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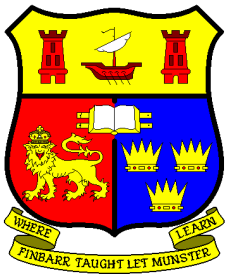
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Studies on the Texture, Functionality, Rheology and Sensory Properties of Cheddar and Mozzarella Cheeses

Thesis presented by

Anna Christina Moynihan, B.Sc. ***(NUI)***

for the degree of

Doctor of Philosophy

in

Food Chemistry

April, 2015

DECLARATION BY THE CANDIDATE
Studies on the Texture, Functionality, Rheology and Sensory of Cheddar and Mozzarella cheeses

Anna Moynihan

I hereby declare that work described in this thesis is my own and has not been submitted for another degree, either in Universtiy College Cork, or elsewhere

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Anna Moynihan

April 2015

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Summary

The effect of fortification of skim milk powder (SMP) and sodium caseinate (NaCn) on Cheddar cheeses was investigated. SMP fortification led to decreased moisture, increased yield, higher numbers of NSLAB and reduced proteolysis. The functional and texture properties were also affected by SMP addition and formed a harder, less meltable cheese than the control. NaCn fortification led to increased moisture, increased yield, decreased proteolysis and higher numbers of NSLAB. The functional and textural properties were affected by fortification with NaCn and formed a softer cheese that had similar or less melt than the control.

Reducing the lactose:casein ratio of Mozzarella cheese by using ultrafiltration led to higher pH, lower insoluble calcium, lower lactose, galactose and lactic acid levels in the cheese. The texture and functional properties of the cheese was affected by varying the lactose:casein ratio and formed a harder cheese that had similar melt to the control later in ripening. The flavour and bake properties were also affected by decreased lactose:casein ratio; the cheeses had lower acid flavour and blister colour than the control cheese.

Varying the ratio of α_{s1}:β-casein in Cheddar cheese affected the texture and functionality of the cheese but did not affect insoluble calcium, proteolysis or pH. Increasing the ratio of α_{s1}:β-casein led to cheese with lower meltability and higher hardness without adverse effects on flavour.

Using camel chymosin in Mozzarella cheese instead of calf chymosin resulted in cheese with lower proteolysis, higher softening point, higher hardness and

lower blister quantity. The texture and functional properties that determine the shelf life of Mozzarella were maintained for a longer ripening period than when using calf chymosin therefore increasing the window of functionality of Mozzarella.

In summary, the results of the trials in this thesis show means of altering the texture, functional, rheology and sensory properties of Mozzarella and Cheddar cheeses.

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This PhD has been a very long and challenging road which was only made possible by the help and support of many people. I gratefully acknowledge the financial funding granted by the Food Institutional Research Measure (FIRM) for project 08RDTMFRC670 Natural Ingredient Cheese Solutions under the Department of Agriculture, Food and the Marine. I would also like to gratefully acknowledge the scholarship from the Fulbright Commission that allowed me to travel to the University of Wisconsin-Madison to study for part of my PhD.

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I am very grateful to all the staff at Wisconsin's Centre of Dairy Research for all their help with my many projects during my time there. I would like to especially thank Rani who continually gave me advice, support and guidance. She has been an amazing support to me while I wrote up my thesis and I am truly grateful for this. I would like to thank Mark for all his help and advice with my work. I am sincerely thankful to Mike for all his help, advice and for teaching me so much about filtration. I truly appreciate the CDR cheese making teams help and time; John Jaeggi, Joey, Ray, Bene and Gina made the long days of cheese making so enjoyable and I truly appreciate all their help. I would also like to thank Carol and Luis for all their help and expertise in

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CHAPTER 1

Literature Review: Cheese Texture and Rheology

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1.1 Rheology

Rheology is the study of the deformation and flow of matter when exposed to stress or strain (Fox et al., 2000; Gunasekaran and Ak, 2003). Properties of foods can be measured by rheology (Lucey et al., 2003); rheology examines relationships between stress, strain and time (Foegeding et al., 2003; Stokes et al., 2013). Rheology is used in food science to gain an understanding of processing effects on foods, of system structure and to characterize food structure (Foegeding et al., 2003). Rheological properties of cheese relate mainly to its composition, structure and strength of attractions among its structural elements in cheese (O'Callaghan and Guinee, 2004).

1.2 Rheology of cheese

Deformation is a measure of displacement in size or shape of a material when it is subjected to a force (Gunasekaran and Ak, 2003). This change can be temporary, partly recoverable or permanent and, when related to the force applied during measurement of materials under specific test conditions, can describe the rheological characteristics of the material (O'Callaghan and Guinee, 2004).

Stress is a measurement of pressure and therefore expressed in units of Pascals (Pa) (Daubert and Foegeding, 1998); it is defined as the distribution of force over an area of material (O'Callaghan and Guinee, 2004). Two types of stress exist: normal stress (σ) where force is applied perpendicularly to a surface and shear stress (τ) where the force is applied parallel to the surface plane (Gunasekaran and Ak, 2003; O'Callaghan and Guinee, 2004). Applying stress

to a solid-like material results in deformation (Fox et al., 2000). An example of normal stress is chewing of cheese and spreading cream cheese is an example of shear stress (Lee and Lee, 2013)

Strain has been defined as the fractional displacement that occurs under an applied stress (O'Callaghan and Guinee, 2004). It is a dimensionless measurement; if a normal or shear stress is applied the associated strain is called normal (ε) or shear strain (γ), respectively (Daubert and Foegeding, 1998).

When strain is directly proportional to applied stress, a material is described as a perfect elastic or Hookean solid; in contrast, an ideal fluid or Newtonian liquid will not support a stress (Fox et al., 2000). Basically, a Hookean solid will deform and Newtonian liquid will flow (Gunasekaran and Ak, 2003). Cheese, like most foods, is a viscoelastic substance (Lucey et al., 2003) meaning that it is a material that has both viscous (or fluid) and elastic (or solid) like characteristics (Foegeding et al., 2003). Cheese characteristics such as composition, microstructure, macrostructure and physicochemical aspects of its components will consequently affect rheology (Fox et al., 2000). Rheological properties of cheeses are important not only due to their impact on sensory quality for the consumer but they are also related to characteristics such as handling, stacking and suitability of cheese as an ingredient for industrial purposes (O'Callaghan and Guinee, 2004).

Unlike ideal elastic and viscous materials, time is an important characteristic to take into consideration when performing rheological tests on viscoelastic foods (Foegeding et al., 2003; Lucey et al., 2003). When a viscoelastic material is

subject to deformation, the energy is partly stored (elastic) and partly dissipated (viscous) and the timescale of applied stress can cause different responses on the measurements of rheological properties of the food (Lucey et al., 2003). The time dependence of cheese rheology can be shown using a simple creep experiment (Daubert and Foegeding, 1998) where a constant stress is applied to a sample and the time-related change in strain is examined (Figure 1.1). When a strain is applied to a viscoelastic material that is so small it does not cause permanent damage to the material, a linear relationship is observed between stress and strain; hence, the material exhibits elastic behaviour (Fox et al., 2000).

The cheese matrix absorbs the energy from the stress and this energy is instantly lost on removal of the stress and the cheese maintains its original

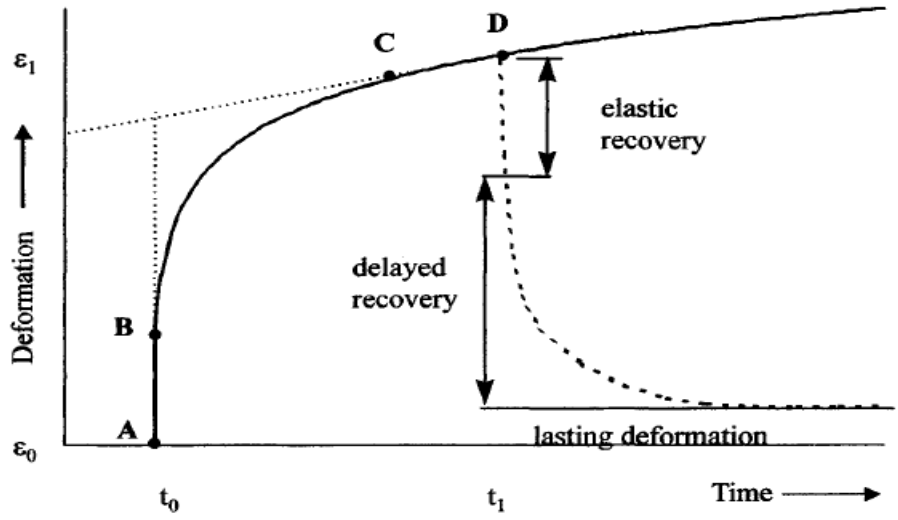


Figure 1.1 Time related change in deformation of cheese after subjecting it to a constant stress at t_0 and deformation recovery upon removal of the stress after t_1 (- - -). Three types of deformation are apparent, the elastic region (AB), viscoelastic region (BC) and viscous region (beyond C). Upon removal of the stress (D) three areas of recovery are evident elastic, viscoelastic (delayed) and lasting deformation as a result of viscous deformation (Fox et al., 2000).

structure. The characteristics of cheese and the degree of stress affect the size of this elastic region. Strains beyond this elastic region show non-linear relationships between stress and strain; the cheese displays viscoelastic deformation where bonds in the cheese structure break and it exhibits both elastic and viscous properties. On removal of stress the recovery of the structure is delayed. At high strains the cheese fractures and hence the damage to the structure is permanent causing the cheese to flow in the viscous region (Fox et al., 2000; O'Callaghan and Guinee, 2004). Therefore viscoelastic foods can be characterised into three regions where the first region refers to the elastic (linear) region, the second region is the viscoelastic (nonlinear) region and the third occurs at sample fracture which is the viscous region (Foegeding et al., 2003).

1.3 Small Amplitude Oscillatory Rheology

Dynamic low amplitude oscillatory shear rheology (DLAOR) or small amplitude oscillatory shear rheology (SAOS) are small strain rheological tests involve using low stresses or strains so that permanent damage is not caused to the cheese; hence, these tests are performed in the linear viscoelastic region of the material (Gunasekaran and Ak, 2003; Foegeding and Drake, 2007; Tunick, 2011; Lee and Lee, 2013). Instruments can be either controlled stress or controlled strain where stress amplitude is constant and strain measured or strain is constant and stress measured, respectively. The stress or strain varies sinusoidally with time (Steffe, 1996). Most rheometers use sinusoidal oscillatory shear and utilizes a constant stress or strain at a certain frequency which produces a certain wave response for stress or strain (Foegeding and

Drake, 2007). Exposing the material to a sinusoidal shear strain with a constant amplitude, γ_0, and frequency, ω, where the shear strain varies with time so that:

$$\gamma(t)= \gamma_0 \sin(\omega t) \quad \text{Equation (1)}$$

Using a strain that is small enough gives a sinusoidal stress response where σ_0 is the stress amplitude:

$$\sigma(t)= \sigma_0 \sin(\omega t+\delta) \quad \text{Equation (2)}$$

These waves vary depending on the rheological characteristics of the material being studied and the phase angle (δ) can relate to characteristics of the material being studied (Figure 1.2). For a perfectly elastic material, stress and strain waves are in phase and δ is 0; in contrast for a Newtonian liquid stress and strain are 90° out of phase. Therefore, depending on the viscous and elastic behaviour of a material, phase angle is $0<\delta<90°$ (Brown et al. 2003; Gunasekaran and Ak, 2003; Foegeding and Drake, 2007). Considering this, the phase angle (δ) or the loss tangent (tan) of δ is a measure of viscous to elastic behaviour of the sample being measured. The stress component that is in phase with the applied strain is called the storage or elastic modulus (G'):

$$G' = \frac{\sigma_0}{\gamma_0}\cos\delta \quad \text{Equation (3)}$$

The stress component that is 90° out of phase with the applied strain is called the loss or viscous modulus (G''):

$$G'' = \frac{\sigma_0}{\gamma_0}\sin\delta \quad \text{Equation (4)}$$

The phase angle can be a measure of viscous to elastic characteristics of materials, therefore:

$$\tan \delta = \frac{G''}{G'} \qquad \text{Equation (5)}$$

Another parameter, complex modulus (G^*), which relates stress amplitude to strain amplitude can be calculated:

$$G^* = \frac{\sigma_0}{\gamma_0} = \sqrt{(G')^2 + (G'')^2} \qquad \text{Equation (6)}$$

The relationships between these equations can be shown in a trigonometrical representation (Figure 1.3).

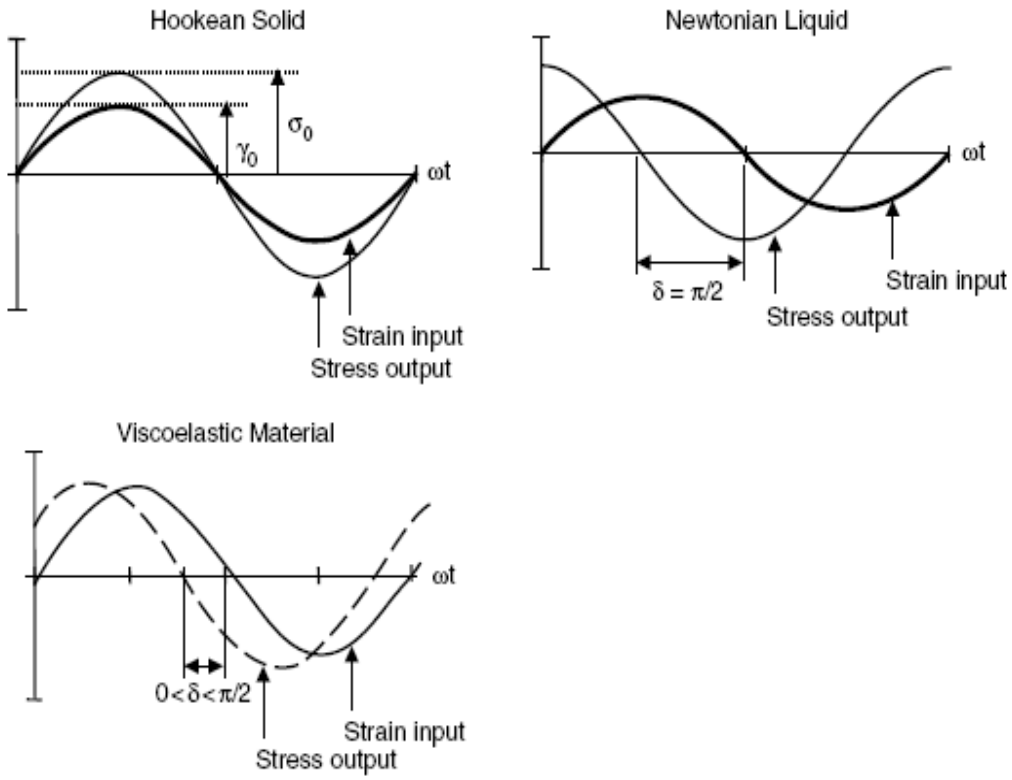


Figure 1.2 Sinusoidal strain input and typical stress-strain responses of elastic solid, viscous liquid and viscoelastic materials (Gunasekaran and Ak, 2003).

A strain or stress sweep can be performed to determine the linear viscoelastic region of a material using an oscillatory rheometer which varies amplitude (stress or strain) at a constant frequency giving the critical stress or strain limit which when exceeded, is outside the linear viscoelastic region of the material (Steffe, 1996; Tunick, 2011). This should be carried out before frequency sweeps to determine the appropriate stress or strain to be used (Gunasekaran and Ak, 2003).

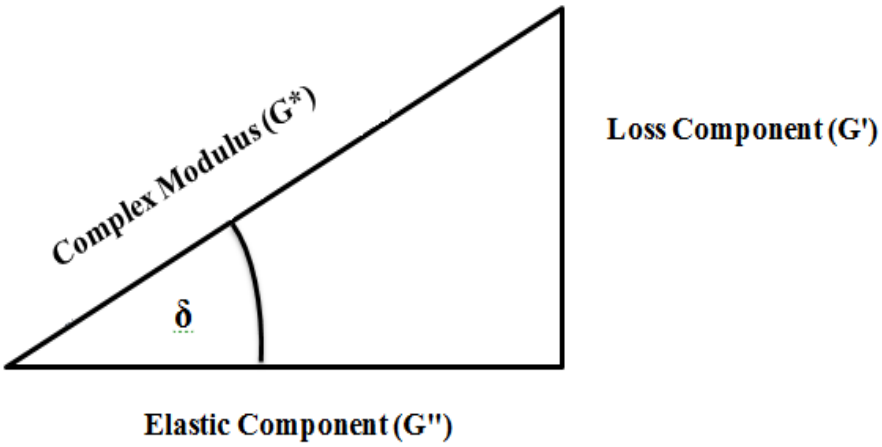


Figure 1.3 Trigonometrical representation to show the relationship between G', G'', G* and δ (adapted from O'Callaghan and Guinee, 2004).

Frequency sweeps vary the frequency while keeping the amplitude constant and within the linear viscoelastic range of the material; this gives information on the viscous and elastic characteristics or a "mechanical spectrum" of a material at different frequencies (Steffe, 1996; Gunasekaran and Ak, 2003) and how the structure responds to various experimental time-scales (Tunick, 2011).

Temperature and time sweeps are used a great deal in cheese research. These tests keep a constant frequency and amplitude over a certain time. Keeping a

constant temperature during this test is a time sweep (Tunick, 2011) and could look at characteristics of gels while it forms, such as during the rennet coagulation of milk (Gunasekaran and Ak, 2003). Temperature sweeps heat materials and from this the thermal properties of cheese can be assessed (Steffe 1996; Gunasekaran and Ak, 2003; Tunick, 2011).

1.4 The application of DLAOR in milk and cheese

Rheological properties of milk gels and cheese have been studied using DLAOR (Guinee et al., 2002). Rheological analysis of milk gels during rennet coagulation detects an increase in gel firmness at point of coagulation, as measured by an increase in G'; this hydrolysis and removal of the macropeptide affects steric stabilization leads to hydrophobic attraction of micelles and decreased electrostatic repulsion. These interactions, and consequently gel formation, are strongly governed by pH (Lucey et al., 2003).

The total number and strength of bonds in a cheese system can be indicated by G' and G" (Udyarajan et al., 2007). These tests can distinguish characteristics of different cheeses and also the rheological behaviour of cheese can vary with ripening time. As the rheological properties of cheese vary with temperature, measuring these properties at various temperatures can help to classify its properties (Gunasekaran and Ak, 2003).

Figure 1.4 shows typical graphs for cheese when subjected to DLAOR during heating. The storage modulus and viscous modulus typically decrease for cheese as the temperature increases which can relate to a decrease in the

number or strength of bonds holding the cheese matrix together (Lucey et al., 2003). As temperature increases, hydrophobic interactions increase in strength

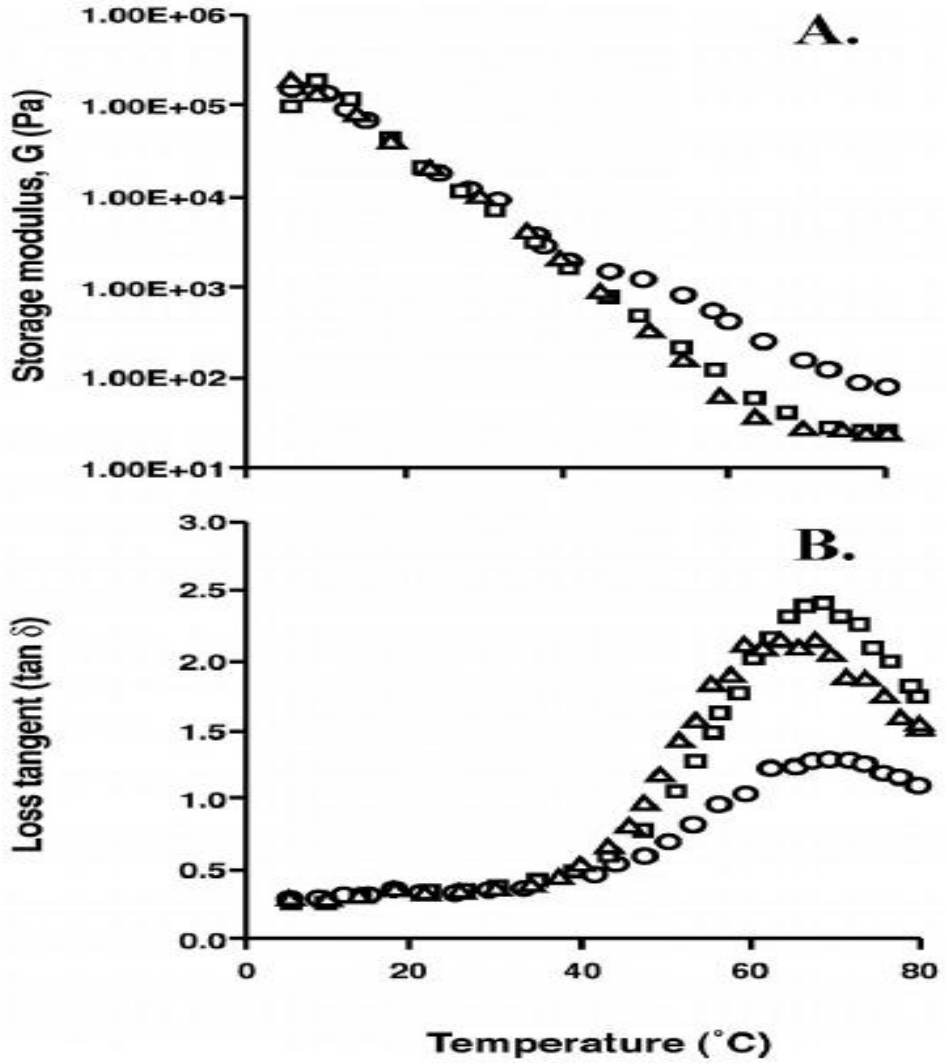


Figure 1.4 Effect of temperature on the storage modulus (a) and tan δ (b) of Cheddar cheese at 2 d (○), 14 d (□) and 1mo (△) (Lucey et al., 2003).

up to 60-70°C; beyond this temperature, they start to lose strength, weakening the cheese matrix and causing cheese to flow (Bryant and McClements, 1998; Johnson and Lucey, 2006). At low temperatures, hydrophobic bonds are also weakened (Horne, 2003); electrostatic interactions increase with increasing

temperature (Bryant and McClements, 1998). The number of bonds per unit volume, and bond type or strength, also contribute to G'. Weakened bonds at lower temperature ranges could cause the caseins to swell causing increased contact area and bonds between particles; the G' value at lower temperatures can indicate the strength of bonds within the cheese system (Choi et al., 2008).

Changes in the G' and tan δ during heating indicate a change in phase from a large elastic rheological response with a high G' and low tan δ to a melted more viscous and fluid cheese with a low G' and higher tan δ (Guinee et al., 2002). As this phenomenon occurs, the tan δ begins to increase; as tan δ is the ratio of G"/G', this increase shows that at higher temperatures the cheese becomes a more fluid-like material (Lucey et al., 2003).

When tan δ=1 the elastic- and viscous-like characteristics of the cheese are equal; this can be considered the crossover point (Figure 1.5) or an index of the softening point of cheese (Gunasekaran and Ak, 2003). The maximum tan δ has been highly correlated with the meltability of cheese (Mounsey and O'Riordan, 1999) it has been used in numerous studies as an index of cheese melt. Once cheese reaches its tan δ_{max}, a decrease can occur in the tan δ along with an increase in G' indicating that the cheese matrix is strengthening even though the temperature is still increasing. It has been hypothesised that this is due to heat- induced formation of colloidal calcium phosphate (CCP). This could be caused if calcium solubility in the serum phase begins to decrease at higher temperatures and crosslinking reactions of proteins with this new insoluble calcium or hydrophobic aggregation of casein occur at high temperatures (Udyarajan et al., 2007).

Softening relates to the loss of elasticity and melt is the ability of the cheese to spread and flow (Lucey et al., 2003). Flow may occur when the elastic modulus becomes lower than the viscous modulus or the tan δ has a value greater than 1 during heating of a cheese (Lucey et al., 2003). Elastic and viscous moduli can be used to examine the properties of cheese; higher elastic modulus compared to viscous modulus can indicate a dominance of elastic character in cheeses such as Mozzarella (Joshi et al., 2004).

In cheese, fat is the only component that actually becomes completely viscous or melts on heating but protein interactions can change with temperature, producing a melt effect (Lucey et al., 2003); milk fat in cheese is a viscous fluid before and after heating; however, it is fully liquid at about 40°C (Guinee et al., 2002). The changes in tan δ to its tan δ_{max} during heating occurs beyond the melting temperature of milk fat, indicating that the main contributor to

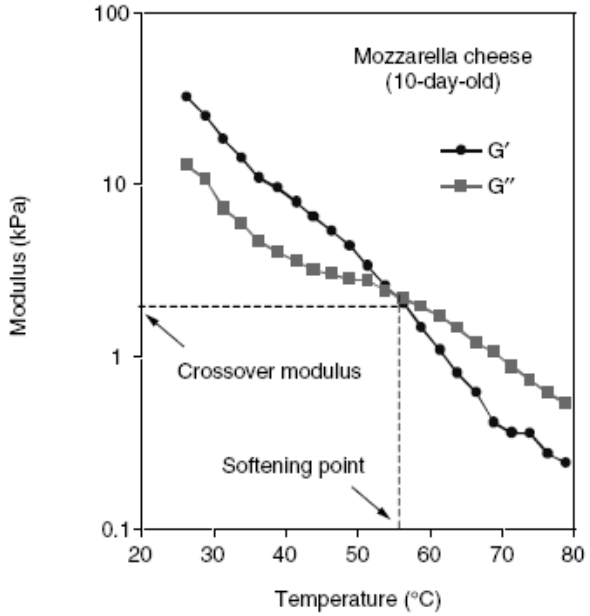


Figure 1.5 Temperature at crossover modulus correspond to cheese softening point (Gunasekaran and Ak, 2003).

differences in rheological properties of cheese on heating are caused by changes to the protein matrix (Udyarajan et al., 2007).

1.5 Large Deformation Rheology

Bourne (2004) offered the definition of texture as: "the textural properties of a food are that group of physical characteristics that arise from the structural elements of the food, are sensed primarily by the feeling of touch, are related to the deformation, disintegration and flow of the food under a force, and are measured objectively by functions of mass, time and distance." Cheese texture is a sensory characteristic assesed by touch and eating (Fox et al., 2000) Texture is important for differentiating cheese varieties and for consumer acceptance (Bourne, 2004). Texture mainly determines food palatability, but it is quite difficult to obtain reproducible quantitative experimental results through a sensory panel when compared to instrumental techniques (Nishinari, 2004). Using sensory evaluation to assess cheese texture requires a trained panel; however, this is time-consuming and expensive, and therefore instrumental measurements are typically used for routine texture analysis (Drake et al., 1999; Fox et al., 2000). Instrumental methods are easier to standardise for texture and are generally based on force-compression tests, the most common of which is texture profile analysis (TPA). As previously discussed, the rheological properties of viscoelastic foods are characterised into three regions. Typical methodology to correlate sensory and rheology properties involve regions two (non-linear region) and three (fracture); these are evaluated using large-strain testing (Foegeding et al., 2003).

Large strain rheology determines properties occurring outside the linear viscoelastic region and indicates permanent deformation where bonds involved in the structural elements of cheese are broken and do not reform. It can characterise nonlinear and fracture properties (Walstra and van Vliet, 1982; Bourne, 2004). Large deformation can be divided into deformation resulting in fracture (non-recoverable) or deformation not resulting in fracture (partial recovery). These measurements used for large deformation studies typically relate to stresses and strains experienced during consumption and size reduction of cheese (O'Callaghan and Guinee, 2004). The energy needed to deform a sample can be stored, used for fracture or dissipated in a way other than by fracture (van Vliet, 2002). Fracture occurs when all bonds on a certain plane in the cheese sample are broken (Walstra and van Vliet, 1982). Deformation rate will govern the stress at which fracture occurs; higher deformation rates will give less time for stress relaxation (Lucey et al., 2003). Instrumental TPA is a two cycle compression technique in which a piece of food is compressed and decompressed twice which mimics the first two bites of food on chewing (Stokes et al., 2013). This test generates a force-time curve (Figure 1.6) which provides texture characteristics such as fracture, hardness, cohesiveness, adhesiveness and springiness (Daubert and Foegeding, 1998; Bourne, 2004). Two other parameters can be calculated from these measured parameters, namely guminess and chewiness.

These parameters are defined (Bourne, 1978) as:

- **Fracturability** is the force at the first significant break in the curve (shown in Figure 1.6)

- **Hardness** is the peak force during the first compression (shown in Figure 1.6)
- **Cohesiveness** is the ratio of the positive force area during the second compression to that during the first compression (A_2/A_1 in Figure 1.6)
- **Adhesiveness** is the negative force area for the first bite (A_3 in Figure 1.6)
- **Springiness** is the height that the food recovers during the time that elapses between the end of the first and start of the second bite
- **Gumminess** is hardness × cohesiveness
- **Chewiness** is gumminess × springiness

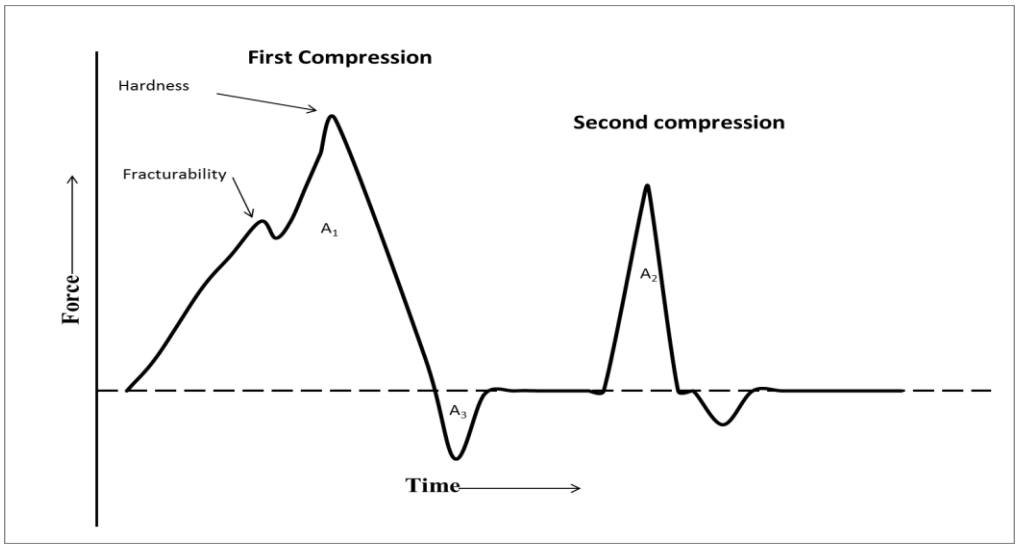


Figure 1.6 A typical force-time curve generated during texture profile analysis. A_1 is the area of the first compression, A_2 is the area of the second compression and A_3 is the negative force area for the first bite (modified from Bourne, 1978).

TPA hardness and springiness have been highly correlated with G' and G". Drake et al. (1999) found that TPA was better at predicting sensory texture measurements than fundamental rheological testing. Brown et al. (2003) found that there were relationships between the rheological and sensory properties of

cheese, mainly rigidity and resiliency. Rigidity was related to complex modulus, storage modulus, fracture modulus and sensory firmness. Resilience was related to phase angle, maximum compliance of creep recovery tests, retardation time, sensory springiness and sensory rate of recovery. Abu-Waar et al. (2013) found that the texture of Cheddar cheese was temperature-dependent; during texture measurement, temperature should be controlled to best correlate sensory and instrumental measurements. Everard et al. (2006) found significant correlations between nine sensory parameters and large strain deformation tests in Cheddar cheese; for example, sensory firmness correlated with instrumental fracture stress and firmness but sensory parameters "grainy" or "moist" could not be correlated significantly with instrumental methods.

1.6 Milk proteins and colloidal calcium phosphate

As previously mentioned, protein in the cheese matrix is the main contributor to rheological properties of cheese. Caseins are phosphoproteins which represents about 80% of protein in bovine milk (Horne, 2002, 2006; Fox and Brodkorb, 2008). Casein in milk exists as colloidally dispersed protein particles with colloidal calcium phosphate (CCP) termed casein micelles (Lucey et al., 2003). Four caseins are present in milk, α_{s1}-, α_{s2}-, β-, and κ-caseins, in approximate ratios of 4:1:4:1 by weight (Liu and Guo, 2008) these can be divided into calcium sensitive (α_{s1}, α_{s2} and β) and calcium insensitive (κ) caseins (Horne, 2006) depending on the level of phosphoserine residues (Horne, 2002). The dry matter of the bovine casein micelle contains about 6% mineral or CCP (Horne, 2006).

CCP plays an important role in the stability of the casein micelle but hydrogen bonds, hydrophobic and electrostatic interactions also govern micelle integrity (Fox and Brodkorb, 2008; Liu and Guo, 2008). Hydrophobic interactions produce an attractive force between non-polar groups (Bryant and McClements, 1998). κ-Casein has a hydrophobic, slightly positive N-terminal region and a highly charged C-terminal peptide (Horne, 2002; Lucey et al., 2003). κ-Casein is the only glycosylated casein; glycosylation occurs at the C-terminus, contributing to the hydrodynamic bulk and hydrophilicity of this casein (Horne, 2002). κ-Casein molecules are found on the surface of the micelle and its C-terminus extends into the surrounding solution, which contributes to the stability of micelles through steric stabilization (de Kruif, 1999; Lucey et al., 2003). The importance of κ-casein in the colloidal stability of the micelle can be seen upon cleavage of κ-casein by enzymes in rennet during cheese-making, lowering the charge density or pH, addition of $CaCl_2$ or ethanol, and also combining these effects, can impact stability (de Kruif, 1999).

Multiple interactions are involved in the binding of caseins together (phosposerine residues and calcium bridging, hydrogen bonding, hydrophobic and electrostatic interactions); therefore, even when the calcium phosphate nanoclusters are dissolved, the surrounding protein structure could remain intact unless disruption of other interactions also occurs (Liu and Guo, 2008; McMahon and Oommen, 2008). Environmental conditions impact protein conformation; for example, electrostatic interactions are very sensitive to pH and ionic strength (Bryant and McClements, 1998). Environmental conditions can also impact on protein functionality; functionality of casein molecules is

dependent on their ability to interact with calcium through phosphoserine groups, hydrophobic regions, hydrophilic interactions with water and also hydrogen bonding and electrostatic interactions (Horne, 2003; McMahon and Oommen, 2008).

All of the caseins are phosphorlylated but κ-casein is distinctive in that any phosphoserine residues are singlets found in the C-terminus. In contrast, residues in α_{s1}-, α_{s2}- and β-casein are usually found in clusters; the number of these residues varies from casein to casein. β-Casein contains a grouping of charged hydrophilic clusters and the phosphoseryl cluster in the N-terminus region, a large hydrophobic region from here to C-terminal also exists. α_{s1}-Casein forms a loop structure; it has hydrophobic regions at each end of the hydrophilic loop where phosphoserine residues are located. Similarly, α_{s2}-casein contains two hydrophobic regions; it has a hydrophilic N-terminus with a phosphoseryl cluster, then a hydrophobic region followed by a hydrophilic loop with clusters of phosphoseryl residues and then a hydrophobic region at its C-terminus (Horne, 2002; Lucey et al., 2003; McMahon and Oommen, 2008). These phosphoseryl clusters act as interaction sites on the caseins (Liu and Guo, 2008).

1.7 The Casein Micelle

Much controversy has existed in forming a model of the casein micelle (Horne, 2006; Fox and Brodkorb, 2008). Recently, McMahon and Oommen (2008) suggested an interlocking lattice model for casein micelle supramolecular structure. This involves an open irregular structure with various linkages

between proteins forming chains or interlocked with calcium phosphate nanoclusters with some protein chains with κ-casein on the outside of the micelle with multiple interactions maintaining micelle integrity. The dual-binding model (Horne, 1998), depicted in Figure 1.7, has been applied to cheese (Lucey et al., 2003; Johnson and Lucey 2006). The dual binding model involves polymerisation of the caseins by two forms of bonding; either crosslinking through hydrophobic regions or bridging across calcium phosphate clusters (Horne, 2002). Caseins polymerize through hydrophobic interactions as they form the casein micelle; hydrophilic regions could cause electrostatic repulsion and limit micelle growth but the negatively charged regions can bind to positively charged CCP nanoclusters causing neutralisation and continued growth of the micelle can occur. κ-Casein can be found throughout the micelle (McMahon and Oommen, 2008) and joins into the micelle assembly through its hydrophobic region; however, due to its lack of a phosphoserine cluster, its hydrophilic area cannot connect into the chain therefore, micelle growth is terminated with a surface of κ-casein (Horne, 2002; Lucey et al., 2003; Johnson and Lucey, 2006). Caseins have a loose tertiary structure where some regions are compact and others more open depending on protein charges (McMahon and Oommen, 2008).

1.8 Introduction to factors affecting cheese texture and functionality

Cheese begins to soften in the early stages of ripening. It was previously considered that proteolysis was one of the main causes of textural changes in cheese (Lawrence et al., 1987; Fox, 1989). Total calcium, proteolysis and pH

were understood to be the critical parameters associated with cheese texture and functionality but now insoluble calcium is recognised as an important index of functionality (Johnson and Lucey, 2006). Chymosin-mediated proteolysis of α_{s1}-casein at the Phe_{23}-Phe_{24} peptide bond was thought to be responsible for the initial softening of cheese (Fox, 1989). O'Mahony et al.

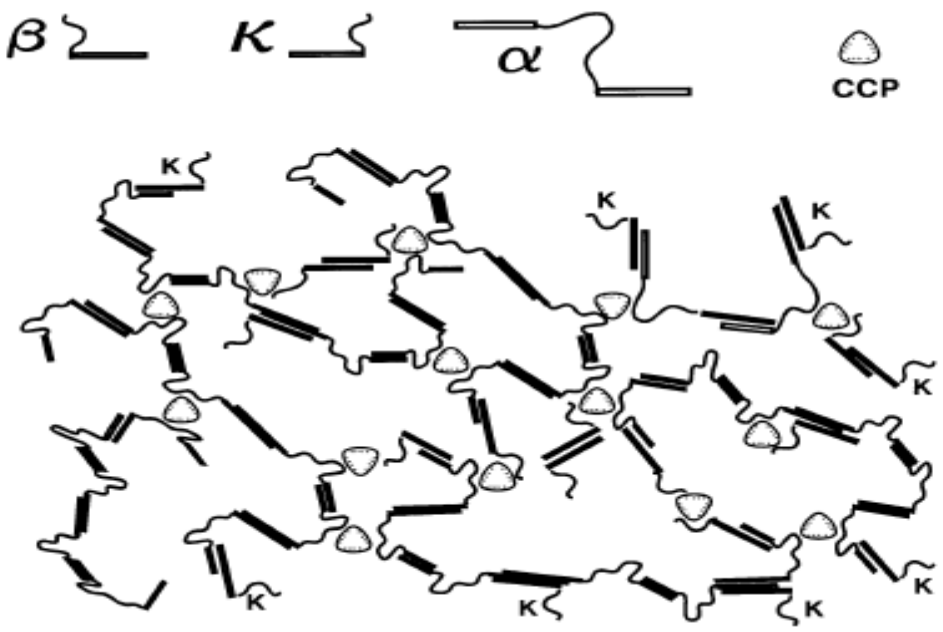


Figure 1.7 The dual binding model of the casein micelle (Horne, 1998).

(2005) inhibited chymosin in Cheddar cheese and found that even when cleavage of the Phe_{23}-Phe_{24} peptide bond of α_{s1}-casein was completely inhibited there was still a reduction in hardness in the early stages of ripening. It was concluded that solubilisation of CCP is the principal cause of softening of the casein network in cheese. These as well as the effect of rate of acid development and pH are probably the main three factors affecting textural and functional properties of cheese.

1.9 Solubilisation of CCP

pH has a strong effect on solubilisation of CCP; decreasing pH solubilises CCP and affects its solubilisation rate (Lucey and Fox, 1993; Pastorino et al., 2003; Johnson and Lucey, 2006). The level of insoluble calcium and pH are key factors relating to cheese meltability. Without a drop in pH during cheesemaking, CCP nanoclusters will remain intact, leading to crosslinking between molecules and limiting the movement or rearrangement of casein molecules (Johnson and Lucey, 2006). There is a high proportion of calcium and phosphate bound to casein in cheese and this is an important structural component of the protein (Lucey and Fox, 1993).

Gastaldi et al. (1996) found that almost all inorganic phosphate was solubilized in milk at pH 5.1 upon bacterial fermentation but around 17% of calcium was still in the micelle. Upon solubilisation of CCP, the positively charged nanoclusters leave a negatively charged site exposed, weakening hydrophobic interactions (Lucey et al., 2003). Along with dissolving CCP nanoclusters during acidification, charge neutralisation of proteins occurs (Horne, 2003). Near the isoelectric point of the caseins, micelles form a compact structure due to a reduction in electrostatic repulsion from low negative charges on the proteins and hydrophobic interactions increase between caseins and hydrophobic regions become more tightly packed (Liu and Guo, 2008). Varying acid concentrations affect gelation profiles of acidified milk systems; the faster the rate of acidification the stiffer the gel obtained. At pH less than 3.0, CCP is essentially completely dissolved; Liu and Guo (2008) found that, below the isoelectric point of casein (pH 2.0-3.0), micelle structure can be influenced by hydrophobic interactions, hydrogen bonding and electrostatic

action. In this pH range, amino acid residues become protonated and are able to form strong hydrogen bonds; therefore, in this pH range, hydrogen bonds and hydrophobic interactions stabilize the structure.

As milk is acidified for cheese making, parameters such as drain pH will have a major impact on the level of retention of minerals in the curd. A decrease in the drain pH during cheese making leads to greater acid production prior to whey drainage and hence increased curd demineralization (Lucey and Fox, 1993). Calcium solubilisation differs in milk and curd; in milk, CCP dissolves between pH 6.0-5.0 but in cheese high levels of CCP can remain in the cheese curd even at pH 4.7 (Lee et al., 2005). As acidification occurs during cheese manufacture, insoluble calcium is converted to soluble calcium; during syneresis, much of the solubilized calcium is lost in the serum phase. Subsequent acidification in the curd leads to a build-up of solubilized calcium; this, in combination with less available water for CCP solubilisation, can hinder further solubilisation (Johnson and Lucey, 2006).

The level of soluble calcium in cheese increases during ripening with most of these changes occurring at the start of ripening (Hassan et al., 2004; Lee et al., 2005, 2010; O'Mahony et al., 2005). The conversion of insoluble to soluble calcium in cheese is thought to be due to an attainment of pseudoequilibrium of these two forms (Lee et al., 2005; O'Mahony et al., 2005). This decrease in insoluble calcium can still occur even when there is little or no change in pH during ripening from formation of lactic acid; however, a large decrease in pH can accelerate the solubilisation of CCP (Lee et al., 2010).

Lower levels of insoluble calcium have been observed in cheese with a lower pH (Lee et al., 2005, 2010). Insoluble calcium within casein in the cheese matrix can affect the viscoelastic properties of cheese as the stress carrying bonds between caseins are affected (Choi et al., 2008). Faster rearrangements of protein-protein bonds occur during acidifaction due to CCP solubilisation, leading to fewer crosslinking bonds in the casein micelle; CCP is a crosslinking agent within the casein micelle and therefore its dissociation can affect rheological and fracture properties of cheese (Fox, 1989; Watkinson et al., 2001; Johnson and Lucey, 2006; Choi et al., 2008). An increased protein density in cheese with greater calcium has been observed to have higher hardness and decreased meltability due to greater crosslinks in the structure (McMahon et al., 2005).

The solubilisation of CCP during the start of ripening in Cheddar cheese has been highly correlated with the reduction in hardness typically observed for this variety (O'Mahony et al., 2005). These changes in the level of insoluble calcium have been highly correlated with changes in the rheological characteristics of Cheddar, such as tan δ_{max} and G' changes during heating. Insoluble calcium has been found to be more significantly correlated with the rheological properties of cheese than proteolysis (Lucey et al., 2005). McMahon et al. (2005) found that, when manufacture was varied to make a Mozzarella cheese that had different pH values but the same level of calcium, the main effect on the meltability of the cheese was calcium and not pH. Lee et al. (2005) observed a decrease in the G' value at 80°C of Cheddar cheese during the first week in ripening that coincided with a reduction in insoluble calcium and pH. Insoluble calcium was also found to be positively correlated

with tan δ_{max} and degree of flow of cheese. Joshi et al. (2003) found that reducing calcium using preacidification but maintaining the same cheese pH value affected the melt properties of Mozzarella cheese. Reduced-calcium cheese had a higher melt, flow rate, extent of flow and softened at a lower time and temperature.

O'Mahony et al. (2006) found that, on heating above 50°C, G' and G" values varied when the level of CCP was changed in Cheddar cheese. In cheese with a greater concentration of CCP, more solid-like characteristics (G'>G") dominated at higher temperatures and the tan δ_{max} value decreased compared to cheeses with lower levels of CCP. Choi et al. (2008) found that the G' and tan δ values at 70°C of cheese made with added EDTA decreased and increased, respectively, as EDTA concentration increased; in this study, pH values were similar and proteolysis was negligible; hence, differences were attributed to the level insoluble calcium. Lower meltability in cheese with a higher calcium level is due to higher protein-protein interactions where more energy is required break bonds and cause flow (McMahon et al., 2005). The attractive forces between caseins associated with high levels of insoluble calcium results in a cheese that will not flow at higher temperatures (Johnson and Lucey, 2006).

Joshi et al. (2004) found that reducing the level of calcium in Mozzarella cheese led to reduced elastic and viscous moduli in the cheese. Reduction of calcium is responsible for weakening of the cheese matrix by increased interactions between protein and water which is present in fat serum channels in Mozzarella, as well as rearrangement of fat particles. In this study, a reduction in viscoelastic nature of the cheese during ripening was also

observed. Higher elastic and viscous modulus in young cheese is believed to be due to protein crosslinking and bonds in the casein network.

1.10 pH

1.10.1 Effect of pH on casein interactions in milk

pH has a marked effect on casein micelles and this can be observed during acidification of milk (Gastaldi et al., 1996). As previously mentioned, pH has an effect on CCP solubilisation by solubilizing ions from the casein micelle during acidification (Gastaldi et al., 1996; Le Graet and Gaucheron, 1999; Pastorino et al., 2003) but not on hydrophobic interactions (Madadlou et al., 2009). During milk acidification, Gastaldi et al. (1996) observed aggregation when there was a pH drop to 5.8 due to reduced steric repulsion, but micelle integrity still remained; this did not affect the rheological behaviour of the milk. As pH decreases, levels of micellar calcium and inorganic phosphate decrease, with solubilisation rate becoming faster below pH 6.0-5.8. The second stage in gelation was observed in the pH range of 5.5-5.0, where upon casein micelles seemed to undergo a transition state; solubilisation of CCP in this pH range may have caused rearrangement of protein conformation and new protein-protein interactions.

Dissociation of CCP is complete at about pH 5.2; faster rearrangements of casein can then occur due to fewer bonds present in the structure (Watkinson et al., 2001). The greatest changes in rheological properties of acid milk gels were observed at pH 4.8-4.7 where viscosity and gel stiffness increased sharply and hence tan δ decreased due to the formation of a network of chains

and clusters of casein aggregates. Adjusting milk to alkaline pH was observed to disintegrate the casein micelle, as measured by decreased turbidity (Huppertz et al., 2008) but readjustment of the pH to 6.6 caused micelle reassociation. In contrast, Madadlou et al. (2009) found that while increased pH leads to decreased turbidity the particle size of the micelles increased and therefore they do not disintegrate. Reformation of casein particles causes a reduction in micelle size without an effect on zeta potential; however, reformed micelles had lower ethanol stability, i.e., stability of milk against ethanol-induced flocculation. No differences in serum or ionic calcium and rennet coagulation time were observed, showing that micelles, while not identical, can experience a largely reversible association and dissociation reaction on alkaline disruption (Huppertz et al., 2008). Higher pH values of casein solutions give micelles with a looser more expanded structure, stronger shear thinning behaviour and a higher apparent viscosity. With an increased particle size at high pH, micelle integrity is maintained but, due to electrostatic repulsive forces among caseins, there is a greater negative charge on the proteins in the micelle (Liu and Guo, 2008; Madadlou et al., 2009).

If neutralisation of proteins and solubilisation of CCP occur in a stepwise fashion during acidification, the micelles maintain their integrity until steric stabilization is reduced enough that aggregation occurs. At a high enough temperature, it is possible that aggregation could begin at a higher pH before CCP has solubilized; this could cause a rearrangement of gel structure on continued acidification towards the isoelectric point (Horne, 2003). In a concentrated casein system, lower pH values are required for total

solubilisation of ions from micelles, which can change the buffering capacity of milk.

1.10.2 Effect of pH on casein interactions in cheese

In concentrated casein systems, more calcium and inorganic phosphate ions remain in the colloidal state when compared to typical casein content of milk during acidification (Le Graet and Gaucheron, 1999). The manufacturing parameters during cheesemaking, such as the rate and extent of acid development, determine the final pH of the cheese and affect final cheese texture by altering protein-protein interactions (Lucey and Fox, 1993; Ramkumar et al., 1998; Pastorino et al., 2003; Johnson and Lucey, 2006). pH is an effective means of monitoring acidification which leads to the loss of insoluble calcium during cheese manufacture and influences the characteristics of the final cheese (Johnson and Lucey, 2006).

When the pH of whey draining is decreased, a greater amount of calcium is solublised in the whey, and this leads to lower calcium content in the cheese (Yun et al., 1995). Rapid acidification prior to rennet coagulation of milk could increase the loss of calcium from the curd due to reduced permeability of the curd particle restricting movement of calcium to the whey (Guinee et al., 2002). Altering the calcium distribution via acidification before rennet addition means that the concentration of calcium in the whey will increase and hence less calcium will be present in the curd (Ong et al. 2012). Cheese pH has an effect on the level of soluble calcium in the curd which is strongly correlated with the initial pH of cheese (Watkinson et al., 2001). Cortez et al. (2008)

varied the pH of Mozzarella cheese by exposure to ammonia or hydrochloric acid to increase or decrease the cheese pH, respectively. The results showed that increased or decreased pH led to decreased hardness and increased melt or increased hardness and decreased melt in the cheeses, respectively. These changes in Mozzarella functionality were attributed to the level of soluble calcium decreasing as the pH increased and increasing as the pH decreased.

Ong et al. (2012) varied the rennetting pH for Cheddar cheese and found that lower pH led to an altered microstructure. The low rennet pH used (pH 6.1) led to cheese with lower porosity and increased volume of protein, probably due to increased protein interactions. Yun et al. (1995) varied drain pH during the manufacture of Mozzarella cheese and attributed a softer cheese texture to a lower level of calcium in the cheese and greater proteolysis at lower drain pH. The effect of pH on the functional properties of cheese above pH 5.0 seems to be associated with CCP solubilisation (McMahon et al., 2005). The pH of cheese curds distinctly affects texture; low pH curds tend to be more crumbly compared to high pH curds, which exhibit a more elastic structure (Lucey and Fox, 1993). This relates to conformation of the proteins at various pH values; protein aggregates in high pH cheese are larger, with a well-defined structure compared to low pH cheese, which has smaller aggregates with less structural uniformity (Lucey and Fox, 1993; Pastorino et al., 2003).

As caseins approach their isoelectric point, their conformation becomes more compact, which can lead to a shorter cheese texture (Lawrence et al., 1987; Lucey and Fox, 1993; Pastorino et al., 2003). Lee et al. (2005) found that low pH Cheddar cheese had a lower fracture and was brittle. Lee et al. (2010) also found that Colby cheese with a pH of about 4.9 was short and brittle.

Decreasing cheese pH can lead to decreased hardness due to calcium solubilisation leading to decreased protein-protein interactions weakening the cheese matrix (Pastorino et al., 2003). McMahon et al. (2005) found similar hardness values in cheese with different pH values but the same calcium contents, emphasising the importance of calcium on cheese texture. Hou et al. (2014a) found that curd washing in Cheddar cheese, which resulted in increased cheese pH, led to increased firmness and fracture stress; the levels of calcium in this study were not tested. However, in further studies, Hou et al. (2014b) used curd washing in cheese with standardised calcium levels and found that increased pH led to increased firmness and fracture stress which as attributed to higher levels of casein bound calcium. Watkinson et al. (2001) found that as pH increased in a model cheese system, fracture properties (fracture stress and strain) generally increased in the pH range of 5.2-6.2 but adhesion area decreased. Everard et al. (2006) observed that increased Cheddar cheese pH leads to increased chewiness, firmness, springiness, cohesiveness, fracture stress and strain, whereas adhesiveness decreases.

Ramkumar et al. (1998) observed that, as cheese pH increased from 5.45-5.9, the G' increased and tan δ decreased for curds acidified with GDL; also, a lower maximum force was observed for lower pH curd samples. The lower modulus was attributed to lower levels of centrifugal serum and hence more water associated with the casein; the serum also contained greater amounts of calcium. Choi et al. (2008) found that the G' value (solid-like character) of cheese at 20 and 70°C was lower for directly acidified cheese made from milk preacidified to pH 5.4 than pH 6.0 and the authors attributed this to loss of CCP crosslinks between caseins. Maldonado et al. (2013) found that the

hardness of Telita cheese was affected by stretching pH; higher pH at stretching led to harder cheese. Hardness was found to be positively correlated with pH and calcium.

pH can have a profound effect on the melt properties of cheese. The rate at which Cheddar cheese flows increases from pH 5.3-5.0 due to reduced interactions between proteins, facilitating flow (Pastorino et al., 2003). McMahon et al. (2005) found that, regardless of pH, Mozzarella cheese had a higher flow at lower calcium levels due to the effect of less CCP crosslinks and attributed the effect of calcium on cheese functionality in the pH ranges of 5.0-5.8.

Below pH 4.9, as the caseins approach their isoelectric point, electrostatic repulsions decrease and hydrophobic interactions increase, leading to increased protein-protein interactions and to a cheese with a poor meltability (Pastorino et al., 2003; Johnson and Lucey, 2006). Lee et al. (2005) found that in Cheddar cheese with a low pH values (pH< 4.9), the increased attractive interactions between proteins as caseins approach the isoelectric point maintained a high G', inhibited meltability and decreased flow during ripening. Lee et al. (2010) found that low pH Colby cheese (pH< 4.9) had a decreased tan δ_{max} and degree of flow; also the cheese with the lowest pH had the highest G' at 5 and 40°C, indicating a more solid-like character. Increasing the pH of Mozzarella cheese from 5.58-5.93 at similar total calcium levels resulted in lower flowability and stretchability during ripening. Also, cheese with a higher pH had a higher G' at 20 and 80°C, resulting in a lower phase an angle at 80°C (Guinee et al., 2002). Choi et al. (2008) found that cheese made with milk preacidified in the pH range of 6.0-5.4 had higher tan δ_{max} values (relating to a higher melt) as pH

decreased due to less insoluble calcium associated with the casein causing higher bond mobility.

1.11 Proteolysis

Proteolysis during ripening can become an important factor when relating to changes in texture and melt of cheese (Johnson and Lucey, 2006). It was found that increased proteolysis was less correlated with rheological and melt properties of Cheddar cheese during ripening than with insoluble calcium content (Lucey et al., 2005). At the start of ripening, α_{s1}-casein is rapidly broken down by residual coagulant at its primary cleavage site (Phe_{23}-Phe_{24}), leading to formation of α_{s1}-casein (f1-23) and α_{s1}-casein (f24-199) (O'Mahony et al., 2005).

Calcium solubilisation can also increase proteolysis (Johnson and Lucey, 2006). pH of cheese can affect the level and type of proteolytic breakdown. Most proteolysis in cheese is due to the coagulant and also possibly plasmin retained in the cheese (Lawrence et al., 1987; Fox, 1989; O'Mahony et al., 2005). As cheese pH increases, the level of intact α_{s1}-casein increases and the level of intact β-casein decreases, due to the low pH optimum of chymosin and high pH optimum of plasmin, respectively (Lawrence et al., 1987; Watkinson et al., 2001). Drain pH during cheese manufacture can affect the level of residual rennet and plasmin retained in the curd and hence the level of proteolysis that occurs during ripening (Lawrence et al., 1987; Fox, 1989; Bansal et al. 2007).

The hydrolysis of casein fractions in cheese by enzymes is related both to the enzyme specificity and accessibility of the peptide bonds to hydrolysis. The hydrolysis of α_{s2}- and β-casein in cheese was dependent on heating temperature and level of inactivation of plasmin during cheese ripening (Benfeldt et al., 1997). The ratio of moisture to casein in cheese can affect the rate of proteolysis (Lawrence et al., 1987). Linear relationships have been observed between the level of intact casein and the salt-in-moisture ratio in cheese. Salt-in-moisture can affect protein conformation which could therefore affect the accessibility of peptide bonds for enzymes (Lawrence et al., 1987).

Joshi et al. (2003) found that reducing calcium in Mozzarella cheese increased proteolysis, possibly from preacidification of milk increasing retention of rennet in the curd. Bansal et al. (2007) found that decreasing the pH of milk to below pH 6.1 before rennet addition led to increased retention of rennet. As casein and rennet (isoelectric point pH 4.75) approach their isoelectric points there are decreased negative charges leading to increased interaction between them and hence increased rennet retention. Børsting et al. (2014) found that the retention of camel chymosin was consistent at 20% between coagulation pH of 6.65 to 6.00, compared to the retention of bovine chymosin which increased from 2 to 21% over the same pH range; at pH 5.60, retention of camel and bovine chymosin was 36 and 47%, respectively. This difference was attributed to the lower negative charge of camel chymosin and thus less variation in charge due to pH. Cheddar cheese with a lower pH has been found to have increased proteolysis due to greater chymosin activity or possibly a greater susceptibility of casein to hydrolysis due to lower crosslinks of CCP (Lee et al., 2005). Hou et al. (2014b) found that reduced-calcium Cheddar cheese was

found to have increased levels of proteolysis which was attributed to increased susceptibility of casein to proteolysis with lower levels of casein bound calcium. Pastorino et al. (2003) observed that decreasing Cheddar cheese pH from 5.3-5.0 resulted in an increase in proteolysis. Inhibiting chymosin significantly reduces primary proteolysis in Cheddar cheese, and breakdown of β-casein is primarily due to plasmin (O'Mahony et al., 2005). The decrease in viscoelastic modulus of Mozzarella during ripening can be related to proteolysis; products of proteolysis can be hydrophobic, which could cause moisture from fat channels to be absorbed into the protein matrix and hydrate the protein network and weaken the structure (Joshi et al., 2004).

Bansal et al. (2007) suggested that casein micelles are saturated with respect to chymosin as it was found that rennet concentration did not impact its retention in curd. Coagulant is the main cause of initial proteolysis in Cheddar cheese (Lane et al., 1997) and hydrolysis of α_{s1}-casein (Benfeldt et al., 1997). Cheese manufacturing method can affect the level of residual rennet in curd; high cook cheese varieties can have lower residual rennet and high moisture cheeses can have higher residual rennet (Bansal et al., 2009a). Lane et al. (1997) examined the use of calf chymosin and porcine pepsin as coagulants in Cheddar cheese. In this study, a modified make procedure was used to inactivate the porcine pepsin by increasing the pH to 7 after cutting the coagulum. Modified cheeses were harder than controls and were more resistant to fracture; these characteristics decreased linearly with increasing level of proteolysis. Taking current knowledge into consideration, a slightly higher pH was observed in modified cheese and the changing of pH during manufacture to inactivate

porcine pepsin could have affected the level of insoluble calcium and hence rheological properties.

CCP solubilisation could cause partial relaxation of protein-protein interactions allowing chymosin greater access to potential cleavage sites on casein (Pastorino et al., 2003; O'Mahony et al., 2005).

Proteolytic specificity of coagulants could contribute to differences in rheological properties of cheese. Lane et al. (1997) found that porcine pepsin when used for the manufacture of Cheddar cheese did not produce β-casein (f1-189/192), and resulted in less α_{s1}-casein breakdown, and no increase in α_{s1}-casein (f102-199). Using camel chymosin in Cheddar cheese was found to cause a lower level of primary proteolysis, no production of β-casein (f1-189/192), higher levels of intact α_{s1}-casein, no further hydrolysis of α_{s1}-casein (f24-199) and lower quantities of peptides, compared to cheese made with calf chymosin as a coagulant (Bansal et al., 2009b). Cheese made with camel chymosin was subsequently harder and chewier towards the end of ripening (Bansal et al., 2009b). Low-fat Cheddar cheese manufactured with camel chymosin also developed lower levels of 12% TCA soluble nitrogen. Low-fat Cheddar cheeses made with camel chymosin were also characterized as having higher hardness, chewiness and lower meltability (Govindasamy-Lucey et al., 2010). Børsting et al. (2012) found that a high stress at fracture in reduced-fat Cheddar cheese made with camel chymosin was positively correlated with a higher level of intact α_{s1}-casein. The camel chymosin cheese had a higher stress at fracture than cheese made with bovine chymosin; pH development during cheese manufacture and calcium levels were kept constant and only levels of intact α_{s1}-casein varied. Guinee et al. (2002) proposed that varying pH

and calcium concentration of Mozzarella cheese was a means to modify its functional shelf-life. In contrast, it has been seen that use of camel chymosin in Mozzarella could possibly extend functional and textural shelf-life by reducing proteolysis and increasing levels of intact casein compared to bovine chymosin; no differences were found in the level of insoluble calcium, or pH after 14 d of ripening (Moynihan et al., 2014).

Proteinases and peptidases of starter bacteria are mainly responsible for secondary proteolysis (Benfeldt et al., 1997). Starter peptidases are the main contributor to the formation of free amino acids in cheese but a low level of substrate polypeptides formed by hydrolysis of casein by the coagulant could affect amino acid levels in cheese (Lane et al., 1997). Wallace and Fox (1997) found that addition of intermediate levels of free amino acids to Cheddar cheese curd gave a cheese with superior flavour and texture compared to the control or cheeses with a high addition. Lane et al. (1997) found that primary proteolysis was more closely correlated with rheological properties than the level of free amino acids present in the cheese. Børsting et al. (2012) found that higher levels of amino acids in reduced-fat Cheddar cheese were positively correlated with a low strain at fracture, which relates to shortness in cheese texture.

1.12 Conclusions

Texture and rheology are important areas in the study of aspects of cheese relating to cheese manufacture, quality and acceptability. As discussed the most important factors that affect texture and rheology of cheese are CCP, pH and proteolysis. As the pH decreases during cheese manufacture, calcium solubilises; the critical pH points during manufacture such as rennet coagulation, drain and mill pH will affect the final calcium concentration in the curd. It is now known that not only total calcium is important for determining cheese texture and rheology but the level of soluble and insoluble calcium present in the curd. This will also be affected by cheese pH during manufacture. It is well known that proteolysis impacts on the texture and rheology of cheese during ripening. The pH of cheese can affect an impact on proteolysis via the pH optimum of enzymes or by affecting rennet retention in the curd. Not only pH can affect proteolysis but the level of solubilised calcium can affect proteolysis, by exposure of peptide bonds to enzymes. These three factors are interrelated and quite complex, and it is difficult to study these independently when trying to determine the effect on cheese rheology and texture. The importance of considering all aspects of pH history during manufacture, cheese pH, total calcium, soluble calcium, proteolysis and the impact they have the casein micelle and protein network in cheese when studying texture and rheology has been highlighted.

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CHAPTER 2

Impact of fortification of cheesemilk with skim milk powder on the composition, microbiology, proteolysis, texture and functionality of Cheddar cheese

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Chapter 2: Fortification of Cheddar cheese milk with skim milk powder

Abstract

Using skim milk powder (SMP) to fortify milk for cheese manufacture offers the potential benefit of increased yield and vat throughput during times when the seasonality of milk may affect yield. The objective of this study was to evaluate the effect of fortification of cheesemilk with SMP on the composition, microbiological, biochemical, textural and functional properties of Cheddar cheese. Cheesemilk was fortified with three different levels of SMP and used to manufacture Cheddar cheese. Treatments were 0 (control), 10 (low), 20 (medium) and 50% (high) increased casein. Coagula were cut based on firmness and Cheddar cheese made therefrom. Fortification affected the moisture content of the cheeses; cheese made from milk with the highest level of added SMP had the lowest moisture. Fortifying milk for Cheddar cheese manufacture with SMP significantly ($P < 0.05$) increased the cheese yield. It was found that higher levels of non-starter lactic acid bacteria were present in fortified cheeses, possibly due to the protective effect of the higher level of total solids in the milk on bacteria during pasteurisation. Proteolysis was affected by fortification level; increasing the level of SMP in the cheesemilk caused a subsequent decrease in proteolysis in the cheese compared to the control. Meltability was lower in the cheese made from milk fortified with SMP and the texture was found to be harder. Using SMP for fortification had effects on the properties of Cheddar cheese but these effects were more evident at medium and high levels of fortification. Using low levels of SMP for fortification could possibly offer a way to manufacture Cheddar cheese with increased yield while maintaining the characteristic properties of the cheese.

Chapter 2: Fortification of Cheddar cheese milk with skim milk powder

2.1 Introduction

Standards of identity for cheese in many countries prohibit the use of milk powders in its manufacture (Pellegrino et al., 2010). Milk concentration techniques have been previously employed for cheesemaking (Acharya and Mistry, 2004). Concentration techniques such as ultrafiltration have been used in the dairy industry for cheese; low concentration factor ultrafiltration allows for consistent protein content throughout the year and higher levels of concentration would increase yield (Rattray and Jelen, 1996). Use of ultrafiltration retentates for supplementation of cheesemilk offer enhanced cheese manufacturing efficiency as well as energy conservation; increased yield efficiency could also be observed when total solids of milk is low (Kosikowski et al., 1985). Using dried dairy ingredients for standardization of milk for cheese manufacture provides the benefit of increased yield but at a lower cost than ultrafiltration and other membrane technologies (Pellegrino et al., 2010). The seasonality of milk means that at certain times of year when milk volumes are at their lowest cheese plants are not utilized fully. The use of SMP to standardize milk during periods of low production could increase plant throughput giving a more economical and uniform cheese production throughout the year (Freeman et al., 1970). Freeman et al. (1970) found that using SMP instead of milk for standardisation of cheesemilk for Cheddar had little effect on cheese quality but increased yield.

St. Gelais et al. (1997) used low mineral retentate powders with cream containing large fat globules to enrich milk for low fat cheese manufacture; this cheese had the best texture, flavour and colour as scored by consumer panellists. To meet the required casein:fat ratio for low-moisture, part-skim

Mozzarella or low-fat cheese, cream either has to be removed or protein added, typically through addition of skim milk powder (SMP), condensed or membrane-concentrated skim milk (Shakeel-Ur-Rehman et al., 2003a,b). Standardizing milk with milk protein concentrate resulted in cheese with increased yield, decreased meltability and increased hardness compared to the control cheese studied (Shakeel-Ur-Rehman et al., 2003a). Shakeel-Ur-Rehman et al. (2003b) found that using a higher total solids milk for the manufacture of reduced-fat cheese with increased levels of starter bacteria formed a cheese with increased yield, lower primary and secondary proteolysis but had enhanced maturity comparable to the control cheese. St. Gelais et al. (1998) found that, when milk was enriched with demineralised microfiltered retentate powder, calcium caseinate powder or ultrafiltered retentate powder while keeping the casein to fat ratio constant, cheese yield increased; the mineral content of these powders played an important role in cheese manufacture. Yun et al. (1998) found that fortification of Mozzarella cheese with SMP affected cheese properties at the highest level of fortification used (3% wt/ wt); this cheese had higher calcium, lower soluble nitrogen (SN), slightly firmer texture and reduced melt. Kosikowski et al. (1985) manufactured Cheddar cheese with ultrafiltration retentate; the supplemented milk gave high quality cheese with increased yield. Govindasamy-Lucy et al. (2005) manufactured pizza cheese using ultrafiltration retentates to increase the total solids of the cheesemilk; it was found that upon keeping the ratio of rennet to casein constant, cheese yield increased without adverse effects on functionality. Dong et al. (2009) manufactured Mozzarella cheese with microfiltration retentate with added cream. The cheese had satisfactory

attributes but differences were observed from typical Mozzarella which was attributed to higher calcium content of cheese made from microfiltered milk included lower proteolysis and higher hardness.

The objective of this study was to investigate the fortification of cheesemilk with SMP at three different levels and to manufacture Cheddar cheese therefrom. The consequence of this fortification was evaluated with regard to cheese composition, yield, microbiology, proteolysis, texture and functionality.

2.2 Materials and Methods

2.2.1 Pretreatment of cheesemilk

Raw milk was obtained from a local dairy supplier (Rockrohan Farm, Carrigrohane Straight, Cork, Ireland). Part of the milk was separated into cream and skim milk.

The protein content of low heat skim milk powder (SMP) (Tipperary Co-operative, Tipperary, Ireland) was determined by macro-Kjeldahl (IDF, 1986) prior to milk fortification. A slurry (Table 2.1; total solids 21.54%) containing 3.75 kg of SMP was mixed into 40 L of skim milk using a Silverson mixer (Silverson AXR, Silverson Ltd., Chesham, Bucks, England). The composition of the slurry and raw milk were determined for milk standardisation using a milkoscan (FT120, Foss Electric, Denmark). The slurry was standardised to a casein:fat of 0.70:1.00 using the separated cream, batch pasteurised at 63°C for 30 min and stored at 4°C overnight to allow the protein to hydrate. The bulk milk was standardised to a casein:fat of 0.70:1.00, pasteurised at 72 °C for 15 s, and stored at 4°C until cheesemaking.

Table 2.1 Composition of slurry fortified with SMP and cheesemilks used for the manufacture of Cheddar cheeses fortified by 0 (CSMP), 10 (LSMP), 20 (MSMP) or 50 (HSMP) % w/v increased casein level using skim milk powder. Values correspond to means (n=3).

	% Fat	Protein %	% Casein (est)	CN:Fat[1]	%Lactose	% Total Solids
Slurry	6.81±0.16	6.16±0.05	4.80±0.04	0.71±0.02	8.16±0.14	21.54±0.07
CSMP	3.73±0.01	3.35±0.07	2.61±0.06	0.70±0.02	4.37±0.08	12.32±0.08
LSMP	4.08±0.03	3.66±0.06	2.86±0.05	0.70±0.01	4.81±0.08	13.36±0.04
MSMP	4.61±0.03	4.13±0.06	3.22±0.04	0.7±0.01	5.45±0.08	14.93±0.03
HSMP	5.49±0.03	4.91±0.05	3.83±0.04	0.70±0.01	6.52±0.08	17.52±0.04

[1]Casein:fat ratio

2.2.2 Cheese manufacture

Cheese milk was adjusted using the slurry to the desired level of fortification to milks with 0 (control), 10 (LSMP), 20 (MSMP) and 50% (HSMP) increased casein compared to the control (Table 2.1). Four vats of Cheddar cheese were manufactured in the food processing facilities at University College Cork, Ireland according to standard protocol at pilot scale (50 L) and replicated on three separate days. Direct-to-vat frozen starter cultures (0.06%) (R604Y, Chr Hansen, Hørsholm, Denmark) was added to the milk at 31°C and milk was ripened for 30 min. After ripening $CaCl_2$ (0.09%) and chymosin (0.03%) (CHY-MAX Plus, 200 international milk clotting units/ml, Chr Hansen) was added to the milk. Coagulation times of the cheese vats varied and were cut subjectively when the curd was sufficiently firm; addition of SMP to cheesemilk led to decreased coagulation times. Curds and whey were cooked to 38°C over a 30 min period and drained at pH 6.2. Curds were cheddared until pH 5.4, milled and salted (2.5% wt/wt). After pressing overnight, the cheeses were vacuum packed and ripened at 8°C.

2.2.3 Compositional Analysis

Cheese whey was analysed for protein and fat by the macro-Kjeldahl (IDF, 1986) and Gerber (IS, 1955) method, respectively. Cheese was analysed at 20 d of ripening for moisture by oven drying (IDF, 1982), protein by macro-Kjeldahl (IDF, 1986), fat by Gerber (IS, 1955) and salt (Fox, 1963). The pH of 10 g of grated cheese and 10 ml of deionized water blended in a stomacher was measured using a pH meter (PHM210, Standard pH meter, Meterlab,

Radiometer, Cohenhangen, Denmark) (Madkor et al., 1987). All analysis was carried out in triplicate.

2.2.4 Cheese Yield

The actual cheese yield was calculated by dividing the weight of cheese after pressing by the total weight of milk. The adjusted yield was calculated to 37% moisture and 1.7% salt using the following formula (Lau et al., 1990):

$$\textbf{Adjusted yield} = \frac{(\text{actual yield})[100 - (\text{actual percentage of moisture} + \text{percentage of salt})]}{100 - (\text{Desired percentage of moisture} + \text{percentage of salt})}$$

2.2.5 Microbiological Analysis

Starter lactococci (SLAB) and non-starter lactic acid bacteria (NSLAB) were enumerated in duplicate throughout cheese ripening. Starter lactococci were enumerated on LM17 agar (Merck, Darmstadt, Germany) (Terzaghi and Sandine, 1975) after 3 d incubation at 30ºC and NSLAB on double layered Rogosa agar (Merck) (Rogosa et al., 1951) after 5 d incubation at 30ºC.

2.2.6 Assessment of proteolysis

pH 4.6-soluble and insoluble factions of cheese were prepared in triplicate as described by Kuchroo and Fox (1982). The nitrogen content of the pH 4.6-soluble extract was determined by macro-Kjeldahl (IDF, 1986).

Freeze-dried pH 4.6-insoluble fractions were assessed for proteolysis by using urea-polyacrylamide gel electrophoresis (urea-PAGE) (12.5% total acrylamide, 4% cross linking agent, pH 8.9) was performed according to Andrews (1983) with modifications (Shalabi and Fox, 1987). Samples were run through the stacking gel at 280 V and separating gel at 300 V. Gels were stained using Coomassie Brilliant Blue G250 (Blakesely and Boezi, 1977) and destained using distilled water. For free amino acids (FAA) analysis fractions of cheese soluble at pH 4.6 were deproteinised by mixing with equal volumes of 24% (w/v) trichloroacetic acid (TCA), allowing to stand for 10 min and centrifuging at 14,400 *g* for 10 min (Microcentaur, MSE, London, UK). The supernatant was diluted with 0.2 M sodium citrate buffer (pH 2.2) to give approximately 250 nmol of each amino acid residue per ml. The samples were then diluted 1:2 with an internal standard, norleucine, to give an approximate final concentration of 125 nmol of each amino acid residue in 1 mL of injection solution. Samples were then analysed using a Jeol JLC-500/V amino acid analyser (Jeol Ltd., Welwyn Garden City, Herts, UK) fitted with a Jeol Na^+ high performance cation-exchange column. Individual free amino acids were separated by ion exchange chromatography with post-column ninhydrin derivatization and visible colorimetric detection at 570 nm. Results were recovered using an Aminotaq data handling system (Joel Ltd.).

2.2.7 Meltability

Meltability of cheese samples was determined in triplicate by the Schreiber test with some modifications. Cheese samples were cut to 5 mm in height using a

food slicer and 35 mm diameter discs using a cork borer. Cheese discs were placed in the center of a glass Petri dish and covered. Samples were then melted at 232°C for 5 min in an oven (Altan et al., 2005) and allowed to cool for 30 minutes at room temperature. Five readings of the diameter were measured and averaged.

2.2.8 Texture analysis

Cylindrical samples (20 mm height, 20 mm diameter) were cut using a stainless steel cork borer and wire cutter. Samples were wrapped in plastic wrap and stored at 8°C overnight. Samples were compressed at 8°C to 25% of their original height at a rate of 1 mm s^{-1} using a texture analyser (TA-XT2i Texture Analyser, Stable Micro Systems, Godalming, England). Hardness was defined as the force required to compress the sample to 25% of its original height.

2.2.9 Statistical Analysis

Statistical analysis was carried out using one-way analysis of variance (ANOVA) and Tukey's HSD *post hoc* test with PASW Statistics for Windows version 18 (SPSS Inc., Chicago, IL) to compare significant differences between trials, treatments and ripening time. The probability level used for statistical significance was $P < 0.05$.

2.3 Results and Discussion

2.3.1 Cheese composition

Composition of Cheddar cheese made from milk fortified with SMP is shown in Table 2.2. Differences in protein, fat, MNFS and fat in whey were not significant ($P > 0.05$) between the control and experimental treatments. Varying the casein to fat ratio of milk can affect composition of cheese (Guinee et al., 2007). Casein to fat ratio was kept constant between each treatment (Table 2.1) for cheesemaking and hence there were no significant differences ($P > 0.05$) between the cheese in terms of protein and fat. MNFS was not significantly different ($P > 0.05$) between treatments and is an important quality descriptor of Cheddar cheese (Fox et al., 2000). It is also an important parameter to keep constant when assessing texture and functionality of cheese (Bogenrief and Olson, 1995). There were significant differences ($P < 0.05$) between certain compositional parameters of the cheeses. Differences were observed between treatments for moisture, and salt in cheese and protein in the whey. As powder addition increased, the moisture content of the cheese generally decreased. HSMP cheese had a significantly ($P < 0.05$) lower moisture content compared to CSMP cheeses. St. Gelais et al. (1998) found that, as casein content increased in milks enriched with calcium caseinate, there was a subsequent decrease in moisture. Brito et al. (2000) also found that upon fortification of milk with SMP for the manufacture of Maribo cheese, the moisture content of the cheese decreased progressively with increasing fortification with SMP. Kosikowski et al. (1985) found similar results for moisture upon supplementation of milk for Cheddar cheese manufactured with ultrafiltration retentates. LSMP cheese had a significantly ($P < 0.05$) lower salt

Table 2.2. Composition of Cheddar cheeses made from milk fortified to 0 (CSMP), 10 (LSMP), 20 (MSMP) or 50 (HSMP) % w/v increased casein using skim milk powder at 20 d ripening. pH values at 1 and 20 d of ripening. Values are means of three replicates and standard deviations with the latter in parentheses.

	Treatment			
	CSMP	LSMP	MSMP	HSMP
% Protein	24.19^{a} (0.70)	24.11^{a} (0.54)	24.15^{a} (0.34)	25.05^{a} (1.39)
% Fat	32.36^{a} (0.70)	32.47^{a} (1.39)	33.44^{a} (0.59)	33.83^{a} (0.93)
% Moisture	39.58^{b} (1.23)	38.64^{ab} (0.18)	38.45^{ab} (1.06)	36.58^{a} (0.77)
% Salt	1.42^{ab} (0.07)	1.32^{a} (0.08)	1.45^{ab} (0.05)	1.49^{b} (0.05)
% MNFS*	58.53^{a} (2.43)	57.23^{a} (0.94)	58.51^{a} (3.00)	55.37^{a} (1.43)
% Protein (whey)	0.94^{a} (0.04)	0.99^{ab} (0.04)	1.19^{bc} (0.15)	1.31^{b} (0.02)
% Fat (whey)	0.50^{a} (0.16)	0.44^{a} (0.13)	0.46^{a} (0.02)	0.47^{a} (0.04)
pH (day 1)	5.00^{a} (0.02)	4.98^{a} (0.05)	5.05^{a} (0.04)	5.19^{b} (0.02)
pH (day 20)	5.04^{a} (0.03)	5.02^{a} (0.10)	5.09^{ab} (0.06)	5.21^{b} (0.00)

*Moisture in nonfat substance of the cheese
^{a,b,c}Means within the same row not sharing a common superscript differ (Tukey's HSD, $P < 0.05$)

content compared to HSMP cheese.

As powder addition increased, the amount of protein lost in the whey also increased; whey from CSMP and LSMP cheese treatments had significantly ($P < 0.05$) less protein in the whey compared to MSMP and HSMP. This could be a direct effect of greater powder addition releasing a higher level of whey protein during cheese manufacture as total protein content was the cheese was not significantly ($P > 0.05$) affected. Similar results for protein in whey were found upon fortification of Mozzarella cheese with SMP (Yun et al., 1998). Freeman et al. (1970) found that milk standardised with SMP had higher levels of fat, protein and total solids in the whey.

The pH of Cheddar cheese fortified with SMP is also shown in Table 2.2. The pH of HSMP cheese at d 1 and 20 was significantly ($P < 0.05$) higher than those of CSMP and LSMP cheese. Govindasamy-Lucey et al. (2005) found that cheese manufactured with higher total solids milk had a higher pH during ripening than the control cheese; this was attributed to the higher buffering capacity owing to increased casein and insoluble calcium contents.

2.3.2 Yield

Cheese yield corrected to 37% moisture and 1.7% salt is shown in Figure 2.1. The cheese yield showed a linear relationship when correlated with total solids of milk (R^2=0.9946) as can be seen in Figure 2.1. There were significant ($P < 0.05$) differences in yield between each of the cheeses; as fortification level of cheese with SMP increased, the cheese yield also increased. Higher total solids in cheese milk increased cheese yield due to higher amounts of casein and fat being incorporated into the cheese (Govindasamy-Lucey et al., 2005). Brito et al. (2000) found that cheese manufactured from milk fortified with SMP affected the cheese yield which increased proportionally with increased non-fat-solids. Kosikowski et al. (1985) found that Cheddar cheese manufactured with milk supplemented with ultrafiltration retentates had an increased moisture-adjusted yield as the level of supplementation increased. Using milk protein concentrate for milk standardisation resulted in cheese with higher yields due to the high total milk solids recovery in the cheese (Shakeel-Ur-Rehman et al., 2003a, b).

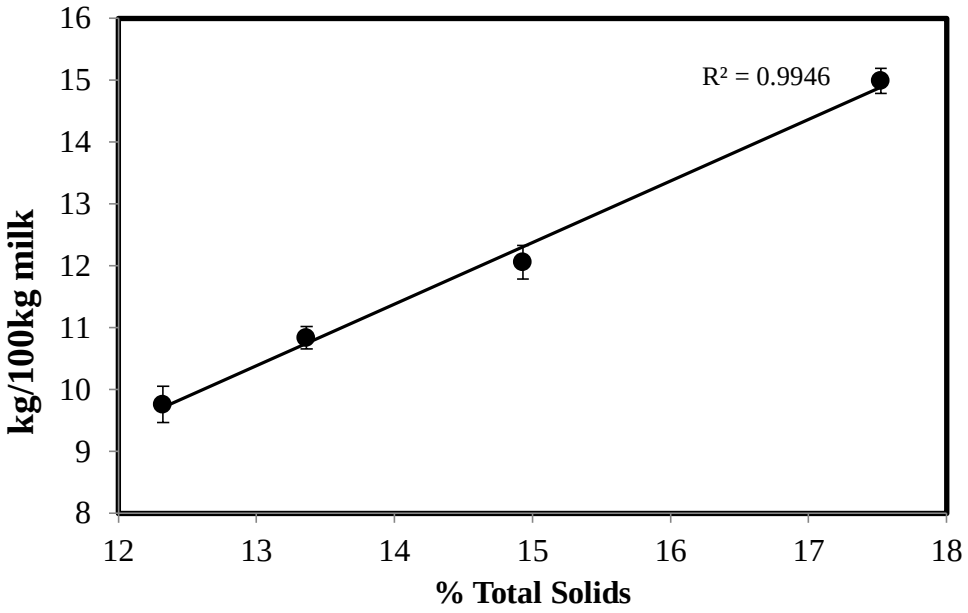


Figure 2.1 Cheese yield per 100 kg of cheese milk corrected to 37% moisture and 1.7% salt for cheese made from milk fortified with 0 (12.32% total solids), 10 (13.36% total solids), 20 (14.93% total solids) or 50 (17.52% total solids) % w/v increased casein using skim milk powder. Values are means of three replicates. Error bars indicate ± one standard deviation.

2.3.3 Microbiology

Starter and non-starter bacterial counts are shown in Tables 2.3 and 2.4, respectively. SLAB counts for cheese treatments show similar trends at d 193 of ripening for each trial. At d 193 of ripening counts of MSMP and HSMP cheeses were significantly ($P < 0.05$) higher than CSMP cheese. No clear trend was evident across the ripening for each treatment; LM17, the agar used to measure SLAB in this study, counts total numbers of lactic acid bacteria and therefore NSLAB counts in the cheese may have a confounding effect on the total SLAB counts later in ripening. However, the SLAB numbers in the control cheese (CSMP) generally decreased significantly throughout ripening (Table 2.3). In Trial 1, MSMP and HSMP cheeses had significantly ($P < 0.05$)

higher NSLAB counts than CSMP and LSMP cheeses from d 35-104 of ripening. At d 193 of ripening for this trial, HSMP cheese had significantly ($P < 0.05$) higher counts than CSMP and LSMP. Trial 2 showed a similar trend throughout ripening with all treatments being significantly ($P < 0.05$) different at d 193 of ripening. Trial 3 showed that HSMP was significantly ($P < 0.05$) higher than all treatments throughout ripening from d 22-55. At d 104 counts of MSMP and HSMP were significantly ($P < 0.05$) higher than the CSMP cheese. All SMP treatments were significantly ($P < 0.05$) higher than the control (CSMP) in this trial at d 193 of ripening. As can be seen in CSMP cheese NSLAB counts are typically low in Cheddar cheese after manufacture (Fox et al., 2000). It is probable that the NSLAB counts in the cheeses fortified with SMP at the beginning of ripening came from the raw milk as number of NSLAB in the powder were <10 cfu g^{-1} (results not shown). Acharya and Mistry (2004) found that starter and NSLAB numbers in cheese manufactured from concentrated milk were not affected by treatment; in this study milk and cream were pasteurised before concentration of the cheese milk. The "Grade 'A' Pasteurized Milk Ordinance" (PMO) states that "if the fat content of the milk product is 10% or greater or a total solids of 18% or greater, or is it contains added sweeteners, the specified temperature shall increase by 3°C" (FDA, 2009). In the current study, milk enriched with SMP to make a stock for fortification was pasteurised after addition of powder. The total solids of this milk stock was on average 21.54%; according to the PMO the temperature of pasteurisation should have increased to 66°C for 30 min to give equivalent microbial death rates. In this study, the higher number of NSLAB counts in cheese enriched with SMP may have come from the fortified milk with higher

total solids that was pasteurised at 63°C for 30 min. The higher total solids could have perhaps offered a protective effect on NSLAB already present in the raw milk allowing them to survive pasteurisation. Another factor to take into consideration is that the higher total solids could have led to a concomitant increase in the viscosity of the milk which could have changed the heat transfer within the milk affecting the temperature-time effect on the bacteria allowing a greater chance of bacterial survival. Thermal conductivity of milk decreases with increasing total solids due to increased viscosity (Fox and McSweeney, 1998).

2.3.4 Proteolysis

Table 2.5 shows the level of pH 4.6-SN/TN (%) of Cheddar cheese manufactured from milk fortified with SMP. pH 4.6 SN/TN is an index of proteolysis (Sousa et al., 2001). As ripening time progressed, the level of pH 4.6-SN/TN increased in all cheeses and there was a significant difference between each ripening time. This is a typical characteristic of Cheddar cheese ripening (O'Mahony et al., 2005). There was a significant difference ($P < 0.05$) between the levels of pH 4.6-SN/TN in each cheese; the cheese fortified with the highest level of SMP (HSMP) developed the lowest level of pH 4.6-SN/TN in all trials at each ripening time point. For Trials 1 and 2, HSMP cheese had significantly ($P < 0.05$) lower levels of pH 4.6-SN/TN compared to CSMP and LSMP cheese at all ripening times measured. For Trial 3 after d 6 of ripening, HSMP cheese had significantly ($P < 0.05$) lower levels of pH 4.6-SN/TN

Table 2.3 Numbers of starter lactic acid bacteria (cfu g^{-1}) for Trials 1, 2 and 3 of Cheddar cheeses fortified with 0 (CSMP), 10 (LSMP), 20 (MSMP) or 50 (HSMP) % w/v increased casein using skim milk powder at 1, 22, 35, 55, 104 and 193 d ripening. Values correspond to means (n=2).

		Ripening time (d)					
		1	22	35	55	104	193
Trial 1	CSMP	$9.3\times10^{9\ a,C}$	$1.3\times10^{10\ a,C}$	$9.4\times10^{9\ b,D}$	$5.9\times10^{9\ b,B}$	$4.7\times10^{9\ b,B}$	$1.7\times10^{7\ a,A}$
	LSMP	$2.8\times10^{10\ c,C}$	$8.0\times10^{9\ a,B}$	$7.2\times10^{9\ ab,B}$	$9.4\times10^{9\ c,B}$	$9.0\times10^{8\ a,A}$	$3.5\times10^{7\ a,A}$
	MSMP	$3.2\times10^{10\ d,E}$	$1.9\times10^{10\ a,D}$	$1.5\times10^{10\ c,C}$	$3.2\times10^{9\ a,B}$	$1.8\times10^{9\ a,AB}$	$3.7\times10^{8\ b,A}$
	HSMP	$1.5\times10^{10\ b,D}$	$9.5\times10^{9\ b,C}$	$5.9\times10^{9\ a,BC}$	$3.7\times10^{9\ a,AB}$	$2.4\times10^{9\ a,AB}$	$6.4\times10^{8\ c,A}$
Trial 2	CSMP	$1.2\times10^{10\ a,B}$	$6.0\times10^{9\ b,AB}$	$5.0\times10^{9\ a,AB}$	$7.0\times10^{9\ a,AB}$	$2.1\times10^{8\ a,A}$	$1.7\times10^{8\ a,A}$
	LSMP	$6.0\times10^{9\ a,C}$	$1.3\times10^{9\ a,AB}$	$3.8\times10^{9\ a,BC}$	$6.0\times10^{9\ a,C}$	$1.1\times10^{9\ a,AB}$	$1.4\times10^{8\ ab,A}$
	MSMP	$3.5\times10^{9\ a,AB}$	$9.7\times10^{9\ c,BC}$	$1.4\times10^{10\ b,AB}$	$5.7\times10^{9\ a,C}$	$1.0\times10^{9\ a,A}$	$2.3\times10^{8\ b,A}$
	HSMP	$8.1\times10^{9\ a,C}$	$3.3\times10^{9\ a,B}$	$4.4\times10^{9\ a,B}$	$2.9\times10^{9\ a,B}$	$2.0\times10^{9\ a,AB}$	$4.5\times10^{8\ c,A}$
Trial 3	CSMP	$6.6\times10^{9\ a,AB}$	$1.3\times10^{10\ a,B}$	$8.2\times10^{9\ a,AB}$	$4.5\times10^{9\ ab,AB}$	$3.8\times10^{8\ a,A}$	$3.5\times10^{6\ a,A}$
	LSMP	$8.1\times10^{9\ ab,B}$	$7.1\times10^{9\ a,B}$	$8.0\times10^{9\ a,B}$	$7.0\times10^{9\ b,B}$	$1.4\times10^{9\ c,A}$	$1.4\times10^{8\ b,A}$
	MSMP	$1.3\times10^{10\ b,A}$	$1.0\times10^{10\ a,A}$	$4.2\times10^{10\ b,B}$	$5.0\times10^{9\ ab,A}$	$2.4\times10^{9\ d,A}$	$2.6\times10^{8\ c,A}$
	HSMP	$4.0\times10^{9\ a,AB}$	$4.5\times10^{9\ a,B}$	$1.1\times10^{10\ a,C}$	$2.1\times10^{9\ a,AB}$	$1.2\times10^{9\ b,AB}$	$6.1\times10^{8\ d,A}$

[a,b,c,d]Means for each trial within the same column not sharing a common superscript differ in treatment (Tukey's HSD, $P < 0.05$)
[A,B,C,D,E]Means for each trial within the same row not sharing a common superscript differ in ripening time (Tukey's HSD, $P < 0.05$)

Table 2.4 Numbers of non-starter lactic acid bacteria (cfu g^{-1}) for Trials 1, 2 and 3 of Cheddar cheeses fortified with 0 (CSMP), 10 (LSMP), 20 (MSMP) or 50 (HSMP) % w/v increased casein using skim milk powder at 1, 22, 35, 55, 104 and 193 d ripening. Values correspond to means (n=2).

		Ripening time (d)					
		1	22	35	55	104	193
Trial 1	CSMP	$0^{a,A}$	$0^{a,A}$	$0^{a,A}$	$2.3\times10^{4\ a,A}$	$6.4\times10^{5\ a,B}$	$1.5\times10^{7\ a,C}$
	LSMP	$5.3\times10^{4\ a,\ A}$	$5.5\times10^{5\ a,A}$	$8.7\times10^{5\ a,A}$	$3.6\times10^{6\ a,B}$	$3.3\times10^{7\ b,C}$	$6.0\times10^{7\ a,D}$
	MSMP	$2.4\times10^{5\ a,A}$	$1.3\times10^{6\ ab,A}$	$4.0\times10^{6\ b,A}$	$1.4\times10^{8\ b,AB}$	$1.7\times10^{8\ c,B}$	$4.0\times10^{8\ ab,C}$
	HSMP	$1.4\times10^{6\ b,A}$	$3.1\times10^{6\ b,A}$	$9.0\times10^{6\ c,A}$	$3.0\times10^{8\ c,A}$	$9.9\times10^{8\ d,C}$	$6.5\times10^{8\ b,B}$
Trial 2	CSMP	$0^{a,A}$	$0^{a,A}$	$0^{a,A}$	$0^{a,A}$	$3.1\times10^{3\ a,A}$	$2.0\times10^{5\ a,A}$
	LSMP	$4.0\times10^{2\ a,A}$	$7.5\times10^{2\ a,A}$	$6.1\times10^{3\ b,A}$	$9.0\times10^{4\ b,A}$	$2.0\times10^{7\ b,B}$	$1.9\times10^{8\ b,C}$
	MSMP	$7.5\times10^{2\ a,A}$	$3.2\times10^{3\ a,A}$	$1.4\times10^{4\ c,A}$	$2.5\times10^{5\ c,A}$	$1.9\times10^{7\ b,A}$	$3.1\times10^{8\ c,B}$
	HSMP	$8.5\times10^{2\ a,A}$	$1.4\times10^{4\ b,A}$	$9.0\times10^{4\ d,A}$	$3.7\times10^{6\ d,A}$	$1.8\times10^{8\ c,B}$	$4.7\times10^{8\ d,C}$
Trial 3	CSMP	$0^{a,A}$	$0^{a,A}$	$0^{a,A}$	$0^{a,A}$	$2.5\times10^{3\ a,A}$	$8.5\times10^{5\ a,B}$
	LSMP	$7.5\times10^{2\ a,A}$	$7.0\times10^{2\ ab,A}$	$7.0\times10^{3\ a,A}$	$3.5\times10^{4\ a,A}$	$5.0\times10^{6\ ab,A}$	$1.4\times10^{8\ b,B}$
	MSMP	$8.5\times10^{2\ a,A}$	$3.0\times10^{3\ b,A}$	$1.6\times10^{4\ a,A}$	$1.1\times10^{5\ a,A}$	$1.5\times10^{7\ b,A}$	$2.4\times10^{8\ b,B}$
	HSMP	$1.1\times10^{3\ a,A}$	$8.0\times10^{3\ c,A}$	$1.7\times10^{5\ b,A}$	$3.3\times10^{5\ b,A}$	$1.3\times10^{8\ c,B}$	$6.8\times10^{8\ c,C}$

[a,b,c,d]Means for each trial within the same column not sharing a common superscript differ in treatment (Tukey's HSD, $P < 0.05$)
[A,B,C,D]Means for each trial within the same row not sharing a common superscript differ in ripening time (Tukey's HSD, $P < 0.05$)

Table 2.5 Levels of pH 4.6 soluble-nitrogen (SN) as a percentage of total nitrogen (TN) for Trials 1, 2 and 3 of Cheddar cheeses made from milk fortified with 0 (CSMP), 10 (LSMP), 20 (MSMP) or 50 (HSMP) % w/v increased casein using skim milk powder at 6, 55, 104 and 193 d ripening. Values correspond to means ± one standard deviation (n=3).

		Ripening time (d)			
		6	55	104	193
Trial 1	CSMP	$7.42 \pm 0.20^{b, A}$	$15.38 \pm 0.42^{c, B}$	$19.46 \pm 0.39^{c, C}$	$24.02 \pm 0.23^{c, D}$
	LSMP	$8.02 \pm .26^{c, A}$	$16.05 \pm 0.11^{d, B}$	$18.95 \pm 0.34^{c, C}$	$22.85 \pm 0.32^{c, D}$
	MSMP	$5.94 \pm 0.14^{a, A}$	$12.41 \pm 0.22^{b, B}$	$15.71 \pm 0.16^{b, C}$	$20.90 \pm 1.22^{b, D}$
	HSMP	$5.47 \pm 0.14^{a, A}$	$10.99 \pm 0.09^{a, B}$	$13.57 \pm 0.19^{a, C}$	$17.35 \pm 0.04^{a, D}$
Trial 2	CSMP	$7.66 \pm 0.24^{c, A}$	$15.65 \pm 0.10^{d, B}$	$18.96 \pm 0.16^{d, C}$	$23.03 \pm 0.02^{c, D}$
	LSMP	$8.01 \pm 0.43^{c, A}$	$14.52 \pm 0.07^{c, B}$	$17.88 \pm 0.20^{c, C}$	$23.07 \pm 0.33^{c, D}$
	MSMP	$6.72 \pm 0.10^{b, A}$	$13.02 \pm 0.13^{b, B}$	$16.78 \pm 0.22^{b, C}$	$21.04 \pm 0.37^{b, D}$
	HSMP	$5.44 \pm 0.14^{a, A}$	$11.35 \pm 0.33^{a, B}$	$13.92 \pm 0.16^{a, C}$	$16.85 \pm 1.38^{a, D}$
Trial 3	CSMP	$7.74 \pm 0.44^{ab, A}$	$15.05 \pm 0.35^{c, B}$	$20.46 \pm 0.25^{d, C}$	$26.38 \pm 0.17^{d, D}$
	LSMP	$8.78 \pm 0.26^{b, A}$	$14.86 \pm 0.16^{c, B}$	$19.10 \pm 0.22^{c, C}$	$24.77 \pm 0.17^{c, D}$
	MSMP	$5.95 \pm 1.32^{a, A}$	$12.87 \pm 0.46^{b, B}$	$17.02 \pm 0.33^{b, C}$	$22.96 \pm 0.16^{b, D}$
	HSMP	$5.84 \pm 1.18^{a, A}$	$9.09 \pm 0.81^{a, B}$	$12.89 \pm 0.16^{a, C}$	$16.53 \pm 0.04^{a, D}$

^{a,b,c,d}Means for each trial within the same column not sharing a common superscript differ in treatment (Tukey's HSD, $P < 0.05$)

^{A,B,C,D}Means for each trial within the same row not sharing a common superscript differ in ripening time (Tukey's HSD, $P < 0.05$)

compared to CSMP and LSMP cheeses. CSMP and LSMP cheeses had the highest level of pH 4.6-SN/TN for all trials throughout ripening. LSMP had higher levels of pH 4.6-SN/TN in all trials at d 6 of ripening even compared to the control, but later in ripening levels were similar or lower than the control.

The difference in proteolysis between each cheese could be attributed to fact that the same amount of chymosin was added to each cheese vat but the yield of each cheese was different. Therefore cheeses with higher level of fortification of SMP had a lower enzyme to substrate ratio. Yun et al. (1998)

also found that there was a decrease in the level of pH 4.6-SN/TN in Mozzarella cheese when it was fortified with SMP (3%). This was attributed to the lower moisture in Mozzarella fortified with 3% SMP; similarly, in this study, the level of moisture was lower in HSMP (Table 2.2) which would have contributed in part to the lower level of pH 4.6-SN/TN. When Acharya and Mistry (2004) made cheese from vacuum-condensed or ultrafiltered milk it was found that it had lower levels of proteolysis compared to the control. Brito et al. (2000) also found lower levels of proteolysis in cheese manufactured from milk fortified with SMP. In their study, delayed proteolysis was attributed to heat treatment applied to the milk powder (medium heat treatment); in the current study low heat treated SMP was used. When the rennet to casein ratio is kept constant for the manufacture of cheese from milk with varying levels of total solids, no difference in proteolysis was observed (Govindasamy-Lucey et al., 2005).

2.3.5 Urea-Polyacrylamide Gel Electrophoresis

Figure 2.2 shows the electrophoretograms of the freeze-dried pH 4.6-insoluble fractions of each treatment and trial at d 104 of ripening. Some differences can be observed between the breakdown products of each treatment. In all trials MSMP and HSMP cheeses appear to have a higher quantity of α_{s1}-casein (f 24-199) than of (f 102-199) indicating low chymosin activity which could relate to the lower levels of pH 4.6-SN/TN observed in these cheese treatments (Table 2.5). α_{s1}-Casein hydrolysis to α_{s1}-CN (f102-199), α_{s1}-CN (f24-199) and their degradation products is associated with residual chymosin in cheese (Sheehan

et al., 2008). As previously discussed, the level of pH 4.6-SN/TN was affected by SMP fortification level with the highest level of fortification generating the lowest level of pH 4.6-SN/TN possibly due to a lower enzyme to substrate ratio in fortified cheeses. β-Casein was also hydrolysed to β-casein (f29-209), β-casein (f106-209) and β-casein (f108-209) and there appeared to be no difference between cheese treatments; the generation of these breakdown

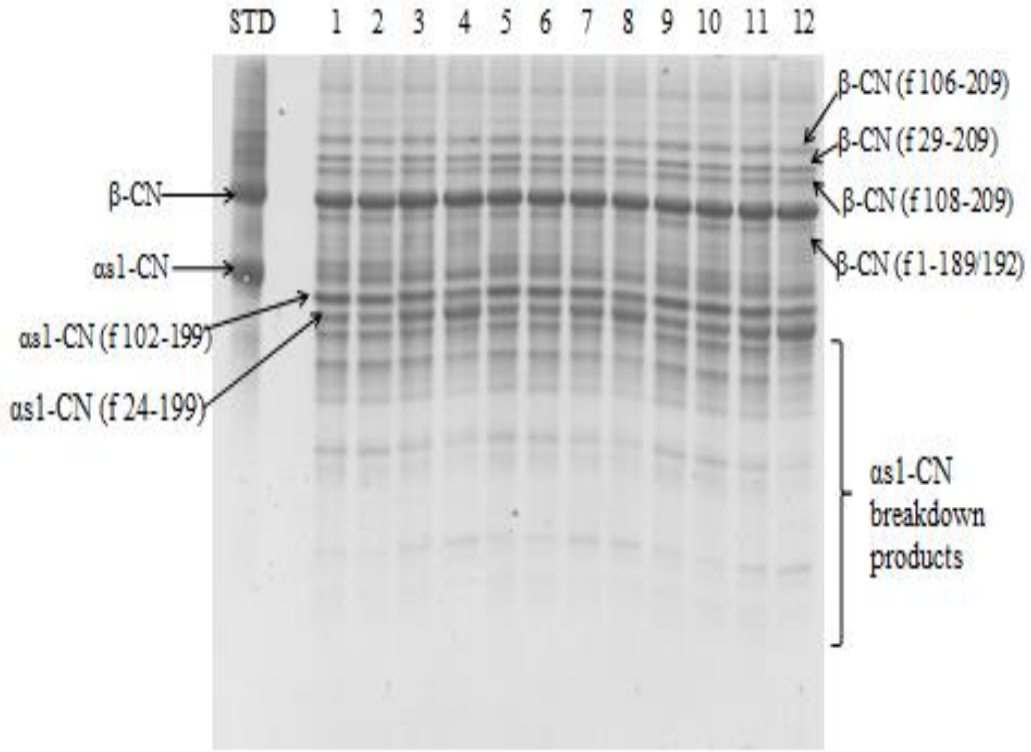


Figure 2.2 Urea-polyacrylamide gel electrophoregrams of sodium caseinate (STD) and the pH 4.6- insoluble fractions of Cheddar cheese made from milk fortified with different levels of skim milk powder at d 104 of ripening. Lanes 1-4, 5-8 and 9-12 represent Trials 1, 2 and 3 respectively. Lanes 1,5 and 9 represent the control CSMP; lanes 2, 6 and 10 represent LSMP; lanes 3, 7 and 11 represent MSMP and lanes 4, 8 and 12 represent HSMP.

products was principally due to the action of plasmin on β-casein (O'Mahony et al., 2005). It is probable that the ratio of plasmin to casein remained constant between cheeses even with fortification with SMP as plasmin is very heat stable (Fox and McSweeney, 1998) could thus be present in the SMP added to milk for fortification.

2.3.6 Individual free amino acids

The concentrations of free amino acids in cheeses fortified with various levels of SMP at d 193 of ripening are shown in Figure 2.3. The fortification of cheese with varying levels of SMP affected the generation of individual free amino acids and significant differences ($P < 0.05$) were observed between cheese treatments. The principal amino acids found in all cheese were glutamic acid, leucine and phenylalanine. MSMP and HSMP cheeses had significantly ($P < 0.05$) higher levels of glutamic acid than CSMP and LSMP cheeses.

HSMP cheese had significantly ($P < 0.05$) lower levels of leucine and phenylalanine compared to CSMP and LSMP cheese. O'Mahony et al. (2005) also reported that the most abundant free amino acids present in Cheddar cheese were glutamic acid, leucine and phenylalanine. The numbers of NSLAB in cheese can have an effect on the level of amino acids present (Shakeel-Ur Rehman et al., 2000; Sheehan et al., 2008). The generation of free amino acids was also affected by the availability of substrates for lactic acid bacterial proteinase or peptidase activity. In this study the levels of NSLAB in the cheese (Table 2.4) and levels of proteolysis (Table 2.5) were significantly different between treatments and may have had an effect on the generation of

free amino acids. Although cheese made from milk with fortified with SMP tended to have higher levels of pH 4.6-SN/TN, MSMP and HSMP cheese had higher levels of glutamic acid. Perhaps the higher levels of NSLAB in these cheese treatments contributed to the generation of higher levels of some free amino acids.

2.3.7 Meltability

Changes in the melt diameter of cheese fortified with different levels of SMP for each trial are shown in Table 2.6. All cheeses increased significantly (P <0.05) in melt diameter as ripening time progressed. Significant differences (P <0.05) were observed between cheese treatments. At the beginning of ripening, cheese manufactured with higher levels of SMP (MSMP and HSMP), had lower meltability in all trials. As ripening time progressed, meltability of HSMP cheeses was significantly lower than the control cheeses. Lower levels of fortification (LSMP cheese) showed little difference in meltability when compared to the control throughout ripening. This shows that the level of fortification used for LSMP cheese did not inhibit meltability as occurred in HSMP cheeses. Yun et al. (1998) found that fortification of Mozzarella cheese with 3% SMP had a slightly reduced melt but the fortification level was relatively low compared to the current study. Acharya and Mistry (2004) found that the manufacture of Cheddar cheese with vacuum-condensed and ultrafiltered milk resulted in cheese with lower meltability. The lower meltability in these cheeses was partly attributed to their lower moisture content. In the present study HSMP cheese had significantly lower moisture

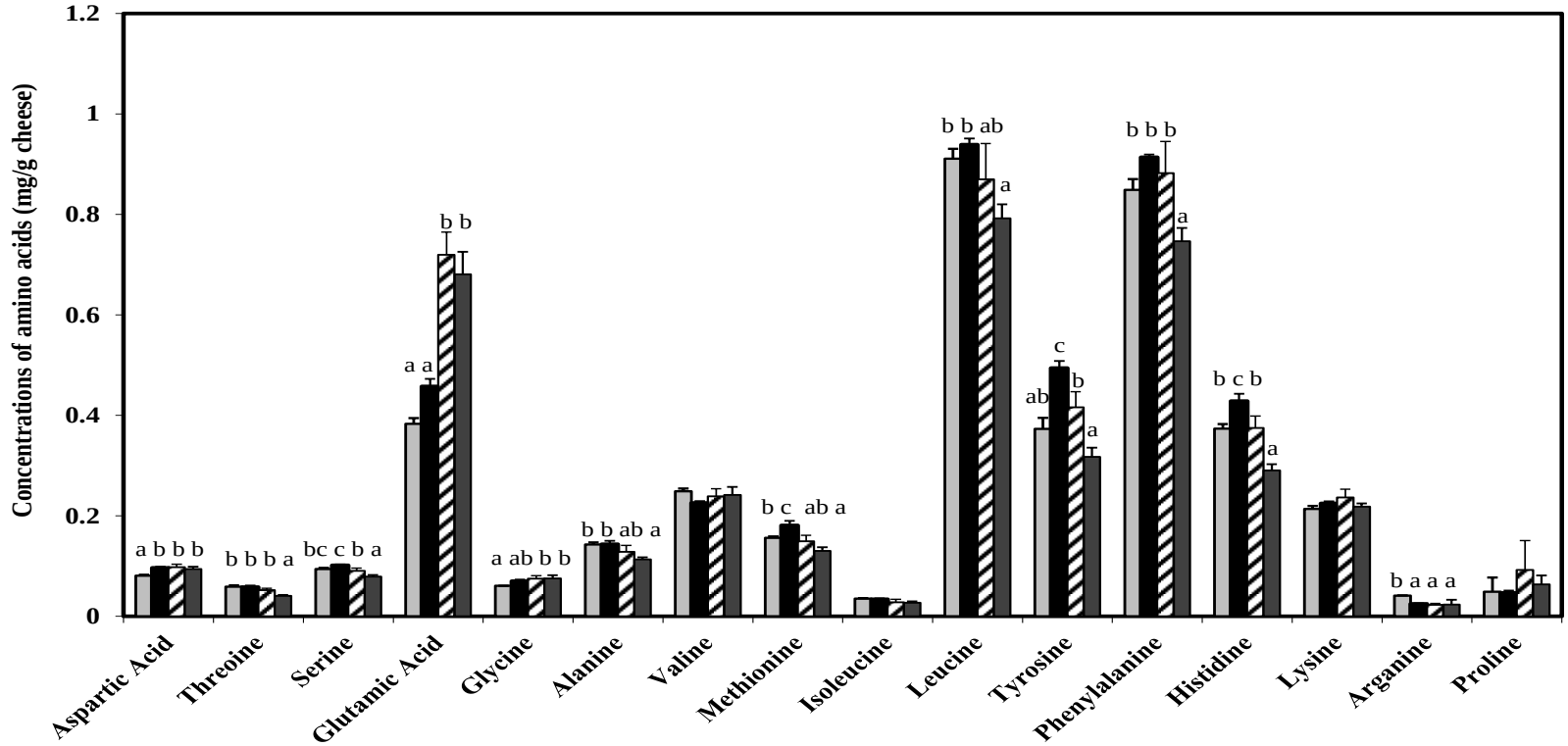


Figure 2.3 Concentrations of individual free amino acids in pH 4.6-soluble extracts from cheese at 193 d made from milk fortified with 0 (CSMP; ■), 10 (LSMP; ■), 20 (MSMP; ■) and 50 (HSMP; ■) % w/v increased casein using skim milk powder. Values represent means from three replicate trials; error bars indicate ± one standard deviation. [a,b,c]Means for each free amino acid not sharing a common superscript differ in treatment (Tukey's HSD, $P < 0.05$)

Table 2.6 Changes in the melt diameter of Cheddar cheeses made from milk fortified with 0 (CSMP), 10 (LSMP), 20 (MSMP) or 50 (HSMP) % w/v increased casein using skim milk powder. Error bars indicate ± one standard deviation.

		Melt diameter (mm)					
				Ripening time (d)			
		7	20	55	104	193	244
Trial 1	CSMP	57.19 ± 2.48 $^{b, A}$	57.31 ± 0.72 $^{ab, A}$	59.94 ± 2.43 $^{a, A}$	71.322 ± 2.38 $^{b, B}$	76.02 ± 3.13 $^{c, B}$	74.476 ± 2.96 $^{c, B}$
	LSMP	58.16 ± 0.55 $^{b, A}$	60.18 ± 3.05 $^{b, A}$	67.41 ± 3.25 $^{b, B}$	67.06 ± 2.41 $^{b, B}$	67.838 ± 1.90 $^{b, B}$	71.39 ± 1.35 $^{c, B}$
	MSMP	54.67 ± 0.32 $^{ab, A}$	55.44 ± 1.99 $^{ab, A}$	59.26 ± 0.62 $^{a, B}$	60.23 ± 0.58 $^{a, B}$	65.84 ± 1.65 $^{b, C}$	66.21 ± 0.76 $^{b, C}$
	HSMP	50.73 ± 2.37 $^{a, A}$	52.91 ± 0.96 $^{a, AB}$	55.42 ± 1.06 $^{a, ABC}$	58.18 ± 3.02 $^{a, C}$	57.72 ± 1.03 $^{a, BC}$	57.72 ± 1.03 $^{a, C}$
Trial 2	CSMP	58.53 ± 1.45 $^{a, A}$	58.11 ± 1.17 $^{b, A}$	63.14 ± 3.94 $^{b, AB}$	67.19 ± 0.91 $^{b, BC}$	69.27 ± 0.99 $^{b, BC}$	73.90 ± 4.06 $^{b, C}$
	LSMP	57.72 ± 2.44 $^{a, A}$	60.44 ± 0.83 $^{c, A}$	61.93 ± 1.86 $^{b, A}$	70.47 ± 1.11 $^{c, B}$	71.51 ± 3.87 $^{b, B}$	80.56 ± 4.66 $^{b, C}$
	MSMP	55.64 ± 3.32 $^{a, A}$	53.72 ± 0.73 $^{a, A}$	58.06 ± 0.70 $^{ab, A}$	57.33 ± 0.30 $^{a, A}$	67.32 ± 1.49 $^{b, B}$	73.85 ± 1.51 $^{b, C}$
	HSMP	53.16 ± 2.79 $^{a, A}$	56.53 ± 0.60 $^{b, AB}$	53.37 ± 0.84 $^{a, A}$	56.62 ± 0.27 $^{a, AB}$	57.96 ± 0.64 $^{a, B}$	57.69 ± 2.10 $^{a, B}$
Trial 3	CSMP	57.51 ± 1.58 $^{bc, A}$	58.92 ± 1.33 $^{ab, A}$	66.52 ± 1.55 $^{c, B}$	70.66 ± 3.70 $^{b, BC}$	72.49 ± 2.50 $^{b, C}$	72.27 ± 0.74 $^{c, BC}$
	LSMP	58.84 ± 0.85 $^{c, A}$	61.60 ± 1.46 $^{b, A}$	63.33 ± 2.81 $^{bc, A}$	69.90 ± 3.49 $^{b, B}$	71.81 ± 2.10 $^{b, B}$	71.00 ± 2.00 $^{c, B}$
	MSMP	55.68 ± 0.96 $^{ab, A}$	57.06 ± 0.40 $^{a, A}$	61.19 ± 1.61 $^{b, B}$	58.22 ± 1.74 $^{a, AB}$	69.17 ± 1.22 $^{b, D}$	65.13 ± 1.22 $^{b, C}$
	HSMP	54.45 ± 1.13 $^{a, A}$	56.19 ± 2.48 $^{a, A}$	54.57 ± 1.91 $^{a, A}$	57.29 ± 1.81 $^{a, AB}$	62.54 ± 0.69 $^{a, B}$	58.85 ± 3.73 $^{a, AB}$

^{a,b,c}Means for each trial within the same column not sharing a common superscript differ in treatment (Tukey's HSD, $P < 0.05$)
^{A,B,C,D}Means for each trial within the same row not sharing a common superscript differ in ripening time (Tukey's HSD, $P < 0.05$)

content (Table 2.2) than CSMP cheese which may have partially attributed to the reduced meltability of HSMP cheese. Levels of insoluble calcium may also affect meltability of cheese (Govindasamy-Lucey et al., 2005); however, this was not measured in this study.

2.3.8 Texture

The texture results for all trials are shown in Figure 2.4. In Trial 1 (Figure 2.4a), fortifying at high levels of SMP (HSMP) resulted in significantly higher (P <0.05) hardness values than CSMP and LSMP cheese up until 35 d of ripening. After 55 d of ripening, both MSMP and HSMP cheeses were significantly (P <0.05) harder than CSMP and LSMP. The same trends for hardness values of treatments were observed in Trial 2 (Figure 2.4b). MSMP was significantly (P <0.05) harder at d 7 and 35 compared to CSMP cheese in Trial 3 (Figure 2.4c). After 104 d of ripening, HSMP and MSMP cheeses were significantly harder than CSMP and LSMP. Addition of relatively low levels of SMP (LSMP) had little effect on the hardness of the cheese when compared to the control. At higher levels of SMP fortification (MSMP and HSMP), cheese texture was harder. High levels of calcium, phosphate and residual lactose in Cheddar cheese has been shown to influence texture attributes (Chevanan and Muthukumarappan, 2007). Increasing the total solids of cheese milk with SMP would consequently increase salts and lactose present in the cheese which could contribute to the higher hardness values observed for high levels of fortification. Yun et al. (1998) also found that Mozzarella cheese fortified with 3% SMP had slightly higher hardness values throughout ripening

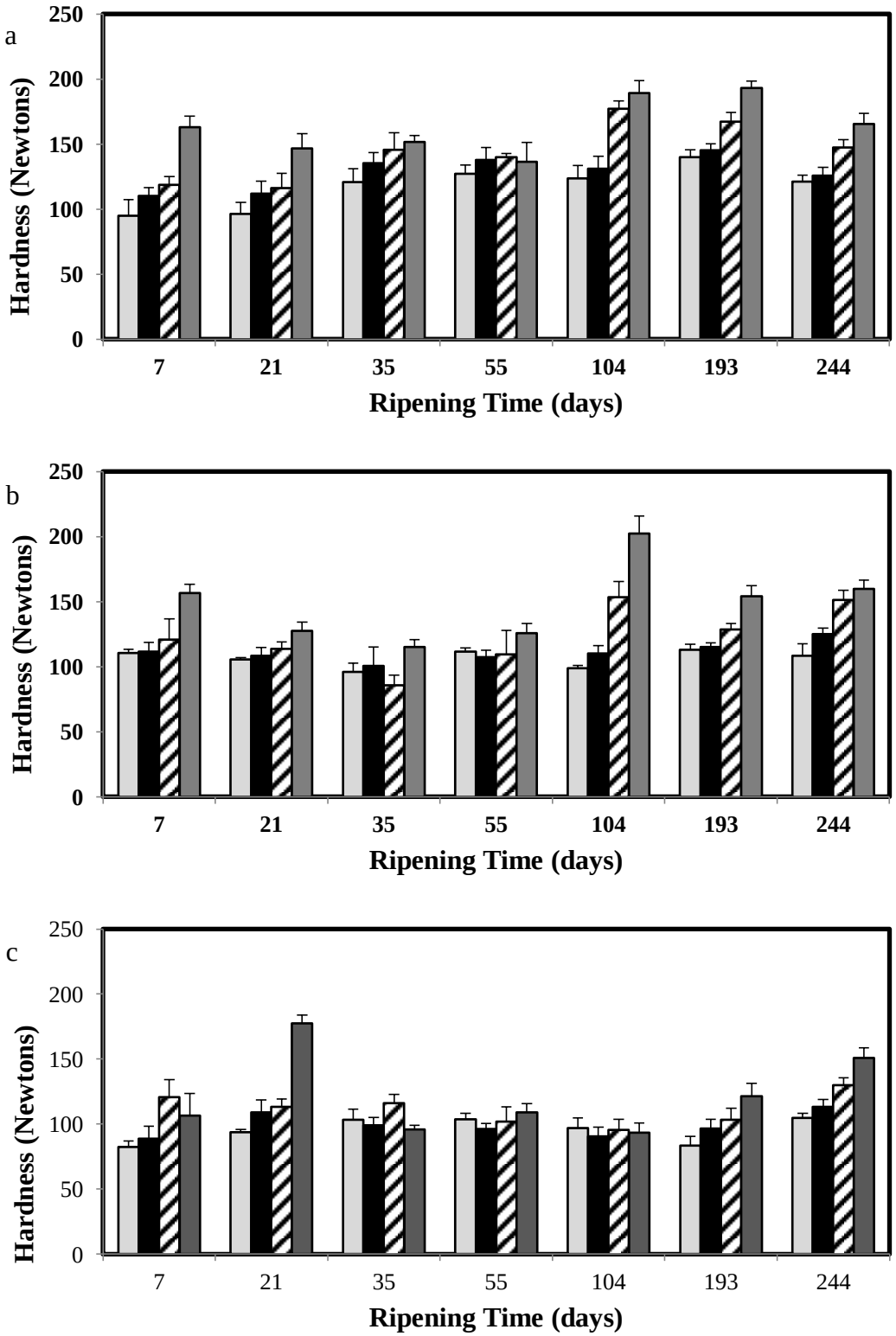


Figure 2.4 Hardness of cheese made from milk fortified with 0 (▫), 10 (■), 20 (◩) or 50 (▪) % w/v increased casein using skim milk powder for trial 1 (a), 2 (b) and 3 (c). Error bars indicate ± one standard deviation.

which was attributed to the higher calcium, lower moisture and lower proteolysis in the cheese. Dong et al. (2009) manufactured cheese from microfiltered milk which had lower levels of pH 4.6-SN/TN, higher calcium and hardness values. Lower pH 4.6 SN was also observed in this study for cheese fortified with high levels of SMP (MSMP and HSMP) (Table 2.5) as well as slightly lower moisture content (Table 2.2) which could have contributed to the higher hardness values observed. Calcium may also have affected the hardness of the cheese; however, this was not measured in this study.

2.4 Conclusions

Use of SMP for fortification of cheesemilk for the manufacture of Cheddar cheese offers some potential benefits of increased yield by increasing the total solids of the milk. SMP fortification significantly ($P < 0.05$) increased moisture adjusted cheese yield. Only slight effects on composition were observed when cheese was fortified with SMP. The moisture was only significantly ($P < 0.05$) lower than the control at the highest fortification level. Whilst lower levels of proteolysis were observed in medium and high levels of SMP probably due to the lower enzyme to substrate ratio in these cheeses, this effect was not clearly observed when low levels of fortification was used. Addition of low levels of SMP had little or no effect on the hardness or meltability of the cheese while high and medium levels tended to give a harder and less meltable cheese.

The results of this study indicate that high levels of SMP may not be ideal when manufacturing typical Cheddar cheese. Taking the results into consideration it may be possible to use higher levels of SMP for fortification when trying to manipulate cheese functionality or fortification may offer a use in ingredient cheese applications such as applications where a less meltable cheese is required. Using low levels of SMP could potentially benefit Cheddar cheese manufacture such as increasing yield and vat throughput while still maintaining properties typical of Cheddar cheese. It is evident from this study that when fortifying milk with powders some considerations should be taken to factors such as milk pasteurisation time and level of rennet addition to the cheese.

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CHAPTER 3

Impact of fortification of cheesemilk with sodium caseinate and calcium chloride on the composition, microbiology, proteolysis, texture and functionality of Cheddar cheese

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Abstract

Fortification of cheesemilk with powders such as sodium caseinate (NaCn) offers potential yield benefits along with improved cheesemaking efficiency. The objective of this study was to evaluate the impact of fortification of cheesemilk with NaCn on the composition, microbiology, proteolysis, texture and functionality of Cheddar cheese. Calcium chloride was added to the cheesemilk fortified with NaCn to improve the rennet coagulation properties. Cheddar cheese was made from cheesemilk fortified with two different levels of NaCn. The treatments were control, 0% NaCn and $CaCl_2$ (Ca), 20% increased casein from NaCn with $CaCl_2$ (L-NaCn) and 40% increased casein from NaCn with $CaCl_2$ (H-NaCn). The casein:fat ratio of the cheesemilk was kept constant for all treatments. The coagula were cut subjectively based on firmness and Cheddar cheese made therefrom. Cheese made from milk fortified with NaCn tended to have lower fat, higher moisture and higher salt compared to the control cheese. Cheeses fortified with NaCn also had higher yield than the control and Ca cheeses. Cheeses fortified with NaCn had higher numbers of non-starter lactic acid bacteria compared to the controls possibly due to the protective effect that higher total solids milk may have on bacterial cell numbers during pasteurisation. Proteolysis was affected by the treatments; Ca cheese tended to have the highest and H-NaCN cheese had the lowest levels of proteolysis compared to the other treatments. Cheese fortified with NaCn and $CaCl_2$ were softer than the control and also tended to have similar or less melt than the control but this difference was not apparent towards the end of ripening. Using NaCn to fortify cheesemilk affected the properties of the

cheese but this may offer a potential to change the characteristics of the cheese for certain applications while increasing the cheese yield.

Chapter 3: Fortification of Cheddar cheese with sodium caseinate

3.1 Introduction

Seasonality of milk causes periods in the cheese industry where plants are idle; a more economical solution is constant cheese production throughout the year (Freeman et al., 1970). Supplementation of cheesemilk using concentration methods offers a potential to improve cheese making efficiency (Kosikowski et al., 1984).

Using ultrafiltration means the milk contains both proteins and colloidal minerals in higher proportions than is normally found in milk giving cheese made from this milk increased yield but this can have negative effects on the cheese (de la Fuente, 1998). Acharya and Mistry (2004) found that concentration of milk with vacuum-condensing or ultrafiltration resulted in Cheddar cheese with lower moisture, higher calcium, lower proteolysis and decreased meltability. Kosikowski et al. (1984) made Cheddar cheese supplemented with ultrafiltration retentates; it was found that the resultant cheese had increased yield and lower moisture.

The use of powder for fortification of cheesemilk is illegal in many countries due to standards of identity of cheese. However, powder fortification offers the benefit of being cheaper than membrane processes but also powder composition can vary greatly depending on the source of milk and manufacturing steps which would affect their reliability (Pellegrino et al., 2010). Brito et al. (2000) used skim milk powder to fortify milk for manufacture of Maribo cheese; fortified cheese had lower moisture, lower proteolysis and higher yield. Yun et al. (1998) fortified milk for the manufacture of Mozzarella with skim milk powder; it was found that fortified

cheeses had lower moisture, higher calcium and at higher fortification levels, lower proteolysis and firmer texture. Shakeel-Ur-Rehman et al. (2003) found that using milk protein concentrate (MPC) to standardise reduced-fat Cheddar cheese increased yield and decreased proteolysis. The level of maturity was also lower in the MPC cheese; however, increased addition of starter bacteria improved maturity. St-Gelais et al. (1998) found that the yield of low-fat Cheddar cheese was affected by the type of powder used for fortification of cheesemilk; yield was higher when diafiltered microfiltration retentate powder was used compared to calcium caseinate or ultrafiltered retentate powders.

El-Shinbiny et al. (1998) suggested the addition of NaCn to whole milk retentate to manufacture Edam cheese to increase yield and improve organoleptic properties as well as increase proteolysis. Lobato-Calleros et al. (2000) found that adding NaCn to milk for the manufacture of fresh cheese led to increased yields and lower syneresis rates.

NaCn is produced by acidification of skim milk to pH 4.6 creating acid casein which is then washed and redissolved by increasing the pH in sodium hydroxide; this is used in industry for its water binding and functional properties (Early, 1998; Pitkowski et al., 2008). During acidification of milk to pH 4.6, as occurs in the process of manufacturing NaCn (Early, 1998), much of the colloidal calcium associated with the micelle is lost (de la Fuente, 1998; Lucey et al., 2003). The objective of this study was to fortify cheese milk with NaCn powder and make Cheddar cheese using traditional manufacturing methods. When cheesemilk was fortified with NaCn, coagulation properties were affected due to the lack of calcium in the system; therefore, calcium chloride was added to the milk to try to replace some of the lost colloidal

calcium. The cheeses were manufactured from control milk, control milk with added calcium chloride and milk fortified with NaCn at high and low levels. Cheeses were then assessed to determine the treatment effects on cheese yield, compositional, microbiology, functionality and proteolysis.

3.2 Material and Methods

3.2.1 Pretreatment of cheesemilk

Raw milk was obtained from a local farm (Rockrohan Farm, Carrigrohane Straight, Cork, Ireland). Part of the milk was separated into skim milk and cream. Prior to milk fortification, the protein content of NaCn powder (Kerry Group, Listowel, Ireland) was determined using the macro-Kejdahl (IDF, 1986) method. 1.15 kg of NaCn powder (88% protein) was added to 30 L of skim milk using a Silverson mixer (Silverson AXR, Silverson Ltd., Chesham, Bucks, England) to form a slurry. The slurry was standardised to a casein:fat ratio of 0.70:1.00, batch pasteurised at 63ºC for 30 min and stored to allow protein to hydrate overnight at 4ºC (Table 3.1). The bulk milk was standardised to a casein:fat of 0.70:1.00 and pasteurised at 72ºC for 15 s and stored at 4ºC. The composition of milks was determined using a milkoscan (FT120, Foss Electric, Denmark).

Table 3.1 Composition of cheesemilk used for the manufacture of Cheddar cheeses made from milk fortified with 0% NaCn (control), $CaCl_2$ (Ca), 20% increased casein with NaCn and $CaCl_2$ (L-NaCn) or 40% increased casein with NaCn and $CaCl_2$ (H-NaCn). Values are means of three replicates with standard deviations in parentheses.

	Fat (%)	Protein (%)	Casein (% est)	CN:Fat[1]	Lactose (%)	Total Solids (%)
Control	3.75(0.05)	3.39(0.07)	2.64(0.05)	0.71(0.01)	4.32(0.07)	12.34(0.11)
Ca	3.75(0.06)	3.39(0.07)	2.65(0.06)	0.71(0.01)	4.32(0.06)	12.35(0.13)
L-NaCn	4.57(0.08)	4.09(0.08)	3.19(0.07)	0.70(0.01)	4.25(0.06)	13.79(0.16)
H-NaCn	5.35(0.08)	4.79(0.09)	3.73(0.07)	0.70(0.01)	4.18(0.06)	15.20(0.17)

[1]Casein:fat ratio

3.2.2 Cheese manufacture

Cheddar cheese (4 vats; Control, Ca, L-NaCn and H-NaCn) were made in triplicate on different days at the food processing facilities at University College Cork, Ireland according to standard protocol at pilot scale (50 L of cheesemilk). Preliminary experiments showed that fortification of milk with NaCn interfered with the coagulation properties of the milk; to form a sufficient gel during coagulation $CaCl_2$ had to be added to the milk that was fortified with NaCn. Cheesemilk was adjusted using the slurry and standardised milk to give the desired fortification levels (Table 3.1) for each vat. The milk treatments were a control, control with added $CaCl_2$ (Ca), a low fortification level of 20% increased casein (L-NaCn) and a high fortification level of 40% increased casein (H-NaCn). All vats were heated for 31ºC. $CaCl_2$ (163 ml of 2 M solution) was added to Ca, L-NaCn and H-NaCn treatment milks and allowed to stir for 30 min. Direct-to-vat frozen starter culture cultures (0.06%) (R604Y, Chr Hansen, Hørsholm, Denmark) was added to all vats and allowed to ripen for 30 min. After ripening chymosin (0.03%v/v) (CHY-MAX Plus, 200 international milk clotting units/ml, Chr Hansen) was added to the milk and allowed to coagulate. The coagulation times of the cheesemilk varied and were cut subjectively based on firmness. Curds and whey were cooked to 38ºC over a 30 min period and drained at pH 6.2. Curds were cheddared until pH 5.4, milled and salted (2.5%). After pressing overnight, the cheese was vacuum packed and ripening at 8ºC.

3.2.3 Compositional analysis

Cheese whey was analysed for fat and protein by the Gerber (IS, 1955) and macro-Kjeldahl (IDF, 1986) method, respectively. Cheese was analysed at 25 d of ripening for moisture by oven drying (IDF, 1982), protein by macro-Kjeldahl (IDF, 1986), fat by Gerber (IS, 1955) and salt (Fox, 1963). Grated cheese (10 g) was blended with 10 ml of deionized in a stomacher and the pH measured using a pH meter (PHM210, Standard pH meter, Meterlab, Radiometer, Copenhagen, Denmark) (Madkor et al., 1987) at 25, 40 and 111 d of ripening. All analysis was carried out in triplicate

3.2.4 Yield

The actual cheese yield was calculated by dividing the weight of cheese after pressing by the total weight of milk. The adjusted yield was calculated to 37% moisture and 1.7% salt using the following formula (Lau et al. 1990):

$$\textbf{Adjusted yield} = \frac{(\text{actual yield})[100 - (\text{actual percentage of moisture} + \text{percentage of salt})]}{100 - (\text{Desired percentage of moisture} + \text{percentage of salt})}$$

3.2.5 Microbiological analysis

Starter (SLAB) lactococci and non-starter lactic acid bacteria (NSLAB) were enumerated in duplicate throughout cheese ripening. Starter lactococci were enumerated on LM17 agar (Merck, Darmstadt, Germany) (Terzaghi and Sandine, 1975) after 3 d incubation at 30ºC. NSLAB were enumerated on

double layered Rogosa agar (Merck) (Rogosa et al., 1951) after 5 d incubation at 30ºC.

3.2.6 Meltability

Meltability of cheese samples was determined using the Schreiber test with some modifications. Three samples for each treatment were cut to 5 mm in height using a food slicer and 35 mm in diameter using a cork borer. Samples were stored overnight at 8ºC to allow the temperature to equilibrate. Cheese discs were placed in the center of a glass Petri dish and covered. Samples were then melted at 232ºC for 5 min in an oven (Altan et al., 2005) and allowed to cool for 30 min at room temperature. Five readings of the diameter were measured for each sample and averaged.

3.2.7 Texture analysis

Cylindrical samples were cut to 20 mm in height and 20 mm in diameter using a stainless steel cork borer and wire cutter. Samples were wrapped in plastic wrap and stored at 8ºC overnight. Samples were compressed to 25% of their original height using a texture analyser (TA-XT2i Texture Analyser, Stable Micro Systems, Godalming, England) at a rate of 1 mm s^{-1}. Hardness was defined as the force required to compress the sample to 25% of its original height.

3.2.8 Proteolysis

pH 4.6-soluble and -insoluble nitrogen fractions of cheese were prepared according to the method of Kuchroo and Fox (1982). The nitrogen content of the soluble fraction was determined (IDF, 1986).

Proteolysis was also assessed using urea-polyacrylamide gel electrophoresis (urea-PAGE) (12.5% total acrylamide, 4% cross linking agent, pH 8.9) was performed according to Andrews (1983) with modifications (Shalabi and Fox, 1987). Samples were run through the stacking gel at 280 V and separating gel at 300 V. Gels were stained using Coomassie Brilliant Blue G250 (Blakesely and Boezi, 1977) and destained using distilled water.

3.2.9 Statistical Analysis

Statistical analysis was carried out using one-way analysis of variance (ANOVA) and Tukey's HSD *post hoc* test with PASW Statistics for Windows version 18 (SPSS Inc., Chicago, IL) to compare significant differences between treatments and ripening times. The probability level used for statistical significance was $P < 0.05$.

3.3 Results and Dicussion

3.3.1 Compositional Analysis

Composition of Cheddar cheese made from milk fortified with NaCn is shown in Table 3.2. Due to differences observed in cheese composition between trials, each trial was assessed individually for all parameters measured. Difference in protein content was not significant ($P > 0.05$) between cheese treatments for Trials 1 and 3. In Trial 2, the protein content of the control was significantly ($P < 0.05$) higher than cheese made from milk fortified with NaCn (L-NaCn and H-NaCn).

The difference in fat content between cheese treatments in each trial was significant ($P < 0.05$) even though the casein:fat ratio was standardised for each treatment (Table 3.1). In all trials, the fat content tended to be lower in cheese made from milk fortified with NaCn when compared to the control. The process of adding powder to the cheesemilk to form a slurry was completed using a Silverson mixer, which may have damaged the fat globules and caused fat to be released into the whey instead of being retained in the cheese; however, this was not measured in this study. The fat in the whey from H-NaCn cheese in all trials was significantly ($P < 0.05$) higher than the control cheeses; this confirms the potential of damage to the fat globules during milk processing. St-Gelais et al. (1998) found that low-fat Cheddar cheese made from milks enriched from calcium caseinate had lower fat content, this was attributed to the need for more calcium addition to improve milk coagulation and curd structure. Conversely, the fat content in the whey of the Ca control in all trials was significantly ($P < 0.05$) lower than all other cheeses. Ong et al. (2013) also found that addition of $CaCl_2$ to cheesemilk lowered fat losses in

Table 3.2 Composition of Cheddar cheeses made from milk fortified with 0% NaCn (control), $CaCl_2$ (Ca), 20% increased casein with NaCn and $CaCl_2$ (L-NaCn) or 40% increased casein with NaCn and $CaCl_2$ (H-NaCn) at 25 d of ripening. Values are means of three replicates and standard deviations with the latter in parentheses.

		Compositional Analysis						
		% Protein	% Fat	% Moisture	% Salt	% MNFS*	% Protein (whey)	% Fat (whey)
Trial 1	Control	24.21^{a} (0.08)	32.25^{c} (0.43)	38.25^{a} (0.30)	1.39^{a} (0.06)	56.46^{a} (0.11)	0.89^{a} (0.01)	0.67^{b} (0.03)
	Ca	24.38^{a} (0.25)	31.75^{bc} (0.25)	40.11^{b} (0.09)	1.54^{ab} (0.12)	58.77^{c} (0.31)	0.87^{a} (0.01)	0.45^{a} (0.05)
	L-NaCn	23.89^{a} (0.25)	30.33^{a} (0.55)	40.20^{b} (0.28)	1.41^{ab} (0.03)	57.70^{b} (0.28)	0.93^{b} (0.02)	0.68^{b} (0.06)
	H-NaCn	24.48^{a} (0.30)	30.83^{ab} (0.58)	40.61^{b} (0.06)	1.58^{b} (0.03)	58.72^{c} (0.43)	1.00^{c} (0.01)	0.87^{c} (0.06)
Trial 2	Control	23.39^{b} (0.22)	32.75^{b} (0.66)	39.20^{a} (0.37)	1.33^{a} (0.01)	58.29^{a} (0.93)	0.98^{ab} (0.03)	0.60^{b} (0.00)
	Ca	23.06^{ab} (0.09)	33.50^{b} (0.50)	38.85^{a} (0.06)	1.37^{a} (0.03)	58.43^{ab} (0.34)	0.93^{a} (0.03)	0.35^{a} (0.05)
	L-NaCn	22.54^{a} (0.52)	31.00^{a} (0.66)	39.93^{ab} (1.16)	1.45^{b} (0.02)	57.98^{ab} (2.19)	1.00^{b} (0.01)	0.63^{b} (0.06)
	H-NaCn	22.32^{a} (0.02)	30.62^{a} (0.13)	41.72^{b} (0.69)	1.58^{c} (0.03)	60.13^{b} (0.96)	1.09^{c} (0.03)	0.72^{c} (0.03)
Trial 3	Control	24.51^{a} (0.21)	32.00^{b} (0.71)	38.54^{a} (0.42)	1.43^{b} (0.01)	56.81^{ab} (0.22)	0.97^{a} (0.01)	0.62^{b} (0.03)
	Ca	23.78^{a} (0.17)	32.17^{b} (0.29)	38.83^{ab} (0.24)	1.41^{b} (0.02)	57.25^{ab} (0.55)	0.91^{a} (0.02)	0.38^{a} (0.03)
	L-NaCn	23.97^{a} (0.36)	32.00^{b} (0.00)	40.02^{b} (0.71)	1.19^{a} (0.00)	60.23^{b} (2.07)	1.00^{ab} (0.02)	0.62^{b} (0.03)
	H-NaCn	23.93^{a} (0.61)	29.50^{a} (0.00)	39.69^{ab} (0.01)	1.53^{c} (0.06)	56.30^{a} (0.01)	1.07^{b} (0.06)	0.80^{c} (0.00)

*****Moisture in nonfat substance of the cheese

^{a,b,c}Means within the same column not sharing a common subscript differ (Tukey's HSD, $P < 0.05$)

the whey but without a change in the final fat composition in the cheese. This was attributed to the addition of $CaCl_2$ leading to similar total fat losses in the whey but with more fat being lost after whey drainage and during the pressing stage due to slight differences in microstructure of the curd. Wolfschoon-Pombo (1997) found that addition of $CaCl_2$ to cheesemilk for the manufacture of Emmenthaler-type cheese led to higher yield which was attributed to a slightly higher but not significant transfer of fat and solids not-fat into the curd.

The moisture content of the control cheese in Trial 1 was significantly ($P < 0.05$) lower than the other treatments. In Trials 2 and 3, the moisture content was lower in the control and Ca cheese when compared to cheese made from milk fortified with NaCn. When cheesemilk is concentrated by ultrafiltration or has added skim milk powder it has typically been seen that moisture in the resulting cheeses decreased (Kosikowski et al., 1985; Brito et al., 2000). The higher moisture in cheese made from milk fortified with NaCn powder may be due to the high water binding capacity of sodium caseinate which could have affected the syneresis properties of the curd during manufacture (Lobato-Calleros et al., 2000). The lower fat observed in cheese fortified with powder compared to the control may also have contributed to the higher moisture. The level of salt in H-NaCn cheese was also higher than the other treatments in all trials. The higher salt may relate to the higher moisture in H-NaCn cheese causing an increased uptake of salt in the cheese. El-Shibiny et al. (1998) also found increased salt in cheese with added NaCn.

In all trials, the protein content of the whey was the highest for the H-NaCn cheese, this may possibly due to unincorporated NaCn being released into the whey. Yun et al. (1998) also found higher levels of protein in whey when

cheesemilk was fortified with skim milk powder. There was no difference between the level of protein lost in the whey between the control and Ca cheeses. Ong et al. (2013) also found that $CaCl_2$ addition to cheesemilk did not affect the amount of protein the whey.

The pH of cheeses made from milk fortified with NaCn is shown in Table 3.3. In Trial 1, the control had a significantly ($P < 0.05$) lower pH than the other cheese treatments at d 25 of ripening. At d 40 of ripening, the control cheese had a significantly lower pH ($P < 0.05$) than H-NaCn; there was no significant ($P > 0.05$) difference between the pH of cheese treatments at d 111 of ripening. In Trial 2, similar trends were observed; control cheese pH was significantly ($P < 0.05$) lower at d 25 of ripening but no significant ($P > 0.05$) difference was observed between treatments measured later in the ripening period. In Trial 3, the control and L-NaCn cheeses had significantly ($P < 0.05$) lower pH compared to the other cheese treatments. Again no significant ($P > 0.05$) differences were observed later in ripening between treatments. Addition of $CaCl_2$ to milk could have caused decreased levels of phosphate and citrate in the soluble phase and therefore association of calcium phosphate and calcium citrate salts into the colloidal phase which could have caused an increased buffering capacity at lower pH (Salaun et al., 2005). Cheese typically manufactured with higher total solids milk (L-NaCn and H-NaCn) has higher pH due to the higher buffering capacity from the added casein (Govindasamy-Lucey et al., 2005); all cheeses had the same level of starter bacteria, which contribute to acidification during cheese manufacture and ripening. For all trials after d 25 of ripening the pH of all treatments significantly ($P < 0.05$) increased. pH increase in cheese during ripening can be due to solubilisation of

colloidal calcium phosphate (Lucey et al., 2003). In all trials, pH increased significantly throughout ripening but no significant ($P < 0.05$) differences were observed between treatments at d 111 compared to the lower pH of the control at d 25.

The control cheese would be expected to have typical levels of colloidal calcium phosphate, as it was not made from fortified milk and hence pH would increase as it solubilises. Ca cheese contained added $CaCl_2$ which would alter the salt balance in milk and cause precipitation of calcium phosphate upon addition of milk (McSweeney et al., 2007). L-NaCn and H-NaCn contained $CaCl_2$ and NaCn which meant it did not have the same level of colloidal calcium phosphate as natural milk. Taking this into consideration, the pH increase during ripening caused by the solubilisation of CCP leading to neutralization of hydrogen ions by phosphate anions (Guinee et al., 2002) would have been greater in the control, in combination with the higher buffering capacity in the other treatments would have led to no significant ($P < 0.05$) difference between the pH of all treatments at d 111 of ripening.

3.3.2 Yield

The moisture and salt adjusted yield of cheese made with milk fortified with NaCn is shown in Figure 3.1. There was no significant ($P > 0.05$) difference between the control and Ca cheeses. Both cheeses that were made from milk fortified with NaCn had significantly ($P < 0.05$) higher yields than the control

Table 3.3 pH values and levels of pH 4.6 soluble-nitrogen (SN) and pH of Cheddar cheeses made from milk fortified with 0% NaCn (control), $CaCl_2$ (Ca), 20% increased casein with NaCn and $CaCl_2$ (L-NaCn) or 40% increased casein with NaCn and $CaCl_2$ (H-NaCn) at 40, 111, 181 and 25, 40, 111 d of ripening, respectively. Values are means of three replicates and standard deviations with the latter in parentheses.

		pH 4.6-SN/TN (%)			pH		
		Ripening Time (days)					
		40	111	181	25	40	111
Trial 1	Control	$15.37^{c,A}$ (0.22)	$20.62^{b,B}$ (0.11)	$24.52^{b,C}$ (0.79)	$5.06^{a,A}$ (0.03)	$5.22^{a,B}$ (0.01)	$5.22^{a,B}$ (0.02)
	Ca	$16.78^{d,A}$ (0.08)	$23.88^{c,B}$ (0.52)	$25.74^{b,C}$ (0.09)	$5.16^{b,A}$ (0.01)	$5.25^{ab,B}$ (0.00)	$5.32^{a,C}$ (0.02)
	L-NaCn	$14.81^{b,A}$ (0.10)	$21.16^{b,B}$ (0.07)	$24.47^{b,C}$ (0.32)	$5.13^{b,A}$ (0.02)	$5.26^{ab,B}$ (0.01)	$5.26^{a,B}$ (0.02)
	H-NaCn	$13.84^{a,A}$ (0.33)	$18.13^{a,B}$ (0.34)	$21.32^{a,C}$ (1.11)	$5.15^{b,A}$ (0.01)	$5.28^{b,B}$ (0.02)	$5.28^{a,B}$ (0.01)
Trial 2	Control	$14.29^{a,A}$ (0.24)	$20.74^{b,B}$ (0.15)	$24.26^{a,C}$ (0.15)	$5.08^{a,A}$ (0.01)	$5.21^{a,B}$ (0.01)	$5.26^{a,C}$ (0.01)
	Ca	$15.91^{b,A}$ (0.40)	$22.29^{c,B}$ (0.40)	$24.56^{a,C}$ (1.40)	$5.16^{b,A}$ (0.01)	$5.27^{a,B}$ (0.03)	$5.33^{a,C}$ (0.01)
	L-NaCn	$14.65^{a,A}$ (0.43)	$20.58^{b,B}$ (0.14)	$22.61^{a,C}$ (0.95)	$5.13^{b,A}$ (0.02)	$5.22^{a,B}$ (0.01)	$5.27^{a,B}$ (0.03)
	H-NaCn	$13.98^{a,A}$ (0.13)	$19.35^{a,B}$ (0.00)	$23.24^{a,C}$ (0.46)	$5.14^{b,A}$ (0.02)	$5.23^{a,B}$ (0.01)	$5.26^{a,B}$ (0.03)
Trial 3	Control	$13.16^{ab,A}$ (0.23)	$17.89^{a,B}$ (0.75)	$22.07^{a,C}$ (0.39)	$5.19^{a,A}$ (0.01)	$5.30^{a,B}$ (0.02)	$5.30^{a,B}$ (0.01)
	Ca	$14.82^{c,A}$ (0.65)	$19.56^{b,B}$ (0.54)	$23.51^{b,C}$ (0.70)	$5.23^{b,A}$ (0.00)	$5.33^{a,B}$ (0.04)	$5.25^{a,B}$ (0.02)
	L-NaCn	$13.35^{b,A}$ (0.34)	$17.79^{a,B}$ (0.73)	$22.05^{a,C}$ (0.36)	$5.17^{a,A}$ (0.01)	$5.28^{a,B}$ (0.06)	$5.30^{a,B}$ (0.01)
	H-NaCn	$12.20^{a,A}$ (0.21)	$16.39^{a,B}$ (0.07)	$20.95^{a,C}$ (0.16)	$5.25^{b,A}$ (0.02)	$5.32^{a,AB}$ (0.02)	$5.38^{a,B}$ (0.06)

^{a,b,c,d}Means for each trial within the same column not sharing a common superscript differ in treatment (Tukey's HSD, P <0.05)
^{A,B,C}Means for each trial within the same row not sharing a common superscript differ in ripening time (Tukey's HSD, P <0.05)

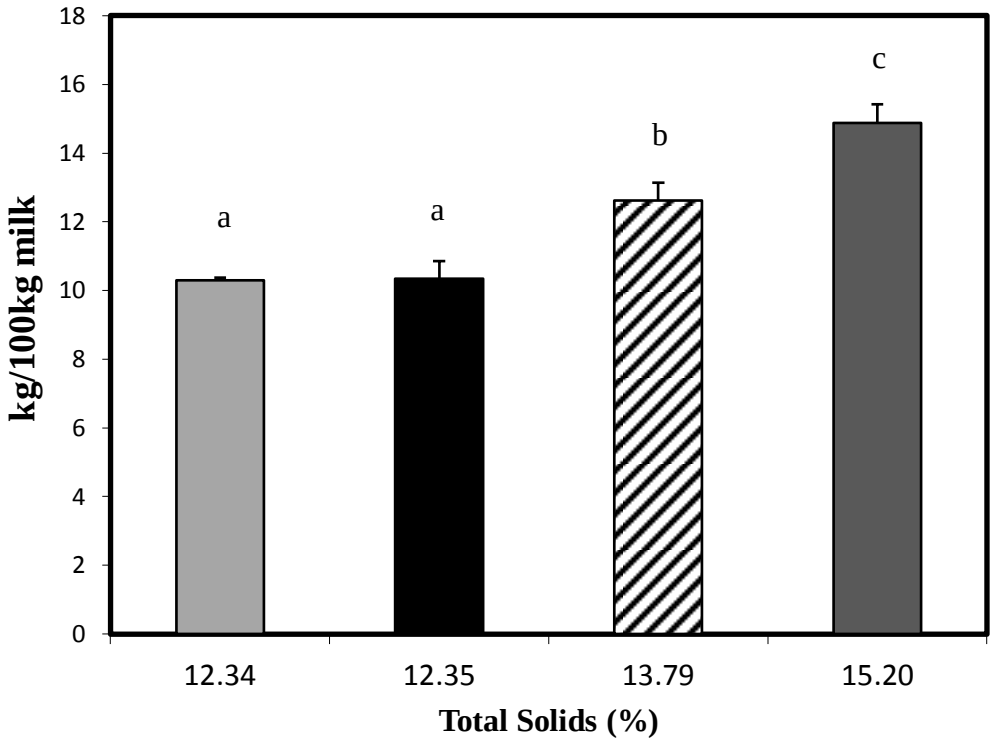


Figure 3.1 Moisture- and salt-adjusted yield (kg/100kg of milk) of Cheddar cheeses made from milk fortified with different levels of NaCn. The cheeses were control (▫), Ca (■), L-NaCn (◪) or H-NaCn (▪) for all trials. Error bars indicate + one standard deviation.

and Ca cheese. H-NaCn cheese had the highest yield of all treatments. Cheese made from milk fortified with NaCn also had higher total solids in the milk when compared to the control and Ca milks. More casein and fat can be incorporated into the cheese when milk with higher total solids is used (Govindasamy-Lucey et al. 2005). It has previously been found that addition of sodium caseinate for cheese manufacture led to increased yield (El-Shinbiny et al., 1998; Lobato-Calleros et al., 2000). Ong et al. (2013) found that addition of $CaCl_2$ to milk had no effect on cheese yield. Conversely, Wolfschoon-Pombo (1997) found increased cheese yield when $CaCl_2$ was added to milk.

3.3.3 Microbiology

Counts of starter and non-starter lactic acid bacteria are shown in Tables 3.4 and 3.5, respectively. LM17 agar was used to enumerate SLAB counts in the cheese; this measured total counts of lactic acid bacteria therefore high NSLAB counts could have a confounding effect on SLAB counts. In general all trials decreased significantly ($P < 0.05$) in SLAB levels from 10^9 at d 1 to 10^6-10^8 cfu g^{-1} at d 181 of ripening. At d 181 of ripening there were significant ($P < 0.05$) differences between SLAB counts in treatments of each trial. For all trials counts in the control cheese were lower than the other treatments. In Trial 1 and 3, both L-NaCn and H-NaCn had significantly ($P < 0.05$) higher SLAB counts than the control and Ca. In Trial 2, L-NaCn had significantly ($P < 0.05$) higher SLAB counts than all other treatments; Ca and H-NaCn were not significantly ($P > 0.05$) different.

For all trials, NSLAB were not present in the control and Ca cheese at the beginning of the ripening period. In Trial 1, NSLAB started to develop in the control and Ca cheese at d 77 and counts significantly ($P < 0.05$) increased throughout the remainder of ripening. L-NaCn and H-NaCn cheeses had NSLAB present at d 1 of ripening which increased significantly ($P < 0.05$) throughout ripening. In Trial 2, the control and Ca cheese had no NSLAB present until d 111 of ripening. In L-NaCn cheese, NSLAB developed at d 25 of ripening but H-NaCn cheese developed NSLAB later at d 77. Counts in both cheeses increased significantly ($P < 0.05$) throughout ripening but L-NaCn cheese typically had higher counts of NSLAB. In Trial 3, the control and Ca only developed NSLAB at d 181 of ripening. L-NaCn and H-NaCn had NSLAB counts present at d 1 of ripening which increased significantly ($P <$

0.05) at the end of ripening. H-NaCn cheese generally had higher NSLAB counts at the beginning of ripening but there was no significant ($P > 0.05$) difference after d 77.

A point to consider is that the powder had <10 cfu g^{-1} (results not shown) of NSLAB; thus, the NSLAB in the cheeses fortified with NaCn probably came from the raw milk. The "Grade 'A' Pasteurized Milk Ordinance" (PMO) states that "if the fat content of the milk product is 10% or greater or a total solids of 18% or greater, or if it contains added sweeteners, the specified temperature shall increase by 3ºC" (FDA, 2009). The fortified slurry that was used in the manufacture of L-NaCn and H-NaCn cheese was batch pasteurised; due to the higher level of total solids, this slurry should probably have been pasteurised at a higher temperature in accordance with the PMO. NSLAB are present in the raw milk; after heat treatment they may remain in the milk in a stressed state after which they recover and begin to grow in the cheese (Beresford, 2007). It is hypothesised that in this study higher number of NSLAB are present in cheese made with milk fortified with NaCn as the higher total solids in the slurry for cheese manufacture offered a protective effect for the bacteria during the pasteurisation. Also, higher total solids in milk would lead to increased viscosity which may affect the heat transfer and hence the time-temperature effect on bacteria survival during pasteurisation. Increasing milk total solids affects thermal conductivity (Fox and McSweeney, 1998). The NSLAB in the cheeses fortified with the slurry were probably in a stressed state but started to recover during the cheese manufacture and ripening. The control and Ca cheese would have developed NSLAB more slowly as these were pasteurised at the correct time x temperature combination for the level of total solids

Table 3.4 Numbers of starter lactic acid bacteria (SLAB; cfg g^{-1}) for Trials 1, 2 and 3 of Cheddar cheeses made from milk fortified with 0% NaCn (control), $CaCl_2$ (Ca), 20% increased casein with NaCn and $CaCl_2$ (L-NaCn) or 40% increased casein with NaCn and $CaCl_2$ (H-NaCn) at 1, 25, 40, 77, 111 and 181 d of ripening, respectively. Values correspond to means (n=2).

		Ripening time (d)					
		1	25	40	77	111	181
Trial 1	Control	$6.8\times10^{9\ b,B}$	$7.0\times10^{9\ a,B}$	$1.7\times10^{10\ c,C}$	$9.5\times10^{8\ a,A}$	$2.0\times10^{8\ a,A}$	$1.1\times10^{7\ a,A}$
	Ca	$5.0\times10^{9\ a,B}$	$6.3\times10^{9\ a,B}$	$1.1\times10^{10\ b,C}$	$3.5\times10^{9\ b,B}$	$1.1\times10^{9\ c,A}$	$8.5\times10^{7\ a,A}$
	L-NaCn	$5.0\times10^{9\ ab,B}$	$6.1\times10^{9\ a,BC}$	$8.0\times10^{9\ b,C}$	$2.4\times10^{9\ ab,A}$	$5.2\times10^{8\ b,A}$	$2.2\times10^{8\ b,A}$
	H-NaCn	$6.5\times10^{9\ ab,C}$	$4.9\times10^{9\ a,C}$	$2.9\times10^{9\ a,B}$	$1.8\times10^{9\ a,AB}$	$3.8\times10^{8\ ab,A}$	$2.7\times10^{8\ b,A}$
Trial 2	Control	$6.3\times10^{9\ b,B}$	$1.0\times10^{10\ b,C}$	$1.2\times10^{10\ c,D}$	$5.2\times10^{9\ a,B}$	$2.1\times10^{8\ a,A}$	$1.1\times10^{6\ a,A}$
	Ca	$6.3\times10^{9\ b,CD}$	$5.7\times10^{9\ a,BC}$	$8.8\times10^{9\ b,D}$	$1.0\times10^{9\ b,AB}$	$2.9\times10^{9\ b,A}$	$1.4\times10^{7\ b,A}$
	L-NaCn	$4.8\times10^{9\ a,B}$	$1.1\times10^{10\ b,C}$	$1.2\times10^{10\ c,C}$	$1.5\times10^{9\ b,A}$	$5.6\times10^{8\ a,A}$	$3.1\times10^{7\ c,A}$
	H-NaCn	$4.7\times10^{9\ a,B}$	$4.9\times10^{9\ a,B}$	$5.0\times10^{9\ a,B}$	$1.4\times10^{9\ b,A}$	$2.8\times10^{8\ a,A}$	$1.2\times10^{7\ b,A}$
Trial 3	Control	$4.2\times10^{9\ a,B}$	$8.0\times10^{9\ b,C}$	$3.8\times10^{9\ b,B}$	$8.1\times10^{9\ d,C}$	$9.7\times10^{8\ c,AB}$	$9.1\times10^{6\ a,A}$
	Ca	$5.3\times10^{9\ a,C}$	$7.1\times10^{9\ ab,D}$	$3.7\times10^{9\ b,B}$	$2.9\times10^{9\ b,B}$	$7.5\times10^{8\ b,A}$	$2.4\times10^{7\ a,A}$
	L-NaCn	$4.5\times10^{9\ a,B}$	$3.2\times10^{9\ a,B}$	$2.6\times10^{9\ a,AB}$	$3.8\times10^{9\ c,B}$	$2.8\times10^{8\ a,A}$	$1.5\times10^{8\ c,A}$
	H-NaCn	$2.9\times10^{9\ a,BC}$	$3.6\times10^{9\ a,C}$	$2.4\times10^{9\ a,B}$	$2.0\times10^{9\ a,B}$	$2.9\times10^{8\ a,A}$	$8.4\times10^{7\ b,A}$

[a,b,c,d]Means for each trial within the same column not sharing a common superscript differ in treatment (Tukey's HSD, P <0.05)

[A,B,C]Means for each trial within the same row not sharing a common superscript differ in ripening time (Tukey's HSD, P <0.05)

Table 3.5 Numbers of non-starter lactic acid bacteria (NSLAB; cfg g^{-1}) for Trials 1, 2 and 3 of Cheddar cheeses made from milk fortified with 0% NaCn (control), $CaCl_2$ (Ca), 20% increased casein with NaCn and $CaCl_2$ (L-NaCn) or 40% increased casein with NaCn and $CaCl_2$ (H-NaCn) at 1, 25, 40, 77, 111 and 181 d of ripening, respectively. Values correspond to means (n=2).

		Ripening time (d)					
		1	25	40	77	111	181
Trial 1	Control	$0^{a,A}$	$0^{a,A}$	$0^{a,A}$	$1.2\times10^{5\ ab,B}$	$1.2\times10^{5\ a,B}$	$3.2\times10^{5\ a,C}$
	Ca	$0^{a,A}$	$0^{a,A}$	$0^{a,A}$	$1.7\times10^{3\ a,A}$	$1.1\times10^{5\ a,A}$	$1.2\times10^{6\ a,B}$
	L-NaCn	$8.0\times10^{1\ a,A}$	$1.3\times10^{3\ b,A}$	$8.3\times10^{3\ c,A}$	$9.5\times10^{5\ c,B}$	$2.3\times10^{7\ c,B}$	$1.7\times10^{8\ b,C}$
	H-NaCn	$7.5\times10^{1\ a,A}$	$1.0\times10^{3\ ab,A}$	$6.6\times10^{3\ b,A}$	$2.7\times10^{5\ b,A}$	$7.7\times10^{6\ b,A}$	$2.1\times10^{8\ c,B}$
Trial 2	Control	$0^{a,A}$	$0^{a,A}$	$0^{a,A}$	$0^{a,A}$	$9.0\times10^{5\ a,B}$	$2.2\times10^{5\ a,C}$
	Ca	$0^{a,A}$	$0^{a,A}$	$0^{a,A}$	$0^{a,A}$	$4.0\times10^{4\ a,B}$	$1.2\times10^{5a,B}$
	L-NaCn	$0^{a,A}$	$6.5\times10^{1\ b,A}$	$2.0\times10^{2\ b,A}$	$7.1\times10^{3\ b,A}$	$5.8\times10^{5\ b,A}$	$3.1\times10^{7\ c,B}$
	H-NaCn	$0^{a,A}$	$0^{a,A}$	$0^{a,A}$	$6.5\times10^{2\ a,A}$	$4.0\times10^{4\ a,A}$	$4.0\times10^{6\ b,B}$
Trial 3	Control	$0^{a,A}$	$0^{a,A}$	$0^{a,A}$	$0^{a,A}$	$0^{a,A}$	$2.0\times10^{3\ a,B}$
	Ca	$0^{a,A}$	$0^{a,A}$	$0^{a,A}$	$0^{a,A}$	$0^{a,A}$	$1.3\times10^{5\ a,B}$
	L-NaCn	$1.0\times10^{1\ ab,A}$	$1.5\times10^{2\ b,A}$	$5.0\times10^{2\ a,A}$	$4.7\times10^{4\ b,A}$	$4.4\times10^{6\ b,A}$	$1.3\times10^{8\ b,B}$
	H-NaCn	$1.0\times10^{1\ b,A}$	$2.8\times10^{2\ c,A}$	$7.5\times10^{2\ b,A}$	$9.7\times10^{4\ c,A}$	$1.7\times10^{6\ b,A}$	$1.1\times10^{8\ b,B}$

^{a,b,c,d}Means for each trial within the same column not sharing a common superscript differ in treatment (Tukey's HSD, P <0.05)

^{A,B,C}Means for each trial within the same row not sharing a common superscript differ in ripening time (Tukey's HSD, P <0.05)

present. The lower numbers of SLAB counts present in the control and Ca at the end of ripening probably related to the lower NSLAB numbers, as previously discussed LM17 agar enumerates all lactic acid bacteria. It is characteristic for typical Cheddar cheese to contain low numbers of NSLAB at the beginning of ripening (Fox et al., 2000).

3.3.4 Proteolysis

The levels of pH 4.6-SN/TN (%) of Cheddar cheeses made from milk fortified with NaCn are shown in Table 3.3. In Trial 1, H-NaCn cheese had significantly ($P < 0.05$) lower levels of pH 4.6-SN/TN throughout ripening. Ca cheese had the highest level of pH 4.6-SN/TN at d 40 and 111. In Trial 2, H-NaCn was significantly ($P < 0.05$) lower at d 111 of ripening and Ca had the highest level of pH4.6-SN/TN at d 40 and 111. At d 181 of ripening no significant ($P > 0.05$) differences existed between any of the treatments. In Trial 3, Ca had the highest level of pH 4.6-SN/TN throughout the entire ripening period measured. In all trials as ripening time progressed there was a significant ($P < 0.05$) increase in the level of pH 4.6-SN/TN in all cheese treatments. Lower levels of pH 4.6 SN/TN in cheese fortified with NaCn could have been due to the fact that the same level of coagulant was added to all vats but the vats with powder addition had a higher yield (Figure 3.1). This would mean that the enzyme to substrate ratio was lower. Lower levels of proteolysis have been seen previously when concentrated milks have been used for cheese manufacture (Yun et al., 1998; Shakeel-Ur-Rehman et al., 2003; Acharya and Mistry, 2004). Higher moisture in cheese made from milk fortified with NaCn may

have led to an increase in rennet activity (McSweeney, 2007) during ripening (Table 3.2). The fact that there was no significant difference in proteolysis at the end of ripening for these cheeses may be attributed to increased enzyme activity in cheeses made with fortified milk which had higher moisture. The higher level of proteolysis that is typically seen in Ca cheese may be due to the $CaCl_2$ addition causing preacidification of the milk which could lead to higher rennet activity (McSweeney, 2007). $CaCl_2$ addition could also cause solubilisation of colloidal calcium phosphate by reducing the pH of the milk (McSweeney, 2007) which might lower the colloidal calcium in the cheese curd. This may lead to a change in the susceptibility of caseins to proteolysis by residual rennet (Joshi et al., 2003). Reduced-salt Cheddar cheese can lead to acceleration of casein breakdown (Møller et al., 2012) therefore perhaps the higher salt levels observed in H-NaCn cheese (Table 3.2) may have led to reduced proteolysis in this cheese.

3.3.5 Urea-polyacrylamide gel electrophoresis

Electrophoretograms of Cheddar cheese made with milk fortified with NaCn are shown in Figure 3.2. Only slight differences could be seen in the breakdown products between cheese treatments. Ca cheese appears to have lower levels of intact α_{s1}-casein and slightly higher levels of α_{s1}-casein degradation products than the other cheeses. The level of α_{s1}-casein breakdown in cheese is related to the level of residual rennet left in the curd (O'Mahony et al., 2005). The Ca cheese also appears to have slightly higher levels of β-CN (f1-189/192) which is hydrolysed by chymosin and the hydrophobic peptide β-CN (f193-209) which may lead to bitterness in cheese (Bansal et al., 2009).

There appears to be no difference in the level of β-casein hydrolysis to β-CN (f29-209), β-CN (f106-209) and β-CN (f108-209), the generation of these peptides is due principally to the action of plasmin on β-casein (O'Mahony et al., 2005; Bansal et al., 2009). Perhaps the level of plasmin to casein remained constant between cheese treatments even with powder fortification as plasmin is a heat stable enzyme (Fox and McSweeney, 1998) and can survive the powder drying process (Kelly and Fox, 2006).

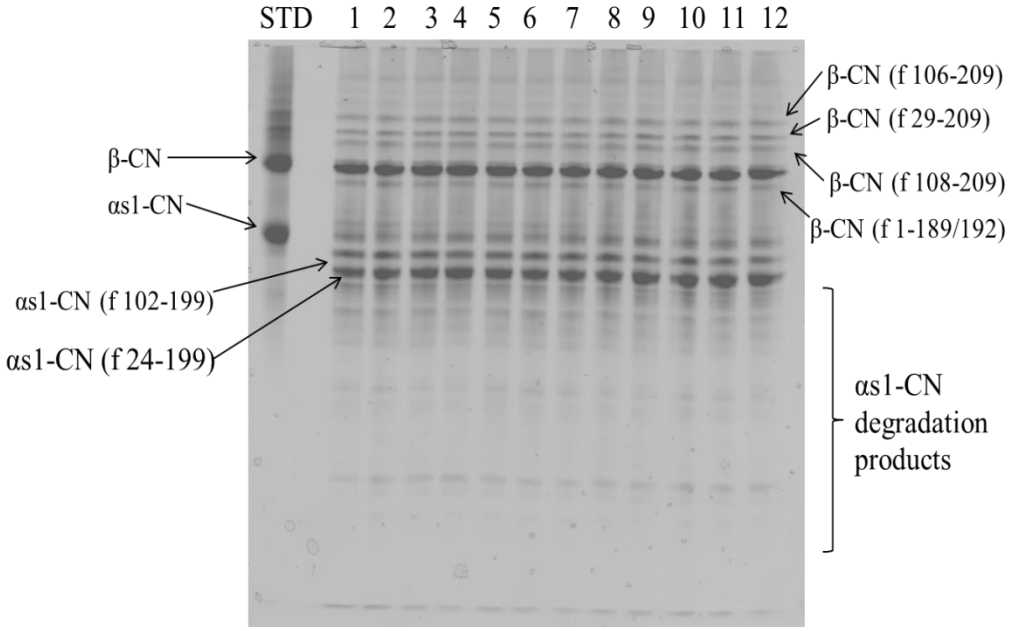


Figure 3.2 Urea-polyacrylamide gel electrophoregrams of sodium caseinate (STD) and the pH 4.6- insoluble fractions of Cheddar cheese made from milk fortified with different levels of skim milk powder at d 40 of ripening. Lanes 1-4, 5-8 and 9-12 represent Trials 1, 2 and 3 respectively. Lanes 1,5 and 9 represent the control ; lanes 2, 6 and 10 represent Ca; lanes 3, 7 and 11 represent L-NaCn and lanes 4, 8 and 12 represent H-NaCn.

As previously discussed the level of pH 4.6-SN/TN was slightly higher in Ca possibly due to the $CaCl_2$ addition leading to milk preacidification and higher rennet activity (McSweeney, 2007). $CaCl_2$ addition could also cause solubilisation of colloidal calcium phosphate (McSweeney, 2007) which may have led to changes in the susceptibility of caseins to proteolysis by residual

rennet (Joshi et al., 2003). The peptide profile of the electrophoretogram (Figure 3.2) confirmed that the increased proteolysis in Ca was due to the action of chymosin in the cheese and not to plasmin activity.

3.3.6 Texture Analysis

The texture results for all trials of cheese made from milk fortified with NaCn are shown in Figure 3.3. In Trial 1 (Figure 3.3a), no significant ($P > 0.05$) differences were observed between treatments on d 7 of ripening. At d 223 of ripening the control and H-NaCn were significantly ($P < 0.05$) harder than Ca and L-NaCn. At all other ripening times measured the control was significantly ($P < 0.05$) harder than all other treatments. In Trial 2 (Figure 3.3b), the control was significantly ($P < 0.05$) harder than L-NaCn. At all other ripening times measured the control was significantly ($P < 0.05$) harder than all other treatments. In Trial 3 (Figure 3.3c), the control cheese was significantly harder than the other treatments at d 7, 111, 181 and 223. At the other ripening times measured in this trial there were no significant ($P >0.05$) differences observed between any of the treatments. All trials had similar trends where the control cheese appeared to have higher hardness values. This could be due in part to the slightly lower moisture content observed in the control cheese compared to L-NaCn and H-NaCn cheese. The lower hardness result is unexpected in L-NaCn and H-NaCn cheese compared to the control as typically in cheese made from concentrated milks are harder due to lower proteolysis and higher calcium (Yun et al., 1998; Dong et al., 2009). The lower hardness in L-NaCn and H-NaCn may be attributed to the higher moisture. Lucey et al. (2003)

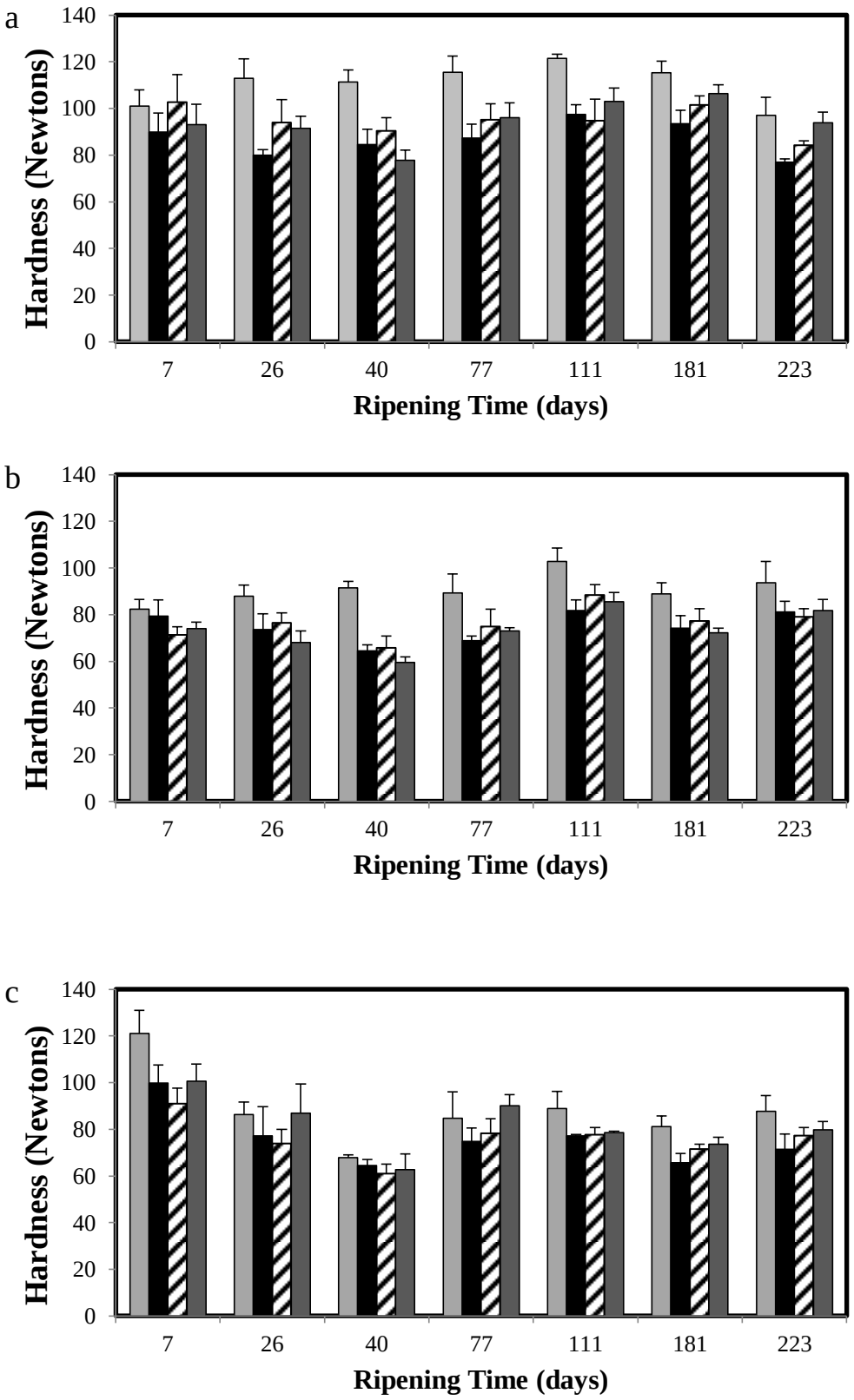


Figure 3.3 Hardness of Cheddar cheeses made from milk fortified with different levels of NaCn. The cheeses were Control (▫), Ca (■), L-NaCn (◪) or H-NaCn (▪) for trial 1 (a), 2 (b) and 3 (c). Error bars indicate ± one standard deviation.

discussed a casein system without colloidal calcium phosphate; decreased calcium associated with casein would form a weaker structure. As previously discussed, casein from sodium caseinate has lost much of its colloidal calcium which may lead to the decreased hardness in these cheeses when compared to the control. Ca also had lower hardness when compared to the control cheese this could be due to the increased level of pH 4.6 SN/TN in this cheese (Table 3.2) which would lead to a softer structure. In contrast, Brickley et al. (2009) found that addition of $CaCl_2$ to Cheddar style cheeses at salting led to a harder cheese which was attributed to increased number of colloidal calcium phosphate crosslinks in the cheese. The differences between these results and the current study may be due to the method of addition of $CaCl_2$ in combination with no observed increase in proteolysis when $CaCl_2$ was added at salting. Ong et al. (2013) also found increased hardness in Cheddar cheese with addition of greater than 50 mg/L of $CaCl_2$ to cheesemilk but the level of proteolysis in this study was not tested.

3.3.7 Meltability

The meltability of cheeses fortified with NaCn is shown in Table 3.6 for Trial 1, 2 and 3. In Trial 1, from d 7-77, H-NaCn and L-NaCn tended to have lower meltability compared to the control and Ca. At d 111, Ca and H-NaCn had the lowest meltability of all treatments. There were no significant ($P > 0.05$) differences between treatments after d 111 of ripening. In Trial 2, no significant ($P > 0.05$) differences in meltability existed between treatments up to d 40 of ripening even though L-NaCn and H-NaCn appeared to be slightly

lower. At d 77 of ripening, H-NaCn cheese had significantly ($P > 0.05$) higher meltability than all other treatments. There was no significant difference between treatments after d 77 of ripening. In Trial 3, there tended to be no difference in meltability up to d 40 of ripening between treatments. At d 77 up until d 181 of ripening, H-NaCn had the lowest melt and L-NaCn had the highest melt of all of the treatments. At d 223 of ripening H-NaCn was significantly ($P < 0.05$) higher than all other treatments.

In general the control cheese either had higher or no difference in meltability compared to the other cheese treatments. The cheeses fortified with NaCn and $CaCl_2$ tended to have lower meltability throughout the start of ripening compared to the control but by the end of ripening these cheeses had similar melt compared to the control. All trials and treatments increased significantly ($P < 0.05$) in meltability during ripening which is a typical attribute of Cheddar cheese (Acharya and Mistry, 2004).

It has been seen previously that cheeses made from concentrated milk systems have reduced meltability (Acharya and Mistry, 2004). Solubilisation of colloidal calcium phosphate and proteolysis lead to increased melt in cheese during ripening (Lucey et al., 2003). Lower meltability initially during ripening can be related to lower moisture in cheese (Govindasamy-Lucey et al., 2005). However, the cheeses fortified with NaCn had higher moisture content (Table 3.2) and reduced meltability was observed at the beginning of ripening compared to the control cheese. Yun et al. (1998) found that Mozzarella cheese fortified with 3% skim milk powder was slightly firmer and had slightly reduced meltability due to lower moisture, decreased proteolysis and higher calcium. In general, H-NaCn cheese had lower levels of proteolysis during

ripening compared to the other treatments which may have contributed to the reduced meltability at the beginning of ripening. Acharya and Mistry (2004) found that when Cheddar cheese was made using either vacuum-condensed or ultrafiltered milk the control had the highest meltability which was attributed to higher calcium, lower moisture and lower fat:protein ratio in the vacuum-condensed and ultrafiltered treated cheeses. Interestingly, condensed milk had lower meltability compared to ultrafiltered milk even though it had higher levels of proteolysis, moisture, higher fat:protein ratio and lower calcium this was attributed to possible higher colloidal calcium. It is surprising that the control had a harder texture (Figure 3.3) and also had similar or higher meltability than the cheeses fortified with NaCn and $CaCl_2$. Chevanan et al. (2006) found good correlations and an inverse relationship between meltability and TPA hardness in Cheddar cheese where lower hardness corresponded to higher melt. The cheeses fortified with NaCn and $CaCl_2$ were softer than the control probably due to the lower moisture in the control; it would also be expected that the control also melt less due to lower moisture content (Acharya and Mistry, 2004). Addition of $CaCl_2$ to milk can lead to an increase in ionic calcium, colloidal calcium and attractive forces between *para*-casein molecules (Law and Tamime, 2010). When attractive forces between *para*-casein molecules increase, meltability decreases (Lucey et al., 2003). Possibly when cheese is heated, heat-induced precipitation of soluble calcium can occur leading to increased cross-linking of caseins, which may lead to reduced melt (Udayarajan et al., 2005; Brickley et al., 2009). The fact that no differences in melt were observed between any of the cheeses as ripening progressed was

probably due to chemical changes in the cheese such as proteolysis and solubilisation of colloidal calcium.

Table 3.6 Melt diameter (mm) for Trials 1, 2 and 3 of Cheddar cheeses made from milk fortified with 0% NaCn (control), $CaCl_2$ (Ca), 20% increased casein with NaCn and $CaCl_2$ (L-NaCn) or 40% increased casein with NaCn and $CaCl_2$ (H-NaCn) at 7, 26, 40, 77, 111, 181 and 223 d of ripening, respectively. Values are means of three replicates and standard deviations with the latter in parentheses.

		Ripening Time (days)						
		7	26	40	77	111	181	223
Trial 1	Control	$59.66^{b,A}$ (1.24)	$60.44^{b,A}$ (0.79)	$65.06^{b,AB}$ (2.89)	$64.53^{b,AB}$ (3.59)	$69.15^{b,BC}$ (2.55)	$71.67^{a,C}$ (0.24)	$72.29^{a,C}$ (1.67)
	Ca	$57.07^{ab,A}$ (2.89)	$58.49^{ab,AB}$ (2.08)	$63.90^{b,B}$ (1.54)	$59.95^{ab,AB}$ (1.47)	$63.57^{a,B}$ (1.52)	$71.10^{a,C}$ (1.34)	$74.69^{a,C}$ (2.39)
	L-NaCn	$57.35^{ab,A}$ (1.52)	$56.98^{ab,A}$ (0.24)	$57.88^{a,A}$ (1.82)	$57.66^{a,A}$ (0.61)	$68.44^{b,B}$ (2.02)	$70.71^{a,B}$ (4.37)	$73.96^{a,B}$ (1.72)
	H-NaCn	$54.97^{a,A}$ (0.76)	$55.58^{a,A}$ (1.95)	$56.41^{a,AB}$ (1.99)	$56.31^{a,AB}$ (2.54)	$61.81^{a,B}$ (0.58)	$70.55^{a,C}$ (4.32)	$70.61^{a,C}$ (0.69)
Trial 2	Control	$59.18^{a,A}$ (1.77)	$63.97^{a,AB}$ (3.04)	$63.73^{a,AB}$ (3.40)	$69.31^{bc,BC}$ (3.29)	$72.47^{a,BC}$ (2.48)	$74.30^{a,C}$ (2.50)	$74.25^{a,C}$ (4.87)
	Ca	$60.38^{a,A}$ (1.00)	$58.62^{a,AB}$ (2.18)	$62.08^{a,AB}$ (3.70)	$62.04^{a,AB}$ (0.66)	$68.70^{a,B}$ (4.41)	$69.11^{a,B}$ (2.05)	$76.61^{a,C}$ (1.53)
	L-NaCn	$60.48^{a,A}$ (2.42)	$58.61^{a,A}$ (2.99)	$60.33^{a,A}$ (2.65)	$64.11^{ab,AB}$ (1.66)	$71.77^{a,BC}$ (3.72)	$75.82^{a,C}$ (4.07)	$77.64^{a,C}$ (2.63)
	H-NaCn	$57.97^{a,A}$ (1.36)	$58.98^{a,A}$ (0.33)	$58.96^{a,A}$ (2.00)	$71.32^{c,B}$ (3.23)	$71.94^{a,B}$ (5.10)	$74.12^{a,B}$ (4.56)	$75.23^{a,B}$ (3.78)
Trial 3	Control	$56.24^{ab,A}$ (1.80)	$57.45^{a,A}$ (0.64)	$58.26^{a,A}$ (1.57)	$60.64^{ab,AB}$ (1.66)	$64.07^{bc,BC}$ (1.99)	$68.29^{bc,CD}$ (1.69)	$69.83^{b,C}$ (1.60)
	Ca	$54.57^{a,A}$ (1.91)	$57.32^{a,B}$ (1.93)	$57.90^{a,AB}$ (1.32)	$60.48^{ab,B}$ (1.19)	$60.35^{ab,B}$ (2.77)	$65.87^{b,C}$ (1.54)	$70.76^{b,C}$ (1.37)
	L-NaCn	$59.11^{b,AB}$ (0.48)	$55.95^{a,A}$ (1.54)	$58.22^{a,AB}$ (2.59)	$62.69^{b,BC}$ (2.00)	$66.06^{c,C}$ (0.61)	$72.10^{c,D}$ (1.95)	$70.89^{b,D}$ (1.65)
	H-NaCn	$56.40^{ab,AB}$ (1.74)	$56.78^{a,AB}$ (1.09)	$54.17^{a,A}$ (1.49)	$57.53^{a,AB}$ (1.00)	$55.88^{a,A}$ (1.89)	$59.96^{a,B}$ (1.31)	$74.49^{a,C}$ (0.39)

^{a,b,c,d}Means for each trial within the same column not sharing a common superscript differ in treatment (Tukey's HSD, $P < 0.05$)
^{A,B,C}Means for each trial within the same row not sharing a common superscript differ in ripening time (Tukey's HSD, $P < 0.05$)

3.4 Conclusions

Using NaCn for the fortification of cheesemilk for Cheddar cheese manufacture impaired the rennet coagulation properties of the milk; $CaCl_2$ had to be added to form sufficient curd firmness during coagulation. NaCn fortification significantly ($P < 0.05$) increased moisture-adjusted cheese yield. Using NaCn to fortify cheesemilk led to changes in the composition of the cheese. Cheeses fortified with NaCn had lower fat, higher moisture and higher salt. Higher numbers of NSLAB were observed in the fortified cheese, possibly due to the protective effect that higher solids milk has on bacteria during pasteurisation. Ca cheese had the highest and H-NaCn cheese tended to have slightly lower levels of proteolysis of all the cheese treatments. The cheeses fortified with NaCn and $CaCl_2$ tended to be softer but the melt was generally lower or similar to the control cheese.

The results of this study indicate that the levels of NaCn and $CaCl_2$ used were not ideal when manufacturing typical Cheddar cheese due to the changes that occurred in composition and chemical changes during ripening. The cheese may be of benefit when used in ingredient cheese type applications due to the changes in functionality and yield of the cheeses. Some consideration needs to be taken into account when using powders for cheesemilk fortification such as the pasteurisation temperature of higher total solids milk and methods to try to reduce the cheese moisture such as a higher cook temperature. Using powder as a method for fortification to increase protein could offer a potential benefit for seasonality issues in milk such as low total solids. Fortification of cheesemilk could also offer the advantage of increasing yield.

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CHAPTER 4

Impact of Lactose Standardisation of Cheesemilk using Ultrafiltration on the Texture, Functionality and Sensory Properties of Low Moisture Part Skim Mozzarella Cheese

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Abstract

Loss of colloidal calcium phosphate through acidification of milk and curd, the extent of proteolysis and the lowest pH obtained in the cheese are recognised as critical parameters influencing the texture, functionality and quality of *pasta filata* Mozzarella cheese. In addition, the colour of the blisters that form during baking on pizza is greatly determined by the amount of residual galactose in the cheese. The objective of this study was to explore an approach to control the amount of residual sugars and pH of the cheese by standardising the ratio of lactose to casein in the milk prior to cheese making. This approach involved the use of both ultrafiltration to remove lactose and water addition to dilute further the remaining lactose. Mozzarella cheese was made from standardised milk (2.5% casein, casein:fat 1.0:1) ultrafiltered to give different lactose:casein ratios: high or control (1.8:1, HL), medium (1.3:1, ML) and low (1.0:1, LL) lactose:casein ratio. The functional and sensory properties of Mozzarella manufactured from these milks were investigated. As expected, LL cheeses had higher pH than ML and HL cheese throughout ripening. LL and ML cheeses also had lower levels of lactose, galactose, lactic acid and insoluble calcium compared to HL cheese. Varying the lactose level had no effect on the levels of proteolysis in the cheeses. Decreasing the lactose also affected the TPA and sensory hardness of the cheeses; LL and ML cheeses were harder than HL cheese during ripening. The maximum loss tangent (LT_{max}) which is an index of cheese melt was lower for LL cheese up until d 28 of ripening; however, after d 28 all treatments exhibited similar meltability. The temperature where the LT=1, which is an index of the softening point, was higher for ML and LL cheese than HL cheese at d 56 and 84 of ripening. LL

cheese also had lower blister colour and stretch than ML and HL cheeses. In conclusion, standardisation of the lactose:casein ratio of cheesemilk for the manufacture of Mozzarella cheese can be a useful technique to prevent excessive acidification and to lower the residual sugar levels in cheese both of which in turn can reduce variability in texture, functionality and sensory properties of Mozzarella cheese.

4.1 Introduction

Lactose is converted to lactic acid by starter bacteria during cheesemaking which results in acidification. The extent of acidification at key processing steps such as at cutting of the coagulum and the lowest pH the cheese obtains are critical parameters involved in creating the texture of the cheese network, and directly influences product quality (Lawrence et al., 1984, 1987; Lucey and Fox, 1993). The rate and extent of acid development influences a wide range of important properties such as moisture content, solubilisation of calcium phosphate, rate of proteolysis during ripening, textural and functional attributes (Johnson and Lucey, 2006). Cheesemakers have developed many different strategies to modify the rate of acidification, including using different types and amounts of starter cultures and temperatures to modify fermentation activity. However, controlling the pH of the finished cheese can be difficult or problematic in *pasta filata* cheeses. The curd must reach a certain pH to be sufficiently pliable prior to the mixing/moulding operation. The potential problem is that acidification of cultured Mozzarella is often very rapid (short make times) which makes it difficult to run all the cheese through the mixer/moulder at the ideal pH. For example, some curd will be run through the mixer/moulder at 5.3 while the final curd maybe run through at pH 5.0. The functional and bake characteristics will be different between the cheeses.

The majority of lactose is lost in the whey but some residual lactose can remain in the curd (Singh et al., 2003). The possibility of a further decrease in cheese pH after salting is dependent upon the residual lactose and this can affect cheese quality (Lawrence et al., 1984). Excessive fermentation of

residual lactose after the end of the manufacturing stage of cheesemaking increases the risk of producing an acidic cheese. The fermentation of residual lactose in cheese is affected by its salt-in-moisture level; a low salt-in-moisture or the use of salt-tolerant starter cultures results in the breakdown of lactose to lactic acid during cheese maturation (Turner and Thomas, 1980; Upreti and Metzer, 2007).

When starter cultures metabolise lactose to produce acid during cheese manufacture, the reduction in pH solubilises insoluble calcium phosphate from the casein micelle; without acid production insoluble calcium phosphate nanoclusters would remain intact and facilitate crosslinking between proteins which would affect cheese functionality (Johnson and Lucey, 2006). The lactose content of milk can vary depending on stage of lactation (Lawerence et al., 1984; O'Brien et al., 1999). Shakeel-Ur-Rehman et al. (2004) suggested that pH is inversely related to the metabolism of lactose during Cheddar cheese ripening; this was due to high residual lactose cheese leading to decreased cheese pH and low residual lactose cheese leading to increased cheese pH during ripening. If cheese pH drops too low (<5.0) cheese can have limited meltability (Lee et al., 2005). High residual lactose in Cheddar leading to low pH was found to give unclean flavours and a coarse body at the end of maturation; the poor sensory score for flavour and body was attributed to low pH and high acidity in the cheese (Shakeel-Ur-Rehman et al., 2004). Cheese can also have a short and brittle texture at low pH which could affect cheese slicability (Lawrence et al., 1987; Lee et al., 2005, 2010).

Reducing sugars are involved in heat induced Maillard browning reaction during cooking of Mozzarella cheese. These reducing sugars can come from

unfermented residual lactose and/or galactose; their levels in cheese can vary depending on the type of starter culture used along with the extent to which the bacteria survive the heat treatment during cooking and stretching (Kindstedt, 2007). Johnson and Olson (1985) suggested means to control lactose levels in curd for Mozzarella manufacture which included curd washing, drainage of curd at high pH or by using *Lactobacillus helveticus* as a component of the starter.

Curd washing is used to control lactose, and hence pH, in many cheese varieties. Curd washing is the process of removing some of the whey during cheese manufacture and replacing it with water, thus reducing the level of lactose in cheese (Guinee and O'Callaghan, 2010; Hou et al., 2014a). Osaili et al. (2010) used curd washing in low-moisture Mozzarella cheese to reduce lactose in the cheese. Increasing the level of curd washing produced cheese with increased moisture, decreased ash, lower galactose, lower proteolysis, decreased meltability and stretch. Lee et al. (2011) found that different washing methods had an impact on the rheological properties of Colby cheese by altering the residual lactose, lactic acid and insoluble calcium levels. Hou et al. (2014a) used curd washing in Cheddar cheese to reduce the lactose levels and found that curd-washed cheese had higher pH and was characterized as harder, less brittle and had different sensory attributes compared to the control cheese. Curd washing can lead to additional issues and problems for industry due to dilution of whey or an additional waste stream for wash water.

Insoluble and soluble calcium phosphate contributes strongly to the buffering capacity of milk (Lucey et al., 1993). The level of calcium and the form that it is present in cheese (soluble or insoluble) plays an important role in the

functionality of cheese (Lucey et al., 2003). In Mozzarella cheese, when insoluble calcium is solubilized there is less crosslinking between proteins which will weaken the structure and increase cheese meltability (Joshi et al., 2003). Upreti and Metzger (2007) found that Cheddar cheese with a low salt-in-moisture and high residual lactose had increased water-soluble calcium. McMahon et al. (2005) found that calcium affected the melt of directly acidified nonfat Mozzarella. It has also been found that the solubilisation of insoluble calcium from the casein micelle is responsible primarily for initial changes in texture of young Cheddar cheese (O'Mahony et al., 2005).

Ultrafiltration is a separation process based on semipermeable membranes, often with a 10,000 Da molecular weight cut off. This is a membrane process that is porous to low molecular weight compounds such as water, lactose and salts which is called permeate. The membrane then retains an enriched high molecular weight portion of fat and protein called retentate (Rattray and Jelen, 1996; Kumar et al., 2013; Marella et al., 2013). During ultrafiltration, partial elimination of soluble compounds occurs but insoluble materials are retained. Ultrafiltration leads to retentate with increased buffering capacity proportional to increased protein content (Salaun et al., 2005).

The objective of this study was to use ultrafiltration to lower the lactose content of milk and thus standardise the milk to different lactose:casein ratios while keeping the casein content constant between treatments for the manufacture of Mozzarella cheese. To create milks with sufficiently low lactose:casein ratios a procedure was employed involving water addition to ultrafiltration retentates. This was performed to determine the ability of reduced lactose milk to control the pH and acidity of Mozzarella. LMPS

Mozzarella was selected as the cheese variety for this study since pH value has a major impact on its functional properties (Guinee et al., 2002; McMahon et al., 2005) and the pH of the cheese often varies considerably based on when the curd is processed through the mixer/moulder. Standardising the lactose of milk using ultrafiltration may have benefits over other traditional methods such as curd washing due to reduced whey dilution or additional waste streams. It may also benefit industry by ensuring pH does not drop too low in the event of delays or problems in the plant. However, ultrafiltration can also remove soluble components such as calcium from milk. Taking into account the importance of pH and calcium in cheese rheology, the impact of lactose:casein ratio on the texture, functionality and sensory properties of LMPS Mozzarella cheese was evaluated.

4.2 Materials and Methods

4.2.1 Ultrafiltration and standardisation of milk

Part skim milk and cream from the University of Wisconsin-Madison Dairy Plant was obtained on the day before cheese manufacture. Ultrafiltration was carried out using 1,500 kg of part-skim milk at ~4°C by recirculating the milk to the feed tank until the desired composition was obtained. The total solids was determined using using Atago refractometers (model-10M and model-20M, Atago Ltd., Tokyo, Japan) and volume reduction. The ultrafiltration process was stopped when the retentate and permeate had reached total solids levels of approximately 11.5% and 5%, respectively. This retentate was named UF retentate 1 (Table 4.1) and part of this was transferred to another tank to be used to standardise cheesemilks. Ultrafiltration of the remaining retentate was continued until a total solids of 14% was reached, this retentate was called UF retentate 2 (Table 4.1).

Three different cheesemilks were standardised by blending of appropriate ingredients to obtain the desired lactose:casein ratios: a control with the highest ratio of 1.8 (HL), a medium ratio of 1.3 (ML) and a low ratio of 1.0 (LL). The HL cheesemilk was standardised by cream removal/addition to obtain a lactose:casein ratio of 1.8 (2.5% casein) and casein:fat ratio of 1.0. The ML cheesemilk was standardised by blending UF retentate 2, cream and permeate to obtain a lactose to casein ratio of 1.3 (2.5% casein) and casein:fat of 1.0. The LL cheesemilk was standardised by blending UF retentate 1, cream and permeate to obtain a lactose:casein of 1.0 (2.5% casein) and casein:fat 1.0. A typical blend for each lactose:casein ratio is listed in Table 4.1. When

necessary, water purified by reverse osmosis (RO) was added to milk after ultrafiltration (Table 4.1).

4.2.2 Rheological analysis of cheesemilk during rennet coagulation

Preliminary cheesemaking trials demonstrated that ML and LL milk samples did not form sufficiently strong gels within an hour after rennet addition which was probably due to the addition of RO water to the milks resulting in decreased calcium addition. RO water was added to the milk during standardisation, after ultrafiltration. Before starting experimental cheese trials rheological analysis of rennet gels was undertaken to achieve similar gelation profiles for each cheesemilk; temperature and $CaCl_2$ addition were varied to obtain a similar G' > 1 (onset of gelation).

The rheological properties of each treatment HL, ML and LL was determined using dynamic low amplitude oscillatory rheometry as described previously by Govindasamy-Lucey et al. (2005). A rheometer (MRC 301, Anton Paar GmbH, Austria) was used to determine the rheological properties of the gels during renneting using an oscillation test with a strain of 1% and a frequency of 0.1 Hz. The measurement geometry used was a concentric cylinder (CC27/T200/SS). Milk was held at the appropriate temperature for 30 min in a water-bath before rennet addition. Fermentation-produced calf chymosin (CHY-MAX Extra, 630 international milk clotting units/ mL; Chr. Hansen Inc., Milwaukee, WI, US) was diluted 1:10 with water and added at a level of 0.05 IMCU/mL of milk. The renneted milk was placed in the cup and the test

Table 4.1 Average composition of skim milk, cream, ultrafiltered milk retentates and permeate used to prepare control (HL), medium (ML) and low (LL) lactose standardised milk treatments. Values represent the means and standard deviations, with the latter in parentheses (n=3).

%		Part skim milk	Cream	UF milk retentate 1	UF milk retentate 2	Permeate	RO Water
Composition	Fat	2.33 (0.24)	29.36 (2.36)	2.61 (0.43)	3.08 (0.43)	0.01 (0.02)	-
	Solids	11.10 (0.42)	35.66 (1.99)	11.09 (0.74)	13.96 (0.39)	5.13 (0.27)	-
	Total Protein	3.23 (0.03)	2.21 (0.19)	4.13 (0.09)	4.90 (0.12)	0.19 (0.05)	-
	Casein	2.44 (0.03)	1.62 (0.17)	3.19 (0.06)	3.64 (0.07)	0.04 (0.04)	-
	Lactose	4.44 (0.07)	3.08 (0.10)	3.24 (0.11)	4.57 (0.04)	4.23 (0.09)	-
Weight	HLC	99.50	0.50	-	-	-	-
	MLC	-	-	-	67.30	8.40	22.90
	LLC	-	-	61.00	-	2.70	34.70

was started 2 min after rennet addition. Readings were taken at one minute intervals for 43 min. The storage modulus (G') was measured.

4.2.3 Cheese manufacture

Four replicates of low moisture, part skim (LMPS) Mozzarella were made with high lactose (HL), medium lactose (ML) or low lactose (LL) milk in the dairy plant at the University of Wisconsin-Madison. The milks were standardised as discussed above (lactose:casein, % casein, casein:fat). Each vat contained 272 kg milk which was pasteurised at 72°C for 19 s and cooled to the appropriate setting temperature for each treatment as determined by gelation experiments; HL, ML and LL were cooled to 31°C, 32°C and 34°C, respectively. Thermophilic culture containing *S. thermophilus* and *Lb. helveticus* (TEMPO 303; Cargill Texturizing Solutions, Waukesha, WI, US) was added to each vat at a rate of 8.46 g/100 kg of milk. 0.01% (w/w) $CaCl_2$ was added to all vats. ML and LL cheese milks were given slightly longer starter ripening times (55-60 min) to compensate for the higher pH of these milks compared to HL milk (45 min). This was to try to maintain a constant pH at rennet addition (~pH 6.70). After ripening fermentation-produced calf chymosin (CHY-MAX ™ Extra, 630 international milk clotting units (IMCU)/mL Chr. Hansen Inc., Milwaukee, WI, US) was added to each vat at a level of 0.05 IMCU/mL of milk. The coagulum of each vat was cut based on a prescribed time to keep a constant inoculation to cut time for each vat, firmness at cutting was not measured; HL was cut at 45 min, ML was cut at 30 min and LL was cut at 35 min. All coagula were cut with 1.9 cm knives and the pH at cutting was constant for all treatments at 6.6. The temperature of the vats was raised to

41°C over a 30 min period. Each vat was cooked at 41°C until the pH reached 5.90, agitation was then stopped, the curd was trenched and the whey drained. The curd was allowed to mat and was cheddared. At pH 5.2, all cheeses were milled and salted at a level of 2.8% (w/w) and stretched in a cooker (Supreme Filata Mixer, Stainless Steel Fabricating Inc., WI, US). After stretching curd was formed into 2.3 kg blocks and kept in ice cold water for 30 min and then brined for 120 min in a saturated salt brine bath. The brine-salted cheese was vacuum-packed and stored at 4°C for 84 d. Analysis was carried out on individual blocks of cheese at d 1, 14, 28, 42, 56 and 84.

4.2.4 Cheese composition, pH and insoluble calcium

All compositional analysis was carried out in triplicate. Milk samples were analysed for fat by Mojonnier (AOAC, 2000), protein (total percentage N x 6.35) by Kjeldahl (AOAC, 2000), casein (AOAC, 2000), lactose (AOAC, 2000), total solids (Green and Park, 1980), and non-protein nitrogen (AOAC, 2000). Buffering was determined by the acid-base titration method (Lucey et al., 1993).

Cheese composition was measured at d 14. Cheese was analysed for moisture (Marshall, 1992), fat by Mojonnier (AOAC, 2000), protein by Kjeldahl (AOAC, 2000) and salt by chloride electrode method (MK II Chloride analyser 926; Nelson and Jameson Inc., Marshfield, WI, US; Johnson and Olson, 1985). Total calcium levels were measured in milk, rennet whey and cheese (d 14) using inductively coupled plasma emission spectroscopy (Park, 2000). Cheese pH was measured at d 1, 14, 28, 42, 56 and 84 using a spear tip pH probe (accuCap Capillary Junction pH combination electrode 13-620-133; Fischer

Scientific, Itasca, IL, US) inserted directly into the cheese. Lactose and galactose were determined at d 42 and lactic acid at d 1, 14 and 42 by HPLC (Zeppa et al., 2001). Acid-base titration (Hassan et al., 2004) was performed to measure the insoluble calcium content of cheese at d 1, 14 and 28.

4.2.5 Proteolysis

Proteolysis was determined in triplicate by measuring levels of pH 4.6-soluble nitrogen (Kuchroo and Fox, 1982) and the nitrogen content determined by Kjeldahl (AOAC, 2000). Proteolysis was measured at d 1, 28 and 56 of ripening.

4.2.6 Texture profile analysis

Cheese was cut into cylindrical samples (16 mm diameter, 17.5 mm height) using a Hobart slicer and steel cork borer. Samples were stored overnight at 4°C until compression testing by texture profile analysis (TPA) was performed at 4°C using a Texture Analyzer TA-XT2 (Stable Micro Systems, Godalming, Surrey, UK). TPA was performed by compressing samples to 80% of their original height; chewiness and hardness were calculated as described by Bourne (1978).

4.2.7 Dynamic small-amplitude oscillatory rheology

Cheese samples were sliced on a Hobart slicer to ~2.3 mm and cut into 50 mm diameter discs. These samples were stored in a refrigerator at 4°C at least 8 h before analysis. Rheological properties of the cheese were assessed with a Paar

Physica Universal Dynamic Spectrometer (UDS 200; Physica Messtechnik, Stuttgart, Germany). Samples were heated from 5-85°C at 1°C min^{-1} on a 50 mm serrated parallel plate and subjected to a strain of 0.5% at a frequency of 0.08 Hz. During heating the parameters measured were G', loss modulus (G") and loss tanget (LT). The maximum LT (LT_{max}), the temperature where the LT_{max} occurred and the temperature where the LT equals to 1 (LT=1) was also measured.

4.2.8 Descriptive sensory analysis

A trained (20 h training) sensory panel consisting of at least 12 panellists used a mixture of sensory Spectrum and Quantitative Descriptive Analysis (Meilgaard et al., 1999) to evaluate the textural and flavour properties of both the unmelted and melted cheese as described by Chen et al. (2009) (Table 4.2). The numerical intensity scale ranged from 0-15 with reference points. Each cheese was designated with a random 3-digit code and assessed in duplicate on 2 separate days. Cheese cubes were tempered at ~12°C before assessment for texture and flavour attributes (acidity, saltiness and butteriness) (Table 4.2). Textural attributes evaluated were firmness and adhesiveness of mass of the cubes (Table 4.2).

Cheeses were mechanically shredded using a food processor (Cuisinart Prep 11 Plus, Madison, WI). A 30.5-cm frozen pizza crust (Arrezzio Thin & Crisp Par-Baked, Sysco Food Services, Baraboo, WI) was thawed, 30 g of tomato pizza sauce (Contadina Roma-style tomatoes pizza sauce, Del Monte Foods Inc., Hanford, CA) was spread over the crust. Approximately 300 g of shredded cheese was added to the crust, which was then baked in a forced-air

commercial oven (Impinger™ Ovens, Lincoln Foodservice Products Inc., Ford Wayne, IN) at 260°C for 5 min. The surface characteristics that were evaluated included free oil release, blister colour, blister quantity and skinning. Stretch characteristics of the cheeses were evaluated by determining the strand length and thickness of the stretched cheese (Table 4.2). Textural properties (i.e., cohesiveness of mass, chewiness and hardness) of the melted cheese were evaluated after cooling to 63°C. Photographs of cheeses showing a range of blister colour, blister quantity and stretch characteristics were used as numerical reference points and were always available to the panellists. Flavour attributes (acid and salt intensities) of melted cheeses were also assessed at 62.8°C.

4.2.9 Experimental design and statistical analysis

Three milk samples each with a different lactose:casein ratio designated (HL, ML and LL) were used to make LMPS Mozzarella on a single day of cheese making and replicated four times. A completely randomized unblocked design was used for analysis of the response variables relating to milk and cheese composition. Analysis of variance was carried out using the PROC GLM procedure of SAS (version 9.1; SAS Institute, 2002-2003). Duncan's multiple-comparison test was used to evaluate differences in the treatments at a significance level of $P < 0.05$ for milk and cheese composition.

The effects of treatment (HL, ML and LL) and ripening time and their interactions on pH, insoluble calcium, proteolysis, functional, textural and sensory properties were evaluated using the MIXED procedure for repeated measurement of the SAS software package (SAS Inst. Inc., Cary, NC, US).

The mean square for cheese, nested within treatment was used as random error term to test treatment.

Table 4.2 Definitions of the attributes used by the trained panelists to evaluate the flavor and texture of the unmelted and melted LMPS Mozzarella cheeses using a combination of Spectrum™ and Quantitative descriptive analysis.

Method of analysis/attribute	Definition/Evaluation procedure	References used/Preparation Instructions/Anchor Points (0-15)
Unmelted Cheese		
Hand firmness	Force to required to compress the cheese between finger and thumb. Place the cheese cube between thumb and fore finger. Compress cheese cube, do not fracture.	Green-colored Thera-Putty (#5075, Sammon Preston) = 4.5 Blue-colored Thera-Putty (#5077, Sammon Preston) = 7.0 Flesh-colored Thera-Putty (Graham-Field, Inc.) = 9.5 Gray Eraser (Primacolor Kneaded Rubber) = 12.0 White Eraser (School Select White) = 15.0
Chewdown: Adhesiveness of Mass	Degree to which mass sticks to the roof of the mouth or teeth. Chew cheese sample between molars 12-15 times. Evaluate cheese adhesive properties.	Polenta (Food Merchants Brand) = 0.0 Quince-paste (La Costena Brand) = 2.5 Rice, converted (Minute Rice Brand) = 3.5 Mashed Potatoes (Hungry Jack Brand) = 7.5, Prepared by boiling 2/3 cup water, ¼ milk, 1 tablespoon butter. Removed from heat, 1 cup of dried potato flakes was added. Brownies (Betty Crocker Dark Chocolate Fudge Brownie Mix; baked using the recipe on the box) = 10.0 American Pasteurized Process Cheese Food, Singles (Kraft Foods) = 14.0

Table 4.2 ***Continued***

Melted Cheese **Surface Characteristics[1] (evaluated at 96°C)**		
Free oil release	The amount of free oil on the surface of the melted cheese.	None to Extreme
Blister Color	The brown color intensity of the blisters.	No brown color to All dark brown color
Blister Quantity	The amount of blisters on the melted surface of the pizza pie.	None – Complete coverage
Skinning	The thickness and toughness of the surface of the melted cheese.	None to Extreme
Stretch Characteristics[1] (evaluated at 91°C)		
Stretch- Strand Length	Stretch the cheese. Insert 1 tine of fork 1 cm into melted cheese. Pull cheese at a controlled constant rate. Measure the height the cheese is stretched to	Height of the stretch was measured in inches
Stretch – Strand Thickness	The thickness of the melted cheese strand. Insert 1 tine of fork 1 cm into melted cheese. Pull up at a controlled constant rate to 6 inches. Stop pulling strand. Observe the melted cheese strand thickness at 3 inches. If strand does not reach 6" – please write down response as NA (Not Applicable).	Reference images used

Table 4.2 ***Continued***

Texture (evaluated at 63°C after heating step)		
Hardness (First Chew)	Force required to bite through the sample with molars. Fold the cheese into 1/4 with inside out, bite with molars.	Philadelphia Full-fat Cream cheese (Kraft Foods) = 0.5 Spam (Hormel Brand) = 2.0 Beef Frankfurters (Best's Kosher Brand) = 5.0 Chewy caramel (Kraft Classic CARAMELS Traditional) = 7.0 Almond (Blue Diamond Brand) = 12.0 Licorice (Starburst Brand) = 15.0
Chewiness (Chewdown characteristics)	The length of time required to masticate the sample to a state pending swallowing. The longer the time required, the chewier the sample is.	Pound Cake (Sara Lee All Butter Pound Cake) = 1.0 Beef Frankfurters (Best's Kosher) = 4.0 Fig Newtons (Nabisco Brand, Kraft Foods) = 7.0 White bread (Wonder Brand) = 9.0 Chewy caramel (Kraft Classic CARAMELS Traditional) = 12.0 Chewing gum (Wrigley's Doublemint) = 15.0
Cohesiveness of Mass (Chewdown characteristics)	Degree to which sample holds together in a mass. Put cheese sample between molars and chew 15 times. Gather to the middle of mouth, evaluate cohesiveness of mass.	Polenta (Food Merchants Brand) = 0.0 Carrots (Metcalfe's Sentry Foods) = 1.0 Beef Frankfurter (Best's Kosher Brand) = 4.5 Wheaties toasted whole wheat flakes (General Mills) = 7.5 Fig Newtons (Nabisco Brand, Kraft Foods) = 11.0 White bread (Wonder Brand) = 14.0

Table 4.2 ***Continued***

Flavor[1] (evaluated at 63°C after heating step)		
Acid	Basic taste sensation elicited by acids	None to Pronounced
Salt	Basic taste sensation elicited by salt	None to Pronounced
Butter	Basic taste sensation elicited by butter	None to Pronounced

[1]Attributes were evaluated using Quantitative Descriptive Analysis (Meilgaard et al., 1999), adapted from Chen et al. (2009).
[2]The following attributes: free oil release, blister colour, blister quantity and strand thickness of the stretched cheeses were evaluated using reference images as described by Chen et al. (2009).

4.3 Results and Discussion

4.3.1 Rheological properties of milk during rennet coagulation

Compositional parameters of the cheesemilks are shown in Table 4.3. Preliminary cheesemaking trials showed that ML and LL cheesemilks did not form a sufficient gel during rennet coagulation in the vat probably due to the loss of calcium during ultrafiltration affecting the rennet coagulation properties of the milk. Rennet coagulation experiments were undertaken to achieve similar gelation curves for all treatments. $CaCl_2$ and temperature were used to adjust the milk rennet coagulation profile. 0.02% $CaCl_2$ was added to all treatment milks and the temperature varied to give similar onset of gelation (G' > 1). The milk gelation curves are shown in Figure 4.1.

At the same rennet and $CaCl_2$ concentration HL milk had a renneting temperature of 31°C, ML had a renneting temperature of 32°C and LL had a renneting temperature of 34°C. The secondary stage of rennet coagulation is dependent on calcium and addition of a certain level of calcium can increase gel strength (Lucey and Fox, 1993). During ultrafiltration soluble components in the milk pass into the permeate (Marella et al., 2013). Changes in insoluble calcium in the casein micelle of milk during ultrafiltration can also affect the renneting properties by lowering the storage modulus during rennet coagulation (Ferrer et al., 2011). Soluble calcium can be lost in the permeate leading to reduced levels of calcium in the treated milk as seen in this study (Table 4.3) which is probably why the milk did not form sufficient coagulum in preliminary cheesemaking trials.

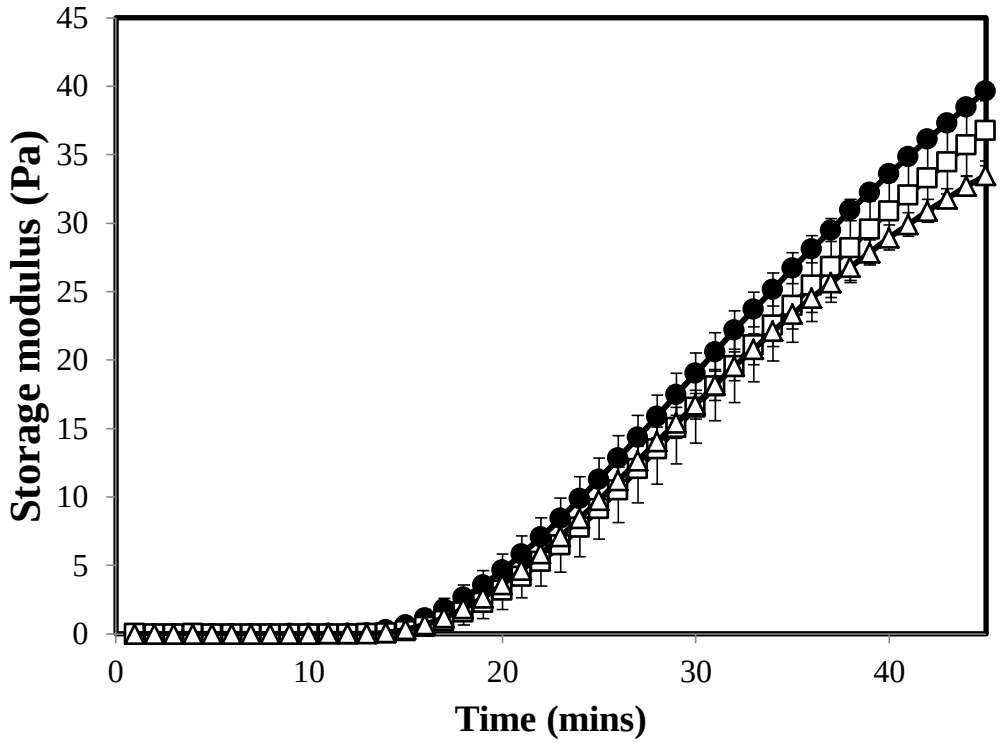


Figure 4.1 Storage modulus of rennet-induced gels for milks with varying levels of lactose, milks were control (HL; ●), medium (ML; □) and low (LL; △) lactose as a function of time after rennet addition. All treatments had 0.01% $CaCl_2$ and temperatures were varied to 31°C (HL), 32°C (ML) and 34°C (LL) during gelation. Values are means (n=2); error bars indicate ± one standard deviation

Addition of 0.01% $CaCl_2$ restored the ability of the treated milks to clot after rennet addition but there was still differences in the gelation time when G' > 1. Increasing the renneting temperature can allow for faster gelation and decreased gelation time (Najera et al., 2003; Moynihan et al., 2014). LL milk had the longest gelation time probably as this treatment had the lowest level of calcium hence it had the highest renneting temperature (34°C) to speed up the gelation process. HL had the shortest gelation time and highest level of calcium therefore the lowest renneting temperature (31°C) was used. ML milk was coagulated at a renneting temperature of 32°C.

4.3.2 Milk composition

The composition of milk treated with ultrafiltration that was used to manufacture cheese treatments HL, ML and LL is shown in Table 4.3. There was no significant ($P > 0.05$) difference between treatments with regards to levels of fat, total protein, true protein, casein, casein:fat ratio and whey protein. The lactose, total solids and lactose:casein ratio was significantly ($P < 0.05$) different between all treatments, as expected, with HL and LL milks having the highest and lowest levels, respectively.

The casein:total protein ratio was significantly ($P < 0.05$) higher for LL cheesemilks than the other treatments. The casein:true protein ratio was significantly higher for LL cheesemilk compared to the ML treatment; no significant ($P > 0.05$) difference was observed for HL milk and the other treatments with regards to casein:true protein ratio. Ultrafiltration can increase the casein to protein ratio (Kumar et al., 2013). There was a significant ($P < 0.05$) difference between the non-protein nitrogen of all treatments; HL milk had the highest and LL milk the lowest level of non-protein nitrogen observed. The reduced levels of lactose, non-protein nitrogen and calcium in ML and LL cheesemilks, was related to the ultrafiltration process. During ultrafiltration water, lactose, NPN and soluble salts (including calcium) pass through the membrane into the permeate (Marella et al., 2013). The levels of calcium in ultrafiltered milks were significantly ($P < 0.05$) different. HL milk had a significantly ($P < 0.05$) higher calcium content (121.36 mg/100g) than ML (115.21 mg/100g) and LL (102.18 mg/100g) milks; ML and LL milks were also significantly ($P < 0.05$) different with respect to calcium.

Table 4.3 Composition and maximum buffering index of milk used to manufacture Mozzarella cheese with varying levels of lactose, cheesemilks were control (HL), medium (ML) and low (LL) lactose. Values represent the means and standard deviations, with the latter in parentheses (n=4).

	Treatment				
	HL	ML	LL	SEM	*P*-value
%Lactose	4.42[c] (0.03)	3.27[b] (0.12)	2.59[a] (0.12)	0.050	<0.0001
%Total Solids	10.90[b] (0.63)	10.21[b] (0.63)	9.15[a] (0.08)	0.259	0.0032
%Fat	2.51 (0.04)	2.42 (0.05)	2.44 (0.05)	0.022	NS[1]
%Total Protein[2]	3.24 (0.06)	3.18 (0.02	3.19 (0.05)	0.024	NS
%True Protein[3]	3.06 (0.07)	3.05 (0.02)	3.09 (0.05)	0.025	NS
%Casein (CN)[4]	2.50 (0.06)	2.47 (0.02)	2.53 (0.05)	0.022	NS
%CN:Total Protein	77.15[a] (0.43)	77.61[a] (0.15)	79.30[b] (0.44)	0.182	<0.0001
%CN:True Protein	81.51[ab] (0.32)	81.03[a] (0.12)	82.02[b] (0.45)	0.164	0.0071
CN:Fat Ratio	1.00 (0.02)	1.02 (0.02)	1.04 (0.03)	0.012	NS
Lactose:CN	1.77[c] (0.05)	1.32[b] (0.04)	1.02[a] (0.06)	0.024	<0.0001
Lactose:Total Solids	0.41[c] (0.02)	0.32[b] (0.03)	0.28[a] (0.01)	0.011	0.0025
NPN	0.028[c] (0.00)	0.021[b] (0.00)	0.017[a] (0.00)	0.0005	<0.0001
Whey Protein[5]	0.57 (0.01)	0.58 (0.00)	0.56 (0.02)	0.006	NS
Ca mg/100g	121.36[c] (3.33)	115.21[b] (2.66)	102.18[a] (3.85)	1.658	<0.0001
Ca:Protein mg/g	37.48[c] (0.50)	36.19[b] (0.90)	31.98[a] (0.87)	0.391	<0.0001
Ca:CN mg/g	48.58[c] (0.67)	46.63[b] (1.16)	40.33[a] (1.13)	0.507	<0.0001
Max dB/dpH[6]	0.030[b] (0.00)	0.029[ab] (0.00)	0.028[a] (0.00)	0.0004	0.0166
pH at max dB/dpH	5.05[a] (0.06)	5.07[a] (0.03)	5.13[b] (0.04)	0.0166	0.0157

[a,b,c] means within the same row not sharing a common subscript differ ($P < 0.05$)

[1] Nonsignificant (F test for full statistical model, $P > 0.05$)

[2]Total % Nitrogen × 6.38

[3](Total % Nitrogen - % NPN) × 6.38

[4](Total % Nitrogen - % Noncasein Nitrogen) × 6.38

[5]True Protein – CN

[6]Buffering Index from acid-base titrations

The ratio of calcium to protein and calcium to casein in the milk also showed the same trend. The total calcium content in milks decreased with a decrease in the lactose:casein ratio, probably due to the removal of soluble calcium along with lactose during the ultrafiltration and by RO water addition. Ferrer et al. (2011) observed a similar trend of decreased calcium to protein ratios during ultrafiltration due to soluble calcium of milk being removed in the ultrafiltration permeate.

The maximum buffering peak of milk and the pH where this occurred during acid-base titrations show significant ($P < 0.05$) differences between treatments. HL milk had the highest maximum buffering index (0.03); this was significantly ($P < 0.05$) higher than LL milk (0.028) (Table 4.3). The pH where the maximum buffering index occurred during acid-base titrations was significantly ($P < 0.05$) higher for LL milk (pH 5.13) compared to HL (pH 5.05) and ML (pH 5.07) milk. The maximum buffering peak in milk during titration occurs typically around pH 5.1. This relates to insoluble calcium phosphate solubilising upon acidification of milk, which is complete about pH 5.1; phosphate ions formed during solubilisation combine with H^+ ions, resulting in buffering. Changes in the colloidal composition of milk can result in changes in its buffering properties (Lucey et al. 2003).

The level of insoluble calcium in milk at pH 6.6 is approximately 68% (Lucey and Fox, 1993) therefore 32% of calcium in milk is in the soluble phase. Ultrafiltration of milk allows soluble components to pass into the permeate (Kumar et al., 2013; Marella et al., 2013); thus, treating the milk with ultrafiltration would have caused soluble calcium in milk to pass into the permeate (Salaun et al., 2005). Alexander et al. (2011) found that diafiltration

or extensive ultrafiltration led to dissolution of calcium clusters from the casein micelle; as the soluble calcium was removed during processing the insoluble calcium solubilized in an attempt to reach equilibrium. Ferrer et al. (2011) found that there was a decrease in the insoluble calcium to casein levels during ultrafiltration. The results from this study seemed to indicate that soluble calcium passed through the membrane into the permeate which could explain the reduced calcium in ML and LL milk. When some of the soluble calcium was removed to the permeate the insoluble calcium probably moved to the soluble phase to find equilibrium; this was indicated by the reduced maximum buffering peak for LL cheese which would indicate lower calcium phosphate.

A shift in the buffering peak towards acidic pH has been seen previously in ultrafiltered retentates; the aqueous phase becomes more saturated in minerals during acidification and, therefore, a lower pH is required to solubilize colloidal minerals (Salaun et al., 2005; Li and Corredig, 2014); the opposite may have occured when retentates were standardised to the same casein concentration using permeate and RO water causing the pH shift towards basic pH as was seen in the current study.

4.3.3 Cheese composition

Compositional parameters of Mozzarella cheese manufactured using milk that was treated with ultrafiltration to vary the lactose:casein ratio is shown in Table 4.4. There was no significant ($P > 0.05$) difference between treatments in terms of moisture, salt, moisture in nonfat substance and salt-in-moisture.

Significant ($P < 0.05$) differences were observed for fat, protein and fat in dry matter. All treatments had significantly ($P < 0.05$) different fat contents; HL cheese had the highest and LL cheese had the lowest level of fat observed. Excessive recirculation of LL and ML cheesemilk through the ultrafiltration unit may have led to damage to the fat globules and reduced fat recoveries in the cheese (Govindasamy-Lucey et al., 2005). Significant ($P < 0.05$) differences were also observed between the protein content of each cheese; LL cheese had the highest and HL cheese had the lowest level of protein. The fat in dry matter of cheese treatments was also significantly ($P < 0.05$) different; HL cheese had the highest and LL cheese the lowest fat in dry matter observed. The differences in fat in dry matter reflect the variations in fat content along with the similar moisture contents between treatments.

There was no significant ($P > 0.05$) difference between the casein concentration and casein:fat ratio of the cheesemilks used to manufacture Mozzarella (Table 4.3). There were significant differences between the NPN levels of milk treatments; ML (0.021%) and LL (0.017%) had lower NPN than HL (0.028%) milk. The NPN was not taken into account when standardising cheesemilks. Perhaps the NPN was lost in the whey during cheese manufacture this would result the HL cheese losing more NPN (as it had a higher level in the milk) and hence its protein content would be lower; consequently ML and LL cheese would have higher protein (N x 6.38).

No significant ($P > 0.05$) differences were observed in the cheese total calcium levels between any of the treatments even though the calcium in the milk was significantly ($P < 0.05$) different (Table 4.3). The HL cheese had higher total calcium than the other treatments, but this difference was small. However, the

ratio of calcium to protein in the cheese was significantly ($P < 0.05$) different between cheeses. The calcium to protein ratio in the LL cheese was lower than that of the HL cheeses. This reflects the differences in calcium content of the milk where HL had the highest and LL had the lowest level of calcium, respectively. The critical pH parameters during cheesemaking (rennet addition, drain pH and salting) were kept constant, which would typically result in cheese with similar calcium levels, but ultrafiltered treatments had lower levels of calcium before cheese manufacture. It would be expected that the ML and LL milk could have less insoluble calcium as previously discussed. Acidification during cheese making would have caused solubilisation of insoluble calcium leading to reduced amounts of calcium in ML and LL treatments due to the reduced amount of calcium in the milk (Table 4.3).

The levels of lactose and galactose in cheese were significantly ($P < 0.05$) different between all treatments (Table 4.4). At d 42 of ripening, lactose was broken down completely in LL cheese. In ML cheese only 0.01% and HL cheese only 0.03% lactose remained. Galactose content was also significantly ($P < 0.05$) different with LL cheese having the lowest galactose levels (0.40%) compared to ML (0.53%) and HL cheese (0.66%). The thermophilic cultures used do not readily ferment the galactose moiety of lactose and it therefore accumulates in the cheese (Johnson and Olson, 1985). Using ultrafiltration to change the level of lactose in Mozzarella cheese had a significant ($P < 0.05$) effect on the level of lactic acid in the cheeses and the age-related changes of lactic acid in cheese (Table 4.5). The concentration of lactic acid as a function of time during ripening is shown in Figure 4.2. At d 1 of ripening LL cheese had significantly ($P < 0.05$) lower lactic acid (0.38%) levels than ML (0.51%)

Table 4.4 Composition of Mozzarella cheese manufactured with milk with varying levels of lactose, cheeses were control (HL), medium (ML) and low (LL) lactose at d 14 of ripening. Lactose, galactose and lactic acid were measured at d 42 of ripening. Values represent the means and standard deviations, with the latter in parentheses (n=4).

	Treatment				
	HL	ML	LL	SEM	*P*-value
%Moisture	45.85 (0.24)	45.85 (0.26)	46.22 (0.39)	0.152	NS[1]
%Fat	23.22[c] (0.27)	22.73[b] (0.23)	22.11[a] (0.38)	0.150	0.0018
%Salt	1.86 (0.23)	1.62 (0.16)	1.86 (0.04)	0.083	NS
%Protein[2]	25.40[a] (0.24)	26.45[b] (0.05)	26.90[c] (0.38)	0.130	<0.0001
MNFS[3]	59.71 (0.38)	59.34 (0.25)	59.34 (0.45)	0.184	NS
FDM[4]	42.88[c] (0.54)	41.98[b] (0.31)	41.10[a] (0.62)	0.255	0.0028
SM[5]	4.05 (0.51)	3.52 (0.37)	4.02 (0.07)	0.184	NS
Ca mg/100g	791.52 (32.28)	781.53 (12.44)	781.01 (50.51)	17.672	NS
Ca:Protein mg/g	31.16[b] (1.01)	29.55[ab] (0.51)	29.03[a] (1.72)	0.595	0.05
% Lactose	0.03[c] (0.01)	0.01[b] (0.00)	0.00[a] (0.00)	0.002	0.0001
% Galactose	0.66[c] (0.01)	0.53[b] (0.01)	0.40[a] (0.02)	0.007	<0.0001

[1]Nonsignificant (F test for full stastistical model $P > 0.05$)
[2]Total % N × 6.38
[3]Moisture in nonfat substance of the cheese
[4]Fat content on a dry weight basis
[5]Salt in moisture phase of the cheese
[a,b,c]Means within the same row not sharing a common superscript differ ($P < 0.05$)

and HL (0.59%) cheeses in agreement with the lower lactose levels in the LL milk (Table 4.3). At d 14 lactic acid levels were significantly ($P < 0.05$) higher than d 1; there was no significant ($P > 0.05$) increase between d 14 and d 42 of ripening. At d 14 of ripening both ML and LL cheese had lower levels of lactic acid than HL cheese. At d 42 of ripening all treatments were significantly ($P < 0.05$) different with HL and LL cheese having the highest (0.89%) and lowest (0.57%) levels of lactic acid, respectively. There were only trace levels of lactose remaining in all treatments at d 42 but HL cheese had the highest levels of residual lactose. Reduced levels of lactose in milk (Table 4.3) resulted in

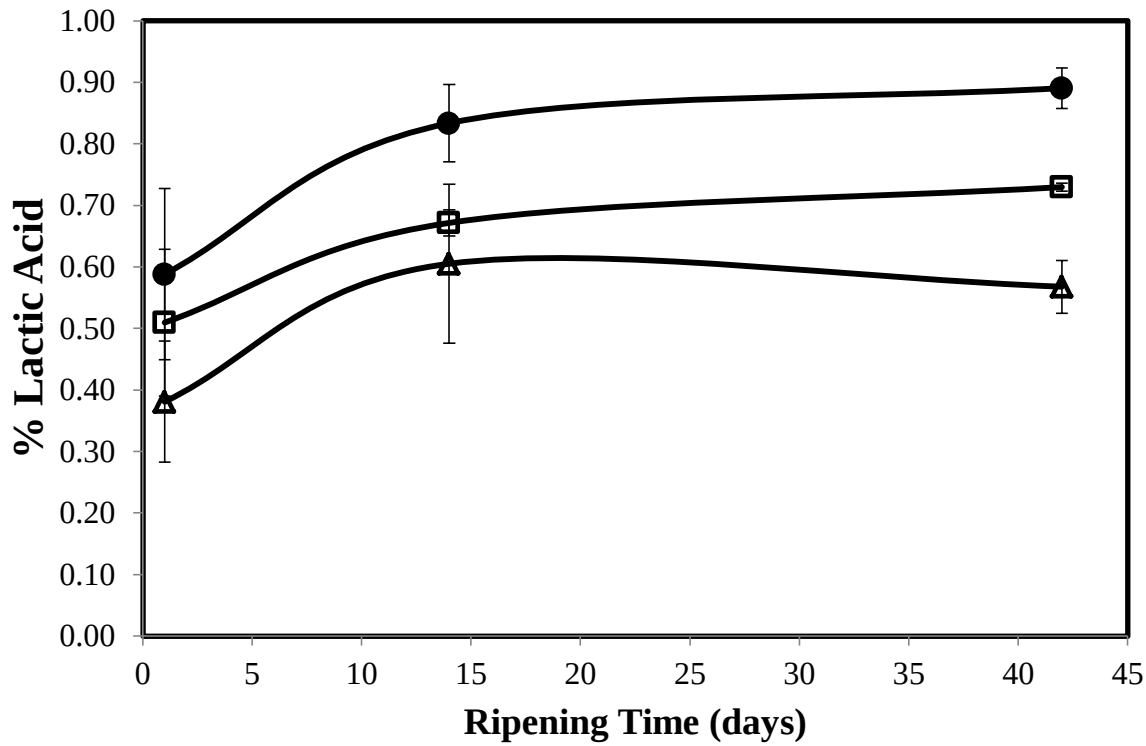


Figure 4.2 Lactic acid as a function of ripening time of Mozzarella cheese made with varying levels of lactose. Cheeses were control (HL; ●), medium (ML; □) and low (LL; △) lactose. Values are means of four replicates; error bars indicate ± one standard deviation.

cheese with lower lactose levels. Shakeel-Ur-Rehman et al. (2004) found that lowering lactose levels in cheese led to reduced lactose during ripening. Compositional factors such as salt-in-moisture can affect the breakdown of lactose and generation of lactic acid (Upreti and Metzger, 2007; Hou et al. 2014b) but there was no significant difference between values of salt, moisture and salt-in-moisture in the current study (Table 4.4). Galactose accumulates in Mozzarella from incomplete metabolism of lactose by Gal^- starters (Johnson and Olson, 1985); the differences in galactose concentration in this study can be attributed to the level of lactose in the cheesemilk. The reduced level of lactose in the LL treatment consequently led to lower levels of galactose. Reducing the level of lactose in cheese also reduced the generation of lactic

acid; therefore, LL cheese had the lowest and HL cheese had the highest level of lactic acid, respectively. Changing residual lactose in cheese can affect the formation of lactic acid (Lee et al., 2010).

4.3.3 pH

Using ultrafiltration to vary the lactose:casein ratio in milks used to manufacture Mozzarella cheese had a significant ($P < 0.05$) effect on treatment and age related changes of the cheese pH (Table 4.5). The pH of the cheeses as a function of time is shown in Figure 4.3. LL cheese had a significantly ($P < 0.05$) higher pH than HL and ML cheese throughout ripening. The pH of HL

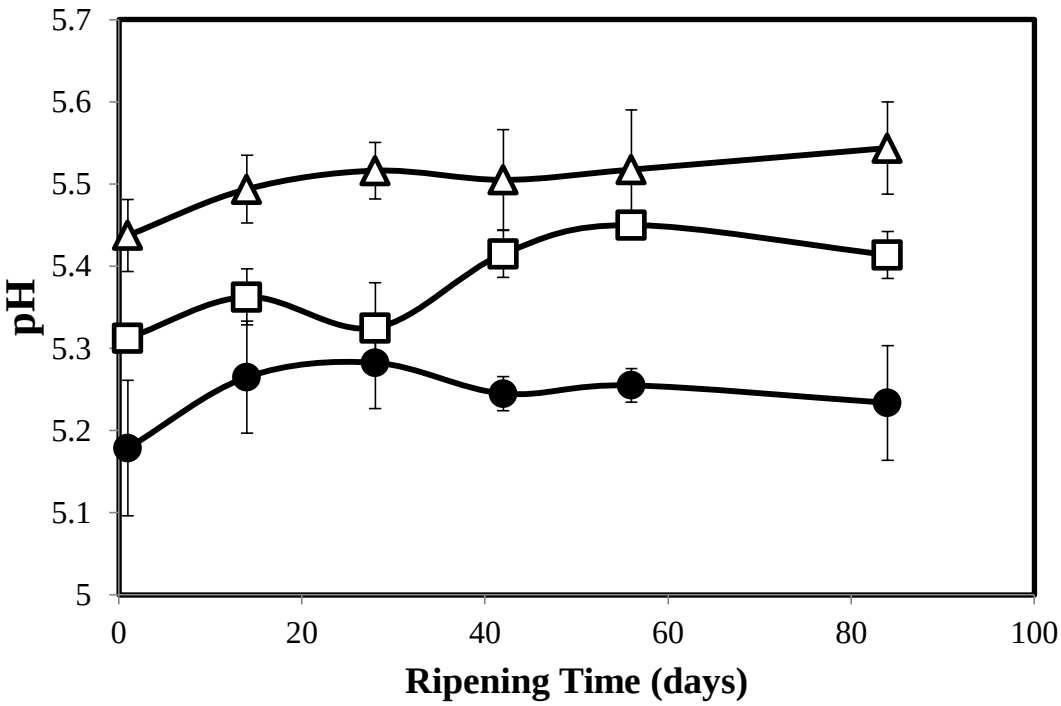


Figure 4.3 pH as a function of ripening time of Mozzarella cheese made with varying levels of lactose. Cheeses were control (HL; ●), medium (ML; □) and low (LL; △) lactose. Values are means of four replicates; error bars indicate ± one standard deviation.

cheese was not significantly ($P > 0.05$) different to that of ML cheese at d 28 of ripening, at all other ripening times the pH of HL cheese was significantly lower than ML and LL cheeses. ML cheese had significantly ($P < 0.05$) lower pH than LL cheese throughout ripening. The pH of HL cheese increased significantly ($P < 0.05$) after d 1 of ripening until d 84. The pH of HL cheese increased from pH 5.17 at d 1 to pH 5.23 at d 84. There was no significant ($P > 0.05$) difference in pH between d 14 and d 84 for HL cheese. The pH of ML cheese also increased significantly ($P < 0.05$) during ripening from 5.31 at d 1 to 5.41 at d 84 of ripening. There was no difference in pH from d 1 to 28 of ripening, the pH increased from d 28 to d 42 of ripening, no further changes in ripening were observed after d 42 for ML cheese. LL cheese increased significantly ($P < 0.05$) from pH 5.44 at d 1 to pH 5.54 at d 84 of ripening. There was no significant ($P > 0.05$) difference in pH between d 1 and 28 of ripening for LL cheese; after d 28 there was a significant ($P > 0.05$) increase but there was no change in pH thereafter.

Starter bacteria added to milk convert lactose to lactic acid during cheese manufacture and ripening (El-Alfy et al., 2008). The level of lactose contributes indirectly to cheese pH by its conversion to lactic acid by bacteria (Upreti and Metzger, 2007). When the concentration of lactose in milk is reduced there is a subsequent reduction in the formation of lactic acid leading to an increased pH. Spangler et al. (1991) found that diafiltration of ultrafiltered retentates used for Gouda cheese had higher pH due to the lower lactose content in the cheese. The primary contributors to the buffering capacity of cheese are proteins, inorganic phosphate and organic acids (Salaun et al., 2005). Typically, the pH of Mozzarella cheese increases slightly during

Table 4.5 Mean squares and probabilities (in parentheses), and R^2 values for insoluble calcium, pH and proteolysis values during ripening of Mozzarella cheese manufactured with different lactose:casein ratios.

	Insoluble Ca	pH	% Proteolysis	Lactic Acid
Treatment (T)	38.073* (0.0478)	0.403** (<0.0001)	0.016 (0.9547)	0.192** (0.0011)
Age (A)	19.165** (0.0008)	0.014** (<0.0001)	79.948** (<0.0001)	0.2019** (<0.0001)
A × T	2.600 (0.1834)	0.004* (0.0194)	0.127 (0.8951)	0.056 (0.4018)
Error	1.4017	0.0016	0.474	0.0052
R^2	0.91	0.93	0.95	0.91

*$0.01 < P \leq 0.05$

**$P \leq 0.01$

ripening due to slow solubilisation of insoluble calcium. If residual lactose in the cheese is converted to lactic acid by starter bacteria, this usual increase in pH can be offset by lactic acid to result in a stable pH (Lucey et al., 2003; Upreti and Metzger, 2007).

The insoluble calcium phosphate in milk relates to its buffering capacity (Lucey et al., 1993). The increase in cheese pH can be due to solubilisation of insoluble calcium phosphate leading to neutralization of hydrogen ions by phosphate anions and increased *para*-casein hydration (Guinee et al., 2002; Lee et al., 2011). The level of protein may also contribute to the buffering capacity of cheese (Upreti and Metzger, 2007).

All cheeses were salted at the same pH. The lower lactose present in the ultrafiltered treatments meant that lower levels of lactic acid were formed after salting compared to the control (Figure 4.2). The insoluble calcium also solubilised at a faster rate in these treatments during ripening compared to the HL cheese (Table 4.6). ML and LL cheese also had higher levels of protein compared to HL cheese. Taking this into consideration, the lower levels of lactic acid in ML and LL cheese in combination with the faster rate of solubilisation of insoluble calcium (which causes an increase in pH) and higher protein led to the increased pH of these treatments compared to HL cheese.

4.3.5 Insoluble calcium

Treatment and age had a significant ($P < 0.05$) effect on the ratio of insoluble calcium to protein in Mozzarella cheese manufactured from milk with varied ratios of lactose:casein (Table 4.5). Calcium is an important structural element

of cheese that is associated with the proteins (Lucey and Fox, 1993); hence, the insoluble calcium was expressed as mg of insoluble calcium per g of protein (Lee et al., 2005). The levels of insoluble calcium in cheese are shown in Table 4.6. HL cheese had significantly ($P < 0.05$) higher insoluble calcium to protein ratio at d 1 of ripening compared to ML and LL cheeses. The concentration of insoluble calcium for HL cheese was 29.48 mg/g protein compared to 27.71 mg/g protein for ML and 27.58 mg/g protein for LL cheese, respectively. LL cheese had significantly ($P < 0.05$) lower insoluble calcium to protein ratio at d 14 of ripening compared to HL and ML cheeses. All treatments had significantly ($P < 0.05$) different insoluble calcium to protein ratios at d 28 of ripening; HL and LL cheese had the highest (26.70 mg/g protein) and lowest (22.17 mg/g protein) ratio of insoluble calcium to protein, respectively. The ratio of insoluble calcium to protein in HL cheese decreased from 29.48 to 26.7 mg/g protein during ripening but this decrease was not significant ($P > 0.05$), the ratio in ML cheese decreased significantly ($P < 0.05$) after d 14 of ripening to 23.57 mg/g protein and LL cheese decreased significantly ($P < 0.05$) after d 1 of ripening from 27.58 to 22.17 mg/g protein.

There was no significant difference ($P > 0.05$) between the total calcium level of each treatment but the calcium to protein ratio was significantly lower in LL cheese compared to HL cheese. The levels of insoluble calcium in LL cheese decreased at a faster rate than the other treatments and the ML cheese decreased at a faster rate than HL cheese during ripening. The calcium to protein ratio was also significantly lower in the LL milk (Table 4.3) presumably reflecting losses of soluble calcium in the ultrafiltration permeate (Li and Corredig, 2014) and dilution of milk by water addition. It was likely

that the losses of soluble calcium in the LL milk sample caused a shift of some insoluble calcium into the serum phase to try to attain and re-establish an equilibrium. Perhaps due to the ultrafiltration process the insoluble calcium decreased faster in the LL cheese to try to attain equilibrium in the cheese. Lee et al. (2005) observed greater solubilisation of calcium after curd washing which removed some of the soluble calcium; this was attributed to the calcium attaining a stable equilibrium between soluble and insoluble calcium. Johnson and Lucey (2006) discussed curd washing and how it removes lactose but also soluble calcium, which can lead to a shift in the equilibrium of soluble and insoluble calcium, leading to greater solubilisation of insoluble calcium. The binding of calcium may also be reduced by a decrease in the ionic strength (Lucey et al., 2003) which may be the case in the ML and LL cheeses with reduced lactic acid (Figure 4.2).

Table 4.6 Insoluble calcium content (mg/g protein) of Mozzarella cheese manufactured with milk with varying levels of lactose, cheeses were control (HL), medium (ML) and low (LL) lactose at d 1, 14 and 28 of ripening. Values represent the means and standard deviations, with the latter in parentheses (n=4).

Ripening Time (d)	Treatment		
	HL	ML	LL
1	$29.48^{a,A}$ (2.37)	$27.71^{b,A}$ (3.20)	$27.58^{b,A}$ (0.69)
14	$27.19^{a,A}$ (1.42)	$27.01^{a,A}$ (0.99)	$24.90^{b,B}$ (0.69)
28	$26.70^{a,A}$ (1.27)	$23.57^{b,B}$ (2.89)	$22.17^{c,B}$ (0.56)

^{a,,b,c}Means within the same row not sharing a common superscript differ ($P < 0.05$).
^{A,B,C}Means within the same column not sharing a common superscript differ ($P < 0.05$).

Both lactose and soluble salts including calcium are removed during ultrafiltration; typically, reducing lactose levels results in reduced lactic acid

production, leading to cheese containing casein with greater level of insoluble calcium (Salaun et al., 2005). pH during cheese manufacture can affect insoluble calcium in cheese; lower pH values during manufacture can lead to lower levels of insoluble calcium or higher levels of soluble calcium in the curd (Lee et al., 2010). In this study, the use of ultrafiltration meant that the soluble and insoluble calcium in the cheesemilk was reduced but the decrease in pH was kept the same during cheesemaking for all treatments. Insoluble calcium typically decreases in cheese during the first few weeks of ripening (Hassan et al., 2004). Upreti and Metzger (2007) observed that solubilisation of calcium partially corresponded with changes in cheese pH. In the current study, no sufficient drop in pH of the treatments was observed in the first few weeks of ripening to induce solubilisation of calcium. Previous studies have also observed no major change in pH during ripening along with solubilisation of calcium which was then attributed to the insoluble and soluble calcium reaching a stable pseudoequilibrium (Hassan et al., 2004; Lee et al., 2010).

4.3.6 Proteolysis

The use of ultrafiltration to vary the lactose:casein ratio levels in milk for the manufacture of Mozzarella cheese had no significant ($P > 0.05$) effect on treatment but had a significant ($P < 0.05$) effect on pH 4.6-SN/TN in terms of age which is an index of proteolysis (Sousa et al., 2001) (Table 4.5). The effect of treatments on proteolysis as a function of ripening time can be seen in Figure 4.4. There was no significant ($P > 0.05$) effect on proteolysis when

varying the level of lactose in Mozzarella but the pH4.6-SN/TN increased significantly during ripening at each of the time points measured.

Govindasamy-Lucey et al. (2005) found that rennet had to be added based on casein level in the milk to get a consistent proteolysis between pizza cheese treatments made with cold ultrafiltered retentates. Da Cunha et al. (2006) found no difference in the level of pH 4.6 SN when using low concentration factor ultrafiltration in Minas Frescal cheese where the protein content was higher. In the current study, the level of protein was higher in cheeses made with ultrafiltered and diafiltered milk and the levels of rennet added to each

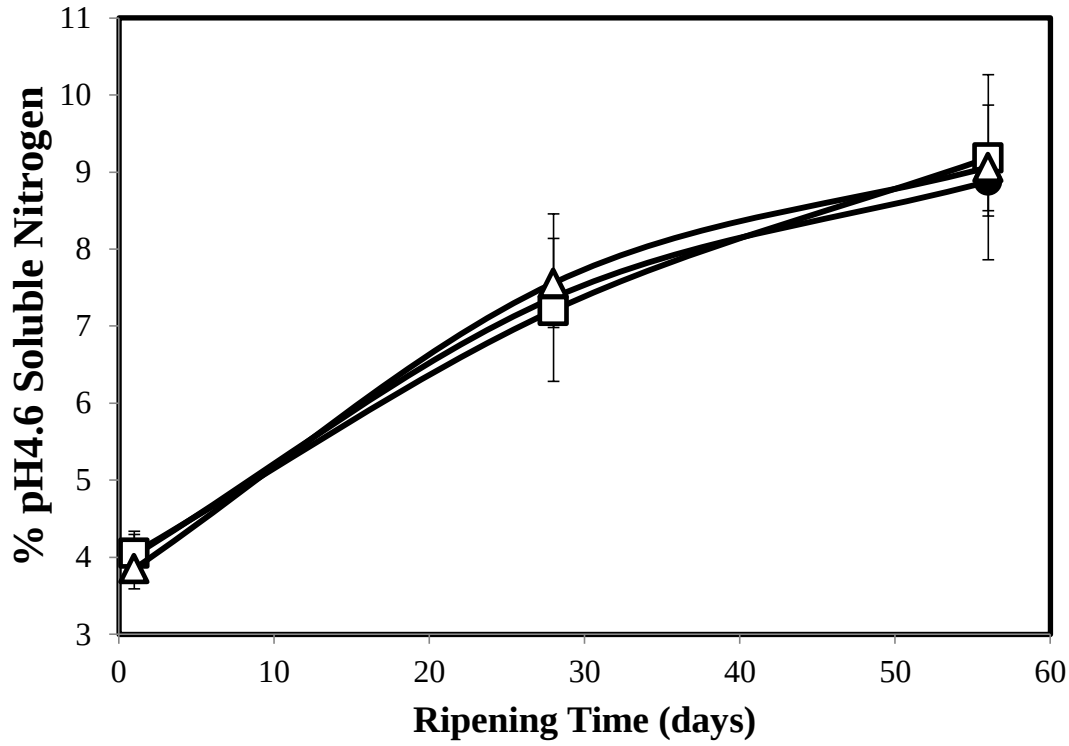


Figure 4.4 pH 4.6 soluble nitrogen as a percentage of total nitrogen as a function of ripening time of Mozzarella cheese made with varying levels of lactose. Cheeses were control (HL; ●), medium (ML; □) and low (LL; △) lactose. Values are means of four replicates; error bars indicate ± one standard deviation.

treatment was the same but the level of proteolysis was not affected. Bansal et al. (2007) found that the level of rennet added to the milk does not affect its retention as casein micelles are saturated with respect to rennet. Perhaps in the current study all protein was saturated with rennet, which would account for no difference in proteolysis even though protein content was higher. Spangler et al. (1990) found that proteolysis was affected by the rennet coagulation temperature; this was attributed to increased coagulation temperature leading to decreased residual rennet. In this study the coagulation temperature was varied but this appeared not to affect proteolysis. It is typical for the extent of proteolysis to increase in cheese during ripening (Joshi et al. 2003; Da Cunha et al., 2006). A higher pH in ML and LL cheese may favour plasmin activity in the cheese and have led to reduced chymosin activity (Lee et al., 2011); however, Hou et al. (2014a) did not see a difference in chymosin activity in washed curd Cheddar cheese which also had reduced lactose and lactic acid leading to the same pH (5.2-5.6) range as the current study.

4.3.7 Texture Profile Analysis

The use of ultrafiltration to vary the level of lactose:casein ratio in milks for the manufacture of Mozzarella cheese had a significant ($P < 0.05$) effect on treatment and age related changes of cheese hardness and chewiness as measured by texture profile analysis (Table 4.7). The effect of treatments on hardness and chewiness as a function of ripening time can be seen in Figures 4.5a and 4.5b, respectively. ML and LL cheeses had significantly higher ($P < 0.05$) hardness values than HL from d 14 to 56 of ripening. At d 84 of ripening

LL cheese was significantly harder than both HL and ML cheeses. At d 14 of ripening ML and LL cheeses had significantly ($P < 0.05$) higher chewiness than HL cheeses. At d 28 and 56 of ripening significant differences ($P < 0.05$) existed between all treatments, HL and LL cheese had the lowest and highest chewiness values, respectively. At d 84 LL cheese had significantly ($P < 0.05$) higher chewiness values than HL and ML cheeses.

All cheeses decreased significantly ($P < 0.05$) in hardness and chewiness throughout ripening. No significant ($P > 0.05$) difference in hardness was observed from d 14 to d 28 of ripening in any of the cheese treatments but there was a significant ($P < 0.05$) decrease in hardness between d 14 and d 56 of ripening. Decreased hardness at the start of ripening is associated with solubilisation of insoluble calcium (O'Mahony et al., 2005). In this study, there was a reduction in insoluble calcium in the first 28 d of ripening (Table 4.6), but this did not seem to be associated with a decrease in hardness values in any treatment. The decrease in hardness over the ripening period may relate to increased levels of proteolysis in the cheese (Figure 4.4). Decreased hardness in Mozzarella during ripening is associated with increased proteolysis and changes in insoluble calcium (Guinee et al., 2002; Moynihan et al., 2014).

Guinee et al. (2002) found that Mozzarella cheese with high pH and typical calcium levels had higher firmness than cheese with lower pH and similar calcium levels but this cheese also had lower levels of intact casein. In the current study there was no significant ($P > 0.05$) difference between levels of proteolysis in cheeses of any treatment (Table 4.5). Da Cunha et al. (2006) found that Minas Frescal cheese made using high concentration factor ultrafiltration had increased firmness compared to the control. This cheese had

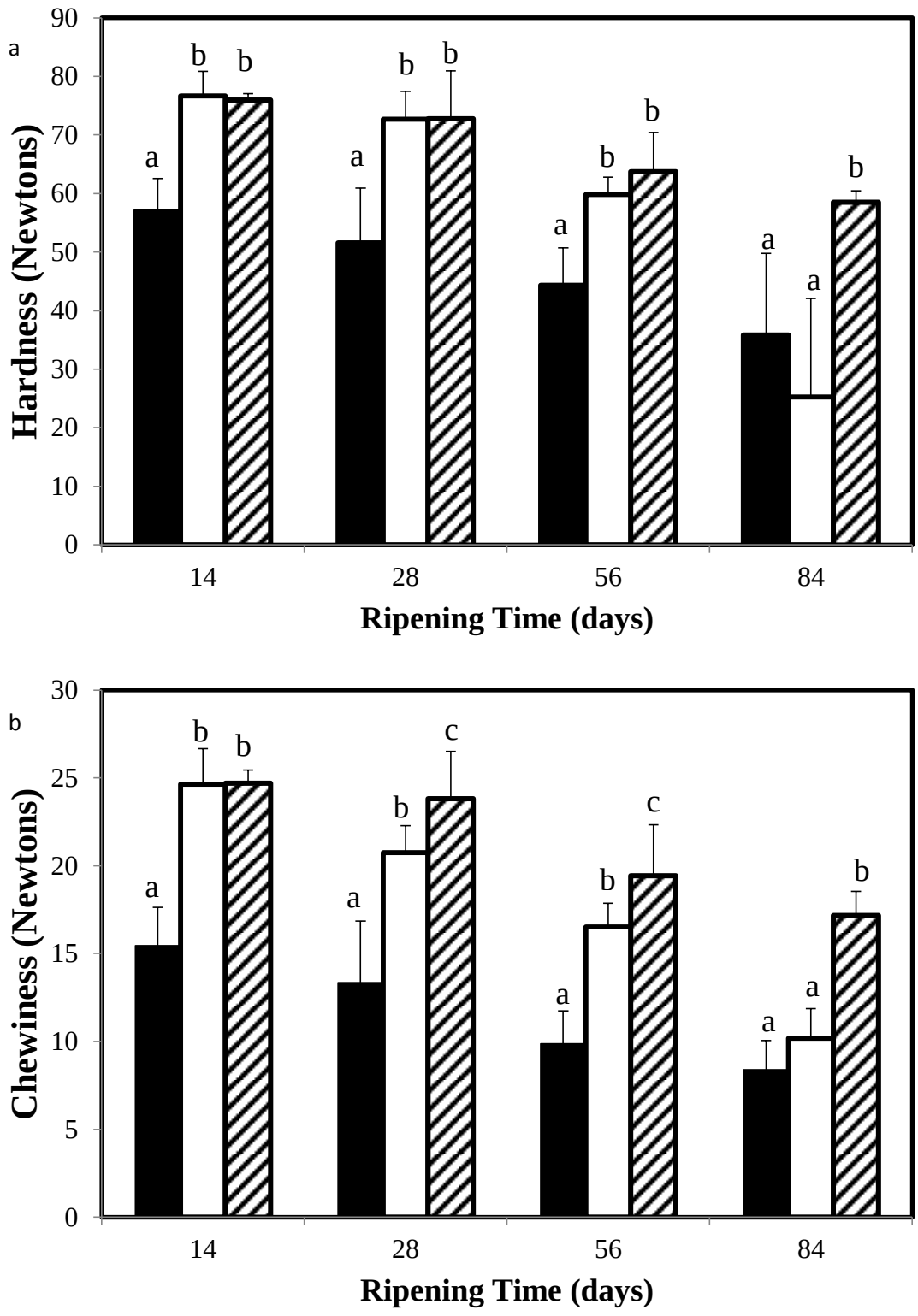


Figure 4.5 (a) Hardness and **(b)** chewiness values as a function of ripening time of Mozzarella cheese made with milk of varying levels of lactose. Cheeses were control (HL; ■), medium (ML; □) and low (LL; ▨) lactose. Values are means of four replicates; error bars indicate ± one standard deviation.

similar levels of proteolysis but lower moisture and higher protein content which may have led to increased cheese firmness. In the current study, there was no significant ($P > 0.05$) difference in moisture between treatments but ML and LL cheeses had higher levels of protein, so this may be linked to the increased hardness values observed for ML and LL cheeses. High protein cheeses have increased cross linking via calcium between proteins leading to firmer texture (Lucey, 2008) but the ML and LL Mozzarella cheese in the current study had decreased insoluble calcium levels (crosslinking material). Low fat cheese is harder than full fat cheese (Lucey, 2008), but the differences in fat levels in this study were relatively small. Everard et al. (2006) found that increased pH in commercial Cheddar cheese led to increased chewiness and firmness; however, these authors did not consider calcium. Ramkumar et al. (1998) found that curd showed an increased solid-like behaviour as the cheese pH increased from 5.45-5.90. Watkinson et al. (2001) found that cheese firmness increased as the pH increased in the range of pH 5.2-6.2. Hou et al. (2014a) found that washed Cheddar cheese which had the same drain pH, mill pH, cheese composition and proteolysis but had lower levels of lactose and lactic acid was overall firmer and less brittle; these cheeses also exhibited a pH increase due to lower levels of lactic acid compared to unwashed Cheddar cheese. Increasing Mozzarella cheese pH by exposure to ammonia led to an increased hardness but also a decrease in soluble calcium (Cortez et al., 2008). The textural differences between cheeses with higher pH may be due to increased levels of insoluble calcium (Lucey et al., 2003). In the current study, ML and LL cheeses had a higher pH and lower levels of insoluble calcium than HL cheese, even at d 1 of ripening, as shown in Figure 4.3 and Table 4.6,

Table 4.7 Mean squares and probabilities (in parentheses), and R^2 values for hardness and chewiness values as determined by texture profile analysis and rheological properties during ripening of Mozzarella cheese manufactured with different lactose:casein ratios.

	Hardness	Chewiness	LT_{max}[1]	LT=1[2]	TLT[3]
Treatment (T)	1746.94** (0.0018)	374.83** (<0.0001)	1.297** (0.0041)	6.585* (0.0308)	6.908 (0.1559)
Age (A)	2168.65** (<0.0001)	221.33** (<0.0001)	4.584** (<0.0001)	16.69** (<0.0001)	71.919** (<0.0001)
A × T	316.75** (0.0004)	12.404** (0.0017)	0.419 (0.0547)	1.417 (0.1495)	0.464 (0.9937)
Error	52.873	2.531	0.175	0.813	4.011
R^2	0.901	0.958	0.809	0.791	0.705

[1]Maximum loss tangent values

[2] Temperature at which loss tangent value is = 1

[3]Temperature at which loss tangent value is a maximum

*$0.01 < P \leq 0.05$

**$P \leq 0.01$

respectively; however, these cheeses were firmer than HL cheese, in contrast to previous studies. The higher pH was due to decreased levels of lactose (Table 4.4) and lactic acid (Figure 4.2) in the cheese treated with ultrafiltration. It would be expected that the lower levels of insoluble calcium in ML and LL cheese would results in a softer cheese but these cheeses had higher firmness values which are in agreement with the studies of Ramkumar et al. (1998), Watkinson et al. (2001) and Hou et al. (2014a) where higher pH led to increased cheese firmness.

4.3.8 Dynamic small-amplitude oscillatory rheology

Treatment and age had a significant ($P < 0.05$) effect on LT_{max} and the temperature at which LT=1 of Mozzarella cheese manufactured from milk with different lactose:casein ratios (Table 4.7). The effect of treatment on the LT_{max} as a function of ripening time can be seen in Figure 4.6. LL cheese had a significantly ($P < 0.05$) lower LT_{max} than HL and ML cheese at d 14 and 28 of ripening. As ripening continued, no significant ($P > 0.05$) differences were observed between the LT_{max} of any of the treatments. The LT_{max} of ML and LL cheeses increased significantly ($P < 0.05$) during the ripening period. The LT_{max} of HL cheeses increased significantly ($P < 0.05$) up to 56 d of ripening; by d 84 the LT_{max} had decreased and was not significantly ($P > 0.05$) different to the other ripening times measured.

The LT_{max} has been highly correlated with cheese meltability (Mounsey and O'Riordan, 1999) and indicates a more liquid-like system (Govindasamy-Lucey et al., 2005) hence LL cheese had a lower meltability than HL and ML

cheese at d 14 and 28 of ripening. The increase in LT_{max} of cheese during ripening is related to the solubilisation of insoluble calcium phosphate and proteolysis (Lucey et al., 2003). The increase in LT_{max} during cheese ripening has been observed to occur in the first 4 weeks of ripening and has been more highly correlated with changes in insoluble calcium than with proteolysis (Lucey et al., 2005). In the current study, the LL and ML cheese had lower levels of insoluble calcium than HL cheese and no difference was observed between treatments with regard to proteolysis (Table 4.7). Lower levels of insoluble calcium typically result in a more meltable cheese. The lower LT_{max} and lower level of insoluble calcium in LL cheese compared to HL cheese does not seem to agree with this hypothesis. However, the LT_{max} was not significantly ($P > 0.05$) different between any treatments after d 28 of ripening which may relate to insoluble calcium in LL cheese solubilising at a faster rate than in HL cheese leading to the cheeses having similar meltability (LT_{max} values) after d 28 of ripening. The observed decrease in the LT_{max} of HL cheese towards the end of ripening may be due to the cheese becoming less meltable, which can happen to Mozzarella cheese during ripening, due to high levels of proteolysis leading to weaker interactions between strands in the protein matrix and, hence, reduced melting properties (Lucey, 2008). ML and LL cheese also seemed to be more meltable (indicated by higher LT_{max}) at the end of the ripening period measured.

The temperature where the LT=1 during ripening can be seen in Figure 4.7. The LT=1 is considered the softening point or where the cheese begins to change from a solid to viscous-like material during heating (Gunasekaran and

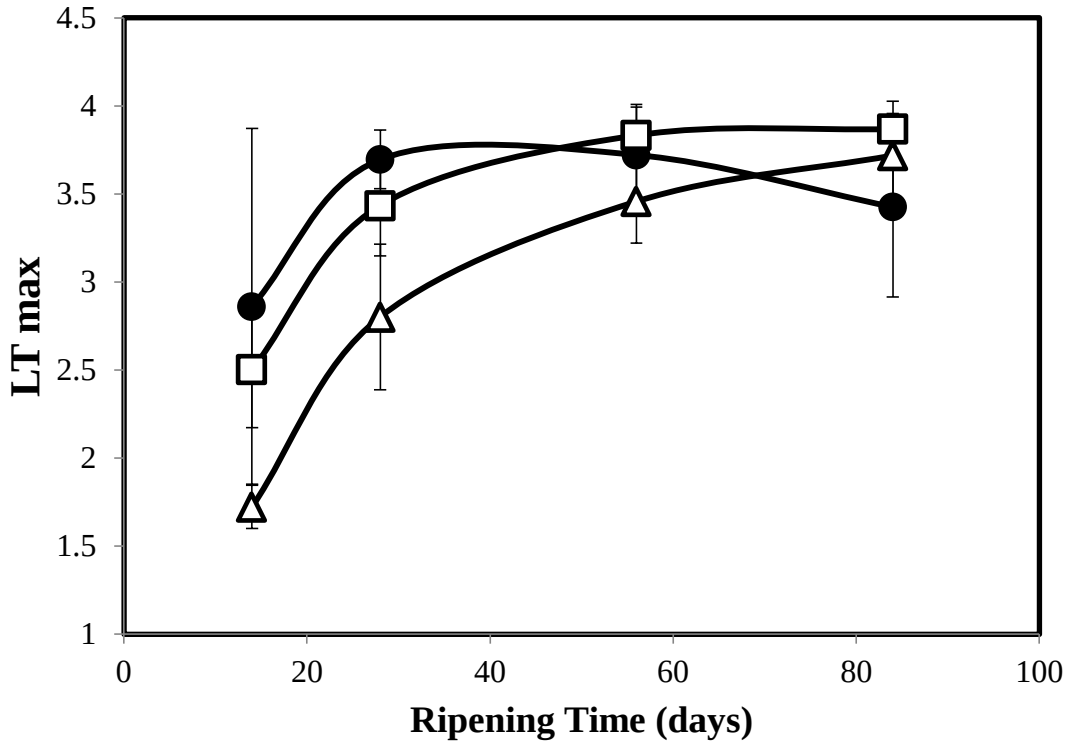


Figure 4.6 Maximum loss tangent as a function of ripening time of Mozzarella cheese made with varying levels of lactose. Cheeses were control (HL; ●), medium (ML; □) and low (LL; △) lactose. Values are means of four replicates; error bars indicate ± one standard deviation.

Ak, 2003). At d 14 and 28 of ripening no significant ($P > 0.05$) differences were observed for the temperature where LT=1 between any of the treatments. At d 56 and 84 both ML and LL cheeses had a significantly ($P < 0.05$) higher temperature where LT=1 than HL cheese. Values for all treatments decreased significantly ($P < 0.05$) during the ripening period measured. The temperature where the LT=1 typically decreases during ripening in Mozzarella cheese due to proteolysis (Moynihan et al., 2014) and solubilisation of insoluble calcium phosphate (Govindasamy-Lucey et al., 2005).

Age had a significant ($P < 0.05$) effect on the temperature of the LT_{max}; however, no significant ($P > 0.05$) difference was found between treatments

for the temperature of the LT_{max} (Table 4.7). Temperature where the LT_{max} occurred can be seen in Figure 4.8. All treatments decreased significantly ($P < 0.05$) during the ripening period measured. A decrease in the temperature of LT_{max} during ripening is typical characteristic of cheese (Govindasamy-Lucey et al., 2005; Lucey et al., 2005; Lee et al., 2010). Lucey et al. (2005) found significant correlations between levels of pH 4.6 soluble nitrogen and the temperature where the LT_{max} occurs during ripening. In the current study the temperature where the LT_{max} occurred during ripening and the level of pH 4.6 soluble nitrogen (Figure 4.4) between treatments were not significantly different.

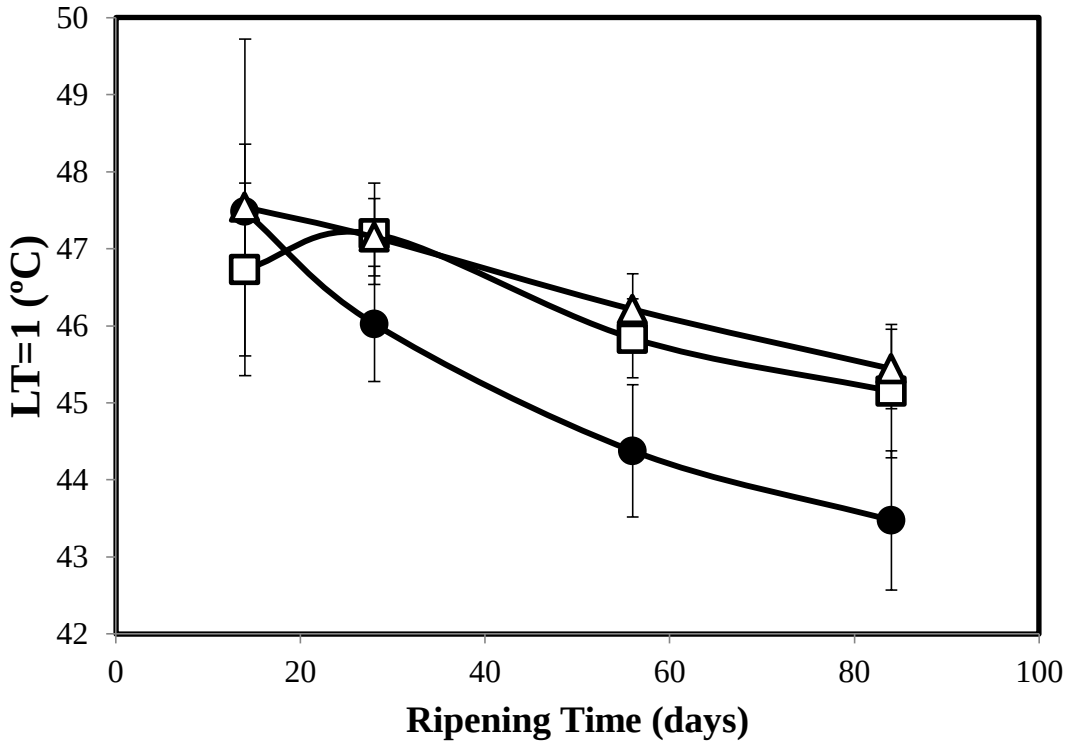


Figure 4.7 Temperature where the loss tangent is 1 as a function of ripening time of Mozzarella cheese made with varying levels of lactose. Cheeses were control (HL; ●), medium (ML; □) and low (LL; △) lactose. Values are means of four replicates; error bars indicate ± one standard deviation.

Joshi et al. (2003) found that as the amount of calcium was reduced in Mozzarella cheese there was an increase in cheese melt and flow which was attributed to changes in the protein matrix due to less cross linking between proteins in reduced calcium cheese. This study also found that reduced calcium Mozzarella had increased levels of proteolysis which may have caused increased meltability. Guinee et al. (2002) found that when the pH of Mozzarella cheese was increased from 5.58 to 5.93 but with no difference in composition or calcium content of the cheeses, the high pH cheese took longer to melt, had decreased flowability and stretchability. The high pH cheese had slightly higher levels of intact casein which may have contributed to this. Cortez et al. (2008) exposed Mozzarella cheese to an ammonia atmosphere, which led to increased cheese pH from pH 5.32 to 5.65; the higher pH led to cheese that had reduced melt. The proteolysis was not different between the cheeses studied; however, increasing the cheese pH using ammonia led to decreased levels of soluble calcium. In the current study no difference was observed in terms of levels of proteolysis in the cheeses but the pH was higher and insoluble calcium was lower for ML and LL cheese.

LL cheese had lower LT_{max} at the beginning of ripening which meant that the cheese exhibited lower melt compared to HL and ML cheese but there was no difference in LT=1 for any treatment. After d 56 of ripening LL cheese had similar LT_{max} and higher LT=1 compared to HL cheese indicating a comparable cheese meltability; however, the cheese required more thermal energy to soften indicated by a higher temperature at which LT=1.

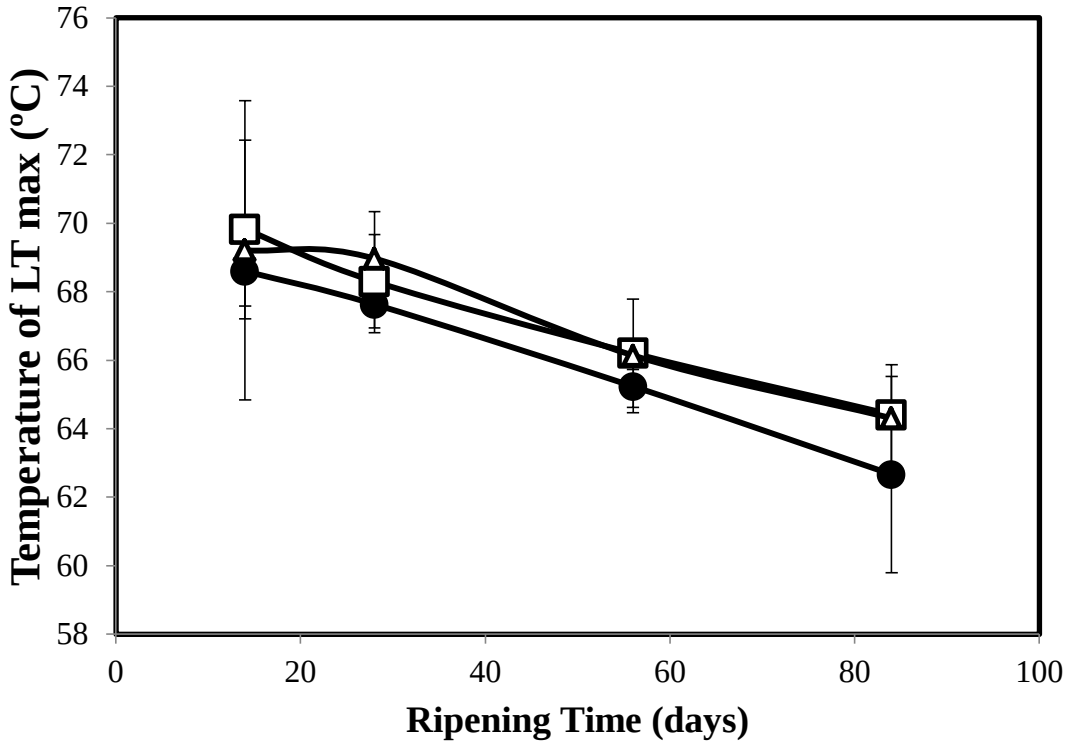


Figure 4.8 Temperature where the loss tangent maximum occurs as a function of ripening time of Mozzarella cheese made with varying levels of lactose. Cheeses were control (HL; ●), medium (ML; □) and low (LL; △) lactose. Values are means of four replicates; error bars indicate ± one standard deviation.

4.3.9 Sensory analysis

The different sensory attributes of unmelted cheese that were significant ($P < 0.05$) for treatment were firmness, adhesiveness of mass and acid; all sensory properties measured were significantly different ($P < 0.05$) for age (Table 4.8). The effects of treatments on the unmelted sensory attributes as a function of ripening time are shown in Table 4.9. HL cheese was significantly ($P < 0.05$) lower in firmness as measured by the sensory panel than ML and LL cheeses at all ripening times. The firmness of all cheese treatments decreased significantly ($P < 0.05$) with age as ripening progressed; these results are in agreement with hardness as measured by texture profile analysis (Figure 4.5).

There was no significant ($P > 0.05$) difference between any of the treatments for adhesiveness of mass at d 14 and 28 of ripening. At d 56 of ripening HL cheese had a significantly ($P < 0.05$) higher adhesiveness of mass than LL cheese and at d 84 this was significantly higher than both ML and LL cheese.

The adhesiveness of mass of all cheese treatments increased significantly ($P < 0.05$) during ripening. Firmness and adhesiveness of mass will typically decrease and increase, respectively during the ripening of Mozzarella cheese (Moynihan et al., 2014).

At d 14 HL cheese had a significantly ($P < 0.05$) higher acid flavour than both ML and LL cheese. At d 56 HL and ML cheese had a significantly ($P < 0.05$) higher acid flavour than LL cheese. The salt flavour of ML cheese was significantly ($P < 0.05$) higher than that of HL and LL cheese at d 14 of ripening but no other differences were observed for any treatment through ripening. The butter flavour intensity of ML cheese was significantly ($P < 0.05$) higher at d 14 and 28 of ripening but again no differences were observed between treatments later in ripening. The lower acid flavour observed in ML and LL cheese may have been due to the reduced lactose level leading to reduced levels of lactic acid in the cheese. Shakeel-Ur-Rehman et al. (2004) found that low lactose Cheddar cheese had a low acid flavour. Hou et al. (2014a) found that curd washing in Cheddar which reduced lactose and lactic acid levels resulted in cheeses that were characterised as less acid, more buttery, creamier, sweeter and saltier than non-curd washed varieties. Lower levels of lactose and lactic acid probably contributed to the differences in flavour of HL, ML and LL cheeses.

Table 4.8 Mean squares and probabilities (in parentheses), and R^2 values for sensory properties of unmelted and melted cheese during ripening of Mozzarella cheese manufactured with different lactose:casein ratios.

	Treatment (T)	Age (A)	A × T	Error	R^2
Firmness	8.850** (0.0058)	8.638** (<0.0001)	0.304 (0.0520)	0.125	0.94
Adhesiveness of Mass	3.152**(0.0021)	5.597** (<0.0001)	0.365 (0.1905)	0.231	0.82
Acid	0.789* (0.0359)	0.299* (0.142)	0.127 (0.1368)	0.071	0.71
Salt	0.439 (0.2350)	0.889** (0.0042)	0.205 (0.2975)	0.160	0.62
Butter	1.554 (0.0996)	2.394** (<0.0001)	0.706* (0.0103)	0.199	0.78
Oxidation	0.248 (0.4538)	3.569** (0.0002)	0.475 (0.3120)	0.380	0.62
Blister Colour	77.45** (<0.0001)	3.024* (0.0187)	0.931 (0.3300)	0.767	0.89
Blister Quantity	6.37* (0.0243)	19.96** (<0.0001)	1.797 (0.0391)	0.685	0.84
Cohesiveness of Mass	2.077 (0.1769)	20.61** (<0.0001)	0.778 (0.1710)	0.47	0.86
Chewiness	0.5698 (0.2048)	0.471 (0.0735)	0.585* (0.0163)	0.182	0.64
Hardness	0.285 (0.3998)	6.62** (<0.0001)	0.778 (0.1710)	0.47	0.86
Acid	1.719** (0.0011)	0.177 (0.1232)	0.047 (0.7602)	0.084	0.70
Strand Length	84.491** (0.0054)	158.02** (<0.0001)	4.544 (0.6742)	6.77	0.80

*$0.01 < P \leq 0.05$

**$P \leq 0.01$

Table 4.9 Sensory properties of unmelted Mozzarella cheese manufactured with milk with varying levels of lactose, cheeses were control (HL), medium (ML) and low (LL) lactose at d 14, 28, 56 and 84 of ripening. Values represent the means and standard deviations, with the latter in parentheses (n=4).

Attribute	Ripening Time (d)	Treatment		
		HL	ML	LL
Firmness	14	$8.62^{a,A}$ (0.75)	$9.95^{b,A}$ (0.22)	$9.70^{b,A}$ (0.50)
	28	$8.53^{a,A}$ (0.89)	$9.84^{c,A}$ (0.60)	$9.17^{b,B}$ (0.47)
	56	$7.77^{a,B}$ (0.65)	$9.14^{b,B}$ (0.45)	$8.72^{b,BC}$ (0.27)
	84	$6.37^{a,C}$ (0.88)	$7.95^{b,C}$ (0.34)	$8.25^{b,C}$ (0.19)
Adhesiveness of Mass	14	$2.25^{a,A}$ (0.41)	$1.60^{a,A}$ (0.19)	$1.95^{a,A}$ (0.19)
	28	$2.47^{a,A}$ (0.68)	$2.04^{a,AB}$ (0.09)	$1.78^{a,A}$ (0.22)
	56	$2.80^{b,A}$ (0.21)	$2.36^{ab,BC}$ (0.27)	$2.04^{a,A}$ (0.40)
	84	$4.41^{b,B}$ (0.77)	$2.94^{a,C}$ (0.77)	$3.01^{a,B}$ (0.75)
Acid Flavour Intensity	14	$5.34^{b,A}$ (0.36)	$4.85^{a,A}$ (0.18)	$4.78^{a,A}$ (0.28)
	28	$5.30^{a,A}$ (0.55)	$5.21^{a,AB}$ (0.35)	$5.17^{a,B}$ (0.34)
	56	$5.53^{b,A}$ (0.22)	$5.48^{b,B}$ (0.21)	$4.82^{a,AB}$ (0.19)
	84	$5.17^{a,A}$ (0.32)	$4.96^{a,A}$ (0.15)	$4.80^{a,AB}$ (0.30)
Salt Flavour Intensity	14	$4.72^{a,AB}$ (0.56)	$5.42^{b,A}$ (0.73)	$4.62^{a,A}$ (0.41)
	28	$5.21^{a,A}$ (0.31)	$5.40^{a,A}$ (0.36)	$5.48^{a,B}$ (0.35)
	56	$5.09^{a,AB}$ (0.34)	$5.20^{a,A}$ (0.19)	$5.33^{a,B}$ (0.30)
	84	$4.53^{a,B}$ (0.31)	$4.85^{a,A}$ (0.61)	$4.91^{a,AB}$ (0.36)
Butter Flavour Intensity	14	$3.53^{a,A}$ (0.51)	$4.24^{b,A}$ (0.84)	$3.44^{a,A}$ (0.26)
	28	$3.70^{a,A}$ (0.86)	$4.94^{b,B}$ (0.81)	$3.39^{a,A}$ (0.30)
	56	$4.63^{a,B}$ (0.52)	$4.71^{a,AB}$ (0.13)	$5.00^{a,B}$ (0.41)
	84	$4.14^{a,AB}$ (0.31)	$4.33^{a,AB}$ (0.31)	$4.32^{a,C}$ (0.41)

^{a,,b,c}Means within the same row not sharing a common superscript differ ($P < 0.05$).

^{A,B,C}Means within the same column not sharing a common superscript differ ($P < 0.05$).

The sensory attributes of melted cheese that were significant ($P < 0.05$) for treatment and age are shown in Table 4.8. The effects of treatment on melted cheese sensory attributes as a function of ripening time are shown in Table 4.10. At each stage of ripening LL cheese had significantly ($P < 0.05$) lower

blister colour than HL and ML cheeses. ML cheese had significantly ($P < 0.05$) lower blister colour than HL cheese after d 14 of ripening. No change in blister colour was observed for HL and LL cheese during ripening but ML cheese decreased significantly ($P < 0.05$) in blister colour during the ripening time. No significant ($P > 0.05$) differences existed between treatments for blister quantity except at d 56 where the HL cheese had significantly ($P < 0.05$) higher blister quantity than the ML and LL cheeses. The blister quantity increased for all treatments during ripening. Johnson and Olson (1985) found that galactose which is a by-product of starter culture metabolism was correlated with browning of Mozzarella cheese. Lactose is broken down into glucose and galactose by starter cultures therefore the lower levels of lactose and galactose (Table 4.4) in the ultrafiltered treatments could have led to the lower blister colour observed in LL and ML cheese. Ma et al. (2013) found that Mozzarella cheeses with similar galactose content had similar browning properties. The higher blister quantity observed in HL cheese at d 56 of ripening may relate to the lower temperature at which LT=1 (Figure 4.7); if the softening point temperature is lower the cheese melts at a lower temperature and has a longer time to flow and form blisters on pizza (Ma et al., 2013). The significant ($P < 0.05$) decrease in LT=1 is probably also related to the significant ($P < 0.05$) increase of blister quantity during ripening.

No clear trend was observed between treatments for the cohesiveness of mass of melted cheese except that LL cheese had significantly ($P < 0.05$) lower cohesiveness of mass than HL and ML cheese at d 84 of ripening. There was a significant ($P < 0.05$) increase in the cohesiveness of mass during ripening for all treatments. At d 14 and 56 of ripening chewiness for ML cheese was

significantly ($P < 0.05$) higher and lower, respectively, compared to LL cheese, at d 84 of ripening the ML cheese was significantly ($P < 0.05$) chewier than HL and LL cheeses. It is unclear why ML melted cheese was chewier than LL cheese. At d 28 and 84 of ripening LL cheese was significantly ($P < 0.05$) harder than HL cheese. Hardness decreased significantly ($P < 0.05$) for all cheese treatments during ripening. Cohesiveness of mass generally increases and hardness decreases during ripening for melted Mozzarella cheese (Moynihan et al., 2014). The LL treatment was probably less cohesive and harder as this cheese appeared to be firmer during ripening as measure by TPA (Figure 4.5).

At d 14 of ripening ML and LL cheeses had significantly ($P < 0.05$) lower acid flavour intensity than HL cheese. LL cheese had significantly ($P < 0.05$) lower acid flavour than HL cheese throughout all of ripening. There was no significant ($P > 0.05$) change in the acid flavour intensity of HL and ML cheese throughout ripening, d 14 and 56 had significantly ($P < 0.05$) lower acid flavour for LL cheese. Like the unmelted cheese the lower acid flavours observed in melted ML and LL cheese was probably due to the lower levels of lactose and lactic acid (Shakeel-Ur-Rehman et al., 2004; Hou et al., 2014a). LL cheese had significantly ($P < 0.05$) lower strand length than HL throughout ripening. The strand length of all treatments increased significantly ($P < 0.05$) throughout ripening. Strand length relates to the ability of the cheese to stretch. The increase in strand length during ripening is probably due to proteolysis and solubilisation of calcium phosphate which reduces protein crosslinking (Lucey et al., 2003). It also indicates that proteolysis was not extensive enough to reduce the level of intact casein molecules that are required for the stretch

characteristics of cheese. The length of stretch typically increases in Mozzarella cheese during the first few weeks of ripening after which it can reduce if the cheese becomes soupy (Lucey, 2008); however, no decrease was observed in any treatment.

Table 4.10 Sensory properties of melted Mozzarella cheese on pizza manufactured with milk with varying levels of lactose, cheeses were control (HL), medium (ML) and low (LL) lactose at d 14, 28, 56 and 84 of ripening. Values represent the means and standard deviations, with the latter in parentheses (n=4).

Attribute	Ripening Time (d)	Treatment		
		HL	ML	LL
Blister Colour	14	$12.65^{b,A}$ (0.92)	$11.55^{b,A}$ (0.41)	$8.84^{a,A}$ (0.79)
	28	$12.94^{c,A}$ (0.42)	$11.10^{b,AB}$ (0.16)	$8.38^{a,A}$ (0.74)
	56	$12.79^{c,A}$ (0.45)	$10.20^{b,BC}$ (0.70)	$7.95^{a,A}$ (0.37)
	84	$12.39^{c,A}$ (1.31)	$9.30^{b,C}$ (1.58)	$8.00^{a,A}$ (0.41)
Blister Quantity	14	$7.26^{a,A}$ (1.52)	$7.72^{a,AB}$ (0.84)	$7.18^{a,A}$ (0.47)
	28	$7.83^{a,A}$ (1.68)	$6.92^{a,A}$ (0.20)	$6.78^{a,A}$ (0.48)
	56	$10.75^{b,B}$ (1.23)	$8.53^{a,B}$ (0.75)	$7.88^{a,A}$ (0.06)
	84	$10.42^{a,B}$ (0.33)	$9.53^{a,B}$ (0.79)	$9.55^{a,B}$ (0.57)
Cohesivenes s of Mass	14	$7.10^{ab,A}$ (1.40)	$7.29^{b,A}$ (0.26)	$6.23^{a,A}$ (0.72)
	28	$7.46^{a,A}$ (0.55)	$7.59^{a,AB}$ (0.50)	$6.94^{a,A}$ (0.88)
	56	$7.91^{a,A}$ (0.34)	$8.43^{a,B}$ (0.71)	$8.59^{a,B}$ (0.45)
	84	$10.29^{b,B}$ (1.10)	$10.22^{b,C}$ (0.90)	$8.99^{a,B}$ (0.67)
Chewiness	14	$7.09^{ab,A}$ (0.16)	$6.63^{a,A}$ (0.42)	$7.32^{b,A}$ (0.49)
	28	$6.73^{a,A}$ (0.31)	$7.13^{a,AB}$ (0.48)	$6.81^{a,AB}$ (0.41)
	56	$6.79^{ab,A}$ (0.27)	$7.31^{b,BC}$ (0.71)	$6.54^{a,B}$ (0.35)
	84	$6.93^{a,A}$ (0.56)	$7.89^{b,C}$ (0.69)	$7.10^{a,AB}$ (0.34)
Hardness	14	$4.22^{a,A}$ (0.49)	$4.20^{a,A}$ (0.23)	$4.62^{a,A}$ (0.29)
	28	$3.76^{a,B}$ (0.53)	$3.90^{ab,A}$ (0.27)	$4.21^{b,A}$ (0.25)
	56	$3.38^{a,B}$ (0.42)	$3.43^{a,B}$ (0.26)	$3.08^{a,B}$ (0.54)
	84	$2.40^{a,C}$ (0.52)	$2.66^{ab,C}$ (0.06)	$2.93^{b,B}$ (0.32)
Acid Flavour Intensity	14	$4.91^{b,A}$ (0.32)	$4.75^{a,A}$ (0.39)	$4.06^{a,A}$ (0.11)
	28	$5.05^{b,A}$ (0.18)	$4.73^{ab,A}$ (0.19)	$4.40^{a,AB}$ (0.42)
	56	$5.15^{b,A}$ (0.36)	$4.82^{ab,A}$ (0.19)	$4.59^{a,B}$ (0.29)
	84	$4.86^{b,A}$ (0.33)	$4.74^{ab,A}$ (0.35)	$4.32^{a,AB}$ (0.29)
Strand Length	14	$12.94^{b,A}$ (1.73)	$10.22^{ab,A}$ (1.56)	$7.44^{a,A}$ (1.14)
	28	$14.25^{b,A}$ (3.53)	$12.58^{ab,A}$ (1.29)	$10.13^{a,A}$ (3.38)
	56	$19.13^{b,B}$ (2.93)	$17.42^{ab,B}$ (4.50)	$15.31^{a,B}$ (3.34)
	84	$18.35^{b,B}$ (2.14)	$19.88^{b,B}$ (2.19)	$14.08^{a,B}$ (2.25)

^{a,,b,c}Means within the same row not sharing a common superscript differ ($P < 0.05$).

^{A,B,C}Means within the same column not sharing a common superscript differ ($P < 0.05$).

4.4 Conclusion

Cheeses made with milk of low lactose:casein ratio had lower levels of lactose, galactose and lactic acid as well as lower levels of insoluble calcium which led to increased pH during ripening. There were some compositional differences between treatments; the cheeses made from ultrafiltered milk had lower fat and higher protein levels. The LL cheese exhibited greater solubilisation of insoluble calcium phosphate during ripening, possibly caused by the processing method of adding RO water to ultrafiltered retentate. Soluble calcium would be lost to the permeate during the ultrafiltration process and the addition of RO water (containing no calcium) would further dilute the amount of serum calcium. Consequently it is possible that calcium leached from the casein to the serum in the cheese to establish an equilibrium. Part of the higher pH observed in the LL cheese was caused by this equilibrium shift and greater solubilisation of calcium phosphate bound to casein. This could be avoided if serum obtained from nanofiltration of the ultrafiltration permeate was used which would maintain serum calcium balance of the original milk There was no effect of treatment on proteolysis as measured by pH4.6-SN/TN.

The functional properties of the cheeses were affected by using ultrafiltration to vary the lactose:casein ratio of cheesemilk. Low lactose:casein ratio cheesemilk led to Mozzarella that was harder and chewier throughout ripening. These cheeses also had differences in the LT_{max} and LT=1. The LL cheese treatment had a low LT_{max} at the beginning of ripening but after d 28 there was no difference in the LT_{max} between any of the treatments. The LT=1 was significantly higher for ML and LL cheese after d 28 of ripening. This showed that ultrafiltration can be used for the manufacture of Mozzarella to produce a

cheese that is both firmer throughout ripening and melts the same as the control after d 28 ripening as indicated by the LT_{max}. This would be highly beneficial as Mozzarella cheese typically has a short shelf life which is related to the cheese becoming excessively soft and soupy. If the hard texture could be maintained with an appropriate functional melt this may increase the shelf life of Mozzarella in addition to its ability to shred.

The sensory properties of unmelted and melted cheese also changed when ultrafiltration was used to vary the lactose:casein ratio of cheesemilk. Ultrafiltration can be utilised to develop Mozzarella that has different properties such as reduced acid flavour, increased hardness and lower blister colour on baking in applications such as pizza where browning may cause issues. In conclusion, using ultrafiltration to standardise the lactose:casein ratio of cheesemilk can offer cheesemakers a way to alter the texture, flavour and functional properties as well as offering the potential to reduce pH variability of low-moisture part-skim Mozzarella.

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CHAPTER 5

Impact of varying ratios of β:α_{s1}-casein on the texture, flavour and functionality of Cheddar cheese during ripening

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Abstract

The objective of this study was to vary the ratio of β:α_{s1}-casein in cheesemilk and determine the texture, flavour and functional properties of Cheddar cheese made from these modified milks. It was hypothesised that cheese made from milk that had a lower proportion of β-casein would be more meltable and develop less bitterness during ripening. Skim milk was processed using cold microfiltration (< 4°C) to produce a milk protein concentrate with a lower proportion of β-casein (LMPC) than a control MPC (TMPC or CMPC). The β-casein removed during the production of low β-casein LMPC was added to another skim milk and processed through warm microfiltration (23°C) to make a MPC with a higher proportion of β-casein (HMPC). Varying the proportion of β-casein in rehydrated MPC solutions at the same casein concentration had an impact on the rheological properties of rennet-induced gels. Gels made with LMPC were stiffer and had a more elastic-like character; the G' values at 60 minutes after rennet addition were 72.9, 63.3 and 54.4 Pa for LMPC, TMPC and HMPC, respectively. Powders were rehydrated in water by mixing overnight and standardized (casein:fat 0.65 and lactose 4.50%) and Cheddar cheese was made from this rehydrated milk using a typical Cheddar manufacturing protocol. Composition, pH, proteolysis, insoluble calcium, texture, rheological and flavour properties of the experimental cheeses were assessed during ripening. Some compositional parameters were different between treatments; LMPC cheese had significantly ($P < 0.05$) lower protein and CMPC cheese significantly ($P < 0.05$) lower FDM. No significant difference was observed for proteolysis as indexed by pH 4.6-soluble nitrogen between cheese treatments. Cheese manufactured with HMPC was less

meltable (lower maximum loss tangent) at days 14, 28 and 84 of ripening. After d 14 of ripening, cheeses made from HMPC and LMPC had higher and lower temperature at which the maximum loss tangent occurred, respectively, compared to CMPC cheese. At all the ripening times evaluated, HMPC cheese had a significantly higher temperature value of loss tangent = 1 (indicating the softening point) than CMPC and LMPC cheese. Throughout ripening, LMPC cheese had lower hardness and chewiness compared to CMPC and HMPC cheeses. No differences between treatments were observed for the flavour attributes assessed by a trained descriptive panel at 84 and 168 d of ripening. Cheddar cheese made from milk with a higher proportion of β-casein was less meltable and firmer but not significantly different in bitterness compared to cheeses made from milk with a typical proportion of β-casein.

5.1 Introduction

β-Casein is one of the principal caseins present in milk and is quite hydrophobic (Fox et al., 2000), containing hydrophilic N-terminal and hydrophobic C-terminal regions (O'Connell et al., 2003). It is well known that β-casein dissociates from the micelle into the serum phase of milk after storage at low temperatures (Downey and Murphy, 1970; Creamer and Berry, 1974) probably due to hydrophobic bonds weakening at low temperatures (Davies and Law, 1983). This property of β-casein has been exploited in numerous studies in an attempt to isolate the protein (Pouliot et al., 1994; Ward and Bastian, 1996; Renner-Nantz and Shoemaker, 1999; Huppertz et al., 2006). Cold membrane filtration of milk has been used as a method to vary the ratio of α_s:β-casein (Van Hekken and Holsinger, 2000; O'Mahony et al., 2007; Holland et al., 2011; Seibel et al., 2015). The dissociation of β-casein from the micelle at low temperatures is reversible on rewarming of milk (Downey and Murphy, 1970; Creamer and Berry, 1974; Davies and Law, 1982).

The types of bonds interacting between caseins in the protein matrix of cheese affect its rheological properties (Lucey et al., 2003). It has been suggested that β-casein plays an important role in the hardening of curd through increased hydrophobic interactions (Yun et al., 1982). Cheese meltability has been more highly correlated with hydrolysis of β-casein than α_s-casein; hence, cheeses with greater β-casein hydrolysis may have increased meltability (Bogenrief and Olson, 1995; Dave et al., 2003). Lowering the level of β-casein in a directly-acidified fat-free cheese system (where proteolysis was minimal) also influenced the rheological properties of the cheese (O'Mahony et al., 2008). Cheese with higher ratio of α_{s1}:β-casein (reduced β-casein proportion) had

greater melt properties and was less firm than cheese made with typical α_{s1}:β-casein levels (O'Mahony et al., 2008). St-Gelais and Haché (2005) found that miniature cheese enriched with β-casein was harder but composition (increased protein and decreased moisture) of these cheeses varied which may have caused confounding effects.

Bitterness is a relatively common defect in cheese usually caused by the accumulation of hydrophobic peptides (Fox et al., 2000). Greater levels of bitterness have been perceived in cheese that had higher levels of β-casein hydrolysis (Bogenrief and Olson, 1995). Hydrophobic peptides generated from hydrolysis of β-casein have been linked to the perception of bitterness in cheese (Jacob et al., 2011). Bansal et al. (2009) found that the peptide β-casein (f1-189/192) was absent from cheese manufactured with camel chymosin, this cheese was also less bitter as the hydrophobic C-terminal bitter peptide β-casein (f193-209) was not released.

The objective of this study was to use cold microfiltration (<4°C) of milk to manufacture powders with decreased level of β-casein based on the method of O'Mahony et al. (2007). β-Casein removed during this process was then added to another batch of skim milk, rewarmed to reassociate the β-casein with the micelle, and processed through warm microfiltration (23°C) to increase the level of β-casein. Cheddar cheese was made with the reconstituted powders to determine the impact of β-casein proportion in cheesemilk on the functional and rheological properties of cheese during ripening. I hypothesised that varying the levels of β-casein in Cheddar cheese may impact the meltability and have an effect on the generation of bitterness in the cheese during ripening. A starter culture strain that is associated with bitterness was used to help

evaluate the impact of varying β-casein level on the development of bitterness in cheese. I wanted to promote bitterness in the cheese by using this starter culture and to determine if the β-casein proportion of the cheese affected bitterness development. O'Mahony et al. (2008) manufactured directly-acidified fat free cheese (which had minimal proteolysis) with typical and low β-casein levels but no ripening was performed. In the current study both low and high β-casein Cheddar cheeses were manufactured and compared to a control cheese during ripening. The effect of varying the level of β-casein on flavour, functionality and rheology of Cheddar cheese during ripening was evaluated.

5.2 Materials and Methods

5.2.1 Powder Manufacture

The microfiltration unit used was custom built and contained two vessels in parallel, each containing a Synder microfiltration element (model FR3B8038, Synder Filtration, CA, USA) with 0.08 µm pore size membrane and 0.12 cm thick spacers. The ultrafiltration unit was also built with two vessels in series containing Synder ultrafiltration elements (model ST2B3838, Synder Filtration, CA, USA) with a 0.001 µm pore size and 0.079 cm thick spacers.

Four types of powders were manufactured on different days. Two control powders were manufactured on two different days; one control was manufactured and used for gelation experiments and preliminary cheese making trials (TMPC powder), the other control powder (CMPC) was used to manufacture the control cheese for ripening studies and analysis. A second control was needed as not enough powder was manufactured initially. Skim milk was pasteurised at 72°C for 19 s. This milk (907 kg) was processed from a balance tank through the microfiltration unit at room temperature (23°C). The retentate was recirculated to the balance tank and the permeate stream went through the ultrafiltration unit. Permeate from the ultrafiltration unit then returned to the retentate balance tank as a method of diafiltrating the retentates (i.e. reducing the protein content). This process continued until the retentate reached total solids of approximately 19-19.5% which was measured using Atago refractometers (model-10M and model-20M, Atago Ltd., Tokyo, Japan).

The low β-casein (LMPC) powder was manufactured by pasteurising skim milk at 72°C for 19 s and cooling to <4°C for 3 days by recirculating chilled

water through a jacketed tank to permit dissociation of β-casein from the casein micelle before processing. Milk (907 kg) was processed through a microfiltration unit at <4°C, the low β-casein retentate was held in a balance tank at ~4°C and the permeate stream was processed through the ultrafiltration unit. The permeate stream from the ultrafiltration unit was pumped back to the retentate balance tank for the microfiltration unit and used for further processing of the low β-casein retentates. The low β-casein retentate was processed until total solids of approximately 19-19.5% were reached. The ultrafiltration retentate containing the β-casein from the ultrafiltration process was maintained at <4°C in a tank for further processing.

The high β-casein (HMPC) powder was manufactured by taking the retentate from the ultrafiltration of permeate from the low temperature microfiltration of LMPC, and adding that retentate to 816 kg of pasteurised skim milk (72°C for 19 s). These were mixed and heated to 50°C for 30 min to try to re-associate the added β-casein back to the casein micelles. The milk was then cooled to 23°C and processed through the microfiltration and ultrafiltration units following the same protocol for the manufacture of the control powders (TMPC and CMPC) until the appropriate total solids of 19-19.5% was reached. All retentates were spray dried (Type PSD 55, APV, Denmark) the day after processing to produce powders for each treatment, dryer inlet and outlet temperature of 188 and 89ºC, respectively.

5.2.2 Powder Analysis

Powders were analyzed for composition after manufacture. Powders were reconstituted by stirring overnight at 4°C in distilled water. A 10% total solids

solution of powder was analyzed for fat by Mojonnier (AOAC, 2000). A 5% total solids solution of powder was analyzed for protein by Kjeldahl (AOAC, 2000), non-casein nitrogen (ISO, 2004) and non-protein nitrogen (ISO, 2001). Powder was analyzed directly for moisture (Marshall, 1992) and ash by heating in a muffle furnace at 550°C for 18 h. Total calcium levels of powders were assessed using inductively coupled plasma emission spectroscopy (Park, 2000).

5.2.3 Quantification of β:α_{s1}-casein levels in the casein micelles

This experiment was performed to determine if β-casein reassociated with the micelle during the processing of the HMPC powders. The powders, CMPC, LMPC and HMPC were reconstituted to 2.5% casein and stirred overnight at 4°C. The following day rehydrated powder solutions were batch pasteurised by heating to 63°C for 30 min to mimic the pasteurisation process prior to cheese making. Rehydrated powder solutions were ultracentrifuged at 100,000 *g* for 1 h at 20°C (Beckman Optima LE-80K Ultracentrifuge, Beckman Instruments Inc., IN, US). The rehydrated solutions and supernatant was analyzed for protein by Kjeldahl (AOAC, 2000). Urea-polyacrylamide gel electrophoresis (urea-PAGE) was then performed according to Andrews (1983) with modifications (Shalabi and Fox, 1987) to determine the level of β:α_{s1}-casein of each sample. Samples were run through the stacking gel at 280 V and separating gel at 300 V. Gels were stained using Coomassie Brilliant Blue G250 (Blaksely and Boezi, 1977) and destained in water. Densitometry was

performed using TotalLab Quant v12.2 (TotalLab Ltd., Newcastle, UK) to quantify the amount of β-casein and α_{s1}-casein.

5.2.4 Rheological Properties of Rehydrated Powder during Rennet Coagulation

The rheological properties of each treatment TMPC, LMPC and HMPC was determined using dynamic low amplitude oscillatory rheometry as described previously by Govindasamy-Lucey et al. (2005). A rheometer (MRC 301, Anton Paar GmbH, Graz, Austria) was used to determine the rheological properties of the gels during renneting using an oscillation test at 32ºC with a strain of 1% and a frequency of 0.1 Hz. The measurement geometry used was a concentric cylinder (CC27/T200/SS). Powders were rehydrated in deionized water overnight at 4°C to 2.5% casein, and the pH of each solution was adjusted to pH 6.5. Rehydrated milk was held at 32°C for 30 min in a water-bath before rennet addition. Calcium chloride was added at a level of 0.005% (v/v); fermentation-produced calf chymosin (CHY-MAX Extra, 630 international milk clotting units/ mL; Chr. Hansen Inc., Milwaukee, WI, US) was diluted 1:10 with water and added at a level of 0.009% (w/w) of milk. The renneted milk was placed in the cup of the rheometer and the test was started 2 min after rennet addition. Readings were taken at one min intervals up to 60 min (after rennet addition). The storage modulus (G') and loss tangent (LT) were measured.

Large deformation properties of the gels were studied to evaluate their resistance to cutting. Apparent yield stress and strain of the gels was determined using a constant shear rate of 0.01 s^{-1} for 500 s. The point where

the shear stress started to decrease was defined as the yield point of the gel (Lucey, 2002).

5.2.5 Milk Standardisation for Cheese Manufacture

Each powder was rehydrated overnight at 4°C to a casein level of 2.7%. The following day samples were taken from each rehydrated solution and analyzed for fat, protein measured as total nitrogen, non-casein nitrogen and non-protein nitrogen as described above. Lactose content was also analyzed (AOAC, 2000). Rehydrated milk powder solutions were then standardised using milk permeate (Prolacta, Sorrento Lactalis, Inc., Nampa, ID, US) and sweet cream (1.47% casein; 30.85% fat). Milks were standardised to a target casein:fat 0.65, casein content of 2.50% and lactose content of 4.50%.

5.2.6 Cheese manufacture

Six vats of Cheddar cheese was manufactured in the dairy plant at the University of Wisconsin-Madison on two separate days. Each day two vats of each treatment was manufactured. Milk (113 kg) from each treatment was batch pasteurised (63°C for 30 min) in the cheese vat and cooled to 32°C. Mesophilic starter culture Cargill Mac71 (Cargill Texturizing Solutions, Waukesha, WI, US) containing *Lactococcus lactis* subsp. *lactis*; was added at a level of 26.9 g/ 100 kg of milk. After ripening for 45 min, fermentation-produced calf chymosin (CHY-MAX Extra) was added at a level of 8.2 mL/100 kg of milk. Coagula were cut subjectively based on firmness as determined by the cheesemaker. The temperature of the vats was raised to 38°C over 30 min. Each vat was cooked at this temperature until the pH

reached 6.10 (approx. 15 min), then the curd was trenched and whey drained. Curd was milled at pH 5.40 and salted at a level of 2.3% (w/w). Curd was pressed for 4 hr at 4.14 bar, and cheese was vacuum-packed and stored at 7°C for 168 d.

5.2.7 Cheese composition, pH, insoluble calcium and electrophoresis

Cheese at d 14 was analyzed for moisture (Marshall, 1992), fat by Mojonnier (AOAC, 2000), protein by Kjeldahl (AOAC, 2000) and salt by chloride electrode method (MK II Chloride analyser 926; Nelson and Jameson Inc., Marshfield, WI, US; Johnson and Olson, 1985). Total calcium levels were measured in milk, rennet whey and cheese (d 14) using inductively coupled plasma emission spectroscopy (Park, 2000). Cheese pH was measured using a spear tip pH probe (accuCap Capillary Junction pH combination electrode 13-620-133; Fisher Scientific, Itasca, IL, US) inserted directly into the cheese. Insoluble Ca content was measured by the acid-base titration method (Hassan et al., 2004) at d 4, 14 and 28 of ripening.

Urea-polyacrylamide gel electrophoresis (urea-PAGE) was performed according to Andrews (1983) with modifications (Shalabi and Fox, 1987) as previously described. Cheese was tested after 4 d of ripening to determine initial levels of β-casein and α_{s1}-casein. Densitometry was performed using TotalLab Quant v12.2 (TotalLab Ltd., Newcastle, UK) to assess total β-casein (including breakdown products) and α_{s1}-casein (f102-199 and f24-199) to determine the level of β:α_{s1}-casein level in the cheese.

5.2.8 Proteolysis

Proteolysis was determined on cheese aged for d 4, 14, 28 and 84 of ripening by measuring levels of pH 4.6-soluble nitrogen (Kuchroo and Fox, 1982) and the nitrogen content determined by (AOAC, 2000). Urea-PAGE was also performed on cheeses for d 4, 14, 84 and 168 of ripening according to Andrews (1983), as previously described, to determine the rate of casein hydrolysation during ripening.

5.2.9 Dynamic small-amplitude oscillatory rheology

Samples were prepared by slicing cheese on a Hobart slicer to ~2.3 mm and cut into 50 mm diameter discs. These samples were stored in a refrigerator at 4°C at least 8 h before analysis. Rheological properties of cheese were assessed using a Paar Physica Universal Dynamic Spectrometer (UDS 200; Physica Messtechnik, Stuttgart, Germany). Samples were heated from 5-85°C at 1°C min^{-1} using a 50 mm serrated parallel plate and subjected to a strain of 0.5% at a frequency of 0.08 Hz. The measured parameters during heating were G', loss modulus (G") and LT. The following parameters; maximum LT value (LT_{max}), the temperature where the LT_{max} occurred and the temperature where the LT equals to 1 (LT=1) were also determined.

5.2.10 Texture profile analysis

Cheese were cut into cylindrical samples (16 mm diameter, 17.5 mm height) using a Hobart slicer and steel cork borer. Samples were stored overnight at

4°C until analysis. Texture profile analysis (TPA) was performed using a Texture Analyzer TA-XT2 (Stable Micro Systems, Godalming, Surrey, UK). TPA was performed by compressing samples to 80% of its original height; chewiness and hardness were calculated as described by Bourne (1978).

5.2.11 Sensory Analysis

A trained sensory panel consisting of at least 12 panellists used Spectrum™ descriptive sensory analysis (Meilgaard et al., 1999) to evaluate flavour attributes of the cheese, was analysed at d 84 and 168 of ripening. Cheeses were cut into cubes and tempered at ~12°C before assessment of flavour attributes. Flavour attributes evaluated were acid, astringent, bitter, milkfat, salt, sulphur and sweet. The numerical intensity scale ranged from 0-15 with reference points, 15 signified a greater intensity of the flavour attribute. Each cheese was designated with a random 3-digit code and assessed on 2 different days.

5.2.12 Experimental Design and Statistical Analysis

Three treatments were used to manufacture Cheddar cheese, in duplicate on each cheesemaking day; each cheesemaking trial was performed on two different days. A 3 × 4 completely randomized unblocked design was used for analysis of the response variables relating to milk and cheese composition. Analysis of variance was carried out using the PROC GLM procedure of SAS (version 9.1; SAS Institute, 2002-2003). Duncan's multiple-comparison test was used to evaluate differences in the treatments and differences between

means for cheese composition and coagulation properties of milk were considered significant at $P < 0.05$.

The effects of treatment and ripening time and their interactions on pH, insoluble calcium, proteolysis, functional, textural and sensory properties were evaluated using the MIXED procedure for repeated measurement with the SAS software package (SAS Inst. Inc., Cary, NC, US). The mean square for cheese, nested within treatment was used as random error term to test treatment.

5.3 Results and Discussion

5.3.1 Powder composition

Different control powders, preliminary trial control (TMPC) and CMPC were manufactured on different days; TMPC was manufactured for use in preliminary cheese making trials and gelation experiments after which CMPC was manufactured to ensure sufficient quantities of powder for the manufacture of Cheddar cheese. Significant differences (P<0.05) were observed between the powders (Table 5.1). CMPC, LMPC and HMPC were reconstituted and standardised before cheese manufacture to account for these differences. TMPC, LMPC and HMPC were used for gelation experiments and preliminary cheesemaking trials. Variation between the compositions of powders was probably due to milk seasonality or manufacturing variables such as temperature of processing, level of diafiltration and a refractometer was used as an indicator of total solids during membrane processing.

The ratio of β:α_{s1}-casein in each powder is shown in Table 5.1. LMPC had a significantly ($P < 0.05$) lower level of β:α_{s1}-casein than the other treatments, as expected. TMPC, CMPC and HMPC did not have significantly different ($P > 0.05$) β:α_{s1}-casein levels; however, HMPC had the highest ratio and hence the greatest level of β-casein (0.82). LMPC had the lowest level of β-casein (0.64) compared to CMPC (0.76) and TMPC (0.78). Ultracentrifugation of rehydrated powders showed a significant difference in the ratio of β:α_{s1}-casein found in the LMPC supernatant compared to CMPC and HMPC. No significant difference was observed between the β:α_{s1}-casein in the CMPC and HMPC powders.

Table 5.1 Composition and rheological properties of rennet-induced milk gels of manufactured powders; control for gelation experiments and preliminary cheesemaking trials (TMPC), control for cheese manufacture (CMPC), low β-casein (LMPC) and high β-casein (HMPC). Values represent the means and standard deviation, with the latter in parentheses (n=3).

	Treatment					
	TMPC	CMPC	LMPC	HMPC	SEM	*P*-value
Powder						
Moisture (%)	3.58^{b}	2.72^{a}	4.5^{c}	4.46^{c}	0.024	<0.0001
Ash (%)	7.77^{a}	8.04^{b}	8.05^{b}	7.72^{a}	0.054	0.0040
Calcium (%)	1.98^{a}	2.29^{b}	1.99^{a}	1.96^{a}	0.052	0.0066
Fat (%)	0.36^{c}	0.21^{a}	0.26^{b}	0.37^{c}	0.005	<0.0001
True Protein (%)	62.97^{b}	61.9^{a}	63.17^{b}	68.48^{c}	0.141	<0.0001
Casein (%)	58.69^{b}	58.76^{b}	58.21^{a}	63.11^{c}	0.095	<0.0001
Ca:protein (%)	3.15^{b}	3.69^{c}	3.16^{b}	2.86^{a}	0.084	0.0008
Ca:casein (%)	3.38^{ab}	3.89^{c}	3.42^{b}	3.11^{a}	0.089	0.0018
Ratio β:α_{s1} casein (Powder[1])	0.78^{b}	0.76^{b}	0.64^{a}	0.82^{b}	0.023	0.003
Ratio β:α_{s1} casein (Supernatant[2])	-	1.14^{b}	0.81^{a}	1.17^{b}	0.047	0.0027
Rheological Properties						
G' at 60 min (Pa)	63.30^{b}	-	72.90^{c}	54.35^{a}	0.676	<0.01
G'>1 (min)	7.00	-	7.00	7.00	0.000	NS
Shear stress (Pa)	55.40^{b}	-	67.50^{c}	44.55^{a}	1.475	<0.01
Shear strain[3]	1.31	-	1.30	1.24	0.040	NS
LT at 60 min	0.36^{b}	-	0.33^{a}	0.39^{c}	0.001	<0.0001

[a,b,c,d]Means within the same row not sharing a common superscript differ ($P < 0.05$).
[1]Ratio of β:α_{s1} casein in the powder as measured by urea-PAGE
[2]Ratio of β:α_{s1} casein in the ultracentrifuged supernatant as measured by urea-PAGE
[3]Shear strain at the point which the shear stress begins to decrease or the yield point of the gel

Ultracentrifugation of the rehydrated powders was carried out at 20°C to determine if the added β-casein in the HMPC treatment actually reassociated with the casein micelle. No difference was observed between CMPC and HMPC for the ratio of β:α_{s1}-casein, so it can be assumed that the added β-

casein in the HMPC sample was associated with the casein micelle and not in the serum phase (supernatant). Upon rewarming of cold milk, β-casein that had dissociated into the serum phase of milk can reassociate back with the micelle (Creamer and Berry, 1974; Davies and Law, 1983).

5.3.2 Rheological properties of rehydrated powder during rennet coagulation

The various MPC powders (TMPC, LMPC and HMPC) were rehydrated to give solutions with 2.5% casein. Differences were observed between the powder compositions for moisture, ash, fat, protein and casein (Table 5.1). LMPC and HMPC powder had significantly ($P < 0.05$) different ratios of calcium to casein; however, TMPC was not significantly ($P > 0.05$) different to either LMPC or HMPC (Table 5.1). Constant casein and calcium are important variables to maintain when trying to observe the effect of β-casein level on gelation properties (O'Mahony et al., 2009).

Gelation profiles for solutions of the rehydrated powders are shown in Figure 5.1. No significant differences ($P < 0.05$) were observed for the gelation time (G'>1 Pa) in any of the treatments (Table 5.1). Previous studies also found no significant difference in the gelation time between samples with different levels of β-casein when the total casein content was similar (O'Mahony et al., 2008; Seibel et al., 2015). At the end of gelation (60 min after rennet addition) the G' value, indicating gel stiffness (Srinivasan and Lucey, 2002), of the different treatments varied significantly ($P < 0.05$). At 60 min the G' values were 72.9, 63.8 and 54.4 Pa for LMPC, TMPC and HMPC, respectively (Table 5.1). The G' value is related to the number and strength of bonds per unit

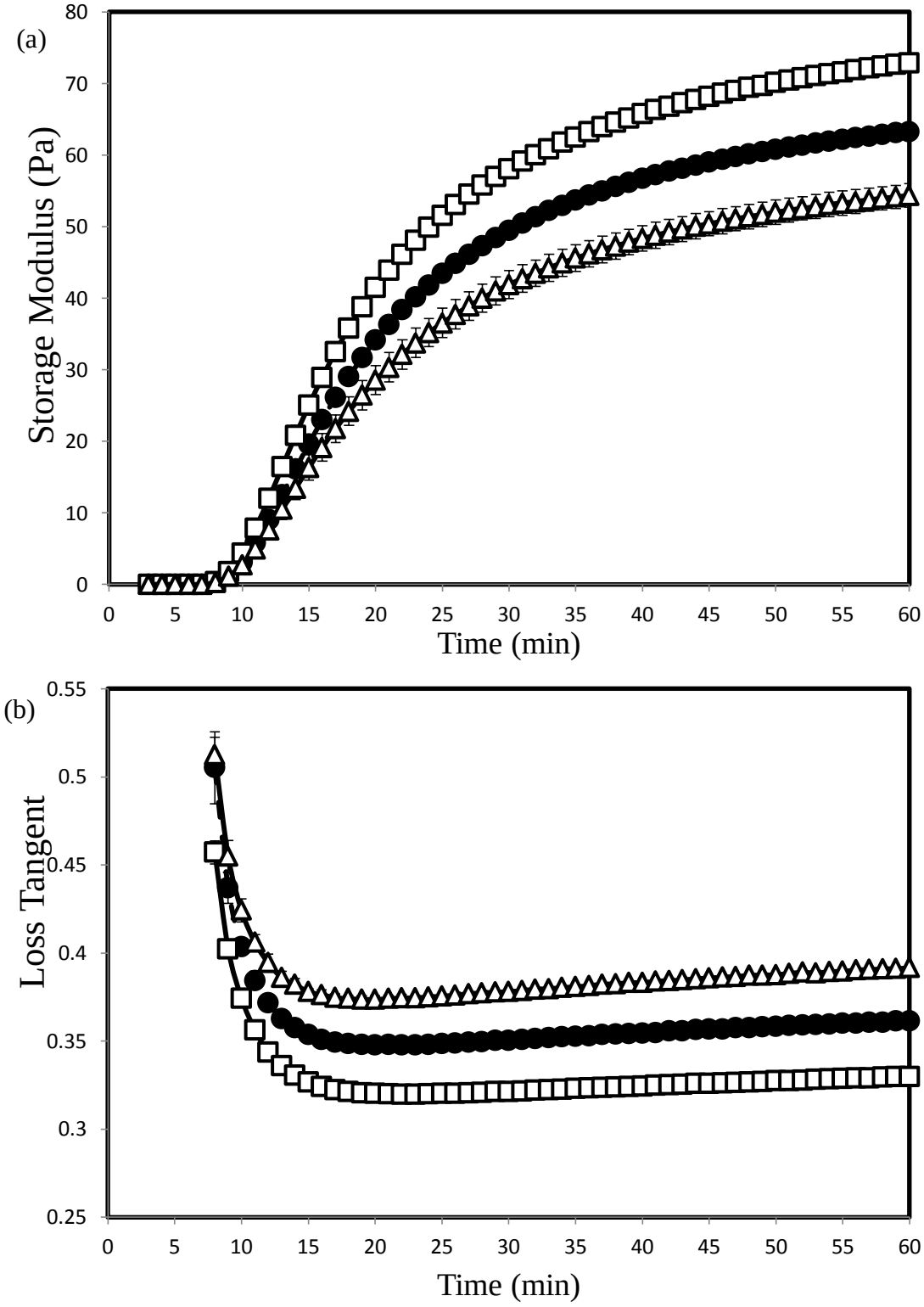


Figure 5.1 (a) Storage modulus and (b) loss tangent for rennet induced gels made with control for gelation experiments and preliminary cheesemaking trials (TMPC) (●), low β-casein (LMPC) (□) and high β-casein (HMPC) (△) powders as a function of time after rennet addition. Values are means (n=2); error bars indicate ± one standard deviation.

volume within a gel network (Lucey, 2002); different levels of β-casein could cause structural changes in the micelle. The increase in G' after the onset of gelation relates to fusion of micelles, particle rearrangements or integration of more particles into the gel network (Lucey, 2002; Mellema et al., 2002). Perhaps a reduction in β-casein reduced some of the repulsive forces involved in the aggregation of rennet altered micelles, suggesting faster aggregation and fusion of micelles leading to higher G' at 60 min.

The loss tangent values during gelation is shown in Figure 5.1b, the loss tangent decreased after the onset of gelation (relating to increases in the G' during gelation (Lucey, 2002)) and leveled off at values ranging from approximately ~0.3-0.4. The loss tangents at 60 min after renneting (Table 5.1) of the treatments were significantly different ($P < 0.05$); values were 0.33, 0.36 and 0.39 for LMPC, TMPC and HMPC, respectively. The loss tangent value is related to the relaxation behaviour of bonds (Lucey, 2002) and to the viscoelasticity characteristics of rennet-induced milk gels (Renner-Nantz and Shoemaker, 1999). The higher loss tangent values observed for HMPC and lower value for LMPC gels compared to TMPC indicate gels with more liquid- and solid-like characteristics, respectively. Loss tangent values can indicate the tendency of networks to rearrange (Srinivasan and Lucey, 2002) with greater values favouring more bond relaxation (Lucey, 2002).

The yield stress is the force needed to yield or fracture a gel network (Govindasamy-Lucey et al., 2005), it is experimentally the point where the shear stress begins to decrease during a shearing process. Significant differences ($P < 0.05$) were observed for the yield stress of all treatments (Table 5.1; Figure 5.2). Yield stress values for treatments were 67.5, 55.4 and

44.6 Pa for LMPC, TMPC and HMPC, respectively. The yield strain was not significantly different ($P > 0.05$) between treatments (Table 5.1). Yield stress and strain relate to the susceptibility of strands in the gel network to break (Lucey, 2002). Increasing the level of β-casein in cheesemilk caused a decrease in yield stress of the rennet gels relating to the lower G' at 60 min which was when the large deformation test began. The yield strain values were not significantly different for treatments indicating similar arrangements of bonds in the gel network but lower yield stress values suggest a weaker gel (Srinivasan and Lucey, 2002) for higher levels of β-casein.

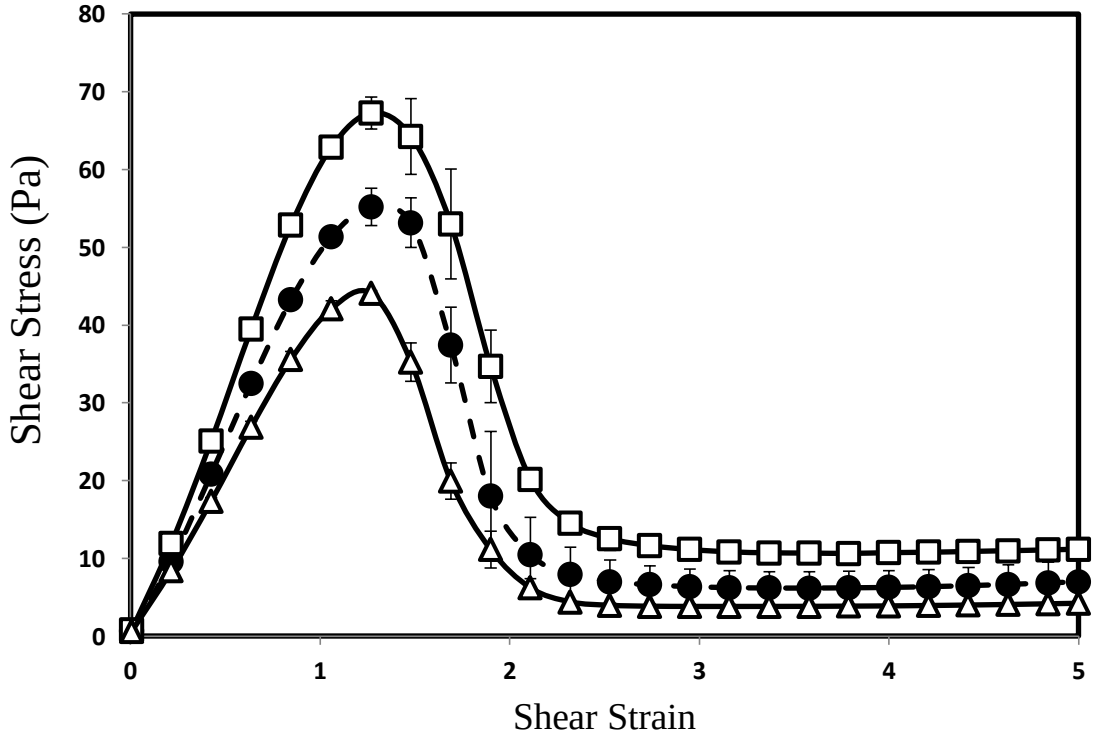


Figure 5.2 Shear stress as a function of shear strain for rennet-induced gels made with reconstituted control for gelation experiments and preliminary cheesemaking trials (TMPC) (●), low β-casein (LMPC) (□) and high β-casein (HMPC) (△) powders as a function of time after rennet addition. Values are means (n=2); error bars indicate ± one standard deviation.

Hydrophobic interactions are an important part of micelle integrity (Horne 1998; Lucey, 2002) and changing concentrations of hydrophobic β-casein could affect gelation properties (Van Hekken and Holsinger, 2000). It has been suggested that dissociation of β-casein from the micelle, or its hydrolysis, can impact on the rennet coagulation properties of milk (Renner-Nantz and Shoemaker, 1999; Bansal et al., 2007). St-Gelais and Haché (2005) found that milk enriched with β-casein had poor coagulation properties, but enriched milks had different casein and calcium contents, which could have confounded these experiments. Yun et al. (1982) found that fortification of milk with β-casein increased curd tension but it is unclear if the added β-casein was reincorporated into the micelle. Van Hekken and Holsinger (2000) used cold microfiltration and various pore-size membranes to enrich milk with β-casein; increased ratio of β:α_s-casein formed milk gels with longer rennet coagulation times and lower gel strength but these results are confounded by changes in the total casein levels. O'Mahony et al. (2009) examined the effect of α_s:β-casein ratio on the rennet coagulation properties of milk protein concentrate solutions where the total casein level was kept constant. It was found that milk gels containing lower levels of β-casein had higher G' values, lower loss tangent and higher yield stress values during renneting; these results were in agreement with those of the current study.

It is clear that the level of β-casein had an impact on the gelation properties of rennet induced gels. Reducing the level of β-casein increased gel stiffness, forming a gel with more elastic-like properties, as indicated by the higher G' value at 60 min. Also lower loss tangent and higher yield stress were observed for LMPC compared to TMPC (although these powder samples had similar

calcium to casein ratios). Increasing the level of β-casein weakened the gel properties for the HMPC sample compared to TMPC.

5.3.3 Cheese composition, pH and insoluble calcium

No significant differences ($P > 0.05$) were observed between compositional parameters of Cheddar cheese made from reconstituted powders analyzed at 14 d except for protein and FDM contents (Table 5.2). LMPC cheese had slightly lower protein content than CMPC or HMPC cheeses. CMPC cheese had lower FDM than LMPC or HMPC cheese but the FDM values were within the expected range for Cheddar cheese (Gilles and Lawrence, 1973). This did not significantly ($P > 0.05$) affect other compositional parameters of the cheese. MNFS of the cheese was not significantly ($P > 0.05$) different between treatments; this parameter is an important compositional parameter that can modify cheese functionality (Bogenrief and Olson, 1995). Lee et al. (2005) reported similar results for the composition of Cheddar cheese manufactured; however, the protein results in this study were also higher than those found for LMPC cheese.

No significant differences ($P > 0.05$) were observed for the pH values of treatments but pH significantly changed during ripening (Table 5.3; Figure 5.3a); pH decreased from d 4 to 14 probably due to fermentation of residual lactose to lactic acid (Lee et al., 2005). After d 14, the pH increased, which could be related to the formation of phosphate anions from solubilisation of CCP, which can neutralize hydrogen ions leading to an increase in pH (Lucey et al., 2003; Hassan et al., 2004). There was no significant differences ($P >$

0.05) observed between treatments for levels of insoluble calcium in Cheddar cheese (Table 5.3) made with different levels of β-casein during ripening (Figure 5.3b).

Table 5.2 Composition (d 14) and ratio of β:α_{s1}-casein (d 4) of Cheddar cheese manufactured using CMPC, LMPC and HMPC powders. Values represent the means of four replicates.

	Treatment				
	CMPC	LMPC	HMPC	SEM	*P*-value
% Moisture	38.09	39.16	38.35	0.382	NS[2]
% Fat	32.60	32.52	32.79	0.155	NS
% Salt	1.37	1.46	1.33	0.043	NS
% Protein[1]	24.12 [b]	22.97 [a]	24.34 [b]	0.166	<0.05
MNFS[3]	56.51	58.03	57.05	0.446	NS
FDM[4]	52.67[a]	53.45[b]	53.18[b]	0.121	<0.05
S/M[5]	3.60	3.73	3.46	0.133	NS
Total Ca (mg/100g)	674	686	659	7.096	NS
Ratio β:α_{s1}[6]	1.34[b]	1.21[a]	1.41[c]	0.009	<0.0001

[1]Total % N × 6.38
[2]Nonsignificant (F test for full stastistical model $P > 0.05$)
[3]Moisture in nonfat substance of the cheese
[4]Fat content on a dry weight basis
[5]Salt in moisture phase of the cheese
[a,b,c]Means within the same row not sharing a common superscript differ ($P < 0.05$)
[6]Ratio of β:α_{s1} casein at d 4 of ripening as measured by urea-PAGE

Age had a significant ($P < 0.05$) effect on the level of insoluble calcium (Table 5.3); the insoluble calcium of all cheese significantly ($P < 0.05$) decreased during the first 14 days of ripening. The level of insoluble calcium remained approximately constant for the rest of the ripening period. Most solubilisation of insoluble calcium occurs in the first few weeks of ripening (Hassan et al., 2004; O'Mahony et al., 2005). The decrease in pH from d 4 to 14 (Figure 5.3a)

Table 5.3 Mean squares and probabilities (in parentheses), and R^2 values for pH, insoluble calcium, pH4.6 SN/TN (% proteolysis), rheological properties, hardness and chewiness values as determined by texture profile analysis during ripening of Cheddar cheese manufactured with different levels of β-casein.

	pH	Insol Ca	Proteolysis	LT_{max}[1]	TLT[2]	LT=1[3]	Hardness	Chewiness
Treatment (T)	0.003	3.57	1.68	0.161**	49.5**	93.3**	161.7**	51.9**
	(0.497)	(0.841)	(0.061)	(0.0009)	(0.0016)	(<0.0001)	(0.0034)	(0.0059)
Age (A)	0.106**	208.7**	218.5**	3.53**	26.2**	68.7**	191.7**	77.2**
	(<0.0001)	(<0.0001)	(<0.0001)	(<0.0001)	(0.0082)	(<0.0001)	(<0.0001)	(<0.0001)
A x T	0.001	4.18	0.205	0.500*	4.65	1.37	3.62*	1.51*
	(0.708)	(0.657)	(0.7839)	(0.0206)	(0.5398)	(0.1208)	(0.0393)	(0.0324)
Error	0.002	6.03	0.390	0.164	5.44	0.729	1.55	0.620
R^2	0.89	0.84	0.98	0.96	0.62	0.95	0.96	0.95

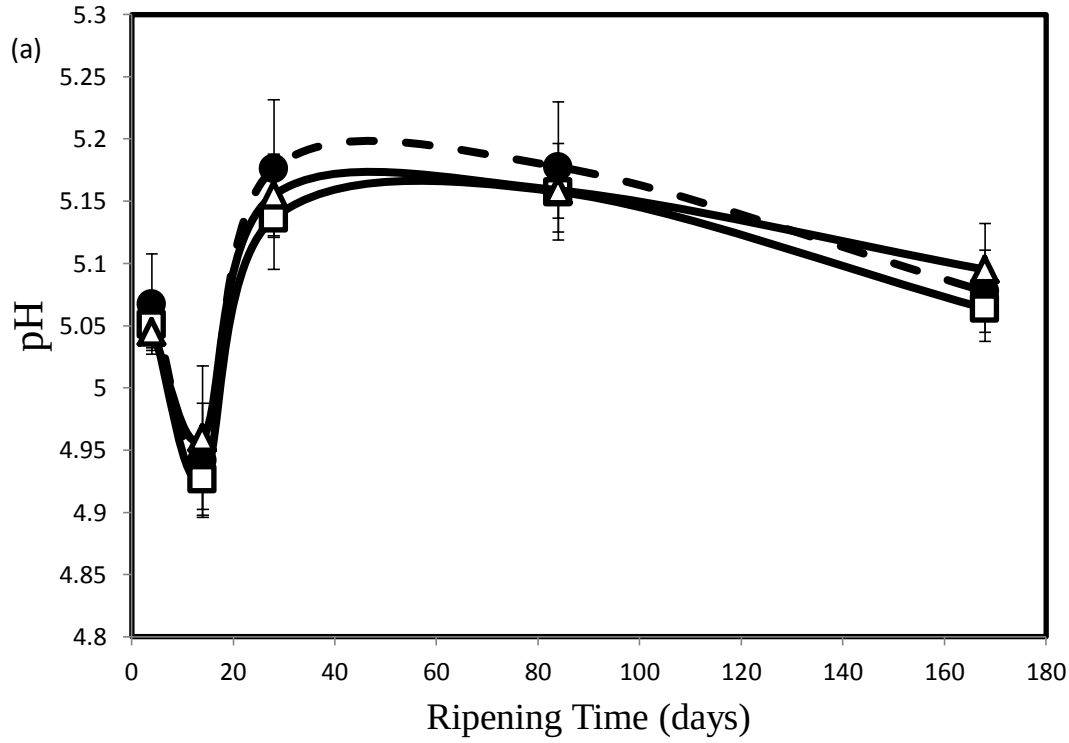
[1]Maximum loss tangent values

[2]Temperature at which loss tangent value is a maximum

[3]Temperature at which loss tangent value is = 1

*$0.01 < P \leq 0.05$

**$P \leq 0.01$



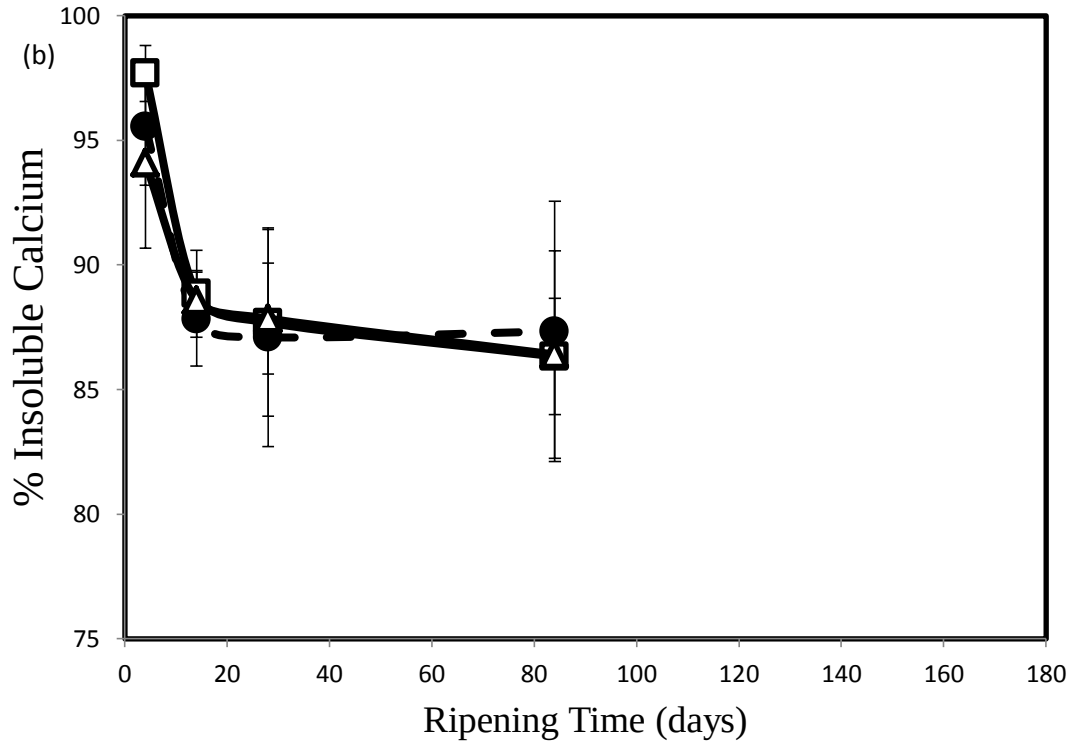


Figure 5.3 (a) pH and **(b)** percentage insoluble calcium as a function of ripening time of Cheddar cheese made with reconstituted powder with varying levels of β:α_{s1}-casein. Powders were CMPC (●), LMPC (□) and HMPC (△). Values are means of four replicates; error bars indicate ± one standard deviation.

probably contributed to the solubilisation of insoluble calcium from d 4 to 14 (Lee et al., 2005).

The ratio of total β:α_{s1}-casein in cheese was significantly different between all cheese treatments (Table 5.2). Results are the ratio of total β-casein and α_{s1}-casein at d 4 as measured by densitometry of urea-PAGE electrophoretograms (Figure 5.4). As can be seen, the β:α_{s1}-casein ratio was lower in LMPC cheese (1.21), compared to the CMPC cheese (1.34), as expected. The β:α_{s1}-casein ratio was increased in the HMPC cheese (1.41) confirming that β-casein was incorporated into the cheese.

5.3.4 Proteolysis

pH 4.6-Soluble nitrogen as a percentage of total nitrogen was used as an index of proteolysis in cheese. There were no significant differences ($P > 0.05$) between the levels of pH 4.6 soluble nitrogen observed between each treatment (Table 5.3) as the level of coagulant addition was kept constant for all treatments during ripening. pH 4.6-Soluble nitrogen increased significantly ($P < 0.05$) with ripening time (Table 5.3; Figure 5.5). Primary proteolysis increases during ripening due to activity of residual coagulant in the cheese (Fox et al., 2000; Bansal et al., 2009).

No clear difference was observed for proteolytic breakdown patterns between treatments as measured by urea-PAGE (Figure 5.4). At each ripening time point monitored the generation of peptides from both α_{s1}- and β-casein for CMPC, LMPC and HMPC cheeses appear to be similar.

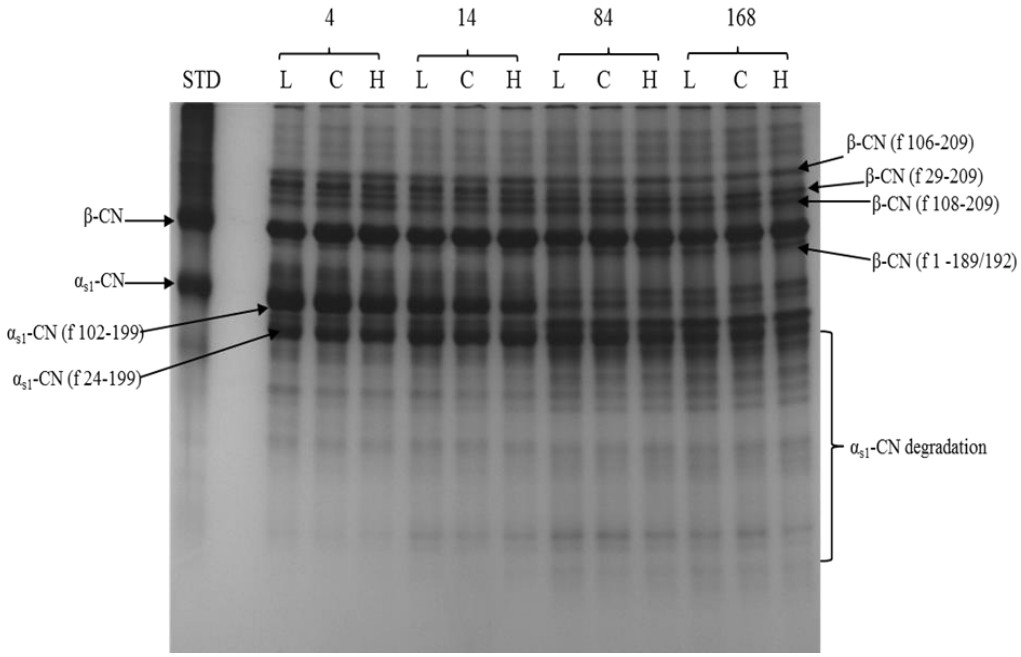


Figure 5.4 Urea-polyacrylamide gel electrophoregram of sodium caseinate (STD), LMPC (L), CMPC (C) and HMPC (H) Cheddar cheese at d 4, 14, 84 and 168 of ripening.

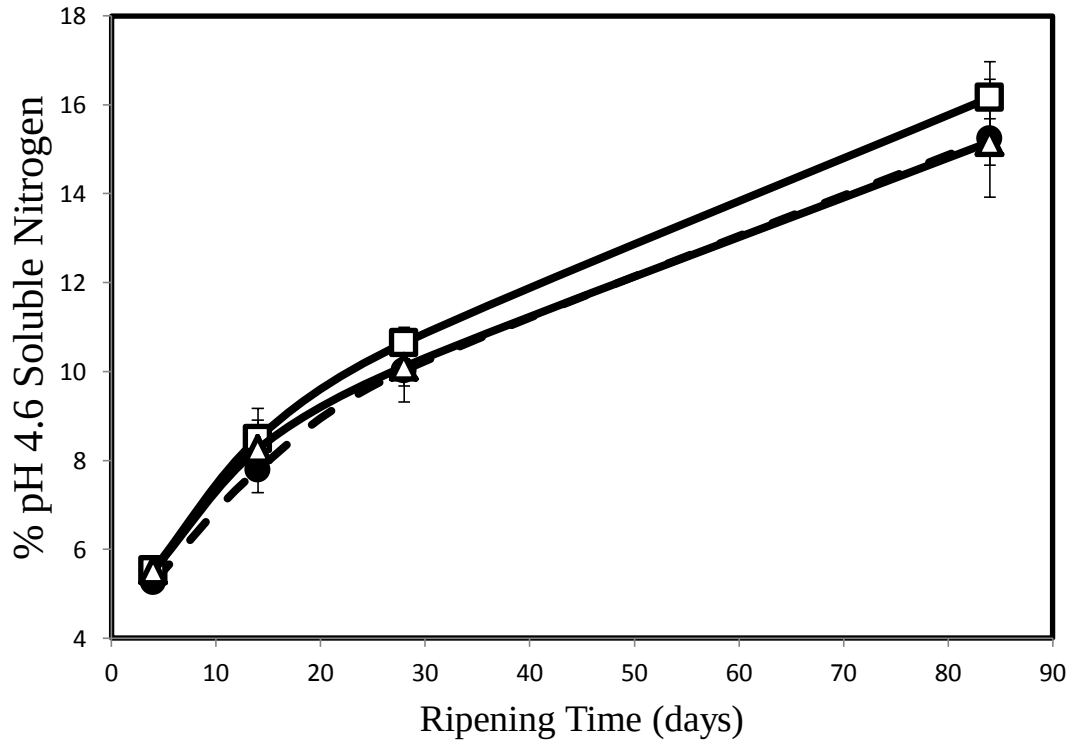


Figure 5.5 pH 4.6 soluble nitrogen as a percentage of total nitrogen as a function of ripening time of Cheddar cheese made with reconstituted powder with varying levels of β:α_{s1}-casein. Powders were CMPC (●), LMPC (□) and HMPC (△). Values are means of four replicates; error bars indicate ± one standard deviation.

5.3.5 Dynamic small-amplitude oscillatory rheology

Treatment and age had a significant (P < 0.05) effect on the rheological properties of Cheddar cheese manufactured with different levels of β-casein (Table 5.3). The effect of treatments as a function of ripening time on the value for the LT_{max} can be seen in Figure 5.6. LT_{max} of cheese made with varying levels of β:α_{s1}-casein increased significantly (P < 0.05) during the ripening period. CMPC cheese exhibited a significant (P < 0.05) increase in LT_{max} values from 14 to 84 d after which no difference was observed. LMPC increased significantly (P < 0.05) from 28 to 84 d after which no difference was observed. HMPC increased significantly (P < 0.05) at all ripening times

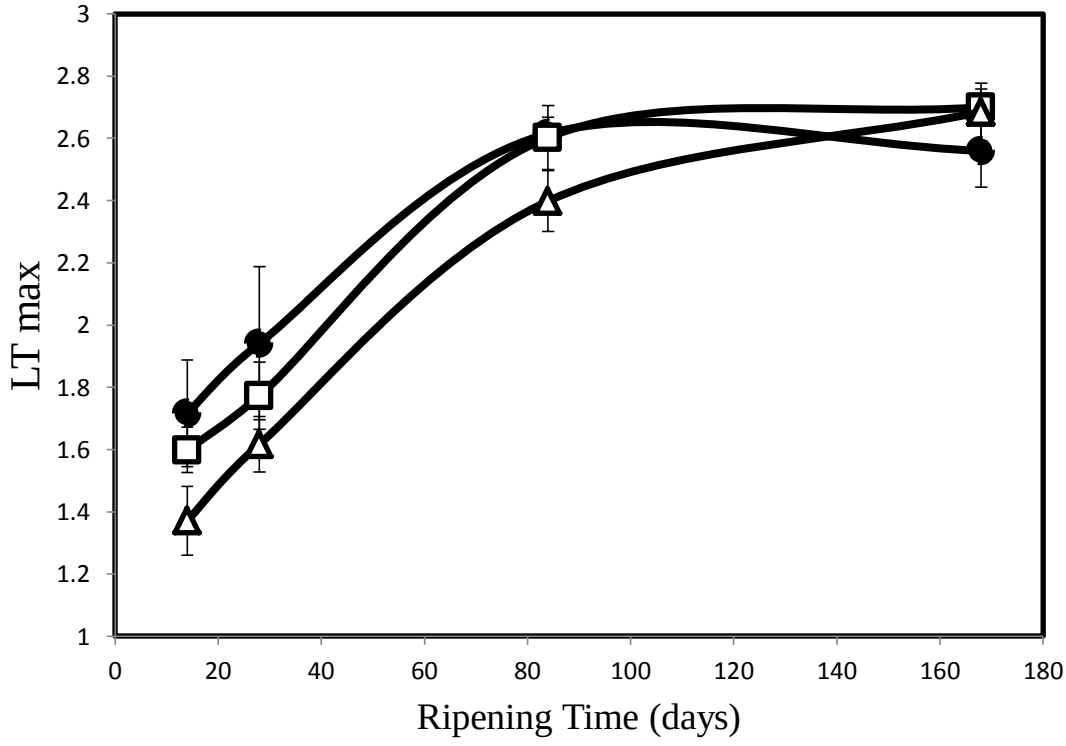


Figure 5.6 Maximum loss tangent as a function of ripening time of Cheddar cheese made with reconstituted powder with varying levels of β:α_{s1}-casein. Powders were CMPC (●), LMPC (□) and HMPC (△). Values are means of four replicates; error bars indicate ± one standard deviation.

monitored. Significant differences ($P < 0.05$) were observed between the LT_{max} of treatments at 14, 28 and 84 d of ripening. At d 14 and 84, LT_{max} of cheeses manufactured with HMPC were significantly lower than CMPC and LMPC cheeses. At d 28 HMPC cheeses was significantly lower than CMPC cheeses. No difference was observed between treatments by 168 d of ripening.

The LT_{max} has previously been used as an index of meltabilty of cheese (Mounsey and O'Riordan, 1999). The meltability of cheese typically increases during ripening due to solubilisation of colloidal calcium phosphate (CCP) and ongoing proteolysis (Lee et al., 2005; Lucey et al., 2003, 2005). O'Mahony et al. (2008) found that directly acidified cheese made with lower levels of β-casein had higher LT_{max} values than the control cheese, in agreement with our results. The higher meltability was attributed to reduced hydrophobic interactions and increased flexibility of caseins in cheese with low β-casein levels which could facilitate melt. In this study, no difference was observed for the LT_{max} of CMPC and LMPC treated cheese, but HMPC cheese had lower values at d 14, 28 and 84 of ripening. This could be due to the presence of higher proportions of β-casein increasing hydrophobic interactions during heating and altering protein-protein interactions, thereby inhibiting cheese melting.

Changes in the temperature of the LT_{max} as a function of ripening time are shown in Figure 5.7. The proportion of β-casein in cheesemilk significantly impacted the temperature of LT_{max} (Table 5.3). No significant differences ($P > 0.05$) were observed for the temperature where the LT_{max} occurred for all treatments at d 14. As ripening time progressed, differences were observed between the treatments. At d 28, 84 and 168 of ripening LMPC cheeses

temperature of the LT_{max} was significantly ($P < 0.05$) lower than the HMPC cheeses. At d 168, HMPC cheese had the highest temperature of the LT_{max} (69.5°C) compared to CMPC (66.6°C) and LMPC (65.0°C).

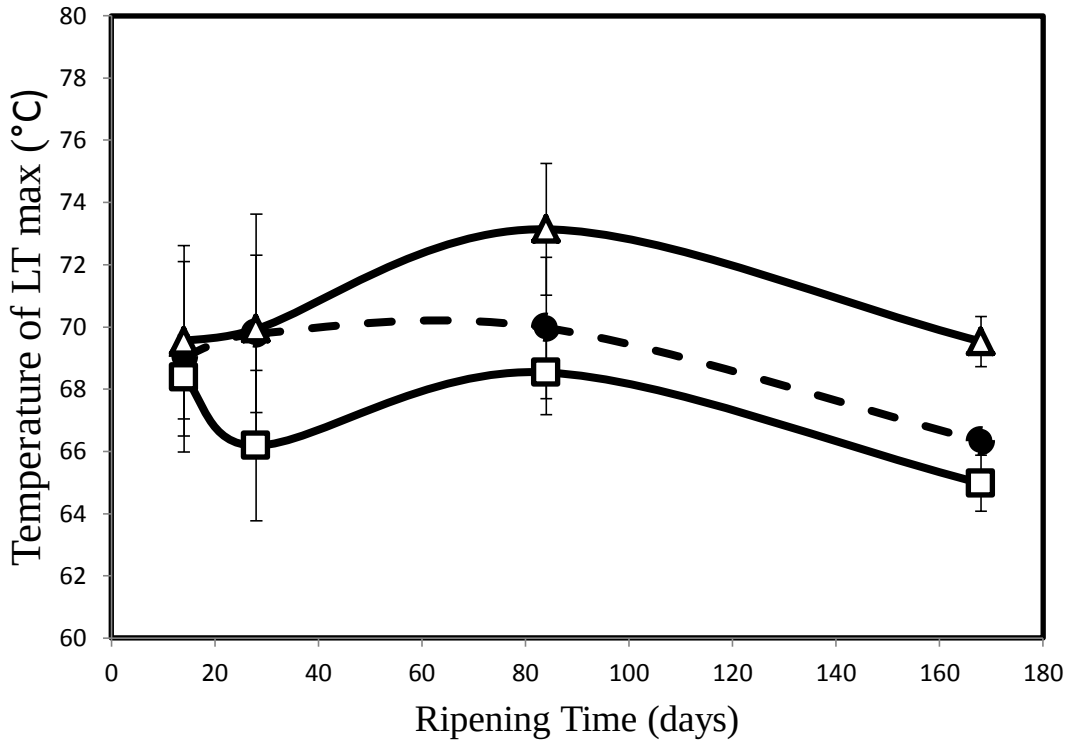


Figure 5.7 Temperature where the loss tangent maximum occurs as a function of ripening time of Cheddar cheese made with reconstituted powder with varying levels of β:α_{s1}-casein. Powders were CMPC (●), LMPC (□) and HMPC (△). Values are means of four replicates; error bars indicate ± one standard deviation.

The temperature of the LT_{max} typically decreases during ripening (Govindasamy-Lucey et al., 2005; Lee et al., 2005). A higher temperature for cheese to reach its LT_{max} value relates to a greater thermal energy required for the cheese to melt (i.e. to attain the highest LT value). Degree of proteolysis, such as the pH 4.6-soluble nitrogen level has been correlated with the temperature where the LT_{max} occurs (Lucey et al., 2005) but in this study no

significant difference was observed for proteolysis between treatments (Table 5.3). Therefore more thermal energy was needed to reach the point where the cheese exhibited the maximum viscous character in cheese with higher levels of β:α_{s1}-casein at d 168.

The strength of hydrophobic or attractive interactions tend to increase with temperature (Lucey et al., 2003). A decrease in these attractive interactions due to reduced β-casein levels would allow greater mobility of bonds and hence greater melt (Lucey et al., 2003; Lee et al., 2005). β-Casein is highly hydrophobic (Horne, 1998). Perhaps the presence of greater numbers of hydrophobic interactions in HMPC cheeses due to higher levels of β-casein required more thermal energy for the cheese to melt (LT_{max}). Similarly, the lower levels of β-casein in LMPC cheese could require less thermal energy for melt, which was in agreement with the lower temperature of the LT_{max}. O'Mahony et al. (2008) also found that cheese with higher levels of β-casein (α_{s1}:β casein ratio of 1.00:1.08) had a higher temperature for the LT_{max}, compared to cheese made with lower levels of β-casein (α_{s1}:β casein ratio of 1.00:1.00).

The temperature where the LT=1 decreased significantly ($P < 0.05$) for all treatments during the ripening period (Figure 5.8). β-Casein proportion had a significant ($P < 0.05$) effect on the temperature where LT=1 occurs (Table 5.3). HMPC cheeses had significantly ($P < 0.05$) higher LT=1 than both CMPC and LMPC cheeses at all ripening times. No significant difference ($P > 0.05$) was observed between CMPC and LMPC treated cheese except at d 84 where LMPC had a lower LT=1 than CMPC cheese. The temperature where the LT=1 relates to the temperature where the cheese begins to transition from

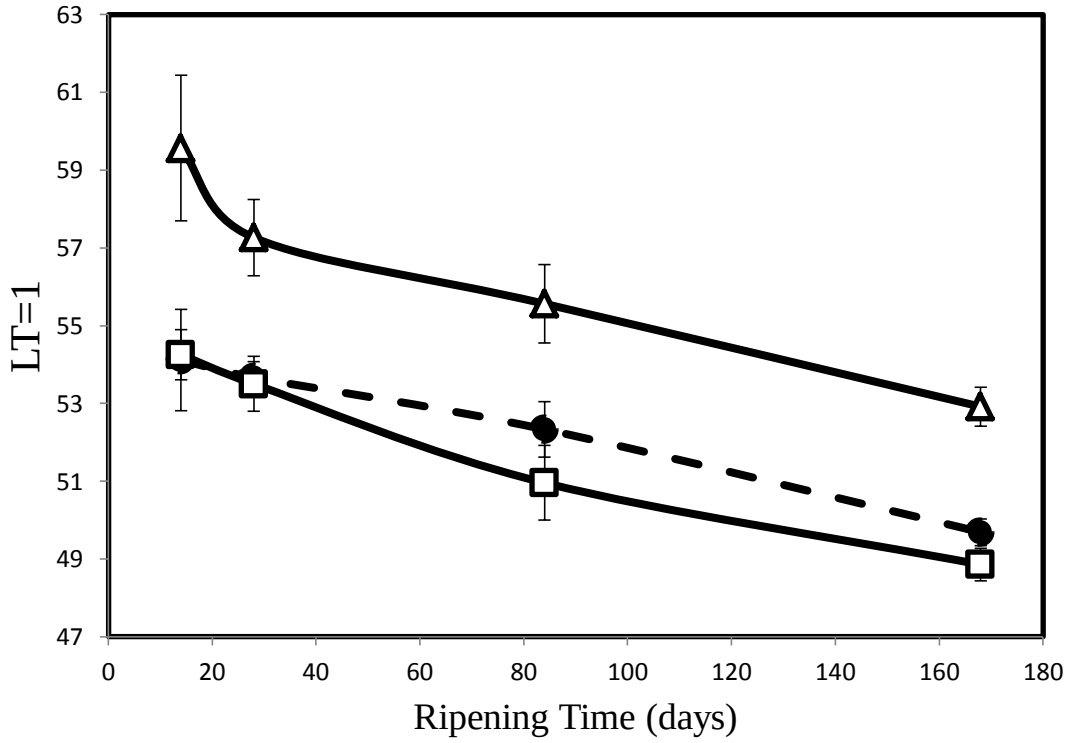


Figure 5.8 Temperature where the loss tangent is 1 as a function of ripening time of Cheddar cheese made with reconstituted powder with varying levels of β:α_{s1}-casein. Powders were CMPC (●), LMPC (□) and HMPC (△). Values are means of four replicates; error bars indicate ± one standard deviation.

a solid to a liquid-like state (i.e. crossover point) or melting point during heating and also correlates with the softening/flow of cheese (Gunasekaran and Ak, 2003). The LT=1 of cheese typically decreases during ripening (Govindasamy et al., 2005), as was observed in this study. Higher levels of β-casein in HMPC cheese lead to a significantly higher temperature where the LT=1 in agreement with the higher temperature of the LT_{max} (Figure 5.7) possibly due to increased hydrophobic interactions. Increased levels of β-casein may require a higher temperature (more thermal energy) to transition from solid to liquid-like character or soften due to the effect of temperature on hydrophobic interactions (Lucey et al., 2003).

5.3.6 Texture profile analysis

The β-casein proportion in cheesemilk and age significantly ($P < 0.05$) affected the cheese textural parameters (Table 5.3). Changes in the hardness and chewiness during ripening of cheese made with varying levels of β-casein are shown in Figure 5.9. Both hardness and chewiness values decreased significantly ($P < 0.05$) during ripening. Differences in hardness values between treatments were significant ($P < 0.05$) throughout ripening. At d 4 and 14 both CMPC and HMPC were harder than LMPC cheeses. At all other time points, significant differences ($P < 0.05$) were observed in the hardness of all cheeses; HMPC cheese had the highest and LMPC cheese had the lowest hardness (Figure 5.9a). Chewiness values showed a similar trend to hardness, with significantly ($P < 0.05$) lower chewiness for LMPC throughout ripening (Figure 5.9b). HMPC cheese had significantly ($P < 0.05$) higher chewiness values than CMPC cheese at d 28 and 168 of ripening. This was not surprising as chewiness is usually proportional to hardness (Bansal et al., 2009). HMPC cheese had the highest chewiness values and LMPC had the lowest observed. O'Mahony et al. (2008) observed that increased cheese firmness was observed with higher levels of β-casein. St-Gelais and Haché (2005) found that cheese enriched with β-casein had higher hardness; however, the casein content of the milk used to manufacture high β-casein cheese was higher than the control and this led to cheese with increased protein and decreased moisture content, which likely had a confounding effect on the hardness results. The significantly ($P < 0.05$) lower protein content of LMPC cheese (Table 5.2) in the current study may also have contributed to the lower hardness and chewiness observed for this cheese. It is well known that textural changes in cheese are correlated with

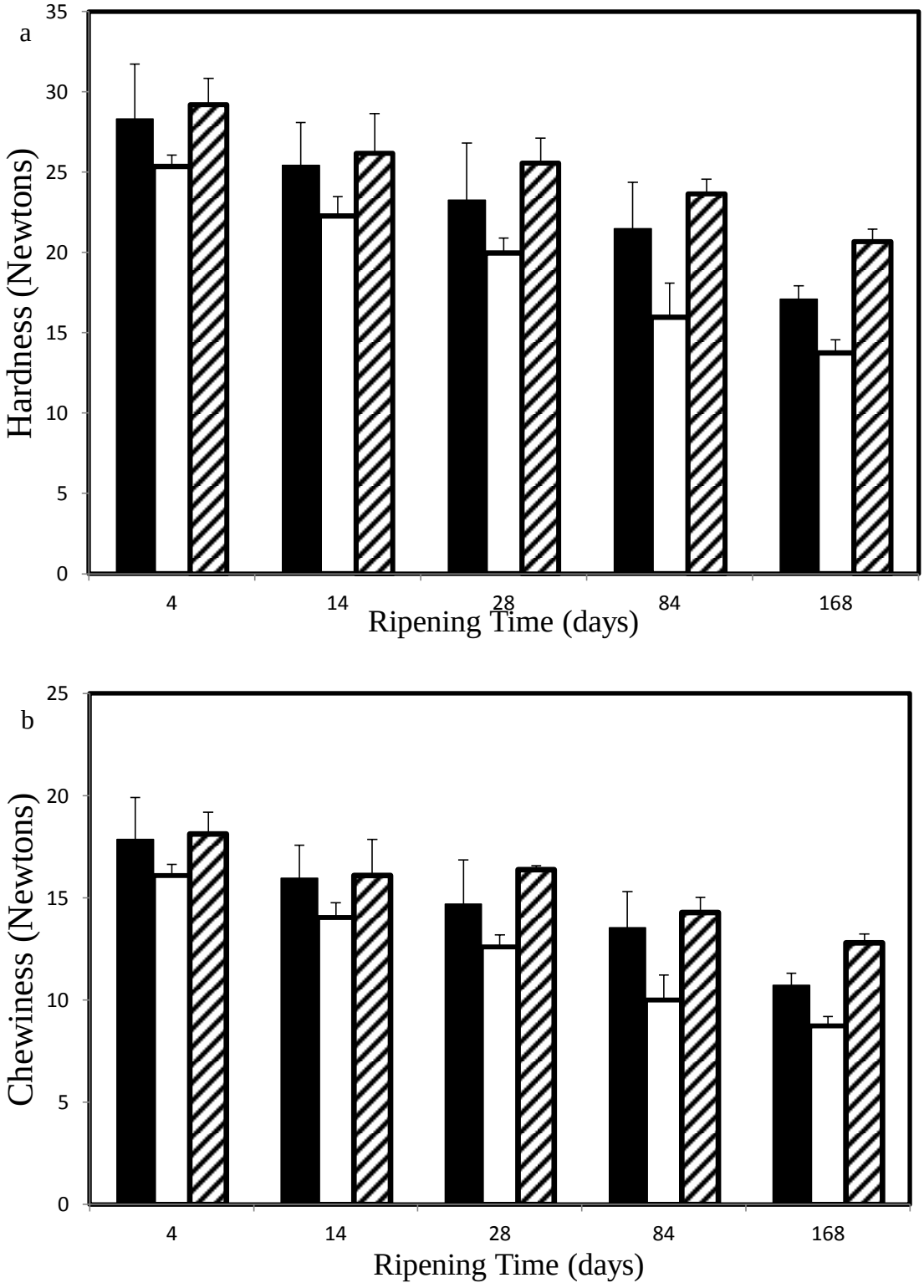


Figure 5.9 (a) Hardness and **(b)** chewiness values as a function of ripening time of Cheddar cheese made with reconstituted powder with varying levels of β:α_{s1}-casein. Powders were CMPC (■), LMPC (□) and HMPC (▨). Values are means of four replicates; error bars indicate ± one standard deviation.

composition, pH, solubilisation of CCP and proteolysis. No difference was observed between these parameters in this study except for the protein content of LMPC cheese; therefore, the presence of higher levels of β-casein seemed to form a harder and chewier structure. TPA analysis was performed at low temperatures (~4°C) and hydrophobic interactions weaken at lower temperatures (Lucey et al., 2005). At lower temperatures, swelling of casein aggregates can occur, causing increased contact area between the particles, which could increase firmness (Lucey, 2002). More swelling of particles with the weakening of hydrophobic interactions could lead to the swelling helping to increase firmness. O'Mahony et al. (2008) hypothesised that casein micelles have a more porous structure upon removal of β-casein. Conceivably increasing β-casein would form aggregates with lower porosity, leading to a less flexible structure and hence the harder cheese structure observed.

5.3.7 Sensory analysis

None of the sensory attributes measured were significantly ($P < 0.05$) affected by treatment but all attributes were significantly ($P < 0.05$) affected by age (Table 5.4). Bitterness could be detected in all cheeses at d 84 and d 168 of ripening (Table 5.5). At d 84 there was no significant difference ($P > 0.05$) between the treatments and cheeses were characterized as having very slight bitterness; at d 168, there was again no significant difference ($P > 0.05$) between treatments. Bitterness increased during ripening for all samples, with cheeses exhibiting only slight bitterness in the scale used. As previously discussed, accumulation of hydrophobic peptides from hydrolysis of β-casein

can result in bitterness in cheese (Fox et al., 2000). While bitterness was observed in all cheeses due to the use of a bitter-producing starter culture strain, proteolysis levels in the cheese were not significantly ($P > 0.05$) different (Figure 5.5). Also the generation of β-casein (f1-189/192), a hydrophobic peptide that can cause bitterness in cheese (Bansal et al., 2009), did not appear to differ between treatments (Figure 5.4). Possibly more extensive hydrolysis of β-casein in the cheese would result in differences in the formation of bitterness between treatments but further cheese ripening (> 6 months) was not evaluated in this study.

Sweetness increased significantly ($P < 0.05$) for LMPC and HMPC cheese but the values were still less than the threshold for sweetness on the scale used at d 84 and 168. Salt increased for LMPC from d 84 to 168 but was still classified as slight on the scale used for salt which was the same classification for CMPC and HMPC. Acid flavour decreased for HMPC from d 84 to 168 but again was still classified as slight. Astringent increased for all treatments from d 84 to 168 but was classified as very slight throughout ripening. Sulphur increased for all treatments from d 84 to 168 from threshold to very slight values on the scale used.

Table 5.4 Mean squares and probabilities (in parentheses), and R^2 values for sensory analysis of Cheddar cheese made with varying levels of β-casein during ripening.

	Sweet	Salt	Acid	Bitter	Astringent	Sulphur
Treatment (T)	0.005 (0.5063)	0.388 (0.0537)	0.002 (0.9951)	0.058 (0.8218)	0.195 (0.4044)	0.003 (0.9713)
Age (A)	0.304** (0.0010)	1.40** (0.0093)	0.184* (0.0254)	13.65** (0.0001)	9.00** (<0.0001)	3.30** (0.0002)
A x T	0.200 (0.2772)	0.329 (0.7805)	0.065 (0.134)	0.040 (0.8811)	0.001 (0.9823)	0.008 (0.9134)
Error	0.135	0.129	0.026	0.315	0.070	0.086
R^2	0.77	0.73	0.94	0.85	0.95	0.84

*$0.01 < P \leq 0.05$

**$P \leq 0.01$

Table 5.5 Sensory properties of Cheddar cheese manufactured using control (CMPC), low β-casein (LMPC) and high β-casein (HMPC) powders at d 84 and 168 of ripening. Values represent the means and standard deviations, with the latter in parentheses (n=4).

	84 d			168 d		
	CMPC	**LMPC**	**HMPC**	**CMPC**	**LMPC**	**HMPC**
Sweet	$0.27^{a,A}$ (0.17)	$0.17^{a,A}$ (0.09)	$0.23^{a,A}$ (0.09)	$0.36^{a,A}$ (0.05)	$0.50^{a,B}$ (0.09)	$0.45^{a,B}$ (0.04)
Salt	$3.96^{a,A}$ (0.13)	$4.22^{a,A}$ (0.36)	$3.94^{a,A}$ (0.24)	$4.35^{a,A}$ (0.51)	$4.83^{a,B}$ (0.33)	$4.41^{a,A}$ (0.38)
Acid	$4.86^{a,A}$ (0.48)	$4.96^{a,A}$ (0.33)	$5.06^{a,A}$ (0.32)	$4.85^{a,A}$ (0.50)	$4.82^{a,A}$ (0.29)	$4.68^{a,B}$ (0.50)
Bitter	$2.78^{a,A}$ (0.72)	$2.97^{a,A}$ (0.68)	$2.93^{a,A}$ (0.56)	$4.39^{a,B}$ (0.56)	$4.53^{a,B}$ (0.39)	$4.29^{a,B}$ (0.21)
Astringent	$2.10^{a,A}$ (0.46)	$2.13^{a,A}$ (0.38)	$2.37^{a,A}$ (0.35)	$3.32^{a,B}$ (0.18)	$3.34^{a,B}$ (0.28)	$3.63^{a,B}$ (0.46)
Sulfur	$1.43^{a,A}$ (0.48)	$1.52^{a,A}$ (0.44)	$1.44^{a,A}$ (0.26)	$2.19^{a,B}$ (0.09)	$2.18^{a,B}$ (0.14)	$2.17^{a,B}$ (0.25)

^{a,b,c,}Means within the same row not sharing a common superscript differ ($P < 0.05$).

^{A,B}Means within the same column not sharing a common superscript differ ($P < 0.05$).

5.4 Conclusions

The level of β-casein had a significant impact on the rheological properties of rennet-induced gels. Decreasing levels of β-casein formed a gel that was stiffer, more elastic-like and had a higher yield stress. Varying the ratio of β:α_{s1}-casein in Cheddar cheese had an impact on the texture and rheological properties during ripening. LMPC cheese had a significantly lower protein content than CMPC and HMPC, and CMPC had slightly lower FDM but this did not affect any other compositional parameters of the cheeses. There was no significant difference in proteolysis, pH or insoluble calcium content between cheeses with different β:α_{s1}-casein levels. The LT_{max} of HMPC cheese was significantly lower than CMPC from d 14 to 84 and LMPC at d 14 and 84, indicating a less meltable cheese. At d 28 to 168 of ripening HMPC cheese had a significantly higher temperature of LT_{max} and throughout ripening HMPC had higher hardness and chewiness values whereas LMPC had the lowest values for these parameters. HMPC had a significantly higher temperature where the LT=1 at all ripening times compared to CMPC and LMPC. These results showed that the use of HMPC in cheese manufacture had a greater effect compared to LMPC and CMPC. The effect observed on the rheological properties of HMPC cheese could have been due to the strengthening of hydrophobic interactions upon heating of the cheese. At lower temperatures, hydrophobic interactions weaken. TPA, which was measured at low temperatures, indicated increased hardness and chewiness in cheese when β-casein level was increased. Possibly changing β-casein level could have an effect on the porosity and swelling or contact area between casein aggregates affecting its textural properties. Although the cheeses contained varying levels

of β-casein, no difference was observed in the flavour attributes measured in this study.

It was clear that β-casein level had an effect on the rheological properties of rennet induced gels. During ripening it was found that higher levels of β-casein (HMPC) seemed to have a greater impact on the rheological and textural properties of Cheddar cheese than observed for LMPC and compared to CMPC. Hydrophobic interactions and possibly changes in the micelle due to incorporation of in β-casein into the micelle could have caused the effects observed in the current study.

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CHAPTER 6

Impact of Camel Chymosin on the Texture, Flavour and Functionality of Low Moisture Part Skim Mozzarella Cheese

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Abstract

The objective of this study was to investigate the impact of coagulant (bovine calf chymosin (BCC) or camel chymosin (CC), on the functional properties and performance shelf-life of low-moisture part-skim (LMPS) Mozzarella. Both chymosins were used at two levels (0.05 and 0.037 IMCU/mL) and clotting temperature was varied to give similar gelation times for each treatment (as this also impacts cheese properties). Functionality was assessed at various cheese ages using dynamic low-amplitude oscillatory rheology, and performance of baked cheese on pizza. Cheese composition was not significantly different between treatments. There was no significant difference ($P > 0.05$) in the level of total calcium or insoluble calcium in the cheeses initially or during ripening. Proteolysis in cheese made with BCC was higher than in cheeses made with CC. At 84 d of ripening, maximum loss tangent (LT_{max}) values were not significantly different in the cheeses, suggesting that these cheeses had similar melt characteristics. After 14 d of cheese ripening, the crossover temperature (loss tangent = 1; indicating the softening point) was higher when CC was used as coagulant. This was due to lower proteolysis in the CC cheeses compared to those made with BCC since the pH and insoluble calcium levels were similar in all cheeses. Cheeses made with CC maintained higher hardness values over 84 d of ripening compared to BCC. Cheeses made with CC maintained higher sensory firmness values and adhesiveness of mass scores during ripening. When melted on pizzas, cheese made with CC had lower blister quantity and the cheeses were firmer and chewier. Since the two

types of cheeses had similar moisture contents, pH values, and insoluble Ca levels, the differences in proteolysis were responsible for the firmer and chewier texture of CC cheeses. When cheese performance on baked pizza was analyzed properties, such as, blister quantity, strand thickness, hardness and chewiness were maintained for longer ripening time than cheeses made with BCC indicating that the use of CC will help to extend the performance shelf-life of LMPS Mozzarella.

6.1 Introduction

The melt and stretch performance of any cheese when baked on pizza is determined by cheese composition, pH history (especially extent of acidification at coagulant addition), insoluble colloidal calcium phosphate content and the amount of intact casein (Johnson and Lucey, 2006). As cheese ages residual proteolytic activity of the coagulant can quickly hydrolyze sufficient casein to greatly increase flowability and decrease the stretch of cheese both to the extent that bake performance may be negatively impacted. During refrigerated storage, low moisture part-skim (LMPS) Mozzarella loses its desired firmness and chewiness. The ability to conveniently slice or shred the cheese for use on pizza is diminished. LMPS Mozzarella cheese also tends to become sticky, clinging to mechanical blades and clumps. Proteolysis and aging therefore limits the window that industrial convertors can slice or shred the cheese but it can also limit retail and home use shelf-life.

The principal proteolytic agent in cheese is the coagulant and limiting its activity in high moisture cheeses will increase shelf-life, which will help to maintain the desirable characteristics consumers are looking for in those cheeses. Historically, various methods have been used by cheese manufacturers to reduce residual coagulant activity in cheeses; such as reducing the amount of coagulant used, or greatly reducing the storage temperature including freezing the cheese. For pasta filata cheeses, the use of less proteolytic coagulants, BCC and *Rhizomucor meihei* rennet, compared to *Rhizomucor pusillus* rennet, and greatly increasing the water temperature and processing time during the pasta filata step have also been used (Sheehan et al., 2004).

Recently, a coagulant became available that has strong clotting activity but reduced overall proteolytic activity. This enzyme is a fermentation-produced coagulant originally derived from camels and is sold by Chr. Hansen's Laboratory (Milwaukee, WI) as Chymax® M. Kappeler et al. (2006) studied recombinant (fermentation-produced) camel chymosin (CC) and studied its properties as a milk coagulant compared to fermentation produced bovine calf chymosin (BCC). Average clotting activity (measured using visual determination of the clotting point in min) on bovine κ-casein was 70% greater for CC compared to BCC but CC had a lower general proteolytic activity on bovine casein. Camel chymosin has 7-fold higher ratio of clotting activity to general proteolytic activity compared with BCC (Kappeler et al., 2006).

Bansal et al. (2009) used fermentation-produced CC and BCC in full-fat Cheddar cheese. The level of coagulant was varied to give comparable gel strengths at cutting, which resulted in the use of 30% less international milk clotting units (IMCU)/mL of CC. Proteolysis was significantly different in cheeses produced from CC and BCC, when monitored by levels of pH 4.6-soluble nitrogen, urea-PAGE and RP-HPLC. Cheese made from BCC showed higher levels of primary proteolysis, which was attributed to the lower usage level of CC added and its lower general proteolytic activity. At the end of ripening, cheese made with CC had higher hardness and chewiness values than cheese made with BCC. Bansal et al. (2009) suggested that CC could be used in cheeses where there was a tendency for bitterness as sensory analysis showed that cheeses made with CC were less bitter, while urea-PAGE analysis indicated β-CN (f1-189/192) was not observed in CC cheeses. This indicated that CC may not be hydrolyzing much of β-CN. This has important

ramifications for the firmness, and perhaps flow of cheese when it is heated. Lucey et al. (2003) described the flow and stretch of cheese when heated as being influenced by 3 competing factors: charge repulsion or attraction, hydrophobic attraction, and degree of intact casein or insoluble Ca levels. If there is no charge repulsion or too much attraction (such as very low pH cheese <4.95) or too much insoluble Ca crosslinking (such as high pH cheese >6.20), then there is no flow. However, regardless of the degree of charge repulsion or hydrophobic attraction, if the amount of intact casein is reduced sufficiently through proteolysis, the cheese will flow when heated and there will be a loss in stretch length (Lucey et al., 2003).

Govindasamy-Lucey et al. (2010) made low-fat Cheddar cheese with starter cultures known to cause bitterness and used 20% less CC compared with BCC. They found that, during ripening, low-fat Cheddar cheese made with CC produced lower levels of soluble nitrogen and had higher hardness and chewiness values. Loss tangent (LT) and degree of flow (DOF) values, which are indices of cheese meltability, were lower at 1 and 3 months of ripening for cheeses made with CC compared to cheese made with BCC. Bitterness developed in both cheeses but lower levels were present in the cheese made with camel chymosin (Govindasamy-Lucey et al., 2010).

The level of residual chymosin activity in Mozzarella cheese depends on the temperature of stretching; proteolysis progressively decreases following the use of higher stretching temperatures (Kindstedt et al., 2004). Kindstedt et al. (1995) varied the level of BCC used in Mozzarella cheese from 60-100% of the amount normally used. Using lower levels of BCC significantly reduced free oil formation and rate of proteolysis, although no significant effect was

observed on the functional properties of the melted or unmelted cheese (Kindstedt et al., 1995). Sheehan et al. (2004) studied the effect of three coagulants: BCC, *Rhizomucor meihei* rennet and *Rhizomucor pusillus* rennet, on the functionality of reduced-fat Mozzarella. The composition of all cheese produced from these coagulants were similar. Coagulant type had no effect on changes in pH and non-expressible serum during cheese ripening. The type of coagulant used had an effect on the level of degradation of caseins and pH 4.6-soluble nitrogen. However, coagulant type did not influence functional properties (firmness and flowability) of the Mozzarella cheese. In contrast, the type of milk-clotting enzyme used to coagulate milk for directly acidified Mozzarella had an effect on the functional properties of the cheese such as melt and stretch (Oberg et al., 1992); cheeses made with BCC had more melt but less stretch, which was consistent with greater proteolysis of α_s-CN by BCC compared with the other milk clotting enzymes studied. In contrast, porcine pepsin gave the most stretch and least increase in melt, which was attributed to porcine pepsin preferentially degrading β-CN over α-CN, causing less weakening of the protein network. Thus, previous studies indicate that coagulant type and usage level can affect some of the properties of Mozzarella cheese.

Optimum functional properties of LMPS Mozzarella are usually observed between 2 and 6 wk for cheeses that are stored refrigerated. Desirable cheese properties include retaining sufficient firmness to allow machinability, sufficient melt and stretchability (Alvarez, 1986); although the exact specific functionality required varies according to the needs of the end-user. Several reports have attributed the softening of Mozzarella cheese and other changes in

the functional properties during refrigerated storage to proteolysis (Creamer, 1976; De Jong, 1976; Farkye et al., 1991; Oberg et al., 1991a,b; Tunick et al., 1993). Beyond 6 wk of refridgerated storage, excessive proteolysis can be a contributing factor to decreased machinability, machinability is the ease with which a cheese can slice or shred (Chen et al., 2009). Due to the lower proteolytic activity of CC, it is believed that the use of CC may extend the functional shelf life of LMPS Mozzarella cheese compared to cheese made with BCC. Previous studies (Bansal et al., 2009; Govindasamy Lucey et al., 2010) used lower amounts of camel chymosin for cheesemaking due to its greater clotting activity. However, it is unclear if the lower proteolysis levels in cheeses made with CC was caused by lower general proteolytic activity on bovine caseins or just due to the lower enzyme addition leading to lower level of primary proteolysis. The objective of this study was to compare functional and sensory properties and shelf life performance of LMPS Mozzarella cheese manufactured with different coagulants (BCC and CC). We wanted to compare the properties of cheeses made with similar levels of both BCC and CC. However, clotting time will be faster in the milks renneted with the same amount of CC as that of BCC because of its greater clotting activity (Bansal et al., 2009). Thus, we wanted to experimentally vary the level (IMCU) of both coagulants from a high (0.05 IMCU) to a low (0.037 IMCU) level of coagulant addition. Due to the different clotting activities between CC and BCC, we adjusted the clotting temperature to obtain similar gelation times in all treatments. Therefore, the objective of this current study was to compare LMPS Mozzarella cheese manufactured with different coagulants (BCC and

CC) at 2 coagulant levels (0.05 and 0.037 IMCU/mL) when the cheese compositions were similar.

6.2 Materials and methods

6.2.1. Rheological Properties of cheesemilk during rennet coagulation

Rheological properties of milk was determined using dynamic low amplitude oscillatory rheometry as previously described by Govindasamy-Lucey et al. (2005). The time point where the storage modulus (G') was greater than 1 Pa was defined as the gelation time. A rheometer (MRC 301, Anton Paar GmbH, Austria) was used to determine the rheological characteristics of the gels during renneting using an oscillation test performed at 1% strain and a frequency of 0.1 Hz. A concentric cylinder (CC27/T200/SS) measuring geometry was used. Reconstituted (10% w/v) low heat skim milk powder containing 0.01% (v/v) $CaCl_2$ was used to determine the rennet coagulation characteristics of the rennets. Rennet coagulation temperature and level of $CaCl_2$ addition was varied to give similar gelation times for each treatment (Table 6.1). Milks were held at the appropriate temperature for 30 min in a water-bath and 10 µl of the appropriate diluted rennet; calf chymosin (BCC) (CHY-MAX ^{TM} Extra, 630 international milk clotting units (IMCU)/mL Chr. Hansen Inc., Milwaukee, WI, US) or camel chymosin (CC) (CHY-MAX ^{TM} M, 1000 IMCU/mL Chr. Hansen Inc.) was added to the milk and placed in the cup. To prevent surface dehydration of the milk a layer of vegetable oil was put on the surface. The test was started 2 min after addition of rennet and readings were taken at one minute intervals. The rate at which the gel firmed was evaluated as the rate of change in G' values between the gelation time and cutting time (Pa min^{-1}).

Large deformation properties of gels were studied to assess its resistance to cutting. A constant shear rate test at 0.01 s^{-1} was used to determine an apparent yield stress and strain of the gels. This test was performed at the gelation times used for cheese making. The point where the shear stress started to decrease was defined as the yielding point of the gel (Lucey, 2002).

Table 6.1 Milk gelation conditions for LMPS Mozzarella manufacture made with different enzyme treatments high calf chymosin (HBCC), low calf chymosin (LBCC), high camel chymosin (HCC) and low camel chymosin (LCC).

Gelation Conditions	**HBCC**	**LBCC**	**HCC**	**LCC**
CHY-MAX ™ Extra (IMCU/mL)	0.05	0.037	-	-
CHY-MAX ™ M (IMCU/mL)	-	-	0.05	0.037
Temperature (°C)	33.3	36.0	31.5	33.3
$CaCl_2$ (%)	-	0.006	-	-

6.2.2 Cheese Manufacture

Four vats of low moisture, part skim (LMPS) Mozzarella cheese was manufactured in the dairy plant at the University of Wisconsin-Madison in triplicate on three separate days. The milk clotting conditions and type of coagulant used was varied for each vat as shown in Table 6.1. Milk (272 kg; 2.3% fat, casein:fat=1:1) was pasteurized at 72°C for 19 s and cooled to the appropriate ripening temperature for each vat. A direct-vat-set thermophilic culture comprising *S. thermophilus* and *Lb. helveticus* (TEMPO 303, Cargill Texturizing Solutions, Waukesha, WI, US) was added at a level of 9 g/100 kg starter culture was added to each of the milk vats. After ripening for 60 min, fermentation-produced calf chymosin (CHY-MAX ™ Extra, 630 international milk clotting units (IMCU)/mL Chr. Hansen Inc., Milwaukee, WI, US) or

camel chymosin (CHY-MAX ^{TM} M, 1000 IMCU/mL Chr. Hansen Inc.) was added to the respective milk specified in Table 6.1. Results from preliminary trials showed moisture of LBCC cheese was higher than the other cheeses produced. Some measures during cheese manufacture were taken to bring the moisture of all cheeses to within the same range. The pH at cutting for all cheeses was 6.5. All coagula were cut with 1.9 cm knives but the LBCC sample was cut 25 min after rennet addition compared to 45 min for all other treatments. The temperature of the vats was raised to 41°C over a 30 min period. Each vat was cooked at 41°C until the pH reached 5.90, agitation was then stopped, the curd was trenched and then whey drained. Curd was not cut into slabs for high calf (HBCC), high camel (HCC) and low camel (LCC). LBCC was cut into 4 slabs and stacked. At pH 5.25, all cheeses were milled and salted at a level of 2.6% (w/w) and stretched in a cooker (Supreme Filata Mixer, Stainless Steel Fabricating Inc., Columbus, WI, US). Cheeses were kept in cold water for 30 minutes and then brined for 120 minutes. The brine-salted cheese was vacuum-packed and stored at 3°C for 84 d. Analysis was carried out on d 1, 14, 28, 42, 56 and 84.

6.2.3. Cheese Composition, pH and Level of Insoluble Calcium

Compositional analysis was carried out at d 14. Cheese was analysed for moisture (Marshall, 1992), fat by Mojonnier (AOAC, 2000), protein by Kjeldahl (AOAC, 2000) and salt by chloride electrode method (MK II Chloride analyser 926, Nelson & Jameson Inc., Marshfield, WI, Johnson and Olson, 1985). Total Ca was measured for milk, rennet whey and cheese at d 14 using inductively coupled plasma emission spectroscopy (Park, 2000). Cheese

pH, proteolysis and insoluble Ca content were measured throughout ripening. pH was monitored by grinding 10 g of cheese with 10 mL of distilled water and measuring the pH of the slurry using a pH meter (Accumet AB15 Plus, Fisher Scientific, Singapore; Madkor et. al, 1987). insoluble Ca content was measured by acid-base titration (Hassan et al., 2004).

6.2.4 Proteolysis

Proteolysis was indexed by pH 4.6 soluble nitrogen (Kuchroo & Fox, 1982) and the nitrogen content of this measured by Kjeldahl (AOAC, 2000) at d 1, 14, 28, 56 and 84 of ripening.

6.2.5 Dynamic Small-Amplitude Oscillatory Rheology

The rheological properties of the cheese were studied using a Paar Physica Universal Dynamic Spectrometer (UDS 200 Physica Messtechnik, Stuttgart, Germany). Cheese samples were sliced using a Hobart slicer to ~ 2.3 mm and cut into 50 mm diameter discs. Samples were stored in refrigerator at 4°C for at least 8 h before testing. The cheese was heated from 5-85°C at 1°C min^{-1}. A 50 mm serrated parallel plate was used and the cheese was subjected to a strain of 0.5% at a frequency of 0.08 Hz. The parameters measured were G', loss modulus (G") and loss tangent (G"/G') during heating of the cheese. The maximum LT (LT_{max}), temperature where this occurred and the temperature where the LT is 1 which indicates the transition from a solid to a liquid-like system (i.e., a crossover point) was also measured.

6.2.6 Melt Profile Analysis

The melt and flow properties of Mozzarella cheese was measured using a UW-Meltprofiler (Muthukumarappan et al., 1999). Cheese was cut into slices that were 7 mm in height using a Hobart slicer and 30 mm discs were cut from this using a stainless steel cylindrical borer. Samples were stored in plastic bags at 4°C and allowed to temper for at least 6 h before analysis. Samples were placed in an oven at a constant temperature of 70°C. The height and temperature was continuously measured. DOF was calculated as the change in height of the cheese sample at 60°C compared to the cheese height at the beginning of the test.

6.2.7 Texture Profile Analysis

Cheese was cut into cylindrical samples (16 mm diameter, 17.5 mm height) and stored overnight at 4°C prior to compression. Texture analysis was performed using a Texture Analyzer TA-XT2 (Stable Micro Systems, Godalming, Surrey, UK). Texture profile analysis was performed by compressing samples to 38% of its original height, chewiness and hardness was calculated as previously described by Bourne (1978).

6.2.8 Descriptive Sensory Analysis

A trained (20 h of training) sensory panel consisting of at least 12 panelists used a mixture of sensory Spectrum™ and Quantitative Descriptive Analysis (Meilgaard et al., 1999) to evaluate the textural and flavor properties of both

the unmelted and melted cheese as described by Chen et al. (2009) (Table 6.2). The numerical intensity scale ranged from 0-15 with reference points. Each cheese was designated with a random 3-digit code and assessed in duplicate on 2 separate days. Cheese cubes were tempered at ~12°C before assessment for texture and flavor attributes (saltiness and acidity) (Table 6.2). Textural attributes evaluated were firmness and adhesiveness of mass of the cubes (Table 6.2).

Cheeses were mechanically shredded using a food processor (Cuisinart Prep 11 Plus, Madison, WI). A 30.5-cm frozen pizza crust (Arrezzio Thin & Crisp Par-Baked, Sysco Food Services, Baraboo, WI) was thawed, 30 g of tomato pizza sauce (Contadina Roma-style tomatoes pizza sauce, Del Monte Foods Inc., Hanford, CA) was spread over the crust. Approximately 300 g of shredded cheese was added to the crust, which was then baked in a forced-air commercial oven (Impinger^{TM} Ovens, Lincoln Foodservice Products Inc., Ford Wayne, IN) at 260°C for 5 min. The surface characteristics that were evaluated included free oil release, blister colour, blister quantity and skinning. Stretch characteristics of the cheeses were evaluated by determining the strand length and thickness of the stretched cheese (Table 6.2). Textural properties (i.e., cohesiveness of mass, chewiness and hardness) of the melted cheese were evaluated after cooling to 63°C. Photographs of cheeses at the different reference points were available to the panellists. Flavour attributes (acid and salt intensities) of melted cheeses were also assessed at 63°C.

6.2.9 Experimental Design and Statistical Analysis

Four enzyme treatments were used to manufacture LMPS Mozzarella, in triplicate; each cheesemaking trial was performed on three different days. A 4 × 3 completely randomized block design, which incorporated all 4 treatments and three trials, was used for analysis of the response variables relating to cheese composition. Analysis of variance was carried out using the PROC GLM procedure of SAS (version 9.1; SAS Institute, 2002-2003). Scheffe's multiple-comparison test was used to evaluate differences in the treatments at a significance level of $P < 0.05$ for cheese composition and coagulation properties of milk.

A split-plot design was used to monitor the effects of treatment and ripening time and their interactions on pH, insoluble calcium, proteolysis, functional, textural and sensory properties. In the whole-plot factor, treatment was analysed as a discontinuous variable and cheesemaking day was blocked (Govindasamy-Lucey et al., 2011). For the subplot factor, age and age × treatment were treated as variables. The interactive term treatment × day of cheesemaking was treated as the error term for the treatment effect. The ANOVA for the split-plot design was carried out using PROC GLM of SAS.

Table 6.2 Definitions of the attributes used by the trained panelists to evaluate the flavor and texture of the unmelted and melted LMPS Mozzarella cheeses using a combination of Spectrum™ and Quantitative descriptive analysis.

Method of analysis/attribute	Definition/Evaluation procedure	References used/Preparation Instructions/Anchor Points (0-15)
Unmelted Cheese		
Hand firmness	Force to required to compress the cheese between finger and thumb. Place the cheese cube between thumb and fore finger. Compress cheese cube, do not fracture.	Green-colored Thera-Putty (#5075, Sammon Preston) = 4.5 Blue-colored Thera-Putty (#5077, Sammon Preston) = 7.0 Flesh-colored Thera-Putty (Graham-Field, Inc.) = 9.5 Gray Eraser (Primacolor Kneaded Rubber) = 12.0 White Eraser (School Select White) = 15.0
Chewdown: Adhesiveness of Mass	Degree to which mass sticks to the roof of the mouth or teeth. Chew cheese sample between molars 12-15 times. Evaluate cheese adhesive properties.	Polenta (Food Merchants Brand) = 0.0 Quince-paste (La Costena Brand) = 2.5 Rice, converted (Minute Rice Brand) = 3.5 Mashed Potatoes (Hungry Jack Brand) = 7.5, Prepared by boiling 2/3 cup water, ¼ milk, 1 tablespoon butter. Removed from heat, 1 cup of dried potato flakes was added. Brownies (Betty Crocker Dark Chocolate Fudge Brownie Mix; baked using the recipe on the box) = 10.0 American Pasteurized Process Cheese Food, Singles (Kraft Foods) = 14.0

Table 6.2 ***Continued***

Melted Cheese **Surface Characteristics[1] (evaluated at 96.1°C)**		
Free oil release	The amount of free oil on the surface of the melted cheese.	None to Extreme
Blister Color	The brown color intensity of the blisters.	No brown color to All dark brown color
Blister Quantity	The amount of blisters on the melted surface of the pizza pie.	None – Complete coverage
Skinning	The thickness and toughness of the surface of the melted cheese.	None to Extreme
Stretch Characteristics[1] (evaluated at 90.6°C)		
Stretch- Strand Length	Stretch the cheese. Insert 1 tine of fork 1 cm into melted cheese. Pull cheese at a controlled constant rate. Measure the height the cheese is stretched to	Height of the stretch was measured in inches
Stretch – Strand Thickness	The thickness of the melted cheese strand. Insert 1 tine of fork 1 cm into melted cheese. Pull up at a controlled constant rate to 6 inches. Stop pulling strand. Observe the melted cheese strand thickness at 3 inches. If strand does not reach 6" – please write down response as NA (Not Applicable).	Reference images used

Table 6.2 ***Continued***

Texture (evaluated at 62.8°C after heating step)		
Hardness (First Chew)	Force required to bite through the sample with molars. Fold the cheese into 1/4 with inside out, bite with molars.	Philadelphia Full-fat Cream cheese (Kraft Foods) = 0.5 Spam (Hormel Brand) = 2.0 Beef Frankfurters (Best's Kosher Brand) = 5.0 Chewy caramel (Kraft Classic CARAMELS Traditional) = 7.0 Almond (Blue Diamond Brand) = 12.0 Licorice (Starburst Brand) = 15.0
Chewiness (Chewdown characteristics)	The length of time required to masticate the sample to a state pending swallowing. The longer the time required, the chewier the sample is.	Pound Cake (Sara Lee All Butter Pound Cake) = 1.0 Beef Frankfurters (Best's Kosher) = 4.0 Fig Newtons (Nabisco Brand, Kraft Foods) = 7.0 White bread (Wonder Brand) = 9.0 Chewy caramel (Kraft Classic CARAMELS Traditional) = 12.0 Chewing gum (Wrigley's Doublemint) = 15.0
Cohesiveness of Mass (Chewdown characteristics)	Degree to which sample holds together in a mass. Put cheese sample between molars and chew 15 times. Gather to the middle of mouth, evaluate cohesiveness of mass.	Polenta (Food Merchants Brand) = 0.0 Carrots (Metcalfe's Sentry Foods) = 1.0 Beef Frankfurter (Best's Kosher Brand) = 4.5 Wheaties toasted whole wheat flakes (General Mills) = 7.5 Fig Newtons (Nabisco Brand, Kraft Foods) = 11.0 White bread (Wonder Brand) = 14.0

Table 6.2 ***Continued***

Flavor[1] (evaluated at 62.8°C after heating step)		
Acid	Basic taste sensation elicited by acids	None to Pronounced
Salt	Basic taste sensation elicited by salt	None to Pronounced
Butter	Basic taste sensation elicited by butter	None to Pronounced

[1]Attributes were evaluated using Quantitative Descriptive Analysis (Meilgaard et al., 1999), adapted from Chen et al. (2009).
[2]The following attributes: free oil release, blister colour, blister quantity and strand thickness of the stretched cheeses were evaluated using reference images as described by Chen et al. (2009).

6.3 Results and Discussion

6.3.1 Rheological Properties of Cheesemilks during Rennet Coagulation

Due to differences in the general proteolytic activity of recombinant BCC and CC, the gelation conditions were varied to give milk gels with similar gelation times. CC has a higher specificity for κ-casein than BCC and a 70% higher clotting activity (using visual determination of the clotting point in min) than BCC (Kappeler et al., 2006). The level of CC has previously been lowered to give a coagulum with similar gel strength as when BCC was used (Bansal et al., 2009). In this study, the level of calf and CC (HBCC and LCC) were varied at the same temperature to give similar gelation times, the temperature and level of $CaCl_2$ was also varied to have two reference treatments (LBCC and HCC) with the same gelation time as shown in Table 6.1. Varying gelation conditions lead to no significant difference ($P>0.05$) in gelation time for any of the treatments (Table 6.3). After the onset of gelation, the rate of gel firming was different for each treatment, which can be attributed to the variations in gelation conditions used. Temperature can affect the rate of increase in storage modulus and rheological behaviour of rennet-induced gels (Zoon et al., 1988). LBCC had a significantly ($P > 0.05$) higher rate of gel firming compared to HCC and LCC, although it had the lowest enzyme concentration used (0.037 IMCU/mL), it also had the highest coagulation temperature (36°C) treatment and $CaCl_2$ addition. The higher gelation temperature could cause an increase in rennet activity and fusion of micelles (Zoon et al., 1988; Mishra et al., 2005) leading to a faster rate of gel firming. The rate of gel firming of HBCC was higher but not significantly ($P > 0.05$) different than both the camel treatments

(HCC & LCC) probably because of the higher concentration of enzyme used compared to LCC and the higher temperature used compared to HCC. HCC and LCC had very similar gel firming rates even though their enzyme concentrations were different and this is likely due to the effect of change of temperature on the gelation process. HCC was clotted at a lower temperature (31.5°C), which can slow down the gel firming rate and enzyme activity compared to LCC (33.3°C) resulting in these samples having similar gel firming rates.

Table 6.3 Small and large deformation properties of milk during rennet coagulation using high calf chymosin (HBCC), low calf chymosin (LBCC), high camel chymosin (HCC) and low camel chymosin (LCC) treatments.

	HBCC	**LBCC**	**HCC**	**LCC**
Gelation time[1] (min)	20.50^{a} (0.00)[2]	19.50^{a} (0.71)	19.75^{a} (0.35)	20.75^{a} (0.35)
G' at cutting[3] (Pa)	17.65^{c} (0.49)	5.10^{a} (0.29)	13.70^{b} (0.42)	14.50^{b} (0.57)
Gel firming rate[4] (Pa min^{-1})	0.68^{b} (0.02)	0.71^{b} (0.00)	0.50^{a} (0.01)	0.56^{a} (0.02)
Yield[5] Stress (Pa)	43.80^{b} (0.99)	27.90^{a} (0.28)	53.50^{c} (3.11)	47.60^{bc} (0.71)
Yield Strain	1.64^{a} (0.01)	2.25^{c} (0.03)	1.87^{ab} (0.11)	1.93^{b} (0.01)

[1]Point when the gels had a storage modulus of ≥ 1.0 Pa.
[2]Values represent the mean and standard deviation, with the latter in parentheses (n=2).
[3]Firmness value of the gels at the cutting time selected subjectively by our cheesemakers
[4]Gel firming rate was defined as the rate of change in storage modulus (G') values between the gelation time and cutting time (Pa min^{-1})
[5]Yield was defined as the point when the shear stress started to decrease when gels were subjected to a constant shear rate of ~ 0.01 s-1
[a,b,c]Means within the same row not sharing a common superscript differ ($P < 0.05$)

LBCC had the lowest G' at cutting (Table 6.3) as it was cut earlier than the other milks to reduce its moisture content. HBCC has the highest G' (17.65 Pa)

at cutting compared to both camel treatments due to its higher enzyme concentration and higher temperature compared to the G' values of LCC (14.50 Pa) and HCC (13.70 Pa), respectively. Hydrophobic interactions decrease with decreasing temperature which could weaken the bonds in the micelle (Zoon et al., 1988) this could be related to the lower G' observed for HCC. Higher renneting temperature has previously been seen to produce gels with a higher G' (Lucey, 2002).

The yield stress is the force needed to yield or fracture the gel network (Govindasamy-Lucey et al., 2011). LBCC gave the lowest yield stress as it was cut shortly after gelation and had less time after rennet addition, which could cause a decrease in the number and strength of bonds. Rennet-induced gels exhibit time-dependent behaviour, therefore the time allowed before cutting impacts the rheological properties of the coagulum. The longer a gel is aged the more stress that is required for fracture (Zoon et al., 1989). HBCC, HCC and LCC were all cut at the similar time after rennet addition but had different yield stress values; HCC was the highest but was not significantly different ($P > 0.05$) from LCC, and LBCC had the lowest value. It has previously been reported (Mishra et al., 2005) that yield stress values of rennet induced gels can increase when gelation temperature is raised from 29 to 32°C. Both HBCC and LCC had higher gelation temperature (33.3°C) and the yield stress was lower, possibly from the effect of temperature on the bonds in the network, such as a greater possibility of rearrangements in the network and spontaneous bond breakage (Mishra et al., 2005).

LBCC had the highest yield strain compared to the other treatments as the coagulum was cut earlier. HBCC had a lower yield strain compared to HCC but a similar value to LCC. Longer cutting time resulted in decreased yield strain due to ongoing rearrangements in the network forming a more brittle and shorter texture (Lucey 2002; Mishra et al., 2005; Govindasamy-Lucey et al., 2011). HCC and LCC had similar yield strain values (~1.9) which reflected the same storage modulus at cutting (13.7 and 14.5 Pa, respectively).

6.3.2 Cheese Composition, pH and Level of Insoluble Calcium

In preliminary studies, when the same manufacturing protocol was used to manufacture all the cheeses, the moisture content of the LBCC cheese (49%, data not shown) was much higher than those of the HBCC, HCC or LCC cheeses (~46 to 47%). The manufacturing protocol was then modified to decrease the moisture of the LBCC cheeses to be comparable with HBCC, HCC and LCC cheeses by cutting LBCC coagula faster at 25 min after rennet addition compared to 45 min for all other treatments. Also LBCC curds were cut into 4 slabs compared to the rest, which were not cut into any slabs, this increased the surface area for syneresis of LBCC curd.

The chemical composition of cheese made with different levels of calf or CC are shown in Table 6.4. No significant difference was observed for the composition of the cheeses once the moisture content was corrected. The compositions of the cheeses were similar to previous studies on LMPS Mozzarella (Kindstedt et al., 1995). Treatment did not have a significant effect ($P > 0.05$) on pH (Figure 6.1) and the level of insoluble calcium (Figure 6.2) in

Table 6.4 Composition (14 d) of LMPS Mozzarella cheese made using high calf (HBCC), low calf (LBCC), high camel (HCC) and low camel (LCC) chymosin treatments. Values are means of three replicates.

	Treatment					
	HBCC	LBCC	HCC	LCC	SEM	*P*-value
% Moisture	47.31 [a]	47.84 [a]	47.50 [a]	47.92 [a]	0.193	NS[2]
% Fat	21.85 [a]	21.55 [a]	22.16 [a]	21.75 [a]	0.244	<0.05
% Salt	1.61 [a]	1.46 [a]	1.59 [a]	1.62 [a]	0.039	NS
% Protein[1]	25.41 [a]	25.77 [a]	25.43 [a]	24.95 [a]	0.163	<0.01
% MNFS[3]	60.54 [a]	60.99 [a]	61.02 [a]	61.24 [a]	0.340	NS
% FDM[4]	41.46 [a]	41.31 [a]	42.20 [a]	41.77 [a]	0.519	<0.05
% S/M[5]	3.40 [a]	3.05 [a]	3.35 [a]	3.38 [a]	0.081	NS
Total calcium[6]	601[a]	598[a]	650[a]	658[a]	15.4	NS

[1]Total % N × 6.38
[2]Nonsignificant (F test for full stastistical model $P > 0.05$)
[3]Moisture in nonfat substance of the cheese
[4]Fat content on a dry weight basis
[5]Salt in moisture phase of the cheese
[6]Total calcium (mg/100g)
[a,b,c]Means within the same row not sharing a common superscript differ ($P < 0.05$)

the cheese (Table 6.5). Cheesemaking day or milk source had an effect on the pH (Table 6.5) and some differences were observed at 1 d and 14 d but after 14 d of ripening no significant differences ($P > 0.05$) were observed for the pH values of any of the treatments. A change in pH through ripening was not significant ($P > 0.05$) for each individual treatment but a slight increase was observed in the first 14 d of ripening for all cheeses (Figure 6.1) which could be related to solubilization of colloidal calcium phosphate (CCP). Hydrogen ions can be react with phosphate anions produced during solubilization of CCP but fermentation of residual lactose by starter cultures can cause resistance to pH change in the cheese leading to the slight increase but relatively stable pH observed (Guinee et al., 2002; Lucey et al., 2003). As expected, the level of insoluble Ca in the cheese decreased with age (Figure 6.2). The proportion of

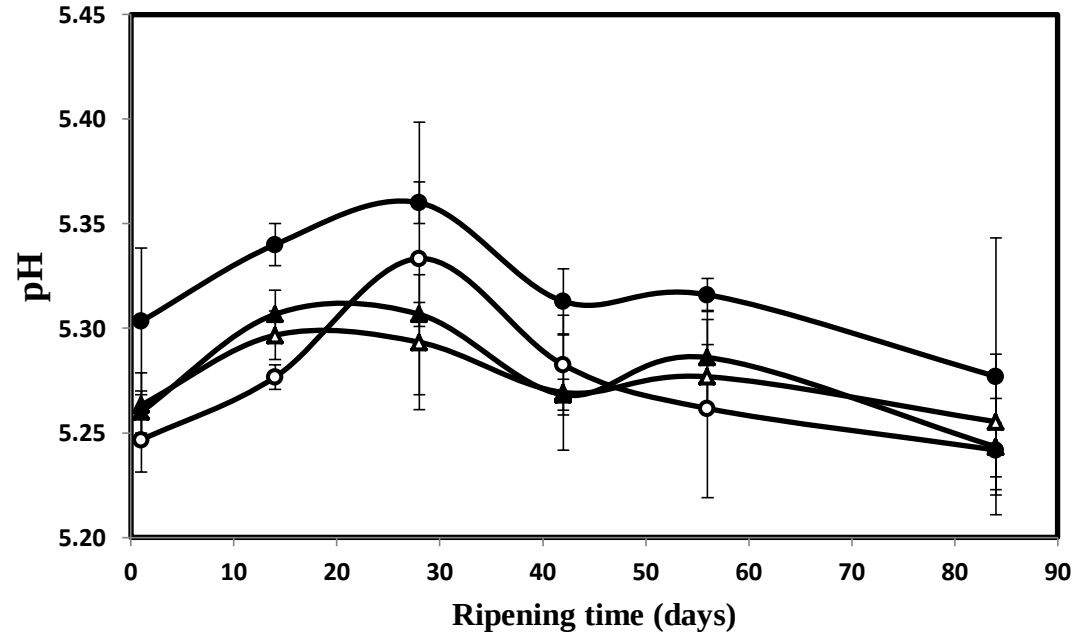


Figure 6.1 pH as a function of ripening time of LMPS Mozzarella made using high calf (HBCC) (○), low calf (LBCC) (Δ), high camel (HCC) (●) or low camel (LCC) (▲) chymosin. Values are means of three replicates; error bars indicate ± one standard deviation.

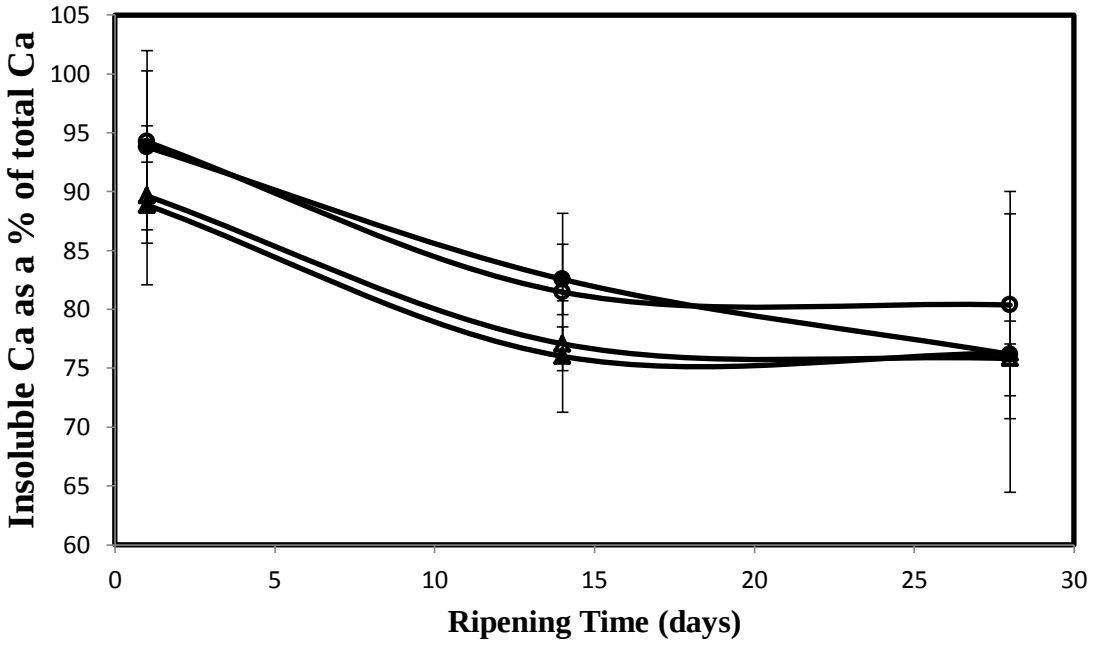


Figure 6.2 Percentage insoluble calcium as a function of ripening time of LMPS Mozzarella made using high calf (HBCC) (○), low calf (LBCC) (Δ), high camel (HCC) (●) or low camel (LCC) (▲) chymosin. Values are means of three replicates; error bars indicate ± one standard deviation.

Table 6.5 Mean squares and probabilities (in parentheses), and R^2 values for pH, pH4.6 SN/TN and insoluble calcium formed during ripening of LMPS Mozzarella cheese.

Factor	df	pH	df	% Proteolysis	df	% Insol Ca
Whole-plot						
Treatment (T)	3	0.008	3	55.033**	3	54.088
		(0.090)		(<0.0001)		(0.2363)
Day of cheesemaking (D)	2	0.001	2	1.335*	2	236.321*
		(0.795)		(0.026)		(0.0195)
Error (T x D)	6	0.002	6	0.186	6	28.999
Subplot						
Age (A)	5	0.007**	4	72.089**	2	732.537**
		(<0.0001)		(<0.0001)		(<0.0001)
A x T	15	0.000	12	1.855**	6	6.939
		(0.668)		(<0.0001)		(0.8946)
Error	40	0.001	32	0.124	16	19.364
R^2		0.797		0.992		0.882

*$0.01 < P \leq 0.05$

** $P \leq 0.05$

insoluble Ca and pH are closely associated with the rate of acid production during cheese manufacture (Lucey et al., 2003).

6.3.3 Proteolysis

Treatment and age of cheese caused significant differences ($P < 0.05$) in the level of pH 4.6-soluble nitrogen as a percentage of total nitrogen (Table 6.5) which is an index of proteolysis. HCC and LCC had a lower level of primary proteolysis after 1 d of ripening and were significantly different ($P < 0.05$) when compared with cheese made with BCC at all ripening times (Figure 6.3). This is probably due to the lower general proteolytic activity of CC compared with BCC (Kappeler et al., 2006). CC has previously been shown to produce a lower level of proteolysis in cheese (Bansal et al., 2009; Govindasamy-Lucey

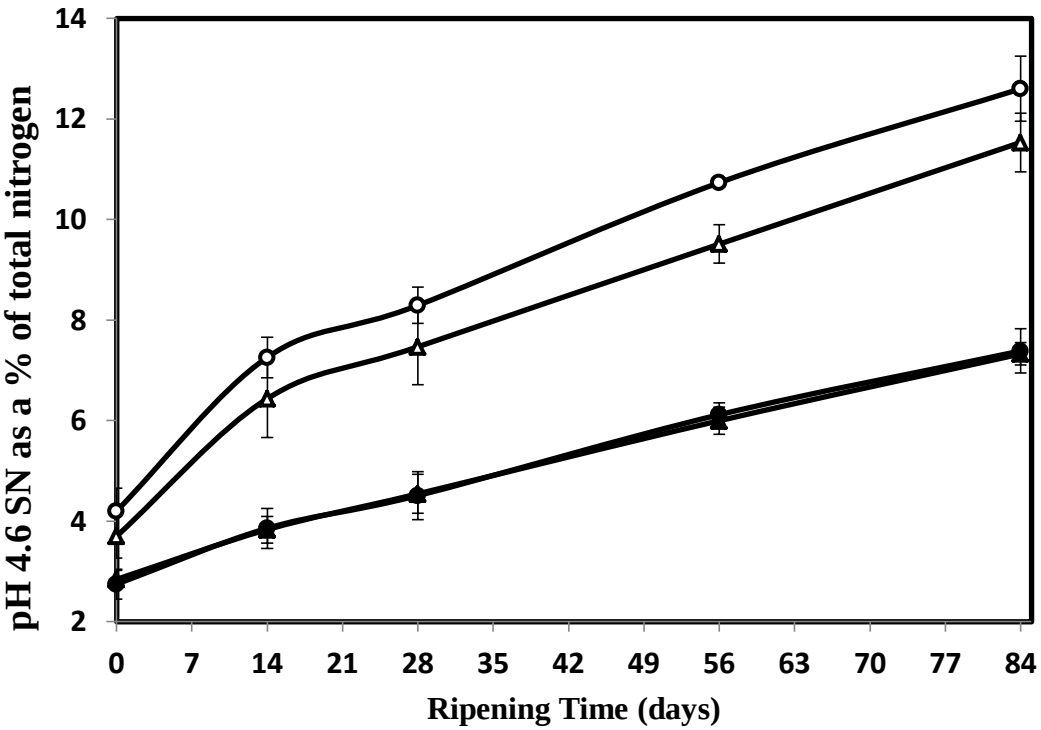


Figure 6.3 pH 4.6 soluble nitrogen as a % of total nitrogen as a function of ripening time of LMPS Mozzarella made using high calf (HBCC) (○), low calf (LBCC) (Δ), high camel (HCC) (●) or low camel (LCC) (▲) chymosin. Values are means of three replicates; error bars indicate ± one standard deviation.

et al., 2010). Proteolysis increased as ripening progressed for all cheeses, as expected. The increase was significantly different ($P < 0.05$) at all ripening times for both BCC treatments but no significant difference was observed for CC cheeses between 14 and 28 d of ripening showing a possible decrease in the rate at which pH 4.6-soluble nitrogen is produced when CC was used in LMPS Mozzarella. Increase in the level of proteolysis throughout ripening is typical in cheese (Kindstedt et al., 1994). The amount of CC added during cheesemaking to the cheese did not have a significant effect ($P > 0.05$) on the level of pH 4.6-soluble nitrogen but the addition of a higher level of BCC produced a significantly ($P < 0.05$) higher level of proteolysis after 28 d compared to the LBCC cheese. Similarily, Dave et al. (2003) found that extent

of overall proteolysis, as determined by 12% TCA-soluble nitrogen and the disappearance of intact caseins during storage, was proportional to the level of BCC used during manufacture of directly acidified Mozzarella cheese. When higher concentrations of rennet are added to cheesemilk, it is expected that more coagulant would be retained in the curd, which could thereby cause a higher level of proteolysis (Kindstedt et al., 1995). This trend was seen for BCC but not for CC. Bansal et al. (2007) suggested that casein micelles are saturated with respect to chymosin, as it was found that rennet concentration did not impact its retention in curd; perhaps CC cheese was saturated with respect to CC at both high and low levels, and therefore the residual level in the cheeses would have been the same, resulting in similar levels of proteolysis regardless of amount added.

6.3.4 Rheological Properties of Cheese

Treatment did not have an effect on the LT_{max} (Table 6.6). The LT_{max} has previously been used as an index of meltability with a higher LT indicating a cheese with more liquid-like property (Govindasamy-Lucey et al., 2005). Treatment did have a significant effect on proteolysis but not on the level of insoluble calcium in the cheese (Table 6.5). Both proteolysis and insoluble Ca play a role in the changes in the rheological properties of cheese during ripening (Lucey et al., 2003); it has previously been found that insoluble calcium is more highly significantly correlated with the LT_{max} than proteolysis (Lucey et al., 2005). The use of different levels of enzyme or different enzyme type did not affect the meltability of the cheese. It has previously been reported that coagulant concentration does not affect the meltability of Mozzarella

Table 6.6 Mean squares and probabilities (in parentheses), and R^2 values for rheological properties, meltprofiler data and texture profile anaylsis for LMPS Mozzarella cheese during 84 d of ripening.

Factor	Df	LT_{max}	Temperature of LT_{max}	Lt= 1	Softening Point	DOF	Hardness	Chewiness
Whole-plot								
Treatment (T)	3	0.469	27.377*	11.918**	16.731*	23.322	462.767*	21.313
		(0.504)	(0.0474)	(<0.0001)	(0.0112)	(0.0649)	(0.0154)	(0.0509)
Day of cheesemaking (D)	2	0.141	4.174	2.527**	8.939	40.042*	705.520**	79.637**
		(0.152)	(0.5140)	(0.0022)	(0.0529)	(0.0258)	(0.0073)	(0.0031)
Error (T x D)	6	0.054	5.602	0.125	1.792	5.597	56.653	4.518
Subplot								
Age (A)	3	0.305**	60.227**	46.35**	9.786*	130.356**	130.974**	18.799**
		(<0.0001)	(0.0003)	(<0.0001)	(0.0207)	(<0.0001)	(0.0033)	(<0.0001)
A x T	9	0.153**	4.770	1.26	3.577	3.859	27.201	1.263
		(0.0006)	(0.6687)	(0.15)	(0.2289)	(0.8352)	(0.3126)	(0.2267)
Error	24	0.293	6.437	0.746	2.494	7.226	21.756	0.877
R^2		0.810	0.673	0.914	0.706	0.782	0.880	0.939

*$0.01 < P \leq 0.05$

** $P \leq 0.05$

cheese (Kindstedt et al., 1995). After 14 d, the LT_{max} for both the BCC treatments began to decrease and the CC treatments increased from 14 to 28 d after which time it decreased until 84 d (Figure 6.4). The LT_{max} for most cheeses typically increases throughout ripening (Govindasamy-Lucey et al., 2007). Govindasamy-Lucey et al. (2010) found that CC caused a significantly lower melt in low-fat Cheddar cheese during ripening compared to BCC; this study did not report the level of insoluble Ca in the cheese which may have contributed to these differences. It has been reported that Mozzarella cheese may increase in stretch up to 1 month but the cheese can become "soupy" beyond this point due to shorter and weaker strands (Lucey, 2008). The decrease in melting (Figure 6.4) could also possibly be due to the extended

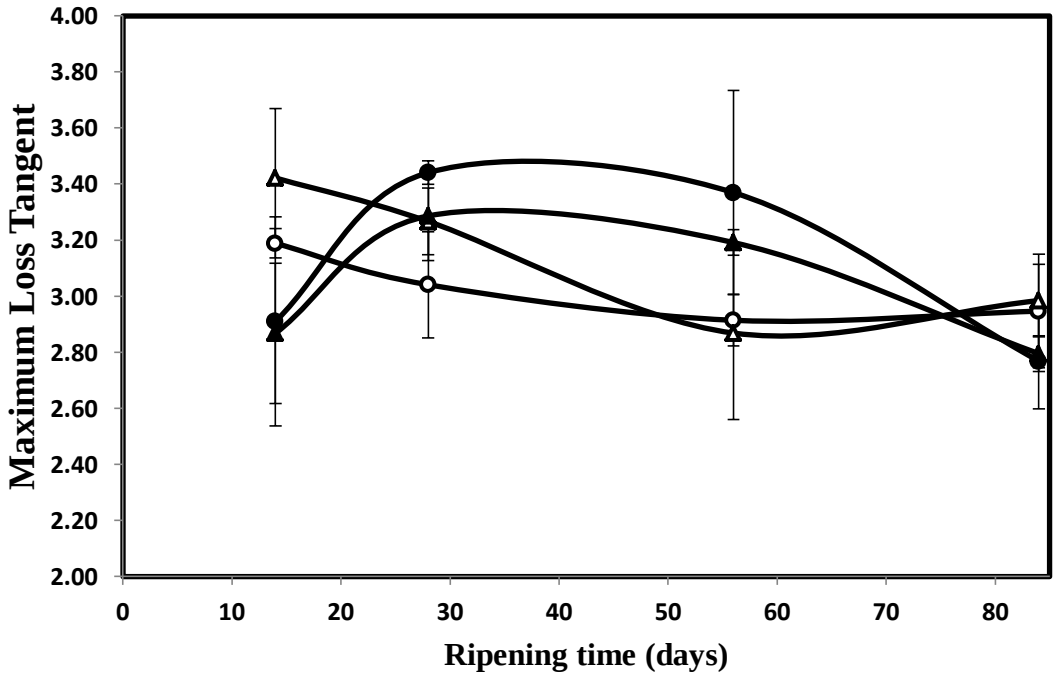


Figure 6.4 Maximum loss tangent (tan δ) as a function of ripening time of LMPS Mozzarella made using high calf (HBCC) (○), low calf (LBCC) (Δ), high camel (HCC) (●) or low camel (LCC) (▲) chymosin. Values are means of three replicates; error bars indicate ± one standard deviation.

storage of 84 d. The increase at the beginning of storage for the CC cheeses and the slower decrease in LT_{max} observed could suggest CC treatment maintains a higher melt and maintaining strong strands due to less proteolysis (Figure 6.3).

Treatment had a significant effect (Table 6.6) on the temperature where the LT = 1 (the crossover point) which is the temperature where the cheese is changing from a more solid to viscous-like material (Gunasekaran & Ak, 2003). No significant effect ($P > 0.05$) on LT = 1 was observed between any of the treatments at 14 d. As ripening progresses HCC and LCC cheeses had a significantly higher crossover point (Figure 6.5) compared to the BCC treated cheese ($P < 0.05$). The temperature where the LT = 1 typically decreases during cheese ripening (Govindasamy-Lucey et al., 2005) and is an index of the softening point of cheese (Gunasekaran & Ak, 2003). A reduction in the LT = 1 during ripening is probably because of the loss of intact CN (caused by ongoing proteolysis) and the loss of cross-linking material (caused by the shift from insoluble to soluble calcium), both of which occur during cheese ripening (Lucey et al., 2003, 2005). As there was no difference in the insoluble calcium levels in all the cheeses (Figure 6.2, Table 6.6), the higher crossover temperature for the cheeses manufactured with CC was due to the lower proteolysis in these cheeses compared to those made with BCC. The CC cheeses decreased in temperature where the LT = 1 at a slower rate than the BCC cheese (Figure 6.5), maintained a higher crossover temperature and required higher temperatures to soften, suggesting that LMPS Mozzarella cheese made with CC had a longer window of acceptable functionality.

Treatment had a significant effect on the temperature where the LT_{max} occurs (Table 6.6). As ripening progresses, the temperature where the LT_{max} occurred began to decrease (Lucey et al., 2005; Govindasamy-Lucey et al., 2005). At 14 d, very little difference was observed for any of the treatments but over the ripening time the cheese made with CC maintained a higher temperature compared to the BCC treated cheese. At 56 d HBCC cheese was significantly ($P < 0.05$) lower than HCC and LCC cheeses.

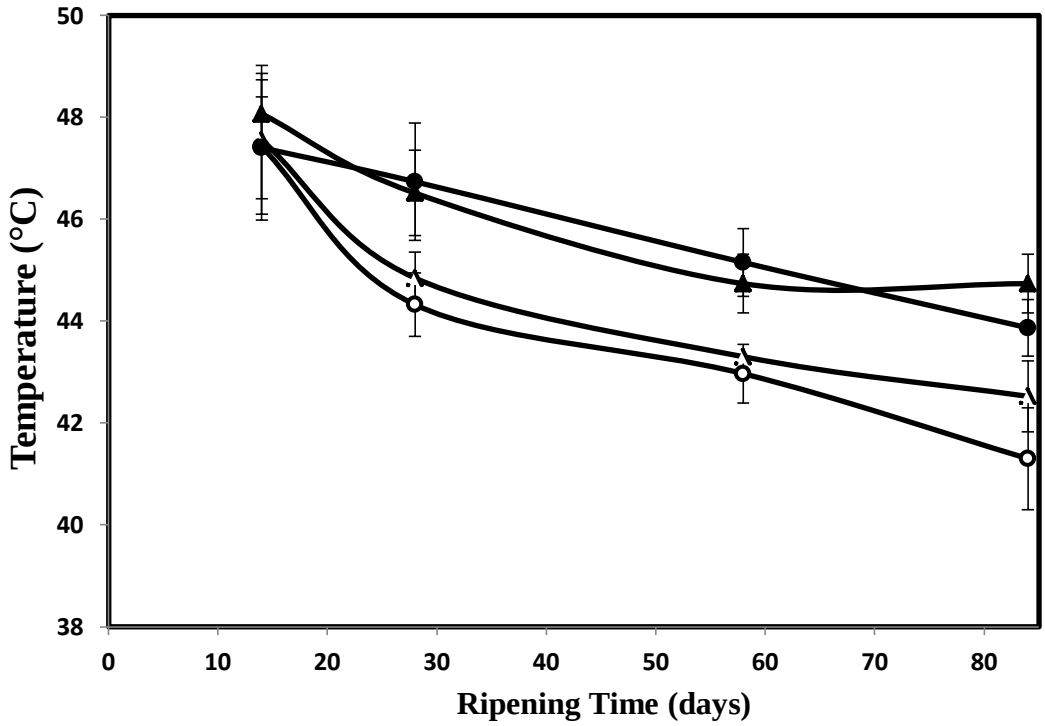


Figure 6.5 Temperature where the loss tangent is 1 as a function of ripening time of LMPS Mozzarella made using high calf (HBCC) (○), low calf (LBCC) (Δ), high camel (HCC) (●) or low camel (LCC) (▲) chymosin. Values are means of three replicates; error bars indicate ± one standard deviation.

The lower values observed for cheese made with BCC (Figure 6.6) could indicate that the higher proteolysis rate affected the temperature at which the LT_{max} occurred; in cheese made with BCC this occurred at a lower temperature and at a faster rate during ripening. Lucey et al. (2005) found that when insoluble Ca was not taken into account proteolysis was weakly correlated with the temperature of the LT_{max}, which could explain the relationship observed in this study.

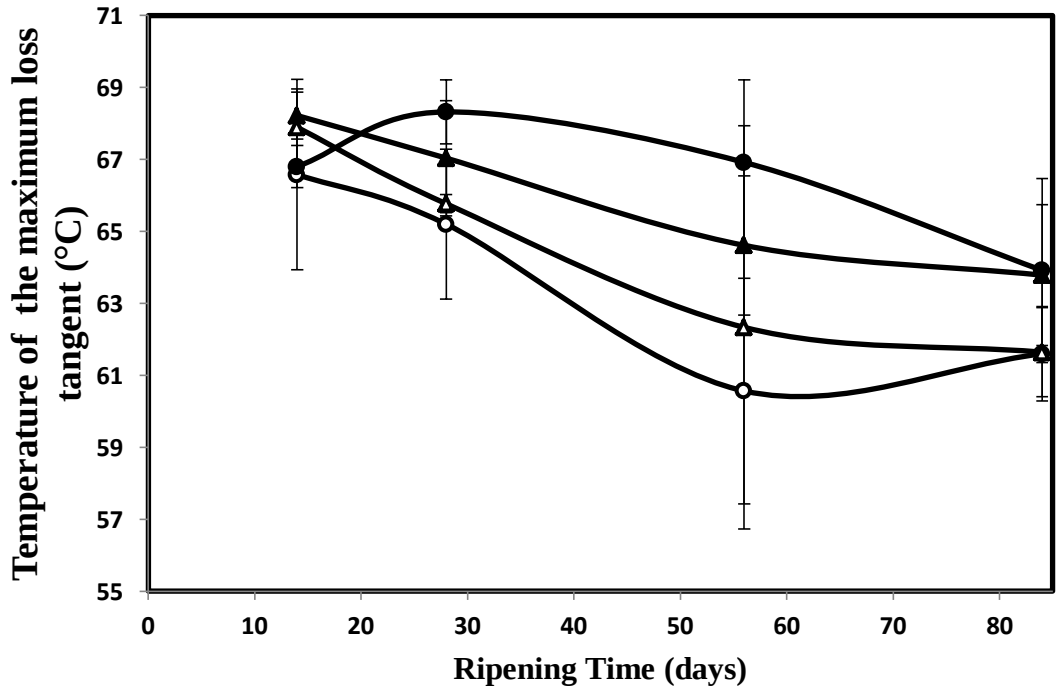


Figure 6.6 Temperature where the maximum loss tangent occurs as a function of ripening time of LMPS Mozzarella made using high calf (HBCC) (○), low calf (LBCC) (Δ), high camel (HCC) (●) or low camel (LCC) (▲) chymosin. Values are means of three replicates; error bars indicate ± one standard deviation.

6.3.5 Melt profile analysis

Degree of flow (DOF) was not affected by treatment (Table 6.6). DOF is the percent change in height of the cheese when heated to 60°C. No significant

differences ($P > 0.05$) were observed for any of the cheeses up to 56 d, possibly due to insoluble Ca being more highly correlated with DOF than proteolysis (Lucey et al., 2005).

The DOF of HBCC treated cheese was significantly higher ($P < 0.05$) than HCC and LCC treatments at 84 d of ripening. The DOF decreased or remained constant from 14 to 28 d and increased for all cheeses from 56 to 84 d (Figure 6.7). The UW-meltmeter test is a destructive measurement, whereas oscillatory is non-destructive; the differences between treatment effect for LT_{max} and DOF could be attributed to squeezing by the top plate used in the UW-meltprofiler causing the cheese to flow whereas for the rheometer, plate gap is kept constant during heating and no flow is observed (Lucey et al., 2005).

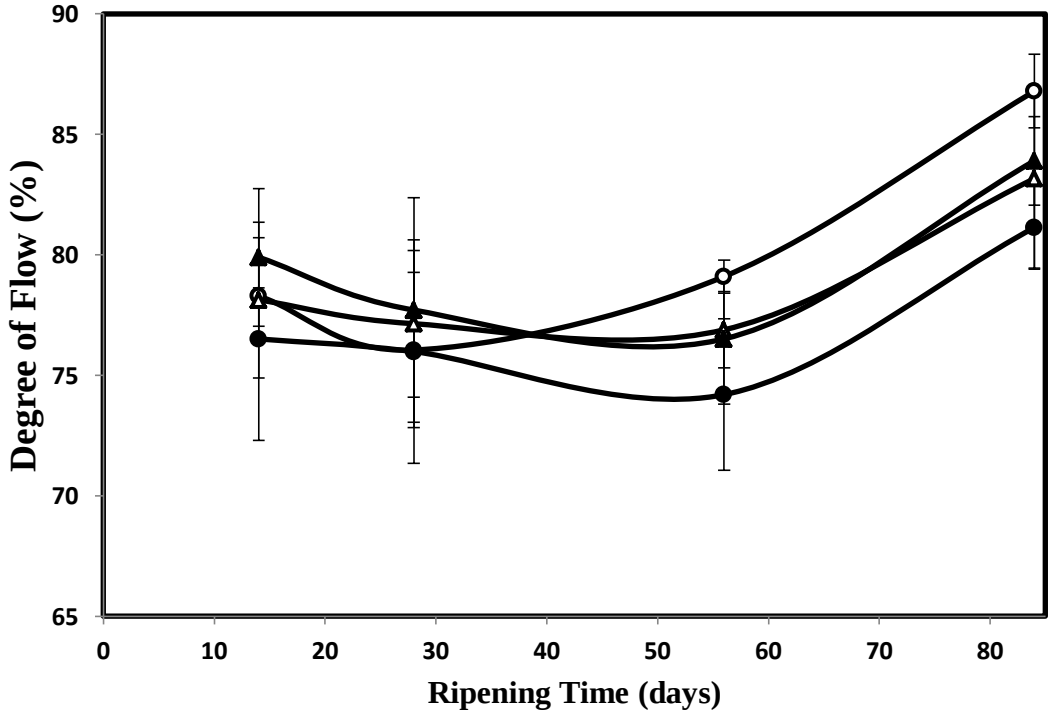


Figure 6.7 Degree of flow at 60°C as a function of ripening time of LMPS Mozzarella made using high calf (HBCC) (○), low calf (LBCC) (Δ), high camel (HCC) (●) or low camel (LCC) (▲) chymosin. Values are means of three replicates; error bars indicate ± one standard deviation.

Also the temperature at which the LT_{max} occurred was above 60°C at all ripening points. The significantly higher DOF for HBCC at 84 d could be due to extensive proteolysis, which may cause more changes in the rheological properties than any minor differences in insoluble Ca. Govindasamy-Lucey et al. (2010) found that, from the beginning of ripening up until 3 months of aging, low-fat Cheddar cheese made with camel chymosin had a lower DOF compared to cheese made with BCC at 6 months.

Treatment significantly ($P < 0.05$) affected the temperature of the softening point (Table 6.6). Essentially no change was observed in the softening point of the cheese up until 56 d of ripening. After 56 d the softening point of cheese made with BCC began to decrease while cheese made with CC maintained a

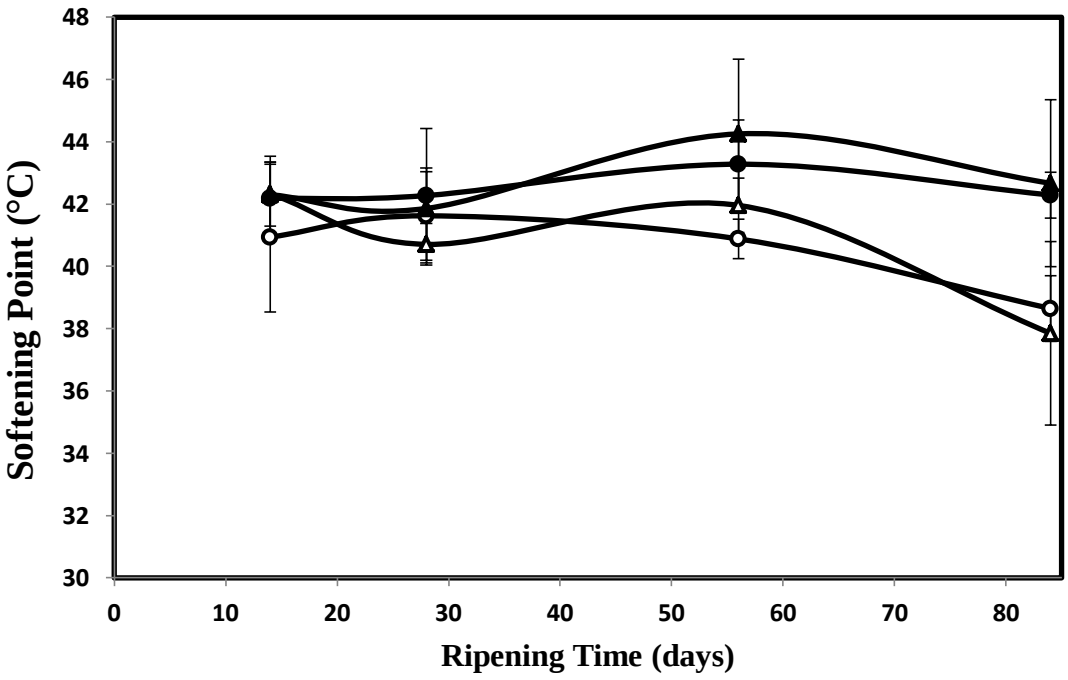


Figure 6.8 Softening point as a function of ripening time of LMPS Mozzarella made using high calf (HBCC) (○), low calf (LBCC) (Δ), high camel (HCC) (●) or low camel (LCC) (▲) chymosin. Values are means of three replicates; error bars indicate ± one standard deviation.

greater softening point. At 84 d both HBCC and LBCC cheeses had significantly lower ($P < 0.05$) softening temperature compared to HCC and LCC cheeses (Figure 6.8). The softening point of cheese typically decreases for cheese during ripening (Muthukumarappan et al., 1999). This decrease was not observed in the present study until 84 d of ripening for the cheese made with BCC. This could indicate that the usage of CC extended the acceptable window of functionality for LMPS Mozzarella by maintaining a higher softening temperature at the end of ripening. The temperature of LT = 1 is also related to the softening point of cheese; as discussed earlier, it was seen that the temperature of the crossover point was lower in the calf cheeses from 28 d of ripening. While this difference is not clear from the UW-Meltprofiler data until 84 d it has been previously reported that proteolysis may not be the only reason for the decrease in softening point of cheese (Muthukumarappan et al., 1999).

6.3.6 Texture Profile Analysis

Treatment had a significant effect on hardness values as measured by texture profile analysis (TPA) (Table 6.6). At 14 d of ripening there was no clear difference between any of the cheeses (Figure 6.9a). As ripening progresses, both BCC treatments decreased in hardness but there was no significant difference between high and low treatments ($P > 0.05$). This decrease in hardness during ripening was significant ($P < 0.05$) for HBCC treated cheeses after 28 d of ripening. The decrease in LBCC treatment was not significant as ripening time progressed further. The hardness of CC treated cheeses did not

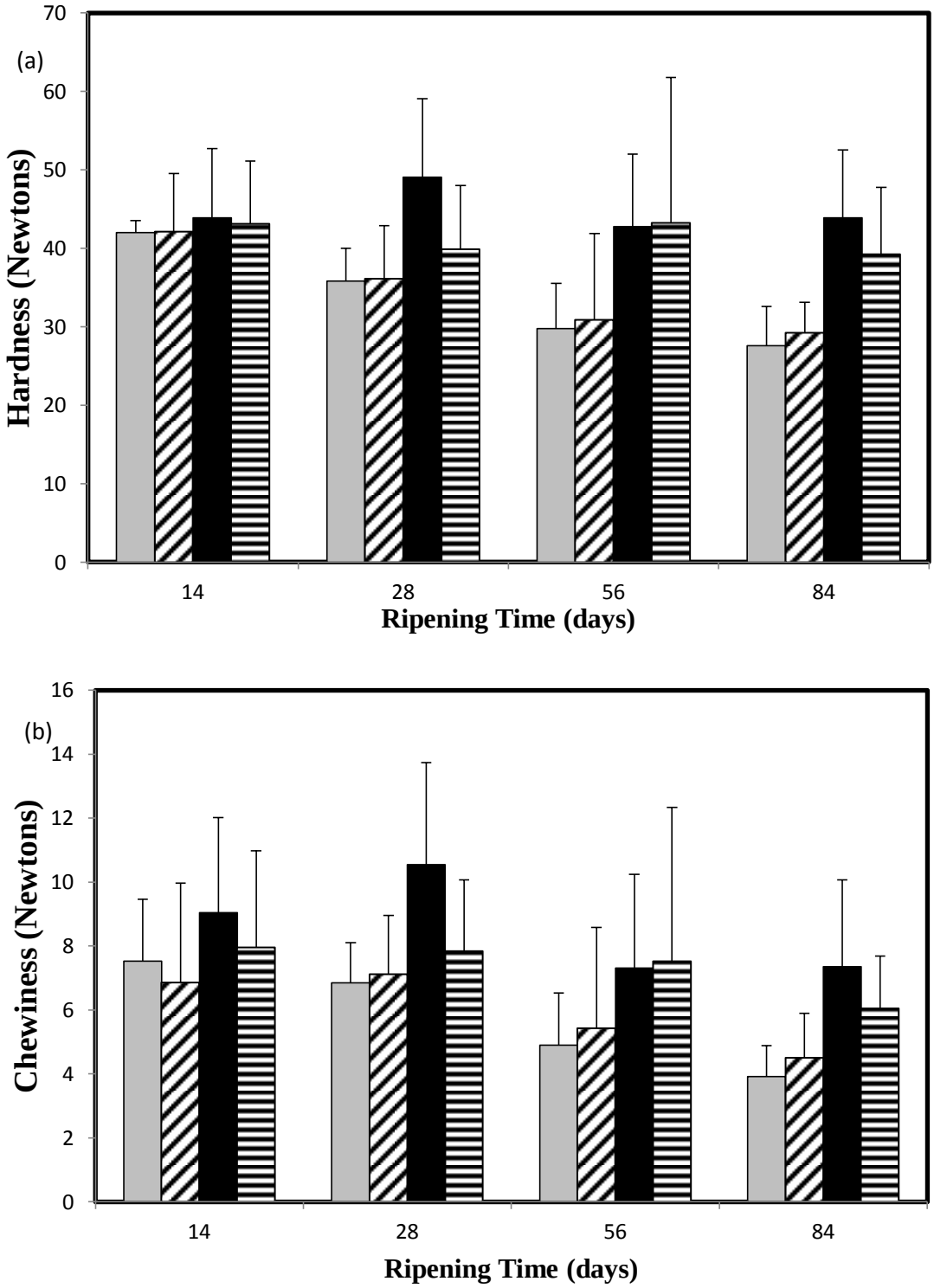


Figure 6.9 (a) Hardness and **(b)** chewiness values of LMPS Mozzarella throughout ripening for cheeses made with high calf (HBCC) (▫), low calf (LBCC) (◪), high camel (HCC) (■) and low camel (LCC) (▬) chymosin treatments. Values are means of three replicated ± one standard deviation.

significantly ($P > 0.05$) decrease during ripening and was essentially unchanged for both treatments (Figure 6.9a). Up until 56 d of ripening there was no significant difference ($P > 0.05$) in hardness for any of the cheeses. In this study hardness value of the CC cheese was higher than BCC cheese but the difference was not significant until 84 d possibly due to the cheesemaking day having a significant effect on hardness values (Table 6.6). At 84 d of ripening HCC was harder even though a higher enzyme concentration was added during cheesemaking and hardness was significantly ($P < 0.05$) higher than both HBCC and LBCC. The observed decrease in the hardness of the HBCC and LBCC cheeses over time and lower hardness values could be due to their higher levels of proteolysis. LMPS Mozzarella typically decreases in hardness during ripening (Kindstedt et al., 1995). Guinee et al. (2002) found that using BCC in Mozzarella resulted in a decrease in hardness throughout ripening which was significantly correlated with the level of unhydrolysed casein. Higher hardness values for CC treated cheeses could be due to their lower level of proteolysis (Figure 6.3) and lower general proteolytic activity of CC (Kappeler et al., 2006). In previous studies using CC in Cheddar cheeses, it was found that cheese made with CC was significantly harder than BCC cheeses at the end of the ripening period (Bansal et al., 2009; Govindasamy-Lucey et al., 2010). There was no significant difference ($P > 0.05$) between high and low treatments (Figure 6.9a). It might be expected that a higher concentration of enzyme would produce a cheese with lower hardness values but there was also no significant difference in the proteolysis of the two types of CC cheeses. The HBCC cheese had significantly higher levels of proteolysis than LBCC cheese at 56 and 84 d of ripening possibly leading to the

significantly lower hardness decrease during ripening (Figure 6.3). Kindstedt et al. (1995) found that the concentration of BCC used for cheesemaking did not affect the TPA hardness value in LMPS Mozzarella.

Treatment did not have a significant effect ($P > 0.05$) on the chewiness values (Table 6.6). The chewiness values for BCC treatments seemed to decrease during ripening and at the end of ripening CC treatments are slightly higher than calf treatments (Figure 6.9b) but this difference was not significant ($P > 0.05$) probably due to the effect of cheesemaking day on TPA results (Table 6.6). Previous studies have observed that cheese made with CC tends to have higher chewiness values proportional to hardness values (Bansal et al., 2009; Govindasamy-Lucey et al., 2010).

6.3.7 Sensory Analysis

Only the attributes that showed a significant difference ($P < 0.05$) between treatments (Tables 6.7 & 6.8) will be discussed here. Firmness, adhesiveness of mass and off flavour intensity showed a significant difference ($P < 0.05$) for treatments when unmelted cheese cubes were evaluated (Table 6.9). At 14 and 28 d of ripening, HBCC and LBCC cheeses had significantly lower firmness values than HCC cheese. At 56 and 84 d, HBCC and LBCC cheeses had significantly lower firmness values than both HCC and LCC cheese. These results are in agreement with the instrumental hardness results as determined by TPA (Figure 6.9a) where cheese made with CC had higher hardness at 56 and 84 d compared to BCC cheeses. The higher sensory firmness values could

Table 6.7 Mean squares and probabilities (in parentheses), and R^2 values for sensory analysis of cheese cubes of LMPS Mozzarella cheese during ripening.

		Cheese Cubes				
Factor	Df	Firmness	Adhesiveness of Mass	Acid Flavour	Salt Flavour	Total Off Flavour
Whole-plot						
Treatment (T)	3	9.399**	6.33**	0.067	0.174	2.611*
		(0.0009)	(0.002)	(0.6895)	(0.4357)	(0.0115)
Day of cheesemaking (D)	2	0.822	0.137	0.012	4.786**	1.506*
		(0.20)	(0.700)	(0.9132)	(0.0008)	(0.0469)
Error (T x D)	6	0.039	0.365	0.132	0.165	0.283
Subplot						
Age (A)	3	4.68**	7.64**	0.050	0.162	4.509**
		(0.0002)	(<0.0001)	(0.7469)	(0.2077)	(<0.0001)
A x T	9	0.294	0.802**	0.073	0.143	0.277
		(0.780)	(0.004)	(0.7868)	(0.2236)	(0.3460)
Error	24	0.489	0.169	0.123	0.099	0.233
R^2		0.806	0.924	0.383	0.844	0.836

*$0.01 < P \leq 0.05$

** $P \leq 0.05$

be related to the lower degree of proteolysis and more intact casein levels for cheese made with CC during ripening (Figure 6.3).

In cheese aged for 14 and 28 d, no significant difference ($P > 0.05$) was observed for any treatment for adhesiveness of mass, which was defined as the degree to which the cheese sticks to the teeth or mouth after chewing (Chen et al., 2009). At 56 and 84 d of ripening HCC and LCC treated cheeses were significantly different ($P < 0.05$) from HBCC and LBCC cheese; CC treatments had a lower adhesiveness of mass than BCC treatments. This showed that with ripening time the cheese treated with CC became less sticky upon chewing and maintained a lower adhesiveness of mass. This could possibly be due to the lower proteolysis giving a more intact structure upon chewing.

A significant difference ($P < 0.05$) between off flavour intensity of treatments was observed at 84 d of ripening where calf treated cheeses had a higher value for perceived off flavour intensity.

Melted cheese characteristics as evaluated by baking shredded LMPS Mozzarella on pizza base are shown in Table 6.10. No significant difference ($P > 0.05$) in blister quantity on pizza was observed at 56 d for LMPS Mozzarella cheese. At 14 d CC had significantly lower blister quantity than the BCC treatments. At 84 d both CC treatments had significantly lower blister quantity. As Mozzarella ages, the quantity of blisters present on the melted cheese surface typically increases (Chen et al., 2009). After 14 d of ripening, strand thickness, as assessed by stretching the melted pizza cheese (Chen et al., 2009), was significantly ($P < 0.05$) lower for BCC treatments compared to CC

Table 6.8 Mean squares and probabilities (in parentheses), and R^2 values for sensory attributes analysed on pizza for LMPS Mozzarella cheese during 84 d of ripening.

		Melted Cheese									
Factor	Df	Free Oil	Blister Colour	Blister Quantity	Strand Thickness	Hardness	Chewiness	Cohesiveness of Mass	Acid Flavour	Salt Flavour	Total Off Flavour
Whole-plot											
Treatment (T)	3	1.217	1.047	18.1**	19.829**	3.89**	3.86*	3.57**	0.213	0.383*	2.057**
		(0.1297)	(0.4434)	(0.0035)	(0.0004)	(<0.0001)	(0.0144)	(0.0027)	(0.2481)	(0.0328)	(0.0027)
Day of cheesemaking (D)	2	4.914*	3.864	41.3**	2.40	0.699**	1.03	13.6**	0.768*	7.190**	0.706*
		(0.0091)	(0.0858)	(0.0005)	(0.0784)	(0.0028)	(0.17)	(0.0001)	(0.0317)	(<0.0001)	(0.0423)
Error (T x D)	6	0.432	1.016	1.22	0.560	0.057	0.419	0.221	0.118	0.066	0.126
Subplot											
Age (A)	3	6.021**	0.906*	14.2**	22.9**	3.93**	0.301	23.7**	0.357*	0.038	5.271**
		(0.0078)	(0.0231)	(<0.0001)	(<0.0001)	(<0.0001)	(0.75)	(<0.0001)	(0.0454)	(0.8800)	(<0.0001)
A x T	9	0.291	0.167	1.20	0.542	0.206	0.229	0.598	0.112	0.108	0.345
		(0.9841)	(0.7024)	(0.3704)	(0.5305)	(0.19)	(0.96)	(0.83)	(0.4808)	(0.7634)	(0.5644)
Error	24	1.203	0.238	1.046	0.595	0.133	0.729	1.11	0.115	0.173	0.397
R^2		0.560	0.788	0.888	0.909	0.885	0.523	0.810	0.643	0.804	0.741

*$0.01 < P \leq 0.05$

** $P \leq 0.05$

treatments. Stretch is the ability of a protein network to maintain its integrity when an elongational stress is applied to cheese (Lucey et al., 2003). The level of proteolysis affects cheese stretch and strand continuity; increased proteolysis can decrease stretch and affect cheese strand characteristics (Chen et al., 2009).

This could possibly be due to the higher levels of proteolysis in calf treatments (Figure 6.3) leading to a lower strand thickness as it has less intact casein and may not maintain strand thickness as well as CC treated cheese (which had a lower proteolysis). Oberg et al. (1992) found that when BCC, bovine pepsin, porcine pepsin or *R. miehei* protease were used in the manufacture of Mozzarella cheese, the type of enzyme used affected the stretch properties of the cheese. At 14 d of ripening the sensory hardness values for melted cheese were significantly ($P < 0.05$) lower for HBCC treatment compared to HCC and LCC. HBCC had the lowest hardness values for melted cheese throughout ripening, and at 28 d this became significantly different ($P < 0.05$) compared to all other treatments. At 56 and 84 d of ripening both BCC treatments were significantly ($P < 0.05$) lower than CC treatments; at these time points no effect of enzyme concentration was observed. These results are similar to those found for the firmness of unmelted cheese and instrumental TPA hardness, which showed that cheese made with camel treatments had higher hardness values than calf treatments especially in aged cheeses (after 56 d). Higher proteolysis and less intact casein is probably the reason for the lower hardness values of the calf treatments. Similarly, low-fat Cheddar cheeses manufactured with CC were also firmer and chewier than those made with BCC (Govindasamy-Lucey et al., 2010).

No significant difference was observed for any of the treatments for the chewiness of the melted cheese at 14 and 28 d of ripening. At 56 d of ripening, HBCC and LBCC were lower in chewiness than both CC treatments. At 84 d of ripening significant differences ($P < 0.05$) were only observed between LCC and both BCC treatments, BCC cheeses had lower chewiness values. At 14 and 28 d cohesiveness of mass (which evaluates the degree to which a melted cheese samples holds together or adheres to itself after chewing) was significantly ($P < 0.05$) higher for HBCC treatments. No significant difference ($P > 0.05$) was observed between enzyme treatments at 56 and 84 d. Cohesiveness increases with cheese age (Chen et al., 2009).

Table 6.9 Sensory analysis results of LMPS Mozzarella throughout ripening for cheese cubes made with high calf (HBCC), low calf (LBCC), high camel (HCC) and low camel (LCC) chymosin treatments. Values represent the mean and standard deviation, with the latter in parentheses (n=3).

Attributes	Ripening Time (d)	Treatment			
		HBCC	LBCC	HCC	LCC
Firmness	14	7.65^{b} (0.38)	7.63^{b} (0.62)	8.91^{a} (0.84)	8.48^{a} (1.18)
	28	6.70^{c} (0.57)	6.98^{bc} (1.23)	8.21^{a} (0.84)	7.90^{ab} (0.33)
	56	5.96^{b} (0.50)	5.56^{b} (0.68)	7.92^{a} (0.24)	7.37^{a} (0.52)
	84	7.07^{b} (0.35)	6.63^{b} (0.72)	8.70^{a} (0.31)	8.81^{a} (0.64)
Adhesiveness of Mass	14	4.17^{a} (0.26)	3.58^{a} (0.19)	3.42^{a} (0.48)	3.62^{a} (0.47)
	28	4.42^{a} (0.62)	4.55^{a} (0.29)	3.62^{a} (0.66)	3.95^{a} (0.85)
	56	5.78^{a} (0.35)	6.13^{a} (0.63)	3.86^{b} (0.05)	3.95^{b} (0.02)
	84	6.46^{a} (0.58)	6.39^{a} (0.77)	4.47^{b} (0.21)	4.60^{b} (0.28)
Off Flavour Intensity	14	2.91^{a} (0.74)	2.69^{a} (0.76)	2.37^{a} (0.29)	2.37^{a} (0.57)
	28	3.42^{a} (0.71)	3.28^{a} (0.54)	2.85^{a} (0.28)	3.22^{a} (0.53)
	56	4.11^{a} (0.53)	3.76^{ab} (0.67)	3.01^{b} (0.50)	3.25^{ab} (0.39)
	84	4.98^{a} (0.75)	4.52^{a} (0.66)	3.27^{b} (0.58)	3.38^{b} (0.20)

[a,b,c]Means within the same row not sharing a common superscript differ ($P < 0.05$)

Off-flavour intensity for melted cheese was not significant ($P > 0.05$) at 14 d of ripening. At 28 and 56 d of ripening, HCC was significantly lower in off-flavour intensity than HBCC and LBCC. At 84 d of ripening both camel treatments were significantly ($P < 0.05$) lower in off-flavour intensities than calf treatments; these were similar results to off-flavours perceived in unmelted cheese. CC extended some of the sensory characteristics for LMPS Mozzarella evaluated in this study. Cheese treated with CC had higher firmness and lower adhesiveness of mass for unmelted cheese. Melted cheese had greater strand thickness, hardness, chewiness and lower blister quantities when evaluated on pizza as ripening progressed. Lower off flavour intensities were also evident in cheese treated with CC. Sensory properties of Mozzarella can be affected by the age of cheese and proteolysis for example extensive proteolysis can affect Mozzarella cheese by causing a loss of stretch properties (Chen et al., 2009). Bansal et al. (2009) found that Cheddar cheese made with CC had lower flavour intensities and sensory texture also showed a lower degree of breakdown which was attributed to lower proteolysis. Govindasamy-Lucey et al. (2010) also found significantly higher firmness and chewiness values for low-fat Cheddar cheese manufactured with CC.

Table 6.10 Sensory analysis results of LMPS Mozzarella throughout ripening for melted cheese made with high calf (HBCC), low calf (LBCC), high camel (HCC) and low camel (LCC) chymosin treatments. Values represent the mean and standard deviation, with the latter in parentheses (n=3).

Attribute	Ripening Time (d)	Treatment			
		HBCC	LBCC	HCC	LCC
Blister Quantity	14	8.77^{a} (1.25)	8.73^{a} (0.82)	7.55^{b} (1.52)	6.59^{b} (1.42)
	28	10.28^{a} (1.13)	9.55^{ba} (2.78)	8.08^{ab} (1.46)	6.10^{b} (2.06)
	56	9.30^{a} (0.96)	10.34^{a} (2.09)	8.69^{a} (1.78)	8.41^{a} (2.43)
	84	11.75^{a} (3.05)	11.59^{a} (2.09)	9.16^{b} (1.25)	9.27^{b} (2.48)
Strand Thickness	14	4.96^{a} (0.61)	5.63^{a} (0.32)	6.93^{a} (1.45)	6.40^{a} (1.05)
	28	3.98^{b} (0.54)	5.00^{b} (1.36)	7.26^{a} (1.28)	7.20^{a} (1.00)
	56	2.86^{b} (0.25)	3.43^{b} (0.62)	5.44^{a} (1.05)	6.05^{a} (0.34)
	84	1.88^{b} (0.25)	2.17^{b} (0.72)	4.11^{a} (0.68)	4.01^{a} (0.43)
Hardness	14	3.91^{c} (0.54)	4.19^{bc} (0.35)	4.62^{a} (0.43)	4.39^{ab}(0.53)
	28	3.33^{b} (0.53)	4.05^{a} (0.43)	4.53^{a} (0.26)	4.48^{a} (0.66)
	56	2.64^{b} (0.21)	3.02^{b} (0.17)	4.15^{a} (0.39)	4.04^{a} (0.33)
	84	2.37^{b} (0.31)	2.44^{b} (0.36)	3.57^{a} (0.55)	3.77^{a} (0.17)
Chewiness	14	7.40^{a} (0.68)	7.69^{a} (0.44)	8.18^{a} (0.73)	8.20^{a} (0.67)
	28	7.71^{a} (0.43)	7.74^{a} (0.79)	8.38^{a} (0.45)	8.46^{a} (0.33)
	56	7.37^{b} (0.91)	7.84^{b} (1.30)	8.86^{a} (0.82)	8.94^{a} (1.28)
	84	7.22^{b} (0.31)	7.81^{b} (0.85)	8.13^{ab} (1.16)	9.08^{a} (1.14)
Cohesiveness of Mass	14	8.93^{a} (1.67)	7.80^{b} (1.22)	8.03^{b} (1.81)	7.75^{b} (1.69)
	28	10.66^{a} (1.28)	9.13^{b} (1.90)	8.35^{b} (0.98)	8.51^{b} (1.96)
	56	10.75^{a} (0.38)	10.36^{a} (1.21)	10.31^{a} (1.88)	9.47^{a} (0.97)
	84	11.75^{a} (0.18)	11.23^{a} (0.73)	11.08^{a} (0.70)	11.53^{a}(0.28)
Off Flavour Intensity	14	2.15^{a} (0.70)	1.96^{a} (0.14)	1.85^{a} (0.27)	1.73^{a} (0.27)
	28	3.07^{a} (0.65)	3.05^{a} (1.05)	2.23^{b} (0.97)	3.27^{a} (0.97)
	56	3.61^{a} (0.33)	3.68^{ab} (0.71)	2.59^{c} (0.76)	2.94^{ac}(0.76)
	84	4.03^{a} (0.19)	4.10^{a} (0.30)	2.69^{b} (0.29)	2.96^{b} (0.74)

^{a,b,c}Means within the same row not sharing a common superscript differ ($P < 0.05$)

6.4 Conclusions

Using CC as the coagulant in LMPS Mozzarella cheese manufacture affected some of its textural, functional and flavour properties. Cheese manufactured with CC had lower proteolysis compared to BCC after d 1 of ripening. This change in proteolytic activity impacted the properties of the cheese. Since the CC cheeses had similar moisture contents, pH values and CCP solubilization the differences in proteolysis can help explain the firmer and chewier texture of cheese made with CC. Proteolysis contributes to a softer texture by weakening the casein network of the cheese. Chymosin hydrolyses the Phe_{23}-Phe_{24} bond of α_{s1}-casein (McSweeney et al., 1993), which is related to the initial softening of cheese (Creamer and Olson, 1982). Shredding and slicing of LMPS Mozzarella cheese requires a firm, nonadhesive texture. CC cheeses had higher hardness, temperature when the LT = 1 (crossover temperature), temperature of the LT_{max} and temperature of the softening point. As ripening time progressed the effect of enzyme treatment became more obvious. Sensory texture descriptive analyses of cheeses at all ripening time points agreed with instrumental texture profile analysis; cheese made with CC were firmer and less sticky than those made with BCC. When cheese performance on pizza was analyzed, properties such as blister quantity, strand thickness, hardness and chewiness seemed to be maintained for longer ripening time than for cheese made with BCC. Off flavour intensities were lower in both melted and unmelted cheese at the end of ripening. The level of each enzymes used (high and low treatment) had little effect on the properties of LMPS Mozzarella; the type of enzyme (BCC or CC treatment) used had a greater effect on the characteristics evaluated in this study. Taking these results into consideration,

CC offers an approach to extend shelf life performance of LMPS Mozzarella and maintain desirable cheese properties for longer periods of time. The shelf life performance of LMPS Mozzarella relates to shredding and slicing of the cheese for the food service market. The machinability of Mozzarella can be affected at the beginning of ripening by high free water present in the cheese and, beyond 6 wk of refrigerated storage, excessive proteolysis can be a contributing factor to decreased machinability; therefore, the use of CC during the manufacture would help to extend the shelf-life for food-service industry.

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CHAPTER 7

Conclusions and Recommendations

7. Conclusions and recommendations

From our trials on the fortification of cheesemilk with skim milk powder (SMP) for the manufacture of Cheddar cheese (Chapter 2) we conclude that:

1. SMP fortification affected the composition of Cheddar cheese and whey; moisture content of the fortified cheese decreased and there was increased protein losses to the whey. Characterisation of the type of protein in the whey may have been useful, as there was a possibility that casein may have transfered to the whey, which may lead to whey processing issues.
2. SMP fortification increased cheese yield; as the total solids of the milk increase the moisture and salt adjusted cheese yield also increased.
3. Higher numbers of NSLAB were found in cheeses fortified with SMP probably due to the high total solids of the milk and SMP slurry offering a protective effect on bacteria during pasteurisation.
4. Primary proteolysis was lowest in the fortified cheeses which led to a harder cheese with decreased meltability.

Based on our research, the following recommendations are suggested:

1. To maintain constant moisture between cheese treatments, steps should be taken during cheese manufacture such as increased firmness at cutting the coagulum and lower cook temperatures to increase the moisture when using SMP.
2. Pasteurisation temperature of the milk and SMP slurry should be increased to ensure inactivation of bacteria or alternatively the slurry

should be divided into its respective milk treatment before pasteurisation which would have lowered the total solids of the milk.

3. The rennet should be added based on the level of casein in the milk to ensure the same enzyme to substrate ratio in the cheese and hence similar levels of primary proteolysis.

In general, using low levels of SMP could offer potential benefits in cheese manufacture by increasing yield while maintaining similar properties in the cheese. This may offer a benefit to the dairy industry by increasing the solids level in milk, and thereby increasing vat throughput at times of the year when milk solids or milk volumes are low. It may also be used to develop cheeses for ingredient purposes with decreased levels of proteolysis and meltability.

From our trials on the fortification of cheesemilk using sodium caseinate (NaCn) and $CaCl_2$ (Chapter 3) we conclude that:

1. Fortification of Cheddar cheese with NaCn affected the cheese composition and led to cheese with lower fat, higher moisture and higher salt.
2. NaCn fortification increased cheese yield; as powder fortification inceased, the moisture and salt adjusted cheese yield increased.
3. Higher numbers of NSLAB were found in cheeses fortified with NaCn probably due to the higher total solids of the milk and NaCn slurry offering a protective effect on bacteria during pasteurisation.
4. Primary proteolysis was affected by $CaCl_2$ and NaCn addition; using $CaCl_2$ and high levels of NaCn led to the lowest and highest level of proteolysis, respectively.

5. $CaCl_2$ and NaCn cheeses tended to be softer and appeared to have similar or less melt than the control cheese.

Based on the research carried out, the following recommendations are suggested:

1. A gentler method for powder addition to milk other than the Silverson may be considered as it was hypothesised that this damaged the fat globules leading to decreased fat and increased moisture in the cheese. Alternatively, slightly higher fat levels could be added to fortified milk to compensate for the losses expected during cheesemaking.
2. Pasteurisation temperature of the milk and NaCn slurry should be increased to ensure inactivation of bacteria or alternatively the slurry should be divided into its respective milk treatment before pasteurisation which would have lowered the total solids of the milk.
3. Rennet should be added to cheesemilk on a casein basis to maintain a constant enzyme to substrate ratio and hence similar levels of proteolysis in the cheese.
4. The total calcium and insoluble calcium of the cheeses should have been determined to better understand the effect of NaCn and $CaCl_2$ fortification on the texture and functionality properties.

In general, NaCn is not an ideal powder for the fortification of Cheddar cheese when trying to maintain similar compositional and functional properties to Cheddar cheese; it may, however, have the potential for the dairy industry to increase yield and alter functional properties in certain applications.

From our trials on standardising the lactose:casein ratio of milk for the manufacture of low-moisture part-skim Mozzarella cheese (Chapter 4) we conclude that:

1. Standardising lactose:casein ratio led to differences in protein and fat of the cheese.
2. Decreasing the lactose to casein ratio caused higher pH, lower lactose, galactose, lactic acid and insoluble calcium:protein in the cheese.
3. Decreasing the lactose:casein ratio led to cheese with higher TPA and sensory hardness.
4. Decreasing the lactose:casein ratio resulted in a cheese with lower melt (LT_{max}) at the start of ripening. The softening point (LT=1) was higher for these cheeses.
5. Decreasing the lactose:casein ratio led to lower acid flavours in the cheese and on pizza the cheese had lower blister colour and stretch.

Based on the research carried out, the following recommendations are suggested:

1. To ensure similar composition between cheeses, the NPN should be taken into account when standardising ultrafiltered milk as NPN can permeate the membrane.
2. The differences in insoluble calcium of the cheeses was due to the ultrafiltration process and the addition of RO water; this could be avoided if serum from nanofiltration of the ultrafiltered permeate was used which would maintain serum calcium balance of the original milk.

In conclusion, using ultrafiltration to standardise the lactose:casein ratio of cheesemilk can offer a way to alter the texture, flavour and functional properties as well as offering the potential to reduce pH variability of low-moisture part-skim Mozzarella. This could be used in the dairy industry to control pH in the event of a delay in production. It may also be used to manufacture low-moisture part-skim Mozzarella with a firmer texture that, in turn, may have better machinability when grating and slicing. This could also be used to manufacture a cheese with reduce blister colour when baking on pizza, as well as lower acid flavour.

From our trials on varying the β:α_{s1}-casein of milk for the manufacture of Cheddar cheese (Chapter 5) we conclude that:

1. By using microfiltration of milk at various temperatures it was possible to vary the β:α_{s1}-casein in cheese. This affected the rheological properties of rennet induced gels; lower β:α_{s1}-casein led to gels with a more elastic-like character.
2. Varying the level of β:α_{s1}-casein did not affect the level of insoluble calcium, proteolysis or pH of cheese.
3. Increasing β:α_{s1}-casein ratio in cheese led to decreased meltability and a higher softening point in the cheese.
4. Varying the level of β:α_{s1}-casein did not affect the flavour attributes of Cheddar cheese.

Based on the research carried out, the following recommendations are suggested:

1. The level of protein in the low β:α_{s1}-casein was lower than the other treatments; this should be standardised as it can have a confounding effect on texture and functional properties.
2. No difference was observed for the development of bitterness in cheeses with various levels of β:α_{s1}-casein as we had hypothesised; perhaps further ripening beyond > 6 months may lead to differences in the generation of bitter peptides from β-casein.

In general during ripening, it was found that higher β:α_{s1}-casein ratio seemed to impact the rheological and textural properties of Cheddar cheese compared to the control. Hydrophobic interactions and possibly changes in the micelle due to incorporation of in β-casein into the micelle could have caused the effects observed in the current study. Varying the level of β:α_{s1}-casein in Cheddar cheese could offer a way to alter the functional properties of the cheese during ripening, such as meltability and hardness, without affecting the flavour of the cheese.

From our trials using camel chymosin in low-moisture part-skim Mozzarella cheese (Chapter 6) we conclude that:

1. Using camel chymosin instead of calf chymosin did not affect the pH and insoluble calcium but it did reduce the level of proteolysis in the cheese.
2. Using a high level of calf chymosin compared to a low level led to increased proteolysis in the cheese but the level of camel chymosin added did not affect proteolysis.

3. Mozzarella made with camel chymosin had a higher softening point, higher TPA and sensory hardness and lower blister quantity when baked on pizza.
4. The textural and functional properties of Mozzarella were maintained for longer ripening periods when camel chymosin was used.

Based on the research carried out the following recommendations are suggested:

1. It may be useful to determine the level of residual rennet present in the cheeses as the amount of camel chymosin did not affect the proteolysis.
2. It would be interesting to study the effect of camel chymosin for further ripening studies (> 3 months) to determine for how much longer the shelf-life of Mozzarella may be increased.

In general the use of camel chymosin offers the potential to extend the texture, functional and sensory shelf-life of LMPS Mozzarella cheese for the food service industry by extending the window of acceptability of this cheese. This could have a major positive impact for the dairy industry by offering a way to control the shelf-life performance of the cheese. By using camel chymosin, the industry could hold cheese for a longer time during periods where cheese is not being produced or when milk is scarce, which offers a longer supply of the cheese.

In conclusion, from the research conducted in this thesis, the most promising approach from an industrial point of view is the use of camel chymosin in low-moisture part-skim Mozzarella to extend the textural, functional and flavour

properties of this cheese which inevitably extends the shelf-life performance of the cheese.

Appendix