	0.04
23	60%	4.33	0.1064	10	9.80	0.08
24	60%	4.11	0.2070	10	10.00	0.00
24	60%	4.11	0.1035	10	10.00	0.00