A STUDY ON THE IMPACT OF PHYSICAL DISRUPTION AND REFORMING ON CHEESE TEXTURE

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SUMMARY

In this study, mechanical reforming of non-fat, low-fat and full-fat cheese was investigated. Our first goal was to understand the mechanisms of cheese reformation. In the context of this study, we adopted the word reform to mean the process of breaking the cheese into pieces and putting it together to form a cheese block, which may or may not, differ from its original form. The resulting cheese from that process may also remain intact or fail to be reformed. Our second goal was to determine how reforming influences the functional properties and texture of cheese. Reforming the cheese is a novel technique. No study has yet been published on how this process influences the texture of low-fat cheese, what factors promote fusion between cheese particles after reforming, and under what conditions cheese will mostly reform.

We wanted to know how, to what extent, and under what conditions, cheese fuses and sticks back together when it is reformed. We know the physical and chemical properties of cheese and how cheese matrix is initially formed from milk. Interactions involved in the transition of milk to cheese can help us to understand the factors that are responsible for the reformation of the cheese network. Cheese is essentially a protein matrix held together by the interactions between caseins (i.e., hydrophobic, electrostatic and Van der Waals interactions, hydrogen bonds and ion bridges). Each casein molecule in cheese can be simply viewed as a block copolymer with their segregated hydrophobic and hydrophilic regions offering several interaction pathways with other caseins to form polymers. Factors that influence the strength of the interactions and bonds between caseins should also determine the level/degree of fusion between cheese particles after

reforming. We wanted to alter the strength of those interactions and bonds to see how they would affect the affinity of caseins to stick back together again when we reform the cheese. The question was; would the caseins assemble again as they did in the initial cheese making process? If all of the interactions and bonds restore, then we assume that there would be no difference in texture between the reformed and the original cheese.

In the course of this study, the impact of grating size, temperature, pH and use of different types of emulsifiers on the reformability of cheese were evaluated by examining the texture, rheology, melt properties and visual appearance of cheese samples before and after reforming process. Cheese was ground up/ shredded by a food processor/ shredder and then pressed back together to be reformed. Reformation was done by cold extrusion under vacuum using an extruder in dairy plant while in laboratory scale experiments by manual pressing.

The impact of the scale of the disruption on cheese protein network was investigated by grating cheese into different sizes (9, 6, 3 and 1.5 mm) before reforming. Non-fat cheese bases were used in this trial to eliminate the contribution of fat on reforming and to see if reforming can help reduce the textural problems caused by fat removal. Grating and reforming the non-fat cheese reduced its hardness. The size of the particles used for grating did influence the texture properties of reformed cheese with bigger shred sizes giving higher hardness values. All reformed cheese samples exhibited higher degree of flow than the cheese base, while size of the grating did not influence the meltability.

Our trials on the impact of reforming temperature (4, 18 or 30°C) on the reformability and texture properties of non-fat cheese showed that, when cheese was

reformed at higher temperatures the cheese had softer texture. A vacuum extruder was used for reforming the cheese samples. Reforming the cheese at higher temperatures produced a smooth cheese with a softer texture.

Reducing the pH of low-fat cheese from pH 6.2 to 5.3 brought about a 93% recovery in hardness and about 86% recovery in dynamic moduli of the reformed cheese compared to the cheese base. Microscopy and texture test results showed that cheese fused and reformed better at low pH values, i.e., with greater levels of colloidal calcium phosphate (CCP) solubilisation. At high pH, i.e. 6.2, reforming the cheese reduced its hardness and storage modulus while making it more meltable which was attributed to the presence of weaker interactions and incomplete recovery of the bonds between caseins after the reforming process.

Impact of different types of emulsifiers (anionic emulsifiers: citric acid esters of monoglycerides (CITREM), diacetyl tartaric acid esters of monoglycerides (DATEM), sodium stearoyl lactylate (SSL), zwitterionic: lecithin and non-ionic: distilled monoglycerides (DM), lactic acid esters of monoglycerides (LACTEM), acetic acid esters of monoglycerides (ACETEM) and sorbitan tristearate (STS) on reforming aged low-fat and full-fat Cheddar cheese was investigated. Non-ionic emulsifiers did not alter the texture of full-fat cheese except for the addition of STS for texture properties at low temperatures, while they increased the meltability of the full fat cheese. Use of non-ionic emulsifiers seemed to make the low-fat cheese more prone to fracture during compression except for STS. As for the anionic emulsifiers, SSL reduced the hardness of low-fat cheese and made it very sticky and soft. CITREM, DATEM and STS appeared to

produce firmer cheese. The use of DATEM and SSL resulted in cheese that had exhibited significantly lower loss tangent maximum compared to control cheese.

The overall results of this study suggested that reforming cheese created a weaker/softer cheese protein network through the physical disruption of bonds and interactions. There was a partial recovery of the bonds and interactions in all cheese samples when shreds were repressed back into cheese blocks. Grating and reforming the cheese imparted some level of discontinuity to the protein matrix that was easier to compress and thus was better to chew down. Temperature used for reforming influenced cheese fusion and the final textural properties of the cheese. Increase in temperature increased the hydrophobic interactions, and accompanied by the greater bond mobility, which promoted the fusion of the cheese particles. Cheeses reformed at higher temperatures (~30ºC) were still softer than cheeses reformed at low temperature after 1 week of cold storage probably due to the incomplete recovery of the bonds and interactions that were broken as heated. At low pH, cheese fusion was greater since the solubilization of CCP crosslinks with the decrease in pH increased bond mobility of caseins. Anionic emulsifiers changed the texture properties of the reformed cheese, probably due to their strong interaction with caseins through their charged groups. Addition of emulsifiers during reforming resulted in differences between the full-fat and low-fat cheese especially with the use of more hydrophobic non-ionic emulsifiers, which was probably due to their higher affinity to interact with fat rather than proteins. While low-fat cheese reformed with non-ionic emulsifiers did not show any difference in meltability as compared to control, they increased the degree of flow in full-fat cheese.

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ABBREVIATIONS

CCP	Colloidal calcium phosphate
GDL	D-(+)-Gluconic-δ-lactone
DOF	Degree of flow (%)
INS Ca	Insoluble Ca
G"	Loss modulus
G'	Storage modulus
LT	Loss tangent
LT_{max}	Maximum loss tangent
FTMS	Fourier transform mechanical spectroscopy
SAOS	Small amplitude oscillatory rheology
n	Power law exponent

Chapter 1

LITERATURE REVIEW AND OBJECTIVES

1.1. INTRODUCTION

Cheese is one of the oldest foods consumed by humans dating back before recorded history. It is not clear where cheese making is originated, either in Central Asia, Middle East or Europe. Most authorities believe that cheese was first made around 8,000 years ago in a region called Mesopotamia located between the Tigris and Euphrates rivers in the Middle East. Apparently, cheese was discovered soon after the domestication of animals and use of their milk; and it was probably when humans accidentally realized that curdling of milk yielded an edible and more stable product (Fox and McSweeney, 2004). The word "cheese" comes from Latin word "caseus", from which "casein" is derived and from the proto-Indo-European root "kwat", which means "to ferment, become sour" (Mallory and Adams, 2006). Essentially, the earliest types of cheese were a form of sour milk. Acid development in milk is due to the growth of lactic acid bacteria which coagulated the milk into a gel; this mixture is then separated into curds and whey when disrupted. Heating raw milk that was held warm for sufficient time, to have a considerable amount of acid development, again caused curdling, separating the milk into two phases. Presumably, those curds were the precursors of today's acid-

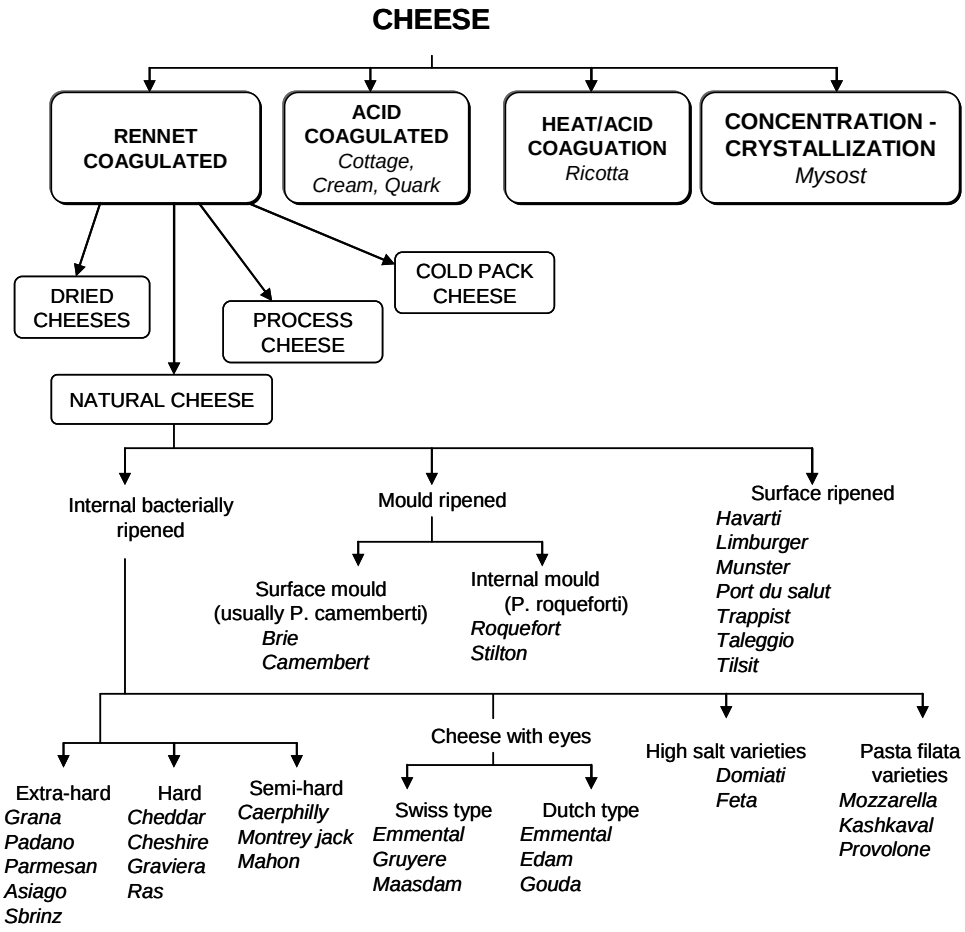
coagulated cheese types such as Ricotta, Cottage, Quark, Karish, Kes and Paneer. Incorporation of proteolytic enzymes in cheese making was believed to first happen again at a very early date, when milk was stored in bags made out of the stomachs of young animals, presumably in an attempt to obtain milk curds observed in the stomach of young mammals after slaughter. It was the residual rennet enzyme that coagulated the milk in those stomach bags (Olson, 1995; Fox and McSweeney, 2004).

Curds produced by rennet are very different from the acid-precipitated curds. Due to its better syneresis properties, rennet coagulation made it possible to produce low-moisture cheese, which was more shelf stable. Therefore, rennet cheese manufacture has become the dominant type over the acid coagulated cheeses, with >75% of the total world production (Fox and McSweeney, 2004). Over the centuries, cheese manufacture has evolved into enormous variety of cheese types with different tastes and textures particular to different countries, regions, climates and milk sources. Today, cheese has the highest diversity among dairy products with about 2000 varieties around the world, yet its manufacturing protocol is based on similar principles for most types (Olson, 1995). Cheese is a complex food product due in part to its biologically and biochemically dynamic nature. Even slight differences in the cheese making protocol, starter culture selection and ripening conditions can produce different types of cheeses. Basic cheese making steps involve pre-acidification of pasteurized milk either by starter culture or acid addition, clotting of the milk by acidification and/or milk clotting enzymes and whey removal where approximately 90% of the water in milk is drained along with lactose, serum proteins and soluble salts. The subsequent curd treatments vary depending

on the cheese type and usually include salting, shaping, pressing of the cheese curds and ripening (Olson, 1995).

Cheese types are classified into several groups based on the method of coagulation, source of milk, composition, firmness, texture, characteristic ripening agents and manufacturing techniques (Table 1.1) (McSweeney et al., 2004).

Table 1.1. Classification of cheese varieties (Adapted from McSweeney et. al., 2004)



Reformed cheese or so called blended cheese is a recent approach in the cheese-making industry used for the incorporation of spices, herbs or fruits and for the

mixing/blending of two or more types of cheese (e.g. cheese with different colors). This approach is mainly used to increase product variety with eye pleasing alternatives to attract consumers and can be applied to many cheese types. The process of reforming the cheese involves breaking up the cheese into pieces, blending with ingredients and forming it back into a cheese block by pressing (Harbutt, 2009). This reformation step is often performed by passing the cheese through an extruder. The cold extrusion of cheese is also used in some types of "cold-pack" cheese varieties produced in the U.S. It is also a convenient way of portioning the cheese for retail packaging (Mueller, 2005).

There are few studies on cheese reforming, where instead of the fresh cheese curds, the final (or aged) cheese is blended. No detailed study has yet been published on how the reforming process influences the overall textural and structural properties of the cheese, what factors promote fusion between cheese particles after reforming, and under what conditions cheese will actually reform. There are patents for extrusion of the cheese into the form of slices, shreds or cheese blocks (Mueller, 2005; Holmes and Rivero, 2007; Reeve and Justiz, 2008; Holmes et al., 2011). A study by Nelson and Barbano (2004) involves cheese reforming to improve the flavor characteristics of reduced fat cheese produced by reforming after grinding and by extracting the fat from full fat cheese. They found that the texture of the reformed cheese was creamier and softer than the original full fat cheese, as evaluated by sensory panelists.

Another way of producing blended cheese is mixing fresh cheese curds of the same or different types with or without spices, herbs or fruits during cheese manufacturing. Curd blending in fact is a traditional step in the making of some cheese varieties to maintain a certain level of acidity and unique texture, e.g. Lancashire

(Robinson and Wilbey, 1998). There are very few studies on curd blending. Chen et al. (1994) studied the sensory properties of reduced fat Cheddar cheeses that are produced either by blending low and high fat curd or by blending aged full fat cheese with low-fat curd. They found that blending aged Cheddar cheese into low-fat cheese curds resulted in higher Cheddar flavor intensity; however, texture scores were low as a result of improper fusion of the cheese curds. Characteristics of reduced fat cheddar cheese produced by blending of full-fat and skim cheese curds at whey drainage was studied by Fenelon et al. (1999) and it was found to be softer than the conventionally made reduced fat Cheddar cheese.

In the manufacture of some cheese varieties, such as, Cheddar, after the whey is drained, curds are allowed to mat together. This curd mass is then cut into blocks and the blocks are piled up and turned around regularly until a chicken-breast like texture is obtained. These cheese curd blocks are then milled, salted and pressed into hoops. Those milled cheese curds fuse together and form a uniform cheese block (Robinson and Wilbey, 1998). Reforming the cheese can be viewed as recurrence of that milling and pressing steps during Cheddar cheese making. What makes the difference is the freshness of cheese curds/particles. Fresh cheese curd is still wet and expels water during pressing. Syneresis (water loss) is not expected to happen when reforming aged cheese particles. The amount and state of the water in the cheese/curd particles can influence their ability to reassociate, however, the mechanism through which those particles fuse is probably similar. Therefore, the chemical and physical changes that occur during cheese making are important as well as its overall textural and biochemical properties of cheese for understanding the mechanisms involved in cheese reforming. Knowledge of the nature of

the interactions involved in the transition of milk to cheese can help understand the factors that are responsible for the reformation of the cheese network.

In this chapter, cheese manufacture, structural and functional properties of cheese and the influence of cheese components on structural and functional properties are reviewed to be able to develop an understanding of the behavior of cheese when it is reformed.

1.2. CHEESE MANUFACTURE

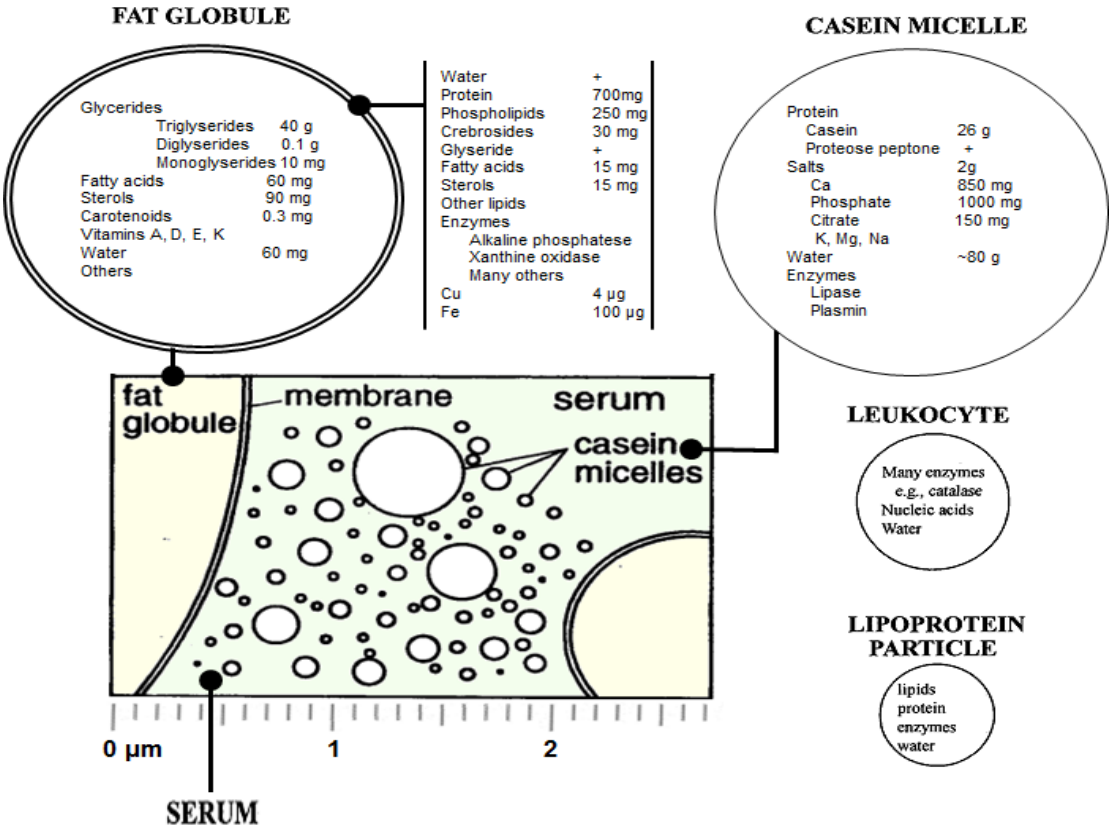
Transition of milk into a gel and then to cheese curd takes place in two main steps; gelation of cheese milk and conversion of the gel into cheese which involves dehydration of the gel and curd treatments. Since milk is the raw material used for cheese, its quality has a direct influence on the quality of the cheese (Fox and McSweeney, 2004).

1.2.1. Milk as a Raw Material for Cheesemaking

Worldwide bovine milk constitutes the majority of the total milk used for cheese manufacture, and the remainder consists of sheep, goat and buffalo milks. The specific set of desired milk properties for cheese manufacture depends on the cheese type. Flavor profiles of certain cheese varieties are obtained from the specific milk type used for their manufacturing, such as, Mozzarella di buffalo, which is made from buffalo milk and Pecorino romano that is made from sheep milk. The whiteness, rennet gelation and the overall compositional differences between the milk types from different animal sources all contribute to the final characteristics of the cheese (Guinee and O'Brien, 2010).

Table 1.2. Composition and organizational structure of milk (adapted from Walstra et al., 2006)

Component	Average content in milk (%, w/w)	Range (%, w/w)
Water	87.1	85.3-88.7
Lactose	4.6	3.8-5.3
Fat	4.0	2.5-5.5
Protein	3.3	2.3-4.4
Mineral substances	0.7	1.7-0.83
Organic acids	0.17	0.12-0.21



Factors that affect the cheese making quality of the milk can be divided into five groups as; composition, microbiology, somatic cell count, enzymatic activity and levels of residues/ contaminants. Composition of milk can vary depending on several factors, such as, animal type and species, season, stage of lactation, age of the animal and feed quality (Guinee and O'Brien, 2010). The chemical composition and structure of the bovine milk is given in Table 1.2 (Walstra et al., 2006).

Cheese is essentially a concentrated form of casein and fat in milk, therefore the ratio of the casein to fat present in milk is of great importance to cheese quality. Figure 1.1 shows an example of the transfer of the components in milk to cheese (Walstra et al., 2006).

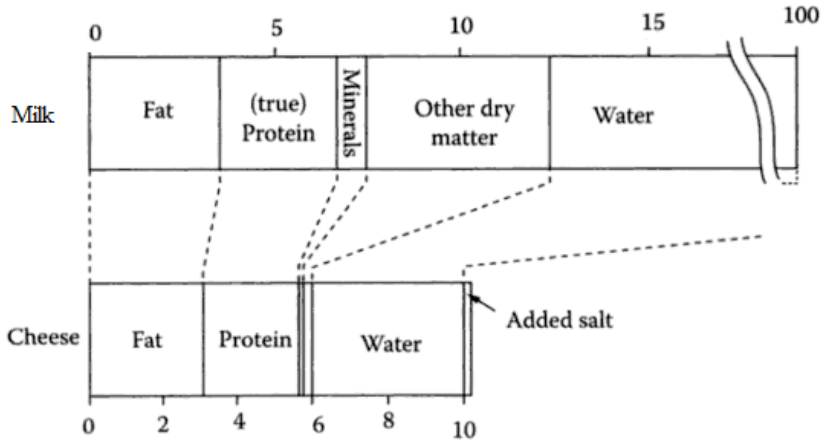


Figure 1.1. Example of the gross composition of milk and cheese (Scales are in kg) (Walstra et al., 2006)

Changes in the fat:casein ratio of cheese milk would require adjustments in the cheese making procedure in order to maintain the same composition and texture properties (Guinee and O'Brien, 2010). The standardization of milk prior to cheese making is performed in order to adjust the milk composition to a target fat:casein ratio, to minimize the compositional variation in milk and to maintain the required fat:protein

ratio in the final cheese (Fox and Cogan, 2004). The rennet coagulability of milk, gel strength, curd syneresis, cheese composition, yield and quality are all dependent on milk composition. Cheese yield estimations are made based on the casein and fat contents of the cheese milk. Composition of milk fat affects its melting point and so the amount of the fat that melts and gets lost from curd during cheese making. The fat in milk contributes to the flavor and texture of cheese. Any treatment of milk that causes damage to the fat globule membrane (shear, turbulence, homogenization) will release free fat and lead to undesirable flavors due to their breakdown. Rancidity in cheese is caused by the formation of free fatty acids due to lipolitic activity and while generally considered as off -flavors, they are desired flavors for some types of Italian cheeses. Different types of fatty acids have distinct flavors, and hence influence cheese flavor. Goat milk has high amounts of short chain fatty acids, which gives the characteristic piquant flavor to some traditional hard Italian cheeses, such as, Parmesan and Romano (Robinson and Wilbey, 1998).

Protein content of milk and its gel forming quality are important in cheese making. Certain genetic variants of κ-casein are associated with higher cheese yield, high fat recovery and less curd fines in cheese whey, probably related to its improved clotting properties (Horne and Muir, 1994). Goat milk shows much slower rennet gelation and forms weaker gels than cow's milk due to its lower ratio of α_{s1}- to α_{s2}-casein than bovine milk and therefore, is more suitable for soft cheese making (Robinson and Wilbey, 1998). Whey proteins are not retained (to any great extent) in most cheese varieties. High heat treatment of milk causes denaturation of whey proteins resulting in their interaction with

caseins, which in turn hinders the rennet coagulation properties of heat treated milk (Robinson and Wilbey, 1998).

Lactose in milk is either lost into cheese whey or fermented by starter culture during cheese making, therefore there is only little or trace amounts of residual lactose left in cheese (Guinee and O'Brien, 2010). Calcium is another important component of milk for cheese making. The amount of colloidal calcium phosphate (CCP) crosslinks between the caseins influence the textural properties of cheese, therefore ratio of soluble to CCP and the total calcium in milk is critical. Increase in milk acidity solubilizes the CCP and as the casein-bound Ca^{++} level decreases in cheese, it becomes softer and more meltable (Lucey et al., 2003). Other factors that influence the quality of milk include microbial load, enzymatic activity and health of the animal (Guinee and O'Brien, 2010). In addition to those factors, milk should be free of chemical residues like antibiotics. Improper hygienic conditions during milking and transport result in microbial contamination and high microbial counts which can cause a dramatic increase in acidity and enzymatic activity. Heat resistant proteolytic and lipolytic enzymes and the bacteria that survive the heat treatment will also negatively affect cheese quality (Guinee and O'Brien, 2010).

1.2.1.1. Milk proteins

Major proteins in milk are caseins and whey proteins and they were initially distinguished by their solubility at pH 4.6. Caseins make up about 80% of the total protein content and precipitate at pH 4.6. Whey proteins are soluble at pH 4.6 and constitute about 20% of the total protein in milk (Walstra et al., 2006).

(a) Caseins

Caseins are described as phosphoproteins owing to their post-translationally phosphorylated serine residues. They have a random-coil structure with extremely open and flexible conformation due to their high proline content. Their lack of secondary and tertiary structures makes them heat stable. Each casein molecule has hydrophobic and hydrophilic regions. This amphiphilic nature of caseins is important in their self-association mechanism. Hydrophilic regions contain phosphoserine groups and phosphoserine clusters form CCP linkages. The number of phosphoserine clusters varies for the different types of caseins. There are four main types of caseins; α_{s1}-, α_{s2}-, β-, κ-casein, present at approximate molar ratio of 4:1:4:1 (Horne, 2002; Walstra et al., 2006).

κ-Casein has no phosphoserine cluster and it is the only glycosylated casein. Glycolysation occurs at the C-terminal. The hydrophobic N-terminal of κ-casein is positively charged, while C-terminal is negatively charged, and usually contains only one phosphate group. The lack of phosphoserine cluster stabilizes κ-casein against precipitation by Ca^{++} ions. There are two cysteine residues in κ-casein which may form intermolecular -S-S- bonds creating oligomers of up to 5-11 κ-casein molecules. κ-Casein also complexes with β-lactoglobulin via disulphide reactions upon heating (Walstra et al., 2006).

The α- and β-caseins are sensitive to calcium to such an extent that the concentration of calcium in milk should be sufficient to precipitate them. α_{s1}-Casein is the fraction of α_s-casein that precipitates at >4 mM $CaCl_2$ solution (pH 7.0, 0-4°C). It has the highest net negative charge and its C- and N-terminals are hydrophobic while the central part of the molecule is very hydrophilic having seven of the eight phosphate

groups present in this molecule (Ng-Kwai-Hang, 2002). α_{s2}-Casein is the most calcium sensitive and the least hydrophobic casein having the highest number of phosphate groups. It has two hydrophobic and two hydrophilic segments and contains two cysteine residues (Walstra et al., 2006). β-Casein is the most hydrophobic casein and has the highest number of proline residues making it very flexible (Walstra et al., 2006). The N-terminal segment has all the phosphate groups and is highly negatively charged while the rest of the molecule is highly hydrophobic and has no net charge (Ng-Kwai-Hang, 2002).

In solution, each type of casein molecule has a strong tendency to self-associate through hydrophobic interactions (Horne, 2002). Hydrophobic regions of caseins only interact intermolecularly. Self-association of β-caseins results in the formation of detergent-like micellar structures that resemble a hedgehog with a central hydrophobic core from which hydrophilic peptides stick out and α_{S1}-caseins form worm-like structures with the hydrophobic segments of one molecule interacting with that of a different molecule (Figure 1.2). α_{S2}-Casein and κ-casein show a similar self-association behavior as for the α_{S1}- and β-casein. The degree of association, and hence the size of the polymers is limited due to localized electrostatic repulsion of negatively charged phosphoserine residues (Horne, 2002).

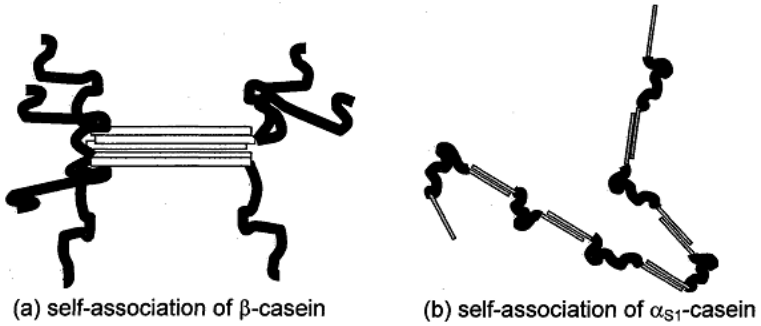


Figure 1.2. Self-association of β-casein and α_{S1}-casein (Horne, 1998).

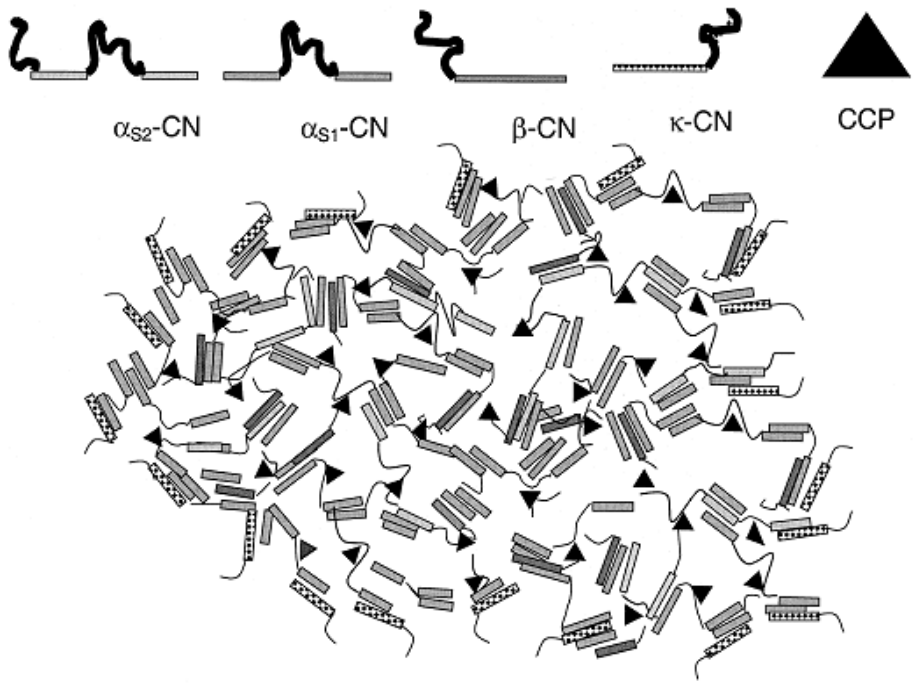


Figure 1.3. Dual-binding model for casein micelle (Horne, 1998)

In milk, caseins are mostly present in the form of micelles that are spherical in shape and have various sizes (50 to 500 nm diameter, average ≈ 150 nm) and molecular weights (10^6 to $>10^9$ Da, average ≈ 10^8 Da). The dry matter of the micelles is ~94% protein and ~6% mineral, which is mainly CCP (Horne, 2006). They are highly hydrated (2–3g H_2O/g protein) and very open having a large voluminosity of ~4 mL/g (Horne, 2002; Horne, 2009). Three-dimensional structure of the casein micelle is not well identified since it cannot be crystallized. Several models have been proposed to explain its structure. According to the dual-binding model (Horne, 1998), one of models that accounts for the behavior of casein micelles under several conditions, casein micelles are formed as a result of the association behavior of caseins with each other hydrophobically and by CCP. The formation of CCP bridges results in a reduction in the net negative charge and hydrophobic attractions become dominant. κ-Caseins terminate the micelle

growth as they do not allow further linkages, and they stabilize the micelle as they are insensitive to calcium ions and their hydrophilic glycosylated residues stick out from the micelle forming a steric protective layer. A schematic representation of the dual-binding model for casein micelle is given in Figure 1.3.

Micelle integrity is maintained mainly by hydrophobic and electrostatic interactions (Horne, 1998). The interaction energy between casein molecules is a product of the balancing act between electrostatic repulsion and attractive interactions as shown in Equation 1.1 (Lucey et al., 2003).

Interaction energy = Electrostatic repulsion + Attractive interactions

{ Multiple negatively charged phosphoserine residues; Charged groups of amino acid residues } { Hydrophobic; CCP crosslinks; Charge bridges }

[Equation 1.1]

Each type of interaction is controlled by temperature, type of the casein and the residual charge on the casein molecule, which is directly influenced by pH, ionic strength and Ca^{++} binding (Lucey et al., 2003). Decrease in temperature reduces the hydrophobic interactions causing a release of caseins from the micelle that are not bound to the casein micelle through CCP crosslinks. While at physiological temperature almost all of the caseins in milk take part in the micelles, cooling the milk to 4ºC reversibly dissociates a considerable part of β-casein into the serum phase. Dissociation of κ-, α_{S1}-, and α_{S2}-caseins occurs at a lesser extent (Walstra et al., 2006). Treatments that disrupt the hydrophobic bonds, e.g. adding urea, or that dissolve calcium, can disintegrate the casein micelle. Removal of Ca^{++} at neutral pH increases the electrostatic repulsion by the exposure of negatively charged phosphoserine residues and results in disintegration of the micelle, however, reducing the pH at the same time compensates for the increase in

the negative charge of the system and keeps the micelle together (Dalgleish, 1997). When the pH of milk decreases, the net negative charge and solvation of the casein micelles are reduced, CCP gets solubilized and at pH ~5.3 complete solubilization of the CCP occurs. At this point, limited casein dissociation is seen when the temperature is >20ºC, at 30ºC there is almost no release of caseins (Lucey, 2004).

There are approximately 10^{14} to 10^{16} micelles per mL of milk at 2.5 g/100 mL casein concentration, meaning that casein micelles are closely packed in milk with a distance of less than one micelle diameter. What prevents them from sticking together is the electrostatic and steric repulsion (Horne, 2009). The stability of the micelle, or its controlled destabilization for cheese and yoghurt manufacturing, is critical in the processing and quality characteristics of a wide range of dairy products (Holland, 2009). The destabilization of casein micelles can occur by one of four methods; 1) enzyme (rennet) action: cheese, 2) acidification: yoghurt, 3) ethanol: cream liqueurs, and 4) combination of acid and heat: Ricotta cheese. Surface properties of casein micelles are very important for the stability or coagulability of milk and the casein interactions both between and within the micelles govern the textural properties of dairy products (Horne, 1998; Lucey et al., 2003).

(b) Whey proteins

Whey proteins are globular proteins and they denature when exposed to heat. They are not phosphorylated, thus they are insensitive to Ca^{++} ions. The two major fractions of whey proteins are; lactalbumins, soluble in 50% saturated $(NH_4)_2SO_4$ or $MgSO_4$, and lactoglobulins, which are salted out under these conditions. Lactoglobulin fraction contains immunoglobulins (Fox, 2009). In bovine milk, the main lactalbumin

fractions, β-lactoglobulin and α-lactalbumin, make up 70% of the whey proteins in which β-lactoglobulins constitutes about 50% of the total whey proteins (Fox and McSweeney, 1998).

β-lactoglobulin has a very compact globular structure and consists of 162 residues per monomer, with a MW of ≈18 kDa. It forms tetramers at pH 3.5 to 5.5, dimers at pH 5.5 to 5.7 and it remains in monomeric form when pH is below 3.5 or above 7.0 (Fox, 2009). It contains two intramolecular disulfide bonds and one thiol group per monomer. The thiol group is very reactive upon thermal denaturation, which allows it to interact with the disulfide groups of other proteins, in particular of κ–casein. The denaturation temperature of β-lactoglobulin is ~73°C (Cayot and Lorient, 1997).

Consisting of 123 amino acid residues, α-lactalbumin is a relatively small protein with a MW ≈14 kDa. It has four intramolecular disulfide bonds but no free thiol group, phosphate or carbohydrate. It contains one Ca^{++} per mole. α-Lactalbumin takes part in lactose syntheses, therefore there is a relationship between the lactose and α-lactalbumin contents of the milk (Fox, 2009).

1.2.1.2. Milk Lipids

Fat in milk mostly exists in the form of large globules of various sizes (0.1-20 μm diameter – average ≈ 3-4 μm) emulsified in the aqueous phase. The globules have a non-polar lipid core consisting of mainly triacylglycerols that is coated by a complex polar bi-layer, namely milk fat globule membrane (MFGM). MFGM has a tripartite structure with an inner layer of phospholipids and proteins, an interstitial protein coat, and an outer membrane layer with an associated glycocalyx (MacGibbon et al., 2006). This membrane comprises the 2-6% of the mass of fat globules (Keenan and Mather, 2002).

Triacylglycerols account for 96 to 98% of the total fat. The structure of the triacylglycerols influences the action of lipolytic enzymes and the flavor of cheeses; it is responsible for the melting point, crystallization behavior, and rheological properties of milk fat. Structural properties of the triacylglycerols depend on their fatty acid composition (Jensen, 2002). Long-chain saturated fatty acids have higher melting point. Due to the variation in the degree of unsaturation and MW of the triacylglycerols, milk fat has a broad range of melting points (from around -40 to 40°C) (Fox and McSweeney, 1998).

1.2.1.3. Lactose

The main carbohydrate of the milk is lactose, a disaccharide consisting of glucose and galactose, and its only known source is milk. Lactose is a reducing sugar and can be found in two anomeric forms (α and β). As with all reducing sugars, it can be involved in the Maillard reaction. Lactose is the carbon source for the growth of lactic acid bacteria, and therefore is important in the acid development and the manufacture of fermented dairy products (Fox, 2009).

1.2.1.4. Minerals

The most important salts in milk are calcium and phosphate. Milk is oversaturated with calcium and phosphorus with no observed precipitation. Their association with caseins in a colloidal state keeps them in solution. They play a vital role in the formation and stability of the casein micelles and they are critically important for both the nutritional and technological aspects of milk. Partition of Ca^{+2} and PO_4^{-3} between the

soluble and colloidal states depends on environmental factors, e.g. pH, temperature, concentration (Lucey and Horne, 2009).

1.2.2. Pre-treatment of cheese milk

Pre-treatments of cheese milk include removal of contaminant debris, killing/removal of pathogenic and spoilage bacteria, standardization of fat:protein ratio and pre-acidification. Adding $CaCl_2$ to aid coagulation is a common practice (Bennett and Johnston, 2004). Depending on the initial milk composition, standardization can be done by removing water and fat, or by the addition of cream, skimmed milk, milk powder, evaporated milk or ultrafiltration retentate (Robinson and Wilbey, 1998). Fat:protein ratio of the milk determines the fat in dry matter content in cheese. Standardization of cheesemilk is necessary to maintain the legally required fat and solids content and to improve solids recovery (Johnson and Law, 2010). Pre-acidification of milk is achieved either by fermentation with starter culture or by direct acidification using food grade acids. It is possible to make cheese without pre-acidification, however, acid development in milk is desired since it aids coagulation and promotes curd syneresis, and also when starter cultures are used, they can prevent the growth of pathogenic and spoilage bacteria as well as contribute to flavor development in cheese (Johnson and Law, 2010).

1.2.3. Coagulation

Coagulation of milk is the central step in cheese making and can be done enzymatically or by acidification or combination of acid-heat (Fox and Cogan, 2004). The vast majority of cheeses are produced by enzymatic coagulation (Guinee and

O'Brien, 2010). Enzymatic coagulation of milk occurs in two overlapping phases; the enzymatic phase, in which hydrolysis of κ-casein takes place, and the aggregation phase, in which destabilized casein micelles aggregate in the presence of Ca^{+2} ions at >15ºC. Figure 1.4 represents the time course of rennet coagulation (Harboe et al., 2010).

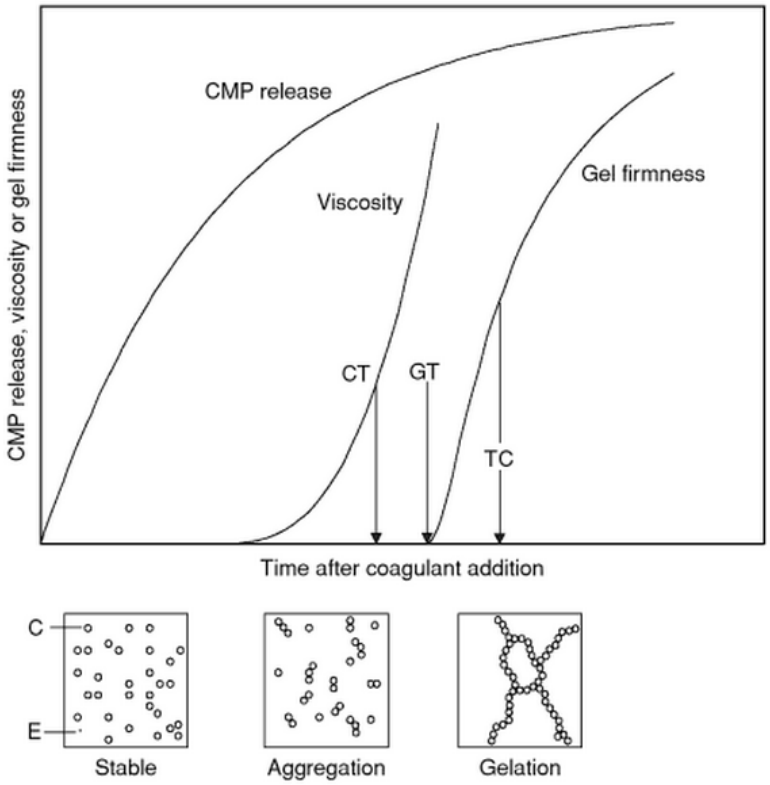


Figure 1.4. Schematic representation of the time course of the events during enzymatic milk coagulation and their relation to the stages of stability, aggregation and gelation. CMP, caseinomacropeptide; C, casein micelles; E, enzyme molecule. Characteristic times: CT, clotting time, GT, gelation time, TC, time to cutting, i.e. to obtain the desired firmness (Harboe et al., 2010).

When the milk clotting enzyme chymosin is added to milk, it specifically cleaves the Phe_{105}-Met_{106} bond on κ-casein molecules and releases glycomacropeptide; the

hydrophilic end of the κ-casein into the serum. Loss of κ-casein hairs reduces the steric stability and the net negative charge of the micelles. Coagulation does not occur until ~87% of the κ-caseins are hydrolyzed (Horne and Banks, 2004). The destabilized micelle, para-casein, binds Ca^{+2} ions strongly. In the absence of free Ca^{+2} ions, coagulation does not occur.

Mechanism of aggregation step is not fully understood. It is suggested that Ca^{+2} ions form bridges, neutralize the charges and with the loss of electrostatic repulsion, hydrophobic interactions dominate. If the temperature is below 15ºC, little aggregation occurs (Lucey, 2004). Hydrophobic interactions are not as strong at low temperatures. It is also claimed that, at low temperatures β-caseins can protrude out of the micelles forming a surface barrier preventing the action of the enzyme on κ-caseins (Dalgleish, 1992). Aggregation starts with the formation of small linear chains of renneted micelles. The time point when the first visible aggregates are seen is referred as the clotting time. As coagulation continues, these small aggregates grow into a three dimensional network, in other words milk proteins form a gel entrapping the fat and serum (Horne and Banks, 2004). Viscoelastic development of the gel during the course of gelation is given in Fig 1.5.

The sharp decrease in the loss tangent corresponds to the visible clotting time. The initially viscous structure of the coagulum rapidly gains elasticity as indicated by the increase in the elastic modulus, G', which then rapidly crosses over the viscous modulus, G" (not visible on the scale of Fig 1.5) (Choi, 2009). If the gel is left undisturbed, gel firmness, as detected by the growth of the dynamic moduli, increases until it reaches a

maximum, and eventually decreases due to macro/microsyneresis and proteolysis (Roefs et al., 1990).

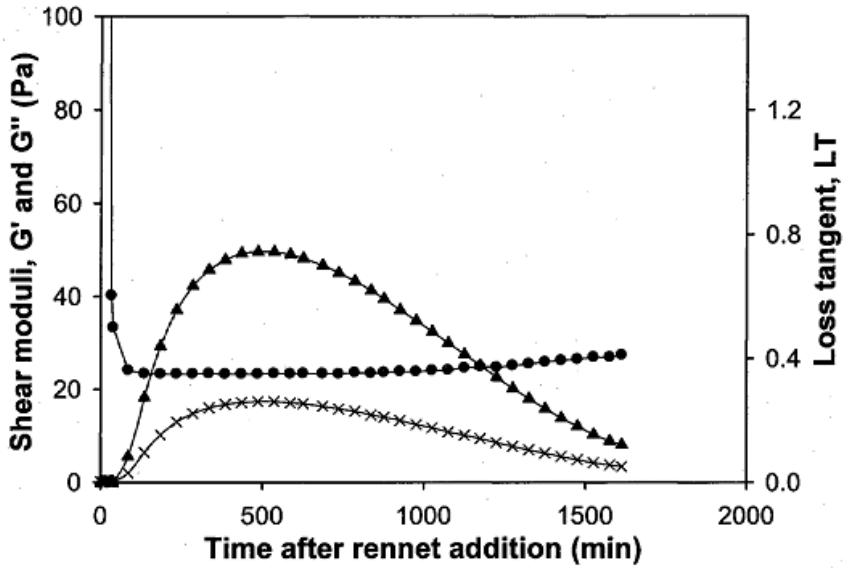


Figure 1.5. A typical example of the change in viscoelastic parameters during rennet gel formation. G': (▲), G": (X), loss tangent: (●) (Choi, 2009)

1.2.4. Post-coagulation treatments

After coagulation, when the gel reaches a sufficient firmness, it is cut into cubes to accelerate syneresis and whey removal. Cutting size influences the moisture retained in cheese with smaller curds holding less moisture (greater surface area). Larger amounts of whey are released if the gel is cut when it is soft, resulting in a lower moisture content in the final cheese. Cutting the gel when it is too weak can create small cheese fines which are lost with whey (Fox and Cogan, 2004). Curd particles expel water, shrink and with the loss of fat and whey on the surfaces, they develop a protein skin (interface). The skin prevents further fat loss but allows whey flow. Skin development is called “healing”,

and is considered necessary for the curd to withstand the subsequent curd treatments (Johnson and Law, 2010). The curd-whey mixture is then stirred while heating to temperatures ranging from 30 to 55ºC depending on the cheese type. Stirring prevents the clumping of curd pieces and facilitates more whey expulsion. Then the whey is drained. Curd treatments vary depending on cheese type. For most varieties, curds are transferred into moulds where further drainage and acidification occur. The curd particles start to fuse together closing up the gaps and forming a continuous mass. Pressure is applied to maintain a good contact between curd particles (Fox and McSweeney, 2004). For adequate curd fusion, the curd particles have to flow, resulting in an increase in contact area between adjacent curd particles and new bonds have to be formed between adjacent particles (Luyten et al., 1991). Curd fusion occurs due to the association of casein molecules on the surfaces of the curd particles. Casein molecules are more flexible below pH 6.0 due to CCP solubilization, and therefore fuse better. Curd firmness increase during the curd treatments with the loss of additional moisture (Lucey et al., 2003).

In pasta-filata type cheeses, the curd is stretched in hot water (>70°C) at a certain pH range. In traditional Cheddar-type cheese processing, the curd is milled and salted before pressing in moulds. Soft cheeses are generally hooped right after draining. Depending on the cheese type, salt may be applied in the form of dry salting where salt is directly added to cheese curds, or brine salting, where cheese blocks are immersed in saturated salt solution. Following manufacturing, most cheese varieties are ripened for a length of time that is dictated by the type of the cheese and desired maturity level.

Initially, cheese has a relatively loose network of para-casein aggregates with interparticle boundaries and openings which then mostly disappear with the ongoing

fusion of the para-casein aggregates. During ripening a complex series of biochemical changes occur resulting in flavor development and textural changes in cheese. These changes are influenced by ripening temperature, cheese pH, manufacturing protocol and the addition of specific enzymes and microorganisms (Fox and McSweeney, 2004).

1.3. STRUCTURAL AND FUNCTIONAL PROPERTIES OF CHEESE

Cheese is essentially a particle gel formed by the calcium phosphate-para-casein aggregates. The para-casein network extends in all directions surrounding the fat globules. There are discontinuities in the matrix at the micro- and macro-structural level. Depending on the manufacturing conditions, fat globules can clump together and coalesce. Curd granule junctions or curd chip (milled) junctions are observed in Cheddar-type and dry salted cheese varieties (O'Callaghan and Guinee, 2004).

Structure of cheese determines its mouth-feel characteristics and functional properties that are perceived as firmness, softness, cohesiveness, springiness, chewiness, brittleness, crumbliness, sliceability, adhesiveness and in baking operations as, meltability, stretchability, browning and oiling off. The physical properties, such as, microstructure, texture and rheology of the cheese are evaluated to predict its functionality and performance (Lawrence et al., 1987; O'Callaghan and Guinee, 2004).

1.3.1. Microstructure

Microstructure of cheese partly reflects its composition, distribution of its components and the treatments it has undergone during manufacture. The state of water (bound, entrapped or bulk), the state of fat and the level of fat coalescence, the extent of

protein association, pH, and the mineral and ionic balance determine the cheese microstructure (Everett, 2007).

There are various microscopic techniques used for visualizing the microstructure of cheese. Light microscopy, fluorescence microscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and confocal laser scanning microscopy (CLSM) have been extensively used. Resolutions for the different types of microscopy techniques and size of the milk components are given in Fig 1.6 (Everett and Auty, 2008).

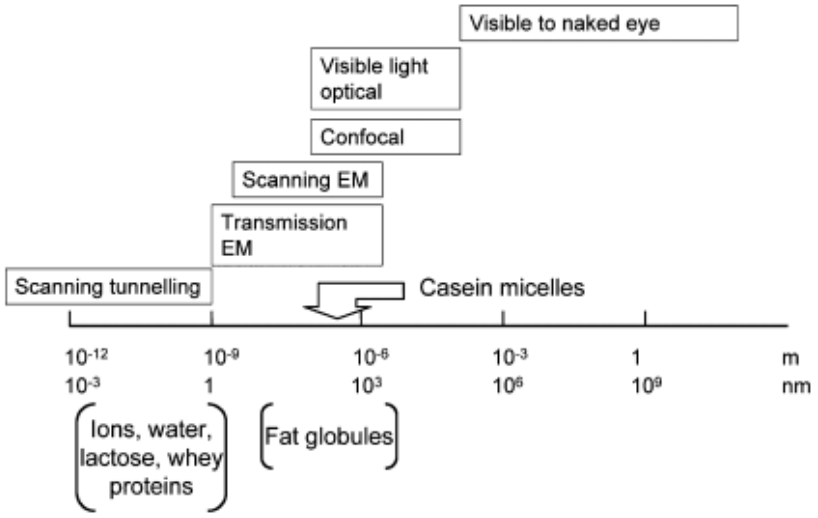


Figure 1.6. Resolution of different types of microscopy and the size of milk constituents (Everett and Auty, 2008)

1.3.2. Texture and Rheology

Texture is defined as the combination of physical properties that are perceived by the senses of touch, sight and hearing (e.g., close, open, gassy, slit-openings, mechanical openness, mealy, grainy) while the term “body” refers to the overall structural properties in relation to consistency, such as, firmness, cohesiveness, rubberiness, elasticity,

plasticity, pastiness, brittleness, curdiness, crumbliness (van Vliet, 1991b; Lucey et al., 2003).

Textural and structural characteristics of cheese show a great variation between, and within, different varieties. There are several factors that influence the textural properties of cheese, such as, composition, pH, ionic balance, level of intact casein and the continuity of the protein matrix, distribution of fat and the macrostructural heterogeneities (e.g., curd granule junctions, cracks, slits, openings), most of which change during ripening (O'Callaghan and Guinee, 2004).

Perception of the physical properties during the consumption of cheese is a highly subjective human experience. Attempts have been made to develop instrumental methods to characterize objectively the physical properties of foods. Basically, any instrumental measurement involves deforming the given sample by applying a force, e.g., by compression or by shear (van Vliet, 1991b; Gunasekaran and Ak, 2003).

The deformation is the measure of displacement in response to the applied force. The term 'deformation' indicates a change in the shape and size which may be temporary, permanent or partly recoverable (Gunasekaran and Ak, 2003).

The amount of the force applied to a material per unit area is defined as stress and expressed with the units of Pascals (Pa) (Bourne, 2002). Two types of stress can be applied to a material; normal stress, if the direction of the applied force is perpendicular to the surface, and shear stress, if it is parallel to the plane of the sample surface. Strain is the change in the size (dimensions) of the sample relative to its initial size. If a shear stress is applied, the material experiences a shear strain (γ), while a normal stress results in a normal strain (ε). The time derivative of the strain is defined as strain rate (shear rate

in simple shear) with the units of sec^{-1} (Daubert and Foegeding, 1998; Gunasekaran and Ak, 2003).

According to the Hooke's law, for a true solid the deformation is proportional to the magnitude of the applied stress. The change in the normal stress (σ) per unit applied strain (ε) is called elastic modulus (E) while the change in the shear stress (σ) per unit applied strain (γ) is the shear modulus (G) (Equations 1.2 and 1.3) (Daubert and Foegeding, 1998).

$$E = \frac{\sigma}{\varepsilon} \qquad \text{[Equation 1.2]}$$

$$G = \frac{\sigma}{\gamma} \qquad \text{[Equation 1.3]}$$

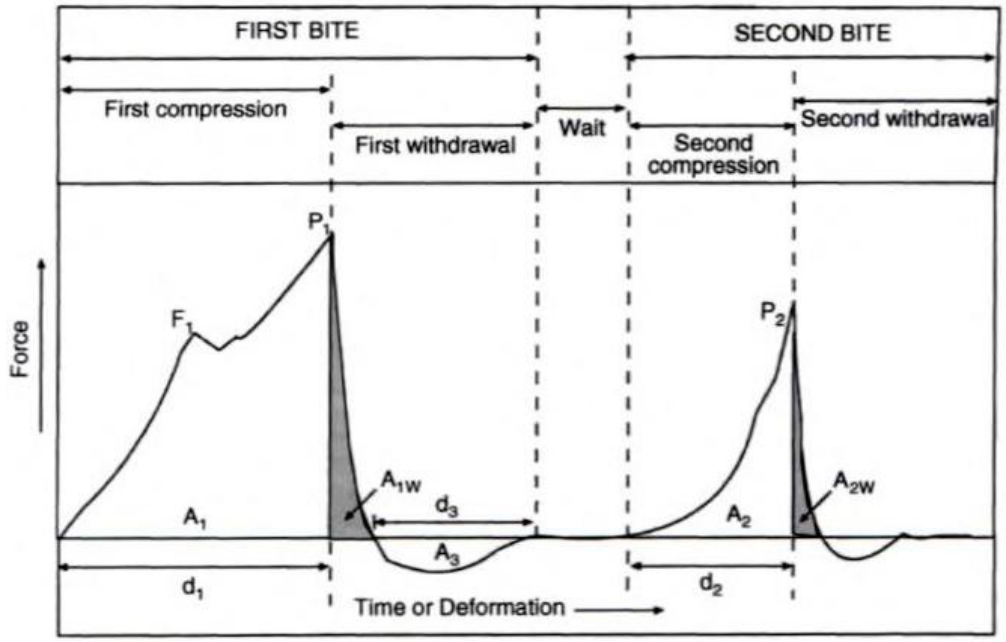
A true elastic material (Hookean solid) will deform instantaneously with the applied stress and will recover to its original shape when the stress is removed. An ideal viscous material (Newtonian fluid) will flow with the applied stress having no recovery in its shape after the stress is removed. In a viscoelastic material, a partial recovery occurs depending on the time scale of the deformation. At short time scales it will behave elastically regaining its original shape almost completely, while at long time scales, the deformation will remain with almost no recovery (Walstra and Peleg, 1991).

The instrumental test methods are categorized into three groups as; empirical, imitative and fundamental methods (Gunasekaran and Ak, 2003).

Empirical methods do not involve a rigorous scientific basis; therefore the results are hard to compare with other instruments. Empirical tests are widely used in the industry because of their simplicity and relatively lower equipment cost. Schreiber test for measuring cheese melt, penetrometer and the puncture tests are examples of empirical tests.

The imitative methods are developed to mimic sensory evaluation by human evaluators and they involve mechanical measurements with some control of experimental variables, such as, probe and product size. An example of imitative test methods is the Texture Profile Analysis. Imitative methods do not measure the true rheological properties, and the results obtained using different testing conditions are not comparable, unless the test geometry is well defined and the data is presented in normalized terms, e.g. stress, strain, moduli. The imitative methods can also be considered as "semifundamental" since they have some control over the test conditions (Gunasekaran and Ak, 2003).

The TPA test, in which a cylindrical cheese sample is deformed by compression with a flat probe twice, tries to mimic the chewing action of the jaw while eating. The first and second cycles of the compression trys to imitate the first and second bites of mastication. A typical TPA curve and the textural parameters obtained from the TPA curve (hardness, cohesiveness, adhesiveness, gumminess, springiness, and fracturability) are shown in Fig 1.7 (Bourne, 2002; Gunasekaran and Ak, 2003).



TPA Texture Terms and Definitions

TPA Term (SI units) [dimensions][a]	Definition	How Measured (see Figure 7.5 for symbol definitions)
Hardness (N) [MLT^{-2}]	Force necessary to attain a given deformation	Force corresponding to P_1
Fracturability (N) [MLT^{-2}]	Force at significant break in the curve on the first bite (originally known as "brittleness")	Force corresponding to F_1
Cohesiveness (–) [–]	Strength of the internal bonds making up the body of the product	A_2/A_1
Adhesiveness (J) [ML^2T^{-2}]	Work necessary to overcome the attractive forces between the surface of the food and surface of other materials with which the food comes in contact	A_3
Gumminess (N) [MLT^{-2}]	Energy needed to disintegrate a semisolid food until it is ready for swallowing	Hardness* Cohesiveness
Chewiness (J) [ML^2T^{-2}]	Energy needed to chew a solid food until it is ready for swallowing	Hardness*Cohesiveness *Springiness
Springiness (m) [L]	The distance recovered by the sample during the time between end of first bite and start of second bite (originally known as "elasticity" — rate at which a deformed material goes back to its undeformed condition after the deforming force is removed)	d_2
Stringiness (m) [L]	Distance traveled by the plunger during the negative force area A_3	d_3
Resilience[b] (–) [–]	Measure of how well a product "fights to regain its original position"	A_{1w}/A_1

[a] L = length (m); M = mass (kg); T = time (s). Appropriate SI units for the dimensions are given in parenthesis.

[b] Defined by (www.texturetechnologies.com); compression and withdrawal speeds should be the same.

[c] A = area under the curve

[d] d = distance

Figure 1.7. Texture Profile Analysis terms and definitions (Gunasekaran and Ak, 2003)

The fundamental methods are performed under controlled conditions, using well-defined rheological, structural and molecular theories; and the data obtained represents the material properties independent of the apparatus used for the measurement (Gunasekaran and Ak, 2003). The relationship between the stress applied on a material and the corresponding deformation as a function of the time scale of the experiment is measured. The sample is stressed either by imposing a constant stress (σ), strain (γ) or strain rate ($\dot{\gamma}$). There are two general groups of test methods: (1) static methods, where the sample is being constantly stressed in the same direction, (2) dynamic methods, where the sample is being stressed in an oscillating way. Fundamental methods include shear rheometry, stress relaxation, creep recovery, uniaxial compression and uniaxial tension tests. Fundamental test results cannot represent the large deformation and fracture properties of the cheese when small strains employed. However, some correlation has been found between the dynamic rheological data and textural attributes (van Vliet, 1991a; Tunick, 2000; Gunasekaran and Ak, 2003).

Rheology is defined as the study of deformation and flow of matters. Rheological measurements demonstrate the relationship between the strain, stress and time. Cheese is a viscoelastic material; therefore time plays an important role on its rheological behavior. The period of time where a stress or strain, of a certain magnitude and direction, is applied on the test sample, is defined as the timescale of the experiment. The time-dependent behavior of foods originates from their structure and interactions. In most food materials, the majority of bonds between structural components are temporary. These bonds can break and reform due to the Brownian motion. The speed of this process determines the viscoelastic character of the material. If the bonds break and reform much

faster than the timescale of the experiment, as in a liquid, all the energy supplied is dissipated as heat, while in an ideal elastic solid all that energy is stored (Lucey et al., 2003; O'Callaghan and Guinee, 2004).

Dynamic or transient test methods are used in determining the viscoelastic behavior of the materials. The dynamic small amplitude oscillatory shear (SAOS) test measures the material response in the linear viscoelastic region (Gunasekaran and Ak, 2003). The amount of the applied strain is small enough to ensure that the sample is not damaged and the stress response is linear. The response of the material to a sinusoidal shear strain or stress is measured. A sinusoidal oscillation has an amplitude (maximum level of stress or strain) and a duration. Duration of a single oscillatory cycle is called frequency (*f* in Hz or cycles/sec) which can also be expressed in terms of radians/sec ($\omega = 2\pi f$, radians/sec) (Daubert and Foegeding, 1998).

In a SAOS test, if the strain applied to an elastic material varies as a function of time according to:

$$\gamma(t) = \gamma_0 \sin(\omega t) \qquad \text{[Equation 1.4]}$$

where (γ_0) is shear strain amplitude, the stress response would be:

$$\sigma(t) = \sigma_0 \sin(\omega t - \delta) \qquad \text{[Equation 1.5]}$$

where (σ_0) is shear stress amplitude and (δ) is the phase angle. Ideal elastic solids have a phase angle of zero since their input and response sinusoidal curves superimpose along the time axes. In a viscoelastic material, the stress response is delayed creating a shift in the phase with an angle of 0 to 90º. The phase angle is 90º for an ideal viscous material.

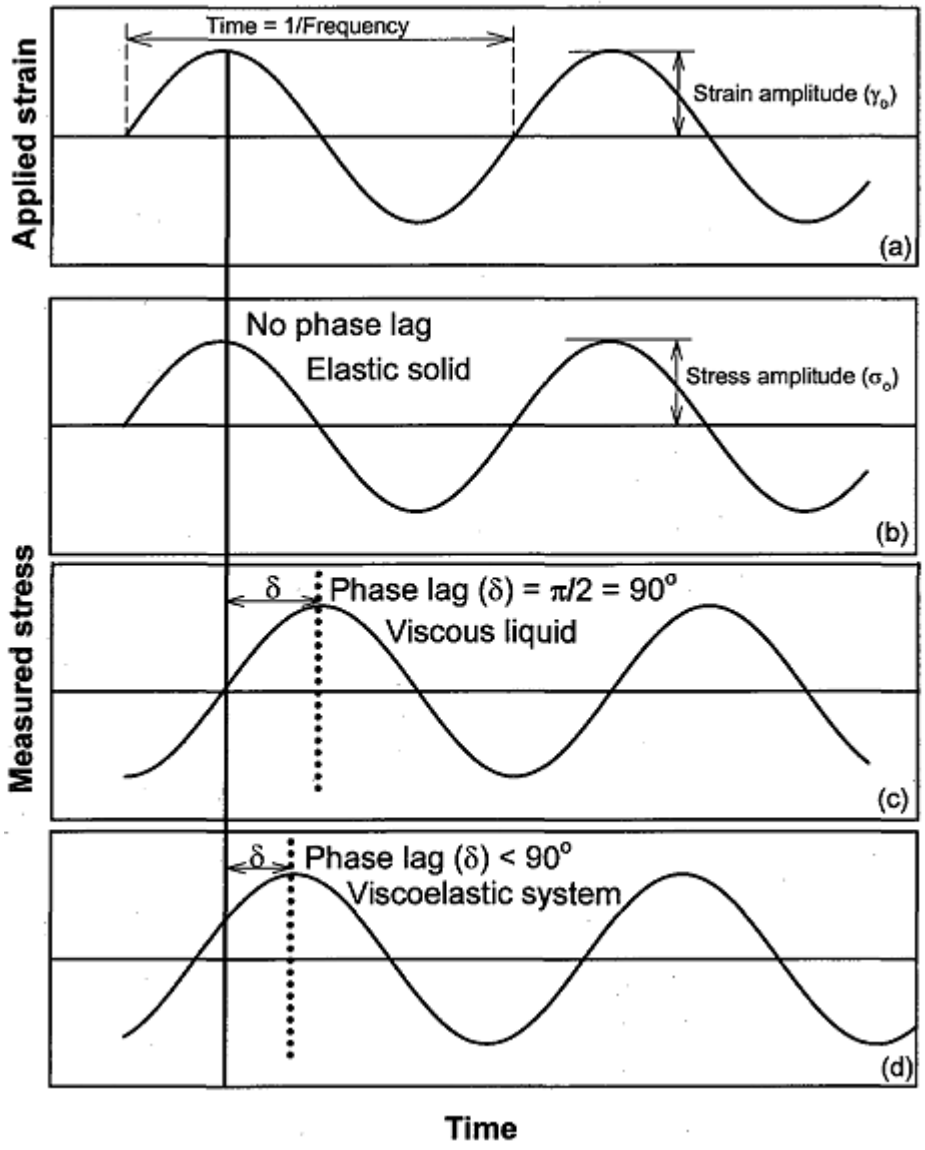


Figure 1.8. Sinusoidal strain input (a) and resultant stress response that is measured in an elastic solid (b), Newtonian liquid (c), and viscoelastic liquid (d) (Bourne, 2002)

Figure 1.8 shows an applied oscillatory strain and the responses obtained from three different types of samples: elastic solid, a Newtonian liquid, and a viscoelastic material (Daubert and Foegeding, 1998).

The storage (or elastic) modulus (G') is the stress component in phase with the applied strain:

$$G' = \frac{\sigma_0}{\gamma_0}.\cos\theta \qquad \text{[Equation 1.6]}$$

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

$$G'' = \frac{\sigma_0}{\gamma_0}.\sin\theta \qquad \text{[Equation 1.7]}$$

G' is the measure of the energy stored and released while G" is a measure of the energy dissipated as heat per cycle of deformation per unit volume. The rate of the deformation process determines the relative amount of viscous and elastic behavior, since G' and G" are angular frequency (ω) dependent functions (van Vliet, 1991a).

The number, strength, and type of bonds between casein molecules determine the rheological properties of cheese. Weak bonds generally break and reform spontaneously by the application of the stress. They contribute to the temporary character of the gel network and contribute largely to the viscous component. The reformation reaction of the bonds occurs at a finite rate determined by the interaction energy profile. In an ideal elastic material this rate is zero. The inverse of this rate is the relaxation time, but since there are so many bonds of different strengths and in different environments in the system it becomes a relaxation spectrum. Strong bonds with high energy content generally have a long relaxation time. They contribute to the permanent or elastic character of the gels. Thus, non-relaxing bonds only contribute to G' whereas very rapidly relaxing bonds only contribute to G". Bonds with relaxation times in the time

scale of the experiment contribute to both G' and G" (Lucey et al., 2003; Horne and Banks, 2004).

The dynamic response of materials can be expressed in terms of the loss tangent (LT) as defined:

$$\tan\delta = \frac{G''(\omega)}{G'(\omega)} \qquad \text{[Equation 1.8]}$$

Higher LT values indicate faster relaxation of bonds/interactions in the gel matrix. When the loss tangent is >1 (G">G'), the material shows liquid-like characteristics (van Vliet, 1991a).

1.3.3. Melt and stretch properties

Meltability is defined as the ability of cheese to flow and lose its discrete structure when heated. Stretchability is the ability of the heated cheese to withstand the amount of stress when pulled, by forming elongated strands that do not break apart, in other terms; stretchability of a cheese is its extent of stretch when it is hot (Gunasekaran and Ak, 2003; Lucey et al., 2003).

In physical terms, melting is the transformation of a material from solid to liquid state by heat. In cheese, fat is the only solid that actually melts when heated. Proteins do not melt, but heat-induced changes in their interaction properties create a melt-like effect on their structure. Heating the cheese reduce the overall number and/or strength of the bonds in cheese protein matrix as indicated by the decrease in the dynamic moduli. While both dynamic moduli (G' and G") decrease in the melting process of cheese, the decrease in G' is greater than the decrease in G". Melt occurs when the viscous character of cheese (as measured by G") dominates over the elastic character (as measured by G'). Thermal

motion of molecules, particles and strands increases at high temperatures. The fat in cheese becomes completely liquid at around 40ºC, however, melt and flow of the cheese is usually observed at higher temperatures (>40ºC). Therefore it is likely that meltablity of the cheese is mainly governed by proteins. The increase in the hydrophobic interactions with temperature can create localized shrinkages at highly hydrophobic regions in the para-casein matrix, making room for motion. Together with the increased electrostatic repulsion and loss of H-bonds, protein matrix weakens and cheese becomes meltable. However, not every cheese is meltable. Acid and heat coagulated cheeses (e.g. cottage, queso blanco), soften with heating but do not melt very much. Acid cheeses have very low pH, thus the electrostatic repulsion between caseins is low, which in turn hinders the mobility of the protein matrix at heating. In heat coagulated cheeses, the formation of disulfide bonds possibly increase the elastic character of the matrix and prevent melting. Melt occurs when the bonding between caseins are reversible enough to relax and allow movement over other casein molecules (Lucey et al., 2003).

Cheese can be stretched when caseins interact with each other and release stress while maintaining sufficient contact. If the protein interactions are too strong, cheese will not stretch but break apart. Stretchability of cheese is related to its viscoelastic properties. There is a critical level of viscoelasticity up to which cheese can be stretched. If the cheese dissipates most of the energy applied to it like a viscous material (soupy), or if it stores most of the energy like an elastic material (fragile), it will not stretch. A continuous para-casein network is necessary for stretching, meaning that caseins should be linked together to form stress carrying fibers and strands (Lucey et al., 2003).

1.4. COMPOSITIONAL PARAMETERS AFFECTING FUNCTIONAL AND TEXTURAL PROPERTIES OF CHEESE

1.4.1. Moisture

The state of water and the water holding capacity in cheese influence its functional properties. Increase in the moisture content of cheese decreases its resistance to deformation and decreases the stress at fracture. Cheese with higher moisture content or with higher moisture to protein ratio is softer due to the decrease in the volume fraction of proteins and the plasticizing effect of water. The hydration of the para-casein matrix reduces the interactions between caseins, thus less energy is needed to disrupt them (Gunesekaran and Ak, 2003). Increase in cheese moisture content can result in poor shredability with an increase in stickiness (Kindstedt, 1995).

1.4.2. Fat

Fat in cheese can be found in the forms of small globules, aggregates of globules or large free fat pools depending on the curd treatments during cheese making. Fat globules coalescence and align along the direction of casein fibers in Cheddar cheese due in the traditional cheddaring process. Such elongations are not seen in Gouda or Edam. It is unclear whether or not fat can be considered as non-interacting inert filler in the protein matrix. It is suggested that fat globules can have some weak interactions with the casein matrix that hold them in place (Everett and Auty, 2008). However, several studies suggest that fat can be considered as inert filler in the para-casein matrix unless milk is homogenized (van Vliet, 1988; Hassan and Awad, 2005). Homogenization of milk

creates smaller fat droplets that are covered mostly with caseins so they can be (actively) involved in gel formation (Horne and Banks, 2004).

The presence of fat in cheese interrupts the continuity of the para-casein matrix and provides weak spots. A reduction in fat content causes a proportional increase in the protein content, which impairs the textural and functional attributes. The relatively low number (also volume fraction) of fat globules results in a denser protein matrix leading to a firm, rubbery cheese that melts poorly. Cheeses with lower fat content have a bland flavor, and their color tends to be pale or translucent (Johnson et al., 2009). According to Code of Federal Regulations, a low fat cheese can contain up to a maximum 6% fat, and a reduced fat cheese refers to a cheese with a 25% fat reduction from its full-fat counterpart (CFR, 2006). The texture of low fat cheese tends to be hard and springy, fractures easily, lacks in cohesiveness, is waxier, less meltable and less smooth than the full-fat cheese (Mistry, 2001). Because of the lack of free oil release during baking, excessive browning occurs with a dry film or skin formation on the surface (Johnson et al., 2009).

Many methods have been developed to overcome the textural problems in reduced or low-fat cheese. However, it remains difficult to make acceptable low-fat (≤6% fat) semi-hard or hard cheeses (Banks, 2004). Increasing the moisture content is a common strategy to reduce the volume fraction of proteins. Allowing the curd to become firmer before cutting, cutting the curd into larger pieces, lower cook temperatures and shorter cooking times and cold water washing of the curd after draining are steps that can help increase the moisture level. Pre-acidification of the milk and use of Ca^{++} chelating acids dissolve the insoluble calcium, increase the hydration of caseins, thus improve the

melt and stretch properties of cheese, in addition to a softening effect. Adjunct cultures are used to improve the flavor of the low fat cheese. Addition of fat replacers is another approach that many studies have used. Incorporation of whey proteins into cheese, high pressure treatment of the cheese milk, extraction of fat from full fat cheese and blending of full-fat and non-fat cheese curds prior to pressing, are some of the recent innovative approaches for improving the textural properties of the reduced and low fat cheese (Johnson et al., 2009).

1.4.3. Protein

Cheese is essentially a protein matrix held together by the interactions between caseins (i.e., hydrophobic, electrostatic and Van der Waals interactions, hydrogen bonds and ion bridges). Each casein molecule in cheese can be simply viewed as a block copolymer with their segregated hydrophobic and hydrophilic regions offering several interaction pathways with other caseins to form polymers (Lucey et al., 2003). Increase in the protein content, increase the strength of the cheese as measured by penetration and compression tests (Gunasekaran and Ak, 2003). Hydrolysis of the proteins during the ripening weakens the structure and reduces the cheese strength. Factors that influence the strength of the interactions and bonds between caseins affect the textural and functional properties of the cheese (Lucey et al., 2003).

1.4.4. pH and mineral content

The cheese pH and calcium content are critical parameters in determining the textural and functional properties of cheese. The rate of acid production and pH values during the manufacture of the cheese has a direct impact on mineral solubilization, hence

the calcium content of the cheese. Therefore, it is difficult to evaluate the influence of pH solely (Lucey et al., 2003).

The Ca^{++} in milk and cheese is present in soluble and insoluble states. It is estimated that in milk around 32% of total Ca^{++} exist in serum phase as soluble complexes (10mM) and free ions (3 mM). The remaining Ca^{++} is associated with casein micelles and called as insoluble calcium (INS Ca) and is found in the forms of CCP and Ca^{++} caseinate. However, in practice, INS Ca^{++} is determined as an indicator of the CCP content due to the difficulty in measuring the actual CCP amount. The amount of the INS Ca^{++} associated with caseins in cheese is more important than the total calcium content in regulating the textural properties of cheese (Lucey et al., 2003).

The texture of the Cheddar cheese changes from springy and elastic (cheese pH ~ 5.3 to 5.5) to brittle and short (cheese pH ~4.8) over various pH ranges. The decrease in pH solubilizes CCP crosslinks between caseins and reduces the electrostatic repulsion with a decrease in the net negative charge. When CCP is solubilized, negatively charged phosphoserine residues cause repulsion. At high pH values (~6.5) curd or cheese texture is firm due to the excessive CCP crosslinks holding the para-casein network tightly. At pH 5.2, protein matrix has the maximum mobility, which provides for good curd fusion and good melting and stretching characteristics. Below pH 5.0 hydrophobic interactions dominates as the electrostatic repulsion greatly decrease. When the pH approaches the pI, cheese texture is brittle and crumbly due to the excessive attraction between caseins. The type of acid used for pre-acidification of cheese milk is important. Use of calcium chelating acids, such as, citric acid, solubilize greater amounts of CCP as compared to other acids, even at the same pH level. Removal of excessive amounts of Ca^{++} by the use

of Ca^{++} chelating agents can make the cheese very tacky as it looses its cohesiveness and becomes very viscous (Lucey et al., 2003).

During aging, some Ca^{++} that is associated with caseins solubilize. The slight increase in the pH often observed after the manufacture of cheeses like Cheddar is due to the slow solubilization of INS Ca^{++} (Hassan et al., 2004). It has also been suggested that the exposure of the phosphoserine residues due to the solubilization of CCP could make casein more susceptible to hydrolysis during cheese ripening (Fox, 1970).

1.5. OVERALL HYPOTHESIS AND OBJECTIVES

We hypothesized that, reforming the cheese physically after breaking it down into pieces will reduce the number of the bonds and protein-protein interactions, unless all of the bonds that were broken are restored upon reformation. The disruption in the continuity of the protein network would improve the textural attributes of the low-fat cheese as this cheese has a much denser protein matrix due to the lack of fat globules. The degree to which the bonds and interactions between caseins will restore after reforming will depend on processing conditions and factors that control the cheese functionality. The recovery of the bonds and interactions and thus the level of cheese fusion will depend on the factors that control protein interactions, e.g. temperature, pH and Ca^{++} binding. Impact of the following parameters on the reformability and texture properties of cheese was investigated:

Objective 1. Impact of grating size

Objective 2. Impact of temperature

Objective 3. Impact of pH and Ca^{++} solubilization

Objective 4. Impact of emulsifiers

1.6. REFERENCES

Banks, J. 2004. The technology of low-fat cheese manufacture. Int. J. Dairy Tech. 57:199-207.

Bennett, R. J., and Johnston, K. A. 2004. General aspects of cheese technology. Pages 23-50 in Cheese: Chemistry, physics and microbiology, 3 edn., eds., P. F. Fox, P. L. H. McSweeney, T. M. Cogan, and T. P. Guinee, Elsevier Academic Press, Netherlands, Amsterdam.

Bourne, M.C. 2002. Food Texture and Viscosity: Concept and Measurement. 2nd edn. Academic Press, San Diego.

Cayot, P., and Lorient, D. 1997. Structure-function relationships of whey proteins. Pages 225-256 in Food Proteins and their Applications, eds. S. Damodaran and A. Paraf, CRC Press, Boca Raton, Florida.

Chen, M. C., Johnson, M. E. and Jaeggi, J. J. 1994. Manufacture of a 25% and a 50% reduced-fat cheddar cheese by blending low and high fat curd. Pages 59-62 in Wisconsin CDR Annual Report July 1, 1993 to June 30, 1994, Wisconsin Center for Dairy Research.

Choi, J. 2009. Colloidal calcium phosphate in bovine casein micelles, and its impact on rheological properties of rennet-induced gels and cheese texture. PhD Thesis, University of Wisconson, Madison.

Code of Federal Regulations. 2006. Food and Drugs: CFR Part 101 Food Labeling. Department of Health and Human Services, Washington, DC.

Dalgleish, D. G. 1992. The enzymatic coagulation of milk. Pages 579-619 in Advanced Dairy Chemistry, 2nd edn, ed. P. F. Fox, Elsevier Applied Science, London, UK.

Dalgleish, D. G. 1997. Structure-function relationships of caseins. Pages 199-224 in Food Proteins and their Applications, eds. S. Damodaran and A. Paraf, CRC Press, Boca Raton, Florida.

Daubert, C. R., and Foegeding, E. A. 1998. Rheological properties for food analysis. Pages 551-569 in Food Analysis, 2nd edn., ed. S. S. Nielsen, Aspen Publishers, Gaithersburg, MD.

Everett, D. W. 2007. Microstructure of natural cheeses. Pages 170-209 in Structure of Dairy Products, ed. A. Tamime, Blackwell Publishing Ltd, Oxford, UK.

Everett, D. W., and Auty, M. A. E. 2008. Cheese structure and current methods of analysis. Int. Dairy J. 18: 759-773

Fenelon, M. A., Guinee, T. P., and Reville, W. J. 1999. Characteristics of reduced-fat Cheddar prepared from the blending of full-fat and skim cheese curds at whey drainage. Milchwissenschaft 54:506-510.

Fox, P. F., 1970. Influence of aggregation on the susceptibility of casein to proteolysis. J. Dairy Res. 37:173–180.

Fox, P. F., and McSweeney, P. L. H. 1998. Chemistry and biochemistry of cheese and fermented milk. Pages 419-421 in Dairy Chemistry and Biochemistry. Blackie Academic and Professional, London.

Fox, P. F., and McSweeney, P. L. H. 2004. Cheese: an overview. Pages 1-18 in Cheese: Chemistry, Physics and Microbiology, Vol. 1. 3rd edn., eds. P. F. Fox, P. L. H. McSweeney, T. M. Cogan, and T. P. Guinee, Elsevier Applied Science, Amsterdam, The Netherlands.

Fox, P. F., and Cogan, T.M. 2004. Factors that affect the quality of cheese. Pages 583-608 in Cheese: Chemistry, Physics and Microbiology, Vol. 1. 3rd edn., eds. P. F. Fox, P. L. H. McSweeney, T. M. Cogan, and T. P. Guinee, Elsevier Applied Science, Amsterdam, The Netherlands.

Fox, P. F. 2009. Milk: an overview. Pages 1-44 in Milk Proteins: From Expression to Food. 1st edn., eds. A. Thompson, M. Boland, H. Singh, Elsevier Applied Science, Amsterdam, The Netherlands.

Guinee, T.P., and O'Brien, B. 2010. The quality of milk for cheesemaking. Pages 1-68, in Technology of Cheesemaking, 2nd edn., eds. B. A. Law, and A. Y. Tamime, Wiley Blackwell, West Sussex, UK.

Gunasekaran, S., and Ak, M. M. 2003. Fundamental rheological methods. Pages 31-112 in Cheese Rheology and Texture, CRC Press, Boca Raton, Florida.

Harboe, M., Broe, M. L., and Qvist, K. B. 2010. The production, action and application of rennet and coagulants. Pages 98-130 in Technology of Cheesemaking, 2nd edn., eds. B. A. Law, and A. Y. Tamime, Wiley Blackwell, West Sussex, UK.

Harbutt, J. 2009. World Cheese Book. Dorling Kindersley Limited, New York, NY, USA.

Hassan, A., Johnson M. E., and Lucey, J. A. 2004. Changes in the proportions of soluble and insoluble calcium during the ripening of Cheddar cheese. J. Dairy Sci. 87:854–862.

Hassan, A.N., and Awad, S. 2005. Application of exopolysaccharide-producing cultures in reduced-fat Cheddar cheese: Cryo-scanning electron microscopy observations. J. Dairy Sci. 88: 4214-4220.

Holland, J. W. 2009. Post-translational modifications of caseins. Pages 107-124 in Milk Proteins: From Expression to Food, 1st edn., eds. A. Thompson, M. Boland, H. Singh, Elsevier Applied Science, Amsterdam, The Netherlands.

Holmes, T.L., and Rivero, O. 2007. Method and system for making sliced cheese. US Patent Application No: 20070178212.

Holmes, T.L., Rivero, O., and Rivero, A. 2011. Method and system for making extruded portions of cheese. US Patent Application No: 20110014311.

Horne, D. S., and Muir D. D. 1994. Influence of κ-casein phenotype on the rennet coagulation time of bovine milk. Milchwissenschaft 49: 386-388

Horne, D. S. 1998. Casein interactions: Casting light on the black boxes, the structure in dairy products. Int. Dairy J. 8: 171–177

Horne, D. S. 2002. Casein structure, self-assembly and gelation. Curr. Opinion Colloid Interface Sci. 7:456-461.

Horne, D. S. 2006. Casein micelle structure: models and muddles. Curr. Opinion Colloid Interface Sci. 11: 148–153.

Horne, D. S. 2009. Casein micelle structure and stability. Pages 133-157 in Milk Proteins: From Expression to Food, 1st edn, eds. A. Thompson, M. Boland, H. Singh, Elsevier Applied Science, Amsterdam, The Netherlands.

Horne, D. S., and Banks J. M. 2004. Rennet-induced coagulation of milk. Pages 47-70 in Cheese: Chemistry, Physics, and Microbiology, Vol. 1 General Aspects, 3rd edn, eds. P. F. Fox, P. L. H. McSweeney, T. M. Cogan, and T. P. Guinee, Elsevier Science and Technology, London.

Jensen, R. G. 2002. The composition of bovine milk lipids: January 1995 to December 2000. J. Dairy Sci. 85:295-350.

Johnson, M. E., and Law, B. A. 2010. The origins, development and basic operations of cheesemaking technology. Pages 68-87 in Technology of Cheesemaking, 2nd edn., eds. B. A. Law, and A. Y. Tamime, Wiley Blackwell, West Sussex, UK.

Johnson, M. E., Kapoor, R., McMahon, D. J., McCoy, D. R., and Narasimmon, R. G. 2009. Reduction of sodium and fat levels in natural and processed cheeses: scientific and technological aspects. Comp. Rev. Food Sci. Food Safety 8: 252–268.

Keenan, T. W., and Mather, I. H. 2002. Milk fat globule membrane. Pages 1568-1576 in Encyclopedia of Dairy Sciences, Vol. 3., eds. H. Roginski, J. W. Fuquay, and P. F. Fox, Academic Press, San Diego, CA.

Kindstedt, P. S. 1995. Factors affecting the functional characteristics of unmelted and melted mozzarella cheese. Pages 27-41 in Chemistry of Structure-Function Relationships in Cheese, eds E.L. Malin and M.H. Tunick, Plenum Press, New York.

Lawrence, R. C, Creamer, L. K., and Gilles, J. 1987. Texture development during cheese ripening. J. Dairy Sci. 70:1748-1760.

Lucey, J. A. 2004. Formation, structural properties and rheology of acid-coagulated milk gels. Pages 105-122 in Cheese Chemistry, Physics and Microbiology Vol. 1: General Aspects, 3rd edn., eds. P. F. Fox, P. L. H. McSweeney, T. M. Cogan, and T. P. Guinee, Elsevier Science and Technology, London.

Lucey, J. A., and Horne, D. S. 2009. Milk salts: technological significance. Pages 351-389 in Advanced Dairy Chemistry, Vol 3., 3rd edn., eds. P. F. Fox and P. L. H. McSweeney, Springer, New York, NY.

Lucey, J. A., Johnson, M. E., and Horne, D. S. 2003. Perspectives on the basis of the rheology and texture properties of cheese. J. Dairy Sci. 86:2725–2743.

Luyten, H., van Vliet, T., and Walstra, P. 1991. Characterization of the consistency of Gouda cheese: Rheological properties. Neth. Milk Dairy J. 45:33–53.

MacGibbon, A. K. H., and Taylor, M. W. 2006. Composition and structure of bovine milk lipids. Pages 1-42 in Advanced Dairy Chemistry, Vol. 2: Lipids, 3rd edn., eds. P. F. Fox and P. L. H. McSweeney, Springer Science, New York.

Mallory, J. P., and Adams, D. Q. 2006. The Oxford Introduction to Proto-Indo-European and the Proto-Indo-European World (Oxford Linguistics), Page 259, Oxford University Press, Oxford, UK.

McSweeney, P. L., Fox, P. F., and Ottogalli, G. 2004. Diversity of cheese varieties: an overview. Pages 1-23 in Cheese: Chemistry, Physics and Microbiology, Vol. 2., 3rd edn., eds. P. F. Fox, P. L. H. McSweeney, T. M. Cogan, and T. P. Guinee, Amsterdam, The Netherlands, Elsevier Applied Science.

Mistry V. V. 2001. Low-fat cheese technology. Int. Dairy J. 11:413–22.

Mueller, A. P. 2005. Cheese extruding machine and process for producing pieces of cheese. US Patent No: US 6,942,888 B2.

Nelson, B. K., and Barbano, D. M. 2004. Reduced-fat Cheddar cheese manufactured using a novel fat removal process. J. Dairy Sci. 87:841-853

Ng-Kwai-Hang, K. F. 2002. Heterogeneity, fractionation and isolation. Pages 1881-1894 in Encyclopedia of Dairy Sciences, Vol. 3., eds. H. Roginski, J. W. Fuquay, and P. F. Fox, Academic Press, San Diego, CA.

O'Callaghan, D. J., and Guinee, T. P. 2004. Rheology and texture of cheese. Pages 511-540 in Cheese: Chemistry, Physics and Microbiology, Vol. 1: General Aspects, 3rd edn., eds. P. F. Fox, P. L. H. McSweeney, T. M. Cogan, and T. P. Guinee, Elsevier Science and Technology, London.

Olson, N. F. 1995. Cheese. Pages 355-384 in Biotechnology, Vol 9., eds. H.-J. Rehm and G. Reed, Verlag Chemie. Weinheim, Germany.

Robinson, R. K., and Wilbey, R. A. 1998. Cheesemaking Practise, 3rd edn, Scott, R., ed, Aspen Publishers, Gaithersburg, MD.

Reeve, J., and Justiz, J. 2008. Reduced fat cheese having enhanced organoleptic properties. US Patent No: 2008/0124428 A1.

Roefs, S. P. R M., van Vliet, T., van den Bijgaart, H. J. C. M., de Groot-Mostert, A. E. A., and Walstra, P. 1990. Structure of casein gels made by combined acidification and rennet action. Neth. Milk Dairy J. 44:159-188.

Tunick, M. H. 2000. Rheology of dairy foods that gel, stretch, and fracture. J. Dairy Sci. 83: 1892-1898.

Walstra, P., and Peleg, M. 1991. General considerations. Pages 3-4, in: Rheological and Fracture Properties of Cheese. Bull. IDF No. 268, International Dairy Federation, Brussels, Belgium.

Walstra, P., Wouters, J. T. M., and Geurts, T. J. A. 2006. Dairy Science and Technology. 2nd edn. CRC Press, Boca Raton, FL.

Van Vliet, T. 1988. Rheological properties of filled gels. Influence of filler matrix interaction. Colloid Polym. Sci. 266:518–524.

Van Vliet, T. 1991a. Inventory of test methods. Pages 16–25 in Rheological and Fracture Properties of Cheese. Bull. IDF No. 268, International Dairy Federation, Brussels, Belgium.

Van Vliet, T. 1991b. Terminology to be used in cheese rheology. Pages 5–15 in Rheological and Fracture Properties of Cheese. Bull. IDF No. 268, International Dairy Federation, Brussels, Belgium.

Chapter 2

IMPACT OF GRATING SIZE ON THE TEXTURE AND MELTING PROPERTIES OF REFORMED NON-FAT CHEESE

Part of this study has been published in: Akbulut, C., Govindasamy-Lucey, S., Lucey, J. A., Jaeggi, J. J. and Johnson, M. E. 2010. Impact of grating size on the texture and melting properties of reformed non-fat cheese. Milchwissenschaft 66 (2): 169-172.

2.1. ABSTRACT

The texture of non-fat cheese tends to be firm and rubbery. We explored the impact of mechanical size reduction of non-fat cheese on the textural properties of reformed cheese after the shreds were pressed back together. Non-fat cheeses were made from skim milk using direct acidification of milk with citric acid. Cheeses were grated into 4 different shred sizes with a food processor using grater heads of different sizes (1.5, 3, 6 and 9 mm diameter). Cheese shreds were filled into plastic syringes that had the nozzles removed and manually pressed. Care was taken to remove air in the pressed cheese. Textural analyses were performed on non-fat cheese base and reformed cheeses that had been stored in syringes for 1 week at 4°C. Hardness was determined by Texture Profile

Analysis and uniaxial compression tests with a Texture Analyzer. Melt properties were determined using UW-Melt-profiler. Smaller shred sizes resulted in softer reformed cheese. Grating disrupted protein interactions, which were only partially reformed as a result of pressing. Thus, grating and repressing reduced the number of interactions in cheese matrix which significantly increased meltability compared to the initial cheese base while there was no significant difference in meltability between different types of reformed cheeses. Grating and reforming reduced the hardness as determined at 80% compression. Mechanical disruption, such as, shredding and repressing, could be used to improve (soften) the poor texture of non-fat cheese.

2.2. INTRODUCTION

Fat makes an important contribution to the characteristic flavor, texture and functionality of cheese. A reduction in the fat content can cause major textural defects in hard and semi-hard cheese varieties, unless corrective measures are taken by the cheese-maker. Lack of desired flavor, pale color and rubbery or firm texture are some of the major defects associated with fat reduction in cheese (Mistry, 2001). These defects become more pronounced with a greater reduction in fat. For that reason, making non-fat cheese with acceptable texture and flavor still presents a challenge to food scientists.

In cheese, a reduction in fat content causes a proportional increase in the protein content, which alters the textural and functional attributes (Guinee et al., 2000). Reducing the fat content of cheese results in a less interrupted (more continuous) protein matrix, which leads to an increase in firmness unless steps are taken to correct this issue (Merrill et al., 1994; Guinee et al., 2000). Many methods have been developed to address textural problems in reduced or low-fat cheese, such as, pre-acidification, use of adjunct cultures

or the addition of fat replacers (Banks, 2004). However, it remains difficult to make acceptable low-fat (≤6% fat) semi-hard or hard cheeses.

Cheeses can be size reduced (e.g. by grinding or shredding) and then extruded at cold temperatures back into a cheese form. These cold extruders include equipment, such as, the Vemag Robot 500 (Reiser, Verden, Germany). As mentioned in Chapter 1, the purpose of this type of operation is usually for the incorporation of spices, herbs or fruits and for the mixing/blending of two or more types of cheese (e.g. cheese with different colors). There have been some studies on repressing cheese curd blends (Chen et al., 1994; Fenelon et al., 1999; Nelson et al., 2004; Whetstine et al., 2006), however, the impact of size reduction and repressing on cheese texture have not been intensively studied. We believe that this approach could also be used to improve the rubbery and firm texture of low or non-fat cheese.

In this study we investigated the textural properties of non-fat cheese that had undergone a physical disruption by grating to different sizes before being manually reformed back into a cheese sample.

2.3. MATERIALS AND METHODS

2.3.1. Cheesemaking

Non-fat cheese bases were made by pre-acidification of skim milk to pH 5.6 with citric acid using the procedure described by Brickley et al. (2008). Citric acid was added to the pasteurized skim milk at 4ºC until pH 5.6 was attained and maintained for 30 min. Then, milk was heated to 33ºC and rennet (Chymax Extra Double Strength, Chr. Hansen, Milwaukee, WI, USA) was added (2g/100kg of milk). The coagulum was cut using 12.7 mm knives approximately 30 min after rennet addition. The curd whey mixture was then

heated up to 37ºC in about 20 min while stirring. Whey was drained, and curd was dry salted (225 g/100 kg milk). The curd was placed into 9 kg Wilson style hoops and pressed for 60 min at 276 kPa. Cheese bases were stored at 4°C for 1 week prior to the grating-reforming treatment. One batch of non-fat cheese base (18 kg) was produced and cut into 250 g blocks and vacuum packed. The manufacturing protocol of non-fat cheese base is given in Table 2.1.

2.3.2 Cheese Reforming

A food processor (Robot coupe R2Dice, Jackson, Mississippi, USA) was used for shredding the cheese blocks. To improve shredability, cheese was cooled overnight to ~1°C. Four sizes of cheese shreds were produced using grater discs having cut sizes with 1.5, 3, 6 and 9 mm diameter which produced 1 mm thick, 20 mm long and 1.5, 3, 6 and 9 mm wide shreds respectively as the cheese block was pushed through the spinning grater disc. The surface area created by shredding 100 g of cheese using those grater discs is calculated and given in Table 2.2. Because of the small quantity of cheese, we used plastic syringes to reform the cheese into cylindrical shapes that would later allow us to easily cut cheese into the correct size for textural testing. Equal amounts of cheese shreds (13 and 38 g of cheese shreds were used for the small and large syringes, respectively) were filled into small (16 mm diameter) and large (30 mm diameter) plastic syringes and manually pressed to the same volume for the same syringe size. The repressed shreds were stored in the syringes for 1 week at 4°C prior to analysis; the 1 week period allowed the cheese shreds to fuse back together again. Trials were replicated 6 times.

2.3.3. Compositional Analysis

Milk samples were analyzed for total solids, fat, protein, and casein (Marshall, 1992). The total solids, fat, protein and pH of cheese were determined (Marshall, 1992). The salt content of the cheese samples measured using Corning Salt Analyzer (Marshall, 1992) and the total calcium content was analyzed by inductively-coupled Argon plasma emission spectroscopy (ICP).

2.3.4. Textural analysis

A TA.XT2 Texture Analyzer (Stable Micro Systems, Godalming, Surrey, UK) with a TA-25 probe (50 mm diameter) and TA-90A flat plate were used for texture testing. Cylindrical cheese samples having 16 mm diameter and 17.5 mm height were used. For the base cheese, a cork borer was used to cut cylindrical samples. Reformed cheese samples were pushed out of the open end (that had the tip cut off) of plastic syringes (16 mm diameter). Cheeses were then cut into samples of 17.5 mm height and kept overnight at 4°C in sealed plastic bags prior to the analysis. Hardness of cheese samples at 4°C was determined at 80% strain by uniaxial compression test and at 62% strain by Texture Profile Analysis. Texture parameters were calculated as described by Bourne (2002).

Melt Profile Analysis was performed using UW Melt-Profiler developed by Muthukumarappan et al. (1999). For melt analysis, syringes of 30 mm diameter were used. Cheeses were cut into 7 mm thick cylinders and held overnight at 4°C in sealed plastic bags. Cylindrical cheese samples at 4°C were placed between two aluminum plates (a dry film lubricant and a layer of oil were sprayed on the plates) in an oven operating at 72°C. A thermocouple was inserted in the center of the cheese slice.

Decrease in cheese height during melting was measured over 15 min by a linear variable differential transformer, which was connected to the top plate. Degree of Flow (DOF) was calculated as the percentage decrease in the original cheese height when cheese reached 60°C. Slope of the linear portion of the flow region of the melt profile was recorded as the average flow rate (Muthukumarappan et al., 1999).

2.3.5. Statistical Analysis

Statistical analysis was performed using Statistical Analysis System (SAS version 9.1). The Analysis of Variance (ANOVA) was performed using PROC GLM procedure to determine the effects of different shred sizes on the texture and melting properties of non-fat reformed cheese with $p \leq 0.05$ significance level. Differences between means were analyzed using Tukey's method for multiple comparisons of means.

2.4. RESULTS AND DISCUSSION

The chemical composition of cheese milk and the non-fat cheese base that was used for reforming is given in Table 2.3. The non-fat cheese base had 56.7 % moisture, 33.2 % protein, 1.7 % salt and 0.9 % fat.

Hardness of cheese samples, as determined by the maximum force obtained at 80% strain, is given in Figure 2.1. Non-fat cheese base, which was not treated, was significantly harder. Grating and reforming reduced the hardness of non-fat cheese and it decreased further with the smaller shredding size. Force values (g) corresponding to different strain (%) levels during compression are given in Table 2.4. There appeared to be no significant difference in hardness between cheese samples when measured at 60% strain level (Table 2.4). At higher strain levels, the cheese base was significantly harder

than the reformed cheese, and reduction in shred size resulted in softer reformed cheese. The reason for the similarity in hardness up to high strain levels (≤60%) was probably due to the fracture of the reformed cheeses at higher strains (reformed cheese were more brittle than cheese base). The force-strain curves obtained from the uniaxial compression test of the cheese samples are given in Fig. 2.2. Cheeses that had been grated to smaller shred sizes fractured at lower stress levels and the resistance to fracture was higher with an increase in grating size.

Hardness values as obtained by TPA did not show any significant difference probably since the applied strain was 62% and lower than the uniaxial compression test (80%) (Table 2.5). The TPA curves of cheese samples showed sharp peaks with no fracture point as seen in Fig. 2.3 showing that cheese did not fracture. Reforming the cheese reduced its chewiness and gumminess significantly for the 1.5 mm grater size, as measured by TPA (Table 2.5). Springiness, adhesiveness and cohesiveness did not show significant difference with reforming (Table 2.5).

Reformed cheese samples exhibited a significantly higher DOF at 60°C as compared to the untreated cheese base (Fig. 2.4). There appeared to be a trend of increasing degree of flow (melt) as the grating size decreased, however, the differences were not statistically significant ($p>0.05$). The maximum flow rate of the cheese samples are given in Table 2.6. Non-fat cheese base had the lowest flow rate and reformed cheeses made with small shred sizes (1.5 and 3 mm) had significantly faster flow rates. Melt profiles of cheese samples are given in Fig 2.5.

Shredding the cheese caused a large increase in its surface area. Shredding produced rectangular shaped shreds (Fig. 2.6) having a width of the grater disc cut

diameter used for shredding. Dimensions of the cheese shreds produced were approximately 1.5x1x20 mm for 1.5mm grater disc; 3x1x20 mm for 3 mm grater disc; 6x1x20 mm for 6 mm grater disc; and 9x1x20 mm for 9 mm grater disc. The approximate surface area created by shredding 100 g of cheese through 4 grater discs having 1.5, 3, 6 or 9 mm diameter cuts is calculated using approximate shred dimensions and given in Table 2.2. As the grating size was reduced, surface area of the cheese increased. Cheese grated at 6 mm had about 5% more surface area than the cheese grated at 9 mm, while the cheese grated at 3 mm had 16%, and 1.5 mm had 32% more surface area than the 9 mm cheese. The surface area created after shredding is a measure of the amount of the disruption and discontinuity in the cheese matrix. The 1.5 mm cheese had 32% more surface that needed to fuse together than the 9 mm cheese when reformed. All shreds fused together well enough to form a cheese piece that held together as seen in the pictures of the cheese samples in Fig. 2.6. All cheese samples including the cheese base exhibited similar resistance to the compression at low strains (about <60%), however, at higher strains reformed cheese was softer than the base and the hardness was reduced as the grating size become smaller. These findings indicated that, many interactions and bonds were formed between the cut surfaces of the cheese after reforming, however, they were not as strong as the original cheese base and/or the number of the new bonds were fewer. In addition to that, when reforming the cheese, mechanical openings, i.e. trapping of visible air gaps, were avoided; however, small gaps at the micro level might have been remained between the shreds. Therefore, shredding and reforming the cheese imparted some level of discontinuity to the protein matrix and smaller shreds created a more open cheese network that was easier to compress (requires less amount of force) and so would

be easier to chew down. Fracture properties of the cheese depend on the size of the weak spots in the cheese matrix (Luyten and van Vliet, 1996). Whether it was the mechanical openings remaining between shreds or the reduction in the number of the bonds and interactions at cut surfaces; shredding cheese and reforming it created weak spots in the protein matrix that can propagate fracture during compression.

Chewiness is defined as the energy needed to chew a semi-solid food until it is ready for swallowing and gumminess is defined as the energy needed to disintegrate it before swallowing (Gunasekaran and Ak, 2003). Reduced chewiness and gumminess values in the reformed cheese were indicators of improvement in the chew-down characteristics of the non-fat cheese.

Melting of the cheese is primarily determined by the number and strength of the casein-casein interactions at high temperatures. Total number and/or strength of the bonds in the cheese matrix decrease when heating, and the cheese softens and finally melts as the viscous character of the cheese dominates over the solid-like, elastic character (Lucey et al., 2003). If the number of the bonds is fewer or if they are weak, less energy is needed for melt. The reason for the higher degree of flow in reformed cheese was probably fewer bonds and interactions in the protein matrix of the reformed cheese as compared to cheese base.

2.5. CONCLUSIONS

Non-fat cheeses that were grated and reformed were softer compared to the original cheese base. Grating cheese into smaller particles lowered the hardness compared to large particle sizes. Melt profile analysis indicated that cheese that had been grated into smaller shreds showed a higher flow rate, which indicated it was easier to

flow during heating. Grating disrupted the cheese matrix, weakened the continuous nature of the network and disrupted many physical bonds between casein particles. Many of these weak interactions reformed during cold storage of the cheese, however, not all interactions were recreated. Grating cheese could be viewed as analogous to the stirring/ shearing of set yogurt gels used to produce stirred yogurt; in both cases incomplete structural reformation occurred. The creation of smaller shred sizes resulted in greater disruption to the cheese matrix with the creation of more cut surface area. Shredding and reforming also resulted in cheese that had a shorter texture, which might be desirable as a common complaint about low-fat/non-fat cheese is its rubbery nature. Mechanical disruption of non-fat cheese produced a softer, more meltable reformed cheese. Overall, the size reduction and repressing process (e.g. using cold extrusion) could have promise in trying to reduce the undesirable textural attributes of non-fat or low-fat cheese.

2.6. REFERENCES

Banks, J. 2004. The technology of low-fat cheese manufacture. Intern J. Dairy Tech. 57:199-207

Bourne, M. C. 2002. Food Texture and Viscosity: Concept and Measurement. 2nd edn. Academic Press, San Diego.

Brickley, C. A., Govindasamy-Lucey, S., Jaeggi, J. J., Johnson, M. E., McSweeney, P. L. H., and Lucey, J. A. 2008. Influence of emulsifying salts on the textural properties of nonfat process cheese made from direct acid cheese bases. J. Dairy Sci. 91:39-48.

Chen, M. C., Johnson, M. E., and Jaeggi, J. J. 1994. Manufacture of a 25% and a 50% reduced-fat cheddar cheese by blending low and high fat curd. Pages 59-62 in Wisconsin CDR Annual Report July 1, 1993 to June 30, 1994, Wisconsin Center for Dairy Research.

Fenelon, M. A., Guinee, T. P., and Reville, W. J. 1999. Characteristics of reduced-fat cheddar prepared from the blending of full-fat and skim cheese curds at whey drainage. Milchwissenschaft 54:506-510.

Guinee, T. P., Auty, M. A. E., and Fenelon, M. A. 2000. The effect of fat content on the rheology, microstructure and heat-induced functional characteristics of Cheddar cheese. Int. Dairy J. 10:277-288.

Gunasekaran, S., and Ak, M. M. 2003. Cheese Rheology and Texture. CRC Press, Boca Raton, Florida.

Lucey, J. A., Johnson, M. E., and Horne, D. S. 2003. Perspectives on the basis of the rheology and texture properties of cheese. J. Dairy Sci. 86:2725–2743.

Luyten, H., and van Vliet, T. 1996. Effect of maturation on large deformation and fracture properties of (semi-) hard cheeses. Neth. Milk Dairy J. 50:295–307.

Marshall, R. T. 1992. Standard methods for the examination of dairy products. 16th edn. Am. Public Health Assoc, Washington, DC.

Merrill, R. K., Oberg, C. J., and McMahon, D. J. 1994. A method for manufacturing reduced-fat Mozzarella cheese. J. Dairy Sci. 77:1783-1789.

Mistry V. V. 2001. Low-fat cheese technology. Int. Dairy J. 11:413–22.

Mueller, A. P. 2005. Cheese extruding machine and process for producing pieces of cheese. US Patent No: US 6,942,888 B2.

Muthukumarappan, K, Wang, Y. C., and Gunasekaran, S. 1999. Modified Schreiber test for cheese meltability evaluation. J. Dairy Sci. 82:1068-1071.

Nelson, B. K., and Barbano, D. M. 2004. Reduced-fat Cheddar cheese manufactured using a novel fat removal process. J. Dairy Sci. 87:841 853

Whetstine, M. E. C., Drake, M. A., Nelson, B. K., and Barbano, D. M. 2006. Flavor profiles of full-fat and reduced-fat cheese and cheese fat made from aged cheddar with the fat removed using a novel process. J. Dairy Res. 89:505-517.

Table 2.1 Cheese manufacturing protocol of the non-fat cheese bases used for reforming

Operation	Time (min)		Temperature/ pH, TA
Initial Skim Milk		TA	0.165
initial milk weight (kg)	272 kg	pH	6.65
Add Citric Acid to Drop pH	0 min	Temp	5 °C
Add diluted citric acid (10% w/w solution pH of ~1.6)		TA	0.41
		pH	5.57
Target: pH - 5.60		g diluted acid	4832 g
Add $CaCl_2$	20 min	TA	0.41
5 oz/1000 lb milk or 81 ml	48 g	pH	5.60
Add Coagulant - 33°C	25 min	Temp	33°C
Ch Hansen, Chymax Extra (dbl str)		TA	0.410
1.9 g / 100 kg milk	5.5 g	pH	5.60
Cutting	55 min	TA	0.269
1/2" knives		pH	5.64
Start Cooking / Begin Agitation	60 min	Temp	32°C
Reach Cooking Temp - 37°C	80 min	Temp	38°C
		TA	0.268
		Whey -pH	5.65
		Curd -pH	5.42
Complete Drain		TA	---
Beginning	85 min	Whey -pH	---
End	95 min	Curd -pH	---
Add Flake Salt	110 min	TA	---
		Curd -pH	---
220 g / 100 kg milk		g salt	613
Hooping (9 kg Wilson)	125 min	Curd -pH	5.32
Pressing - In	130 min		
1 hour, 270 kPa - Out	190 min	Curd -pH	5.40
Storage	Weight	20 kg	
Into cooler after 1 hr press			

Table 2.2 Surface area created by shredding the cheese using grating discs having 1.5, 3, 6 and 9 mm diametric cut sizes (mm^2/100g cheese)

Grating Disc Size			
1.5 mm	3 mm	6 mm	9 mm
1,168,170	941,340	827,930	790,020

Table 2.3 Chemical composition of milk and cheese. Means are for replicates of 6 cheese making trials.

Milk	
Total solids, %	8.63 ± 0.3
Fat, %	0.12 ± 0.0
Casein, %	2.41 ± 0.1
Protein, %	3.13 ± 0.1
Casein:Fat ratio	19.9 ± 0.8
Total Ca, mg/100g milk	118 ± 1.2
Cheese	
Total solids, %	56.79 ± 0.52
Fat, %	0.9 ± 0.01
Protein, %	33.2 ± 0.23
pH	5.68 ± 0.01
Salt, %	1.7 ± 0.09
Total Ca, mg/100g cheese	435 ± 8.21

Table 2.4 Uniaxial compression test results of cheese base and cheeses reformed after shredding into different sizes (1.5, 3, 6 and 9 mm diametric cut). Means are for replicates of 6 cheese making trials.

Cheese Type	Hardness (g) (Maximum Force)	Force at 60% Strain (g)	Force at 70% Strain (g)	Force at 80% Strain (g)
BASE	1.96E+04 ± 317[a]	8.05E+03 ± 523[a]	1.39E+04 ± 277[a]	1.91E+04 ± 778[a]
1.5 mm	1.32E+04 ± 581[b]	7.44E+03 ± 580[a]	1.22E+04 ± 706[b]	1.37E+04 ± 826[b]
3 mm	1.36E+04 ± 805[bc]	8.10E+03 ± 587[a]	1.22E+04 ± 808[b]	1.36E+04 ± 573[bc]
6 mm	1.49E+04 ± 948[cd]	8.31E+03 ± 963[a]	1.23E+04 ± 759[b]	1.44E+04 ± 536[c]
9 mm	1.52E+04 ± 1120[d]	7.69E+03 ± 1308[a]	1.19E+04 ± 2214[b]	1.50E+04 ± 3050[c]

[a-d]Means with different superscript letters within the same column are significantly different ($P<0.05$)

Table 2.5 Texture Profile Analysis results of cheese base and cheeses reformed after shredding into different sizes (1.5, 3, 6 and 9 mm). Means are for replicates of 6 cheese making trials.

	Cheese Type				
	BASE	1.5 mm	3 mm	6 mm	9 mm
Hardness (g)	9.25E+03 ± 6.E+02 [a]	8.08E+03 ± 6.E+02 [a]	8.89E+03 ± 8.E+02 [a]	8.89E+03 ± 8.E+02 [a]	9.40E+03 ± 2.E+03 [a]
Springiness (mm)	1.19E+01 ± 1.E-01 [a]	1.18E+01 ± 4.E-01 [a]	1.17E+01 ± 4.E-01 [a]	1.17E+01 ± 1.E-02 [a]	1.18E+01 ± 2.E-01 [a]
Adhesiveness (g.mm)	-8.70E-05 ± 3.E-05 [a]	-6.80E-05 ± 1.E-05 [a]	-5.70E-05 ± 2.E-05 [a]	-5.70E-05 ± 2.E-05 [a]	-6.70E-05 ± 5.E-01 [a]
Cohesiveness	6.49E-01 ± 1.E-02 [a]	6.53E-01 ± 1.E-02 [a]	6.09E-01 ± 4.E-02 [a]	6.09E-01 ± 4.E-02 [a]	6.09E-01 ± 3.E-02 [a]
Gumminess (g)	6.01E+03 ± 5.E+02 [a]	5.27E+03 ± 4.E+02 [b]	5.40E+03 ± 5.E+02 [ab]	5.40E+03 ± 5.E+02 [ab]	5.68E+03 ± 7.E+02 [ab]
Chewiness (g.mm)	7.13E+04 ± 6.E+03 [a]	6.21E+04 ± 7.E+03 [b]	6.31E+04 ± 6.E+03 [b]	6.31E+04 ± 6.E+03 [b]	6.72E+04 ± 8.E+03 [ab]

[a-b]Means with different letters within the same row are significantly different ($P<0.05$)

Table 2.6 Flow rates of nonfat cheese base and cheeses reformed after shredding into different sizes (1.5, 3, 6 and 9 mm diametric cut). Means are for replicates of 6 cheese making trials

	Cheese sample				
	BASE	1.5 mm	3 mm	6 mm	9 mm
Flow rate	0.27 ± 0.05[a]	0.33 ± 0.03[b]	0.32 ± 0.03[b]	0.31 ± 0.03[ab]	0.30 ± 0.03[ab]

[a-b]Means with different superscript letters within the same row are significantly different ($P<0.05$)

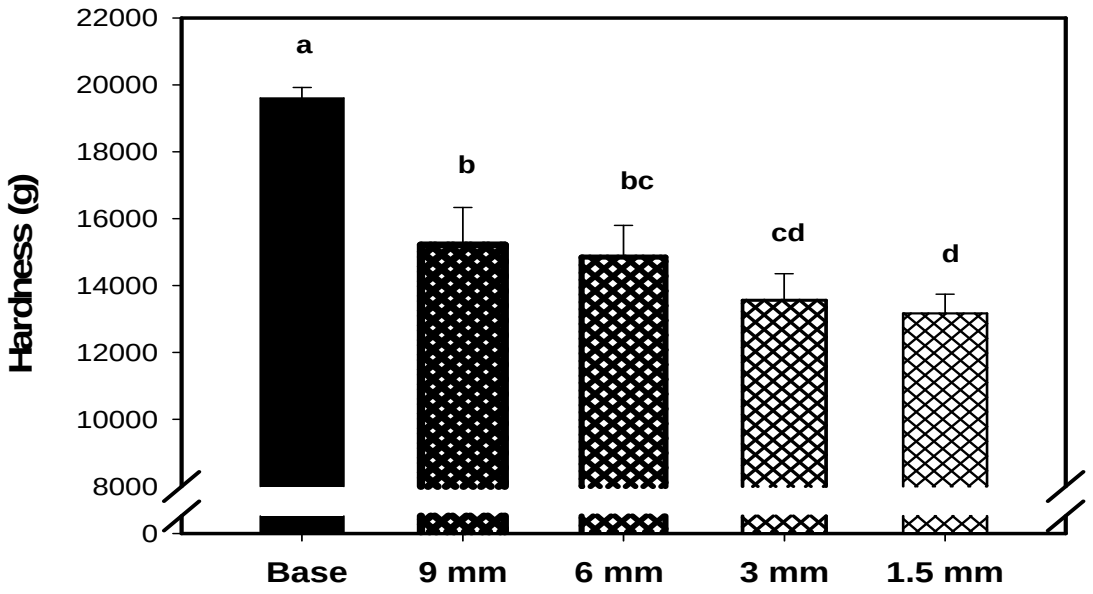


Figure 2.1 Hardness of nonfat cheese base and cheeses reformed after grating into different sizes (9, 6, 3 and 1.5 mm diametric cut) measured by uniaxial compression test. Bars with different letters show significant difference ($P<0.05$). Means are for replicates of 6 cheese making trials.

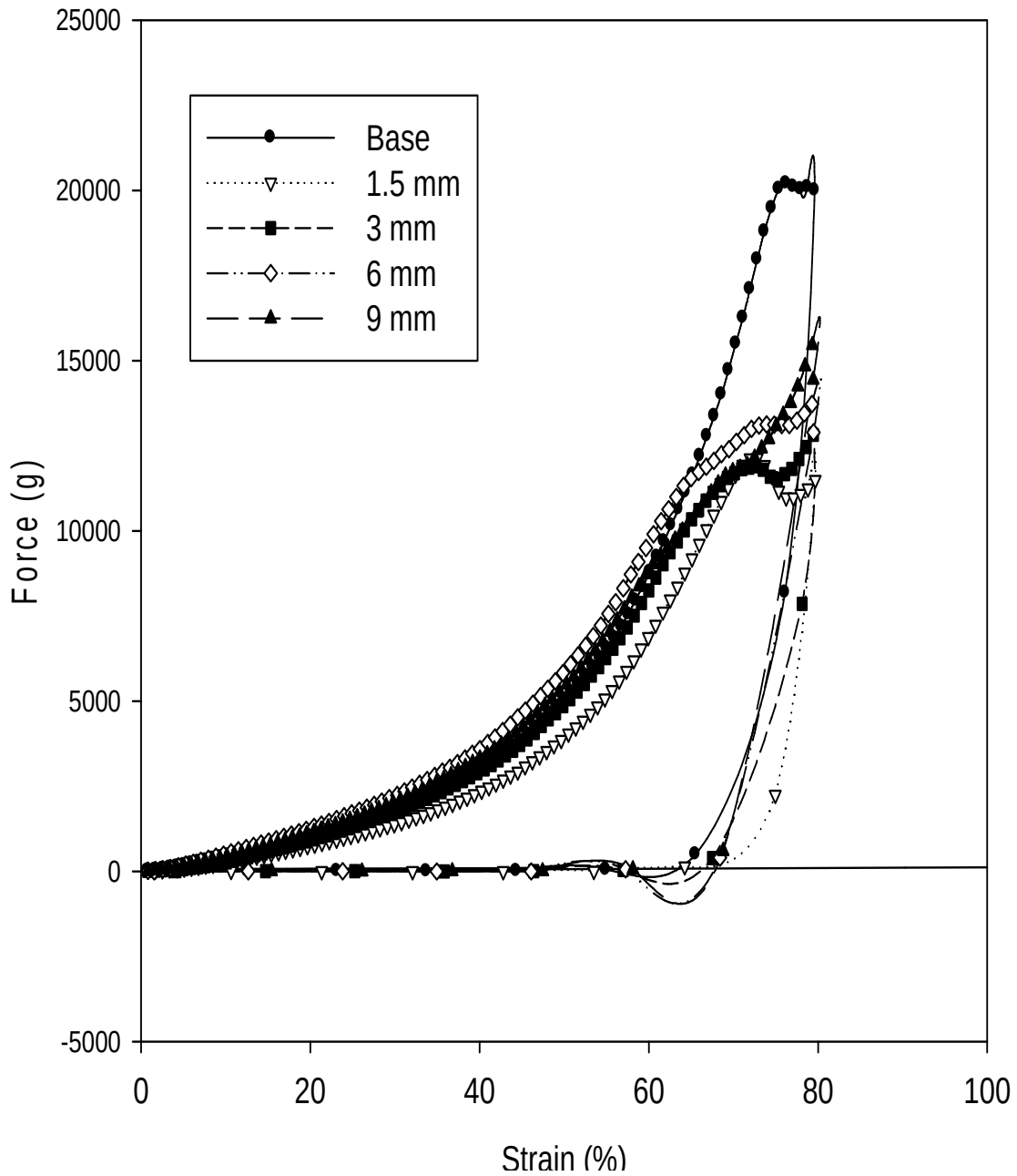


Figure 2.2 Representative uniaxial compression profiles for nonfat cheese base and cheeses reformed after grating into different sizes (9, 6, 3 and 1.5 mm diametric cut)

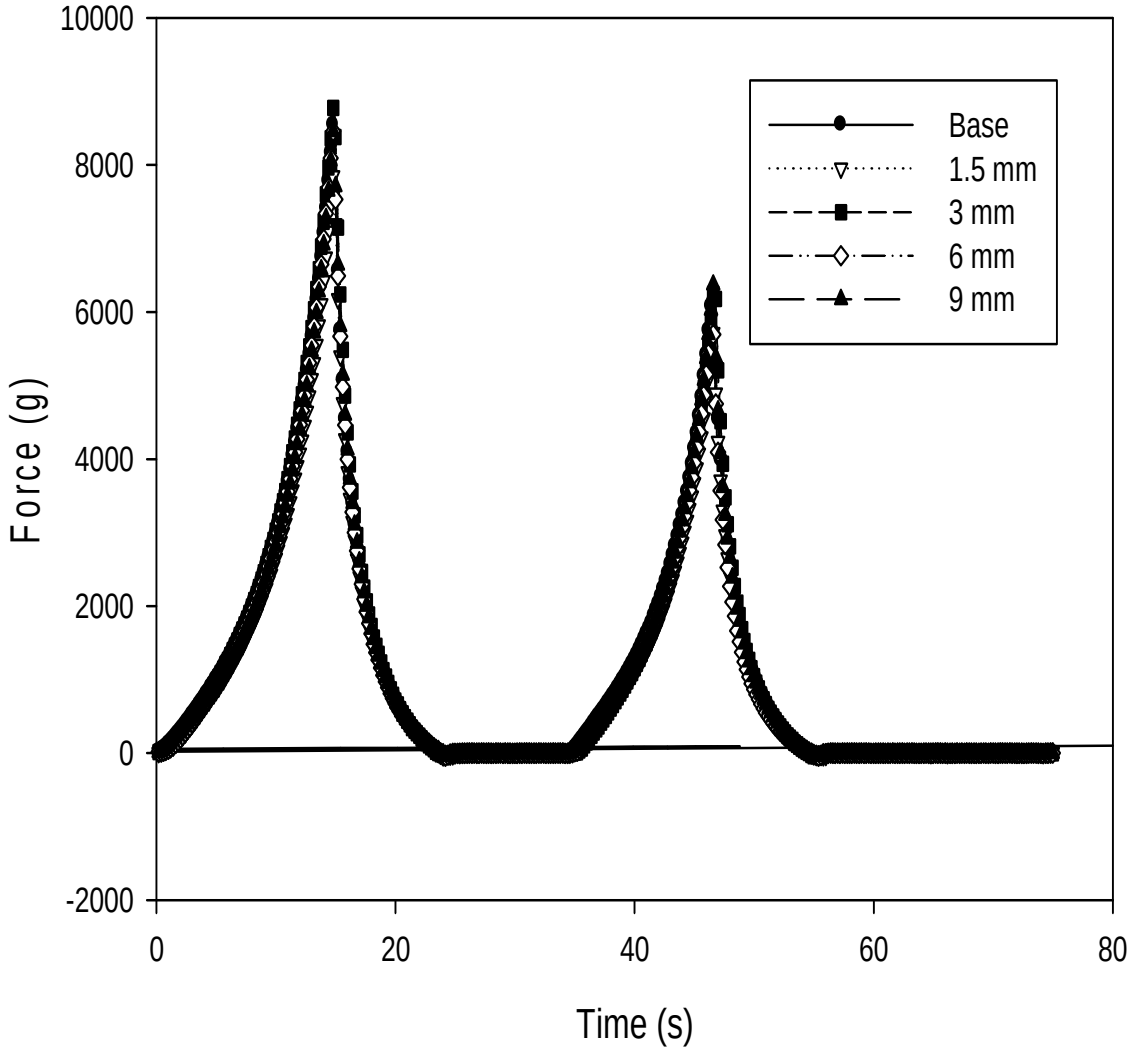


Figure 2.3 Representative Texture profile analysis (TPA) profiles for nonfat cheese base and cheeses reformed after grating into different sizes (9, 6, 3 and 1.5 mm diametric cut)

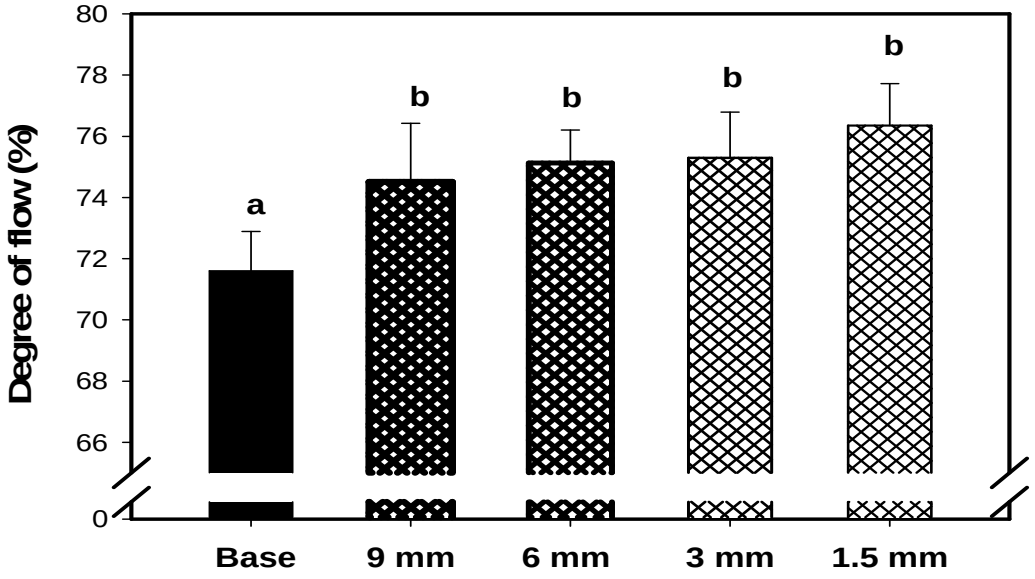


Figure 2.4 Degree of flow at 60°C for nonfat cheese base and cheeses reformed after grating into different sizes (9, 6, 3 and 1.5 mm diametric cut) measured by UW-Melt Profiler. Bars with different letters show significant difference ($P<0.05$). Means are for replicates of 6 cheese making trials.

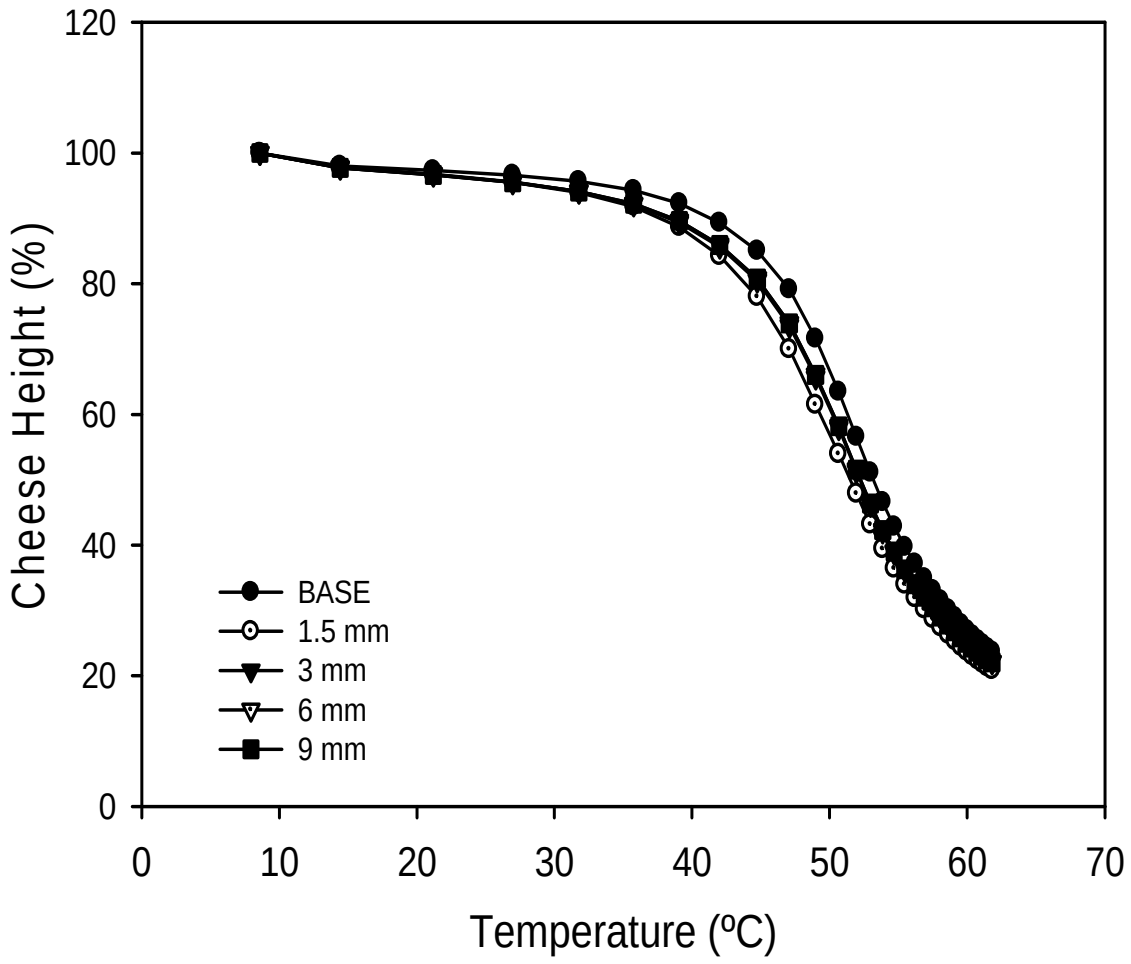


Figure 2.5 Melt profiles for non-fat cheese base and cheeses reformed after grating into different sizes (9, 6, 3 and 1.5 mm diametric cut) measured by UW-Melt Profiler. Means are for replicates of 6 cheese making trials.

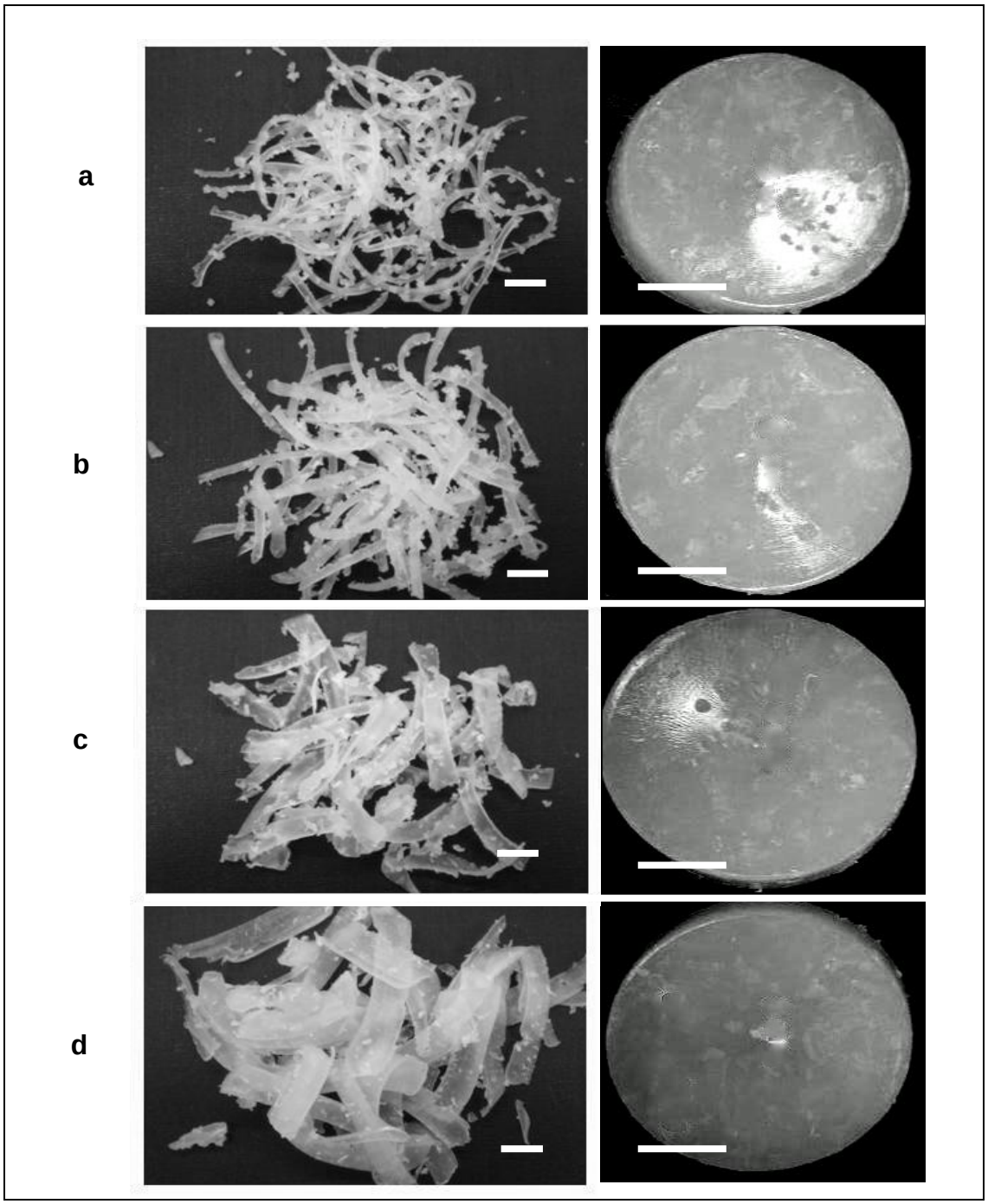


Figure 2.6 Cheese shreds (left-hand column) and cheese samples 1wk after repressing the cheese shreds (right-hand column) obtained by using grater discs of (a) 1.5, (b) 3, (c) 6 and (d) 9 mm diameter. Bars represent 10 mm for both columns

Chapter 3

IMPACT OF REFORMING TEMPERATURE ON THE TEXTURE, RHEOLOGY AND MELTING PROPERTIES OF REFORMED NON-FAT CHEESE

3.1. ABSTRACT

We evaluated the impact of extruding non-fat cheese at various temperatures on the texture of non-fat cheese. Non-fat cheese was brought to 4, 18 or 30°C for 6 h prior to reforming. Reforming was performed using a Vemag vacuum extruder. Textural and rheological analyses were performed on non-fat cheese base and reformed cheeses that had been stored for 2 wk at 4°C. Dynamic rheological properties were measured by small amplitude oscillatory rheology during heating. Textural properties were determined with a Texture Analyzer. Melt properties were determined using UW-Melt-profiler. All reformed cheese samples was significantly softer than the cheese base, except for cheese extruded at 4ºC. Raising the reforming temperature to 30°C reduced the hardness and storage modulus, and increased the meltability of the reformed cheese. Reforming the cheese at higher temperatures produced a smoother cheese having a softer texture. High temperatures increased the mobility of the bonds leading to faster cheese fusion,

however, the net impact was a softening of the cheese structure presumably due to loosening of the para-casein matrix with a decrease in the strength and loss of interparticle bonds.

3.2. INTRODUCTION

Temperature has a profound influence on cheese structure and texture. Visser (1991) studied the rheological properties of Gouda cheese and found that increase in measurement temperature from 14 to 26ºC reduced the compression modulus (E), storage modulus (G') and fracture stress. The melt and flow characteristics of cheese are dependent on casein interactions rather than melting of the fat as cheese melting occurs at temperatures above the point where milk fat has become completely liquid (Lucey et al., 2003). It has been demonstrated that the dynamic moduli of a non-fat cheese also decrease with an increase in temperature (Udayarajan et al., 2007). Caseins cannot denature (unfold) by heat since they already have an open native conformation, but an increase in temperature will change their interaction properties and association behavior since the strength of hydrophobic interactions and electrostatic forces, hydrogen bonding and the solubility of calcium phosphate are all influenced by temperature (Lucey et al., 2003).

In the cheese industry, sometimes cheeses are broken down into pieces and blended together before attempting to fuse the cheese pieces back together again. The attempt to fuse cheese together is called reforming. In the process of reforming, cheese is first physically broken into pieces, which then re-associate to some extent to form a cheese block. Since the structure of cheese is built by the para-casein network, casein interactions are responsible for the ability to re-build a cheese network after reforming.

Reformability of cheese mainly depends on the affinity of caseins to interact. Therefore, it is important to evaluate the impact of parameters that influence the main types of casein interactions that probably occur during the cheese reformation process.

Association of the caseins is driven by hydrophobic interactions and controlled by electrostatic forces (Lucey et al., 2003). When the cheese is heated, the protein matrix adsorbs energy, which influences the interactions that maintain the integrity of the protein network. Interactions under entropic control (e.g., hydrophobic interactions) are strengthened, while those under enthalpic control (i.e., electrostatic, van der Waals interactions and hydrogen bonds) are weakened (Gunasekaran and Ak, 2003).

Hydrophobic interactions occur due to an attempt by nonpolar molecules to reduce their surface area exposed to water. Water tends to form ordered cages around the non-polar molecule. Hydrophobic interactions are weak at low temperatures due to the restricted mobility of the water molecules that form the cage. At high temperatures these cages are no longer any stronger than bulk water and hydrophobic interactions increase (Philips et al., 1994).

Weakening of the hydrophobic interactions, with the decrease in temperature, disrupts the casein micelle in a dilute environment like milk (Lucey et al., 2003). Cheese is a concentrated protein network having about 10 fold more protein than milk. In a study on concentrated casein micelles (~17 to 22% protein content), the concentrated casein micelles exhibited classical viscoelastic gel properties at 5 or 10ºC, however, at 40ºC they flowed freely (Horne, 1998). Horne (1998) suggested that at low temperatures, in the closed packed conditions of the concentrated casein micelles, casein molecules are loosened and tended to link across to neighboring micelles or become entangled with

them causing high elastic moduli and solid-like behavior. However, when the temperature is raised, the micelle structure became tightened up reducing the contact area between adjacent micelles and this resulted in fluid-like behavior (Horne, 1998). In the NMR studies of Rollema and Brinkhuis (1989), with an increase in temperature, there was an increase in the mobility of protons on the amino acid side chains of caseins, suggesting a loosening of the internal structure of the micelle. Therefore, increasing the temperature creates more flexibility in the casein matrix. Increasing temperature reduces protein hydration and water holding capacity as protein-protein interactions become more favorable than protein-water interactions (Teo et al., 1996; Pastorino et al., 2002).

The objective of this study was to determine the impact on temperature of the reforming process and the texture of the reformed cheese.

3.3. MATERIALS AND METHODS

3.3.1. Cheesemaking

Non-fat cheese bases were made by pre-acidification of skim milk to pH 5.6 with citric acid using the procedure described by Brickley et al. (2008). Citric acid was added to pasteurized skim milk at 4ºC until pH 5.6 was attained, and that pH was maintained for at least 30 min. Then, milk was heated to 33ºC and rennet (Chymax Extra Double Strength, Chr. Hansen, Milwaukee, WI, USA) was added (2g/100kg of milk). The coagulum was cut using 12.7 mm knives approximately 1 h after rennet addition. The curd-whey mixture was then heated to 37ºC in about 20 min while stirring. Whey was drained, and curd was dry salted (225 g/100 kg milk). The curd was placed into 9 kg Wilson style hoops and pressed for 60 min at 276 kPa. Cheese bases were stored at 4°C

for 3 wk prior to the grating-reforming treatment. The manufacturing protocol of non-fat cheese base is given in Table 3.1.

3.3.2. Cheese Reforming

Cheese bases were stored at 4°C for 3 wk and then shredded using a large scale shredder (Urschel Shredder - 6 mm crinkle cut, Valparaiso, IN, USA). Shredded cheese were then divided into 3 parts and incubated for 6 h at 3 different temperatures (4, 18 or 30°C) prior to reforming. Temperatures were selected that were below the melting point of the cheese, however, the upper temperature used in this trial was 30°C since incubating the cheese above this temperature caused undesirable changes in cheese, such as, serum release and increased risk of unwanted microbial growth.

Grated cheese was reformed by extruding under 10 MPa vacuum at ambient temperature using a double-screw vacuum filler (Vemag Robot 500, Reiser, Verden, Germany). Reformed cheeses were then vacuum sealed and stored at 4°C for 1 wk prior to analysis.

The vacuum filler (Figures 3.1, 3.3 and 3.4) used for extruding the shredded cheese into cylindrical cheese blocks was composed of an input hopper, a pair of feed screws, a vacuum pump integrated into the double screw unit and an outlet tube extension attached to the double screw housing.

Shredded cheese was manually loaded to the funnel-shaped input hopper and then conveyed into the double screws. A scrapper was attached to the hopper to help force any remaining cheese from the hopper. A rotating spiral attachment was mounted at the bottom part of the hopper to help convey the cheese down. The double screws (Fig. 3.2) had parallel drive shafts driven to rotate together in opposite directions to each other

(counter-rotating) with respective spiral threads at a determined speed, through which cheese pieces were compressed into a uniform cheese flow. The threads of the double screw were thicker towards to the output end in order to decrease the inter-thread spaces, which gradually increased the pressure on the cheese flow as the cheese moved through. The screws were fitted with minimal clearance relative to the inner surface of the cylindrical extruder chamber (barrel) that housed the double screw mechanism.

The feed was conveyed into the extruder chamber with the aid of vacuum and it moved forward by the rotating movement of the double screws. The vacuum formed in the screw housing chamber by vacuum pump helped to draw cheese pieces from the hopper into the screw feed. The double screws comminuted the cheese shreds as it rotated and with the pressure that was build up in the chamber, folded them together into a uniform cheese mass. Formation of air gaps and pockets in the cheese mass was prevented by the vacuum pump, which evacuated air from the extruder chamber where the screw feed was housed.

The friction occurring between the flowing cheese and the outlet tube produced back-pressure against the double screw, and that helped to keep a predetermined extrusion pressure on the cheese. Therefore, the length of the outlet tube was critical for maintaining a sufficient pressure on the cheese to ensure fusion of the cheese mass, and it also provided additional time for cheese to fuse after passing through the double screw. The extension tube of the extruder used in this trial was 1.8 m long.

The inner surface of the extension tube was coated with teflon in order to ease the movement of the cheese used in our trials, as the non-fat cheese was very sticky. Teflon

coating the extension tube improved the flow of the cheese and produced smoother cheese as seen in Fig. 3.7.

Reforming of the cheese was performed at minimum screw speed. It took a few min to build up the vacuum to 10 MPa after the cheese was loaded (This cheese was discarded). The time taken for the cheese to pass through the vacuum filler was approximately 5 to 10 min. The temperature in the extruder was not controlled, therefore the temperature of the cheese changed slightly during the residence time in the equipment. The exit temperature of the cheese that was stored at 4 and 18°C was slightly higher (14 and 22ºC, respectively) probably due to warming up by friction during processing and due to the ambient temperature (~25ºC) of the pilot plant. The temperature of the cheese reformed at 30°C did not exhibit an increase since the temperature at the pilot plant was below that temperature; thus, friction during processing therefore may only make a minor contribution to any temperature increase during processing.

3.3.3. Compositional Analysis

Milk samples were analyzed for total solids, fat, protein, and casein (Marshall, 1992). The total solids, fat, protein and pH of cheese were determined (Marshall, 1992). The salt content of the cheese samples was measured using Corning Salt Analyzer (Marshall, 1992) and the total calcium content was analyzed by inductively-coupled Argon plasma emission spectroscopy (ICP) (Choi et al., 2007).

3.3.4. Textural analysis

A TA.XT2 Texture Analyzer (Stable Micro Systems, Godalming, Surrey, UK) with a TA-25 probe (50 mm diameter) and TA-90A flat plate was used for texture testing. Cylindrical cheese samples having 16 mm diameter and 17.5 mm height were cut using a cork borer and they were kept overnight at 4°C in sealed plastic bags prior to analysis. Texture Profile Analysis (TPA) at 62% compression and uniaxial compression test at 80% compression level was performed on cheese samples at 4°C. Texture parameters were calculated as described by Bourne (2002).

3.3.5. Melt profile analysis

Melt Profile Analysis was performed using UW Melt-Profiler developed by Muthukumarappan et al. (1999). For melt analysis, cylindrical cheese samples having 30 mm diameter and 7 mm thickness were cut with a cork borer and held overnight at 4°C in sealed plastic bags. Cheese sample was placed in the melt profiler oven operating at 72°C immediately after taking out the samples from the refrigerator. A thermocouple was inserted in the center of the cheese disc, which was then placed between two aluminum plates having a dry film lubricant and a layer of oil sprayed on them. Decrease in cheese height during melting was measured over 15 min by a linear variable differential transformer, which was connected to the top plate. Degree of Flow (DOF) was calculated as the percentage decrease in the original cheese height when cheese reached 60°C. Softening point was the temperature that corresponded to the minimum of the first derivative of the change in height with time curve.

3.3.6. Rheological Analysis

Rheological properties were determined using a Paar Physica (UDS 200, Physica Messtechnik GmbH, Stuttgart, Germany) controlled stress rheometer, with a serrated parallel plate geometry. Cheese disks of 50 mm diameter and 3 mm thickness were obtained with a cylindrical stainless steel cork borer and a meat slicer. Cheese discs were then sealed in plastic bags and held at 4°C overnight prior to the test. When loading samples in order to maintain a good contact between plate and cheese, the upper plate was lowered onto cheese not to exceed a normal force of 2 N and then samples were allowed to relax for about 15 min to a relatively constant normal force reading of 0.8 N before starting the test. A thin layer of vegetable oil was applied around the cheese sample to prevent moisture loss.

Small amplitude oscillatory shear (SAOS) test with an applied strain of 0.2% and frequency of 0.1 Hz was used for determining rheological properties of cheese before and after reforming. Temperature sweeps were performed from 5 to 85°C at the rate of l°C/min. We also studied the rheological properties of the cheese base during heating from 5 to 35ºC and immediate cooling from 35 to 5ºC. Storage modulus (G'), loss modulus (G") and loss tangent (LT) were the parameters determined.

A frequency sweep test using Fourier transform mechanical spectroscopy (FTMS) technique, a variant of SAOS, was applied to non-fat cheese base while heating from 10 to 50ºC as described by Udayarajan et al. (2005). In FTMS, the sample is subjected to a complex sinusoidal wave that is a combination of several sine waves of differing frequencies. The frequencies selected for the complex waveform were 0.08, 0.4, 0.8, 4 and 8 Hz and the cumulative strain was adjusted to be within the linear viscoelastic

region (i.e., <0.3%). The digital stress generated were analyzed by rheometer software (US 200, Anton Paar Germany, Ostfildern, Germany) using a Fast Fourier Transform to obtain values of phase angle (δ). The δ values, input strain, geometry factors and amplitude of both waveforms were used by software to calculate G', G" and LT as described by Udayarajan et al. (2005).

3.3.7. Statistical Analysis

Statistical analysis was performed using Statistical Analysis System (SAS version 9.1). Analysis of variance (ANOVA) was performed using PROC GLM procedure to determine the effects of different reforming temperatures on the texture and melting properties of reformed cheese with $p \leq 0.05$ significance level. Differences between means were analyzed using Tukey's method for multiple comparisons of means.

3.4. RESULTS

3.4.1. Milk and cheese composition

The chemical composition of the cheese milk and the non-fat cheese base that was used for reforming is given in Table 3.2. The non-fat cheese base had 58.7% moisture, 32.77% protein, 1.34% salt and 1.22% fat which, according to the Code of Federal Regulations for the labeling of low and nonfat cheese (CFR, 2006), would classify the cheese bases as nonfat (i.e., <1.6% fat, or <0.5 g of fat for a 28-g serving).

3.4.2. Visual attributes

Pictures of the cheese samples prior to reforming (in shredded form), right after reforming and 1 wk after reforming are shown in Figures 3.5, 3.6 and 3.8, respectively.

The cheese shreds held at 30°C were partially fused and matted together by the end of the incubation period as seen in Fig. 3.5. Higher reforming temperatures resulted in more cheese fusion in the extruder while the texture of the cheese incubated at low temperature was still curdy after the reforming process. However, there was no visible difference in the appearance between any of the reforming treatments after 1 wk of storage at 4°C (Fig. 3.7). The initial fluffy appearance on the outer surface of the cheese reformed at 4 and 18°C disappeared during storage and all cheese blocks showed a uniform body. The cross-sectional view of cheese samples shown in Figures 3.6 and 3.8, demonstrated that cheese reformed at 30°C had distorted shape while cheese reformed at 4°C preserved its cylindrical shape. This distortion of cheese blocks reformed at higher temperatures indicated that they were quite soft during reforming.

The exit temperature of the cheese incubated at 4 and 18°C increased during extrusion (to 14 and 22°C, respectively), probably due to warming up caused by friction between cheese and double screws and the outlet tube, as well as the higher temperature of the processing room. The exit temperature of the cheese incubated at 30°C did not increase.

3.4.3. Texture properties

All reformed cheese samples exhibited a softer texture than the untreated cheese base except for the cheese reformed at 4ºC (Fig 3.9). There appeared to be a trend of decreasing hardness as the reforming temperature increased. Cheese that was reformed at 30°C had significantly softer texture as determined by TPA (Table 3.3). On the other hand, uniaxial compression test did not show any differences between the cheese samples (Table 3.4), which might be due to the high strain levels in this test that caused fracture in

all cheeses. Gumminess and chewiness of the cheese were reduced as reforming temperature increased (Table 3.3). No significant differences were found between the chewiness and gumminess values of cheese base and cheese reformed at 4°C. Adhesiveness, cohesiveness and springiness values did not differ between cheese samples.

3.4.4. Rheological properties

Changes in the G' values during heating of cheese samples from 5 to 85°C are shown in Fig. 3.10. At a measurement temperature of 5°C, cheese base exhibited the highest G' values and the G' values decreased with increasing reforming temperatures. The low G' values for cheese reformed at 30°C indicated this sample had lowest number or weakest bonds, in agreement with the low hardness results obtained by TPA test (Fig. 3.9). At high temperatures, e.g., 85ºC, all cheese samples exhibited similar G' values.

Loss tangent curves of the cheese base and reformed cheese samples (Fig. 3.11) were similar except for the temperature at LTmax for cheese base, which was significantly lower than other samples (Table 3.6). The temperature at which LT values are equal to 1 (indicating a melt point) was similar for all cheese samples (Table 3.6).

Changes in the power law exponent (*n*) of non-fat cheese base during heating from 10 to 40ºC are given in Figure 3.12. The *n* values are obtained by plotting the logarithm of G' values against frequency values. The *n* value is an indicator of the frequency dependence of G' values and gives information on the nature of the gel network at any particular temperature (Gunasekaran and Ak, 2000). The increase in *n* values was slight at the low temperature range from 10 to 30ºC indicating that the matrix exhibited strong gel properties.

$\log G' = n \log f + k$

where f is frequency, and n and k are constants.

3.4.5. Meltability

There were no significant differences between the degree of flow and softening temperature of the cheese samples as determined from the melt profile analysis (Table 3.7). The decrease in the height of cheese as a function of time during the melting test showed similar trends for all cheese samples (Fig. 3.13).

3.4.6. Rheological properties of cheese base during heating from 5 to 35ºC and cooling from 35 to 5ºC respectively

Changes in the dynamic moduli of non-fat cheese base during heating from 5 to 35ºC and cooling back to 5ºC is shown in Fig. 3.14. The G' and G" values decreased as cheese sample was heated to 35ºC. Partial recovery of the initial dynamic moduli was observed during cooling back to 5ºC; however, these values were still lower than those initially observed values during heating. Dynamic moduli values of the cheese base at a measurement temperature of 5ºC in the beginning of heating cycle and after heating and cooling are given in Table 3.8.

3.5. DISCUSSION

Non-fat cheese can be viewed as a strong protein gel that is composed of interconnected and overlapping strands of partially fused para-casein aggregates that are held together by physical forces (O'Callaghan and Guinee, 2004). Therefore, the fusion of the cheese particles after physical reformation of the non-fat cheese depends on the interactions between caseins, the building blocks of cheese protein matrix. Fusion takes

place as the casein molecules at the surface of each particle associate with casein molecules of adjacent cheese particles. Factors that influence the strength of the interactions and bonds between caseins would influence the affinity of caseins to stick back together again when the cheese is reformed. Temperature is one of the important factors that influences casein interactions (Lucey et al., 2003), therefore it was expected that cheese temperature during reforming would also influence the cheese reformation and final textural properties after reforming.

Results showed that the incubation temperature used for cheese significantly influenced the texture properties and reformability. Temperature dependent changes in the viscoelastic properties of the non-fat cheese base dictated the final reformed cheese properties. Therefore, for a better understanding of the impact of temperature on used for incubation cheese reforming, we analysed the structure and the behavior of the cheese base over the temperatures used to reform the base.

3.5.1. Viscoelastic properties of non-fat cheese base at different temperatures

There was a loss in the elasticity of the non-fat cheese base with the increase in temperature as indicated by the decrease in G' values (Fig 3.10). The temperatures >40ºC were not in the scope of our study since >40ºC cheese undergoes larger structural changes with increased mobility of the proteins to an extent where the cheese finally melts and flows (Lucey et al., 2003). Cheese exhibits solid-like behavior at temperatures <40ºC where dominated G' over G" values in SAOS studies (i.e. Udayarajan et al., 2005; Muliawan et al., 2007). The highest temperature we used for cheese bases was set to 30ºC in order to prevent any unwanted changes in cheese, such as, serum release. Zhou

and Mulvaney (1998) defined three distinct zones of viscoelastic behavior in a model process cheese system when heating from 5 to 70ºC: (1) Temperatures <10ºC: rubbery solid; (2) From 10ºC to cross-over temperature of G' to G" (LT=1): transition; (3) After the cross-over temperature: viscoelastic melt (Zhou and Mulvaney, 1998). The transition zone is basically where softening of the cheese takes place with a loss in elasticity, which was also observed in our cheese samples when heated (Fig. 3.10). Above the cross-over temperature viscous modulus becomes greater than elastic modulus and the cheese exhibits fluid like behavior (Lucey et al., 2003). In our study, the cross-over temperature for the cheese base was 44ºC (Table 3.6). Zhou and Mulvaney (1998) observed that with an increase in the casein:moisture ratio, the cross-over temperatures changed from 45 to 65ºC in their model cheese system. The relatively low cross-over temperature of our cheese base was an indication of weaker interactions and bonds in our non-fat cheese base, which could be due to its low calcium content since the cheese bases were produced by direct acidification with citric acid, a well known calcium chelating acid. Venugopal and Muthukumarappan (2003) did not observe cross-over (of G' and G") at any temperature during heating from 25 to 60ºC of Cheddar cheese samples made with different fat and moisture levels. It should be noted that crossover points are frequency dependent and also shift to higher temperatures at higher frequencies. Therefore, one should be aware of this aspect and not rely on measurements at a single frequency when defining certain structural changes, such as, transition and melt (Udayarajan et al., 2005). Ross-Murphy (1995) proposed a relationship for the frequency dependence of G', the power law exponent (n) of logG' versus log$f$ is used as a measure of how close the gel is behaving as a strong gel. Changes in the n values could help precisely monitor the

transitions in the cheese structure as a function of temperature. The value of *n* is equal to zero for crosslinked gels (strong gels) showing that G' is independent of frequency. For physical gels (gels that are intermediate between strong and weak gels) the value of *n* is higher than zero and increases as the relaxation times of the bonds in the cheese network becomes shorter (Gunasekaran and Ak, 2000; Udayarajan et al., 2005). The slight increase in the *n* values of the non-fat cheese base with the increase in temperature from 10 to 30ºC indicated that there were some loss in the number and strength of the bonds but those structural changes were small and the G' values were almost independent of frequency (Fig. 3.12). Udayarajan et al. (2005) have also found a similar trend in *n* values of the cheese while heating.

Elasticity of the cheese is important for cheese fusion since the tendency of a food material to stick together or to stick onto a surface is governed by a combined effect of adhesive and cohesive forces and depends on the viscosity of the material (Adhikari et al., 2001). According to the Dahlquist criterion, stickiness does not occur in hard materials; and it states that, for adhesion to occur, the storage modulus of an adhesive must be below 10^5 Pa (Dahlquist, 1969). The decrease in G' values of non-fat cheese base from about $3x10^5$ Pa to around 10^5 Pa as the temperature increased from 5 to 30ºC (Fig. 3.10) indicated that cheese became more adhesive and more susceptible to greater fusion when reformed after heating to 30ºC. It should be noted that, applying Dahlquist criterion to cheese adhesion may not always be relevant due to the fact that cheese is a physical network with cross-links that are transient in nature and the G' values will change depending on the time scale of the measurement (Childs et al., 2007).

Changes in dynamic moduli values of our cheese samples during heating provide information about the interactions that occur in cheese at a molecular level (Lucey et al., 2003). The decrease in the dynamic moduli values was an indicator of a decrease in the total number and/or strength of bonds in cheese matrix (Lucey et al., 2003). When the cheese is heated, hydrophobic interactions increase in strength (Bryant and McClements, 1998), which will induce conformational changes on casein particles that form the para-casein matrix by altering the number of inter- and intraparticle bonds (Roefs and Van Vliet, 1990). Several mechanisms were proposed for explaining the changes in viscoelastic properties and softening of cheese during heating. Guinee et al. (2000) studied the rheological properties of the cheese with various fat contents while heating and they observed a rapid decrease in G' of all cheese samples when the temperature was raised from 20 to 45-50ºC, which was in agreement with the findings of Horne et al. (1994), Guinee et al. (1999) and Joshi et al. (2004). The softening of cheese texture was suggested to be related to an increase in para-casein solvation due to a change in casein conformation or to a pH reduction besides the melting of the fat since the G' of low fat cheese also decreased (Guinee et al., 2000). Studies on the stability and association behavior of casein micelles showed that the voluminosity of the casein micelles decreased with an increase in temperature (Walstra, 1990; De Kruif et al., 2002; O'Connell et al., 2003). Casein particles shrink due to an increase in hydrophobic intraparticle attractions, which in turn reduces their contact area with neighboring particles and that ultimately reduces the number of interparticle bonds (Zoon et al., 1988; Roefs and Van Vliet, 1990; Horne, 1998; Lucey et al., 2003). Increase in temperature will also disrupt the hydrogen bonds. The decrease in the temperature of the non-fat

cheese samples loosened the casein particles, as hydrophobic interactions were weak, and lead to an increase in the number of interparticle bonds between casein particles, which in turn increased the elastic moduli of the cheese samples (Fig. 3.10). The higher G' values of the non-fat cheese base held at 4ºC (Fig. 3.10) indicated that, the protein matrix had more/stronger casein-casein interactions as casein particles loosen and swelled enlarging their contact area. The increased casein interactions at 4ºC resulted in a rigid cheese texture, which was also evident from the pictures of the cheese shreds (Fig. 3.5). The shreds for cheese held at 4ºC appeared to be more intact compared to cheese held at 18 and 30ºC. Cheese shreds that were held at 30ºC were soft. Since this was a non-fat cheese these temperature differences were not due to the degree of solid/liquid fat. A layer of moisture had formed on the surface of the shreds after incubating to 30ºC for 6 h, giving them a shiny appearance as seen in Fig. 3.5. Moisture release was presumably due to decrease in water holding capacity of the proteins with the increase in hydrophobic interactions (Teo et al., 1996; Pastorino et al., 2002).

3.5.2. Reformation of non-fat cheese base at different temperatures

The rigid cheese mass of the cheese held at low temperature resisted flow through the extruder creating circular indentations on the surface as cheese was exiting the extruder. The initial pictures of the reformed cheese samples showed that cheese reformed at 4ºC did not fuse very well with lots of cracks, openings and a grainy texture (Fig. 3.6). On the other hand reformed cheese was smoother for the cheese reformed after holding the cheese shreds at higher temperatures. As the temperature increased, there was a decrease in the total number and/or strength of bonds (e.g. hydrogen bonds) in the matrix resulting in softer cheese (Lucey et al., 2003). Cheese shreds held at 30ºC were

soft making it easier to flow and fold. The moist surface of the shreds that were held at 30ºC could also have promoted fusion during reforming by making the cheese stickier.

All reformed cheese samples were stored for 1 wk at 4ºC before the analysis. Fig. 3.8 shows that the curdy appearance on the outer surface of all reformed cheese samples had disappeared completely and after storage they all looked similar. Cheese samples that were reformed at low temperature (4ºC) showed stiffer texture than the cheese reformed at higher temperatures as indicated by the high hardness values (Fig. 3.9) and G' values at the measurement temperature of 5°C (Table 3.5). Hardness of the cheese is mainly determined by the volume fraction of proteins, strength and number of protein-protein interactions and the continuity of the protein matrix (de Jong, 1978; Chen et al., 1979; Creamer and Olson, 1982; Prentice, 1992; Fox et al., 2000; Gunasekaran and Ak, 2003; Lucey et al., 2003). The stiffer texture of the cheese reformed at cold temperature (4ºC) indicated that the cheese had more numerous interactions between casein particles compared to cheese reformed at 30ºC. Even after storage for one week at 4ºC, the relatively softer structure of the cheese held and processed at 30ºC was still soft compared to the cheese reformed at 4ºC. This could be due to incomplete recovery of bonds and interactions after reforming. Akkerman et al. (1993) studied curd fusion during cheese making and they proposed that mobility of the casein chains and the number of bonds formed between casein particles increased with temperature due to Brownian motion, while the strength of the bonds decrease. Studies on acid milk gels (Roefs and Van Vliet, 1990; Lucey et al., 1997; Peng et al., 2010) and rennet milk gels (Zoon et al., 1988; Lagoueyte et al., 1994; Horne, 1998; Mishra et al., 2005) showed that at low measurement temperatures the G' values and shear moduli of milk gels were high due to

increased contact area and fusion of particles and clusters as a result of increased voluminosity upon cooling. Lagoueyte et al. (1994) studied rennet gelation at 26, 32 or 40ºC. They showed that gel strength increased first with the increase in temperature but decreased with a further increase in temperature. They suggested that bonds between caseins were stronger when temperature was higher leading to quicker formation of strands and clusters of micelles and faster fusion of linked micelles; however, at temperatures above 30ºC the bonds between micelles strands and clusters break and reform leading to a decrease in gel firmness. Horne (1998) has also showed that the gel strength in rennet curds increases linearly until a maximum between 35 and 40ºC and then declines at 45ºC. Loosening of the internal structure of the casein micelle as temperature is increased has been detected by NMR measurements as an increase in the mobility of protons on amino acid side chains of the caseins (Rollema and Brinkhuis, 1989). Even though an increase in temperature promoted interactions between caseins leading to faster cheese fusion, as was observed from the pictures of the cheese right after reforming, the net impact was a softening of the cheese structure due to loosening of the para-casein matrix with a decrease in the strength and loss of interparticle bonds. The question is how even after cold storage at 4ºC for 1 wk, the cheese reformed at 30ºC exhibited softer structure.

Udayarajan et al. (2005) observed that, after heating the non-fat cheese to 90ºC and cooling to 5ºC the gel matrix of the cheese was weaker and less elastic than the original cheese. They suggested that once the protein-protein bonds present in cheese system were disrupted by heating, they did not completely regain their original conformation or strength when cooled back to their initial temperature (Udayarajan et al.,

2005). Subramanian et al. (2006) have also shown that when reduced or regular fat process cheese samples were heated from 10ºC to 40ºC at a rate of 3ºC per min, and held at 40ºC for 30 min before cooling back to 10ºC, the G' values at the end of cooling were less than the values recorded during heating. In our study cheese was only heated up to 30ºC (prior to reforming) and this temperature occurs before the larger transformations that occur during melting. In order to better understand the impact of our temperature range on the rheological properties of cheese, we subjected the base cheese to a heating cycle up to 35ºC and then it was cooled back to 5ºC (Fig. 3.14). Although partial recovery of G' values were observed during cooling back to 5ºC, the G' values were still lower than those initially observed values in the heating step (Table 3.8). This indicated that structural changes occurred even during a mild heating cycle and these changes were not completely restored to the original conformation on cooling. The lower hardness and storage modulus values of the cheese that was reformed at higher temperatures were due to a lower degree of recovery of the initial number and strength of the bonds in the cheese protein matrix. In addition, the softer initial texture of the cheese held at 30°C during processing might have facilitated larger conformational changes in the matrix. It is unlikely that holding the cheese at 30ºC for 6 h before reforming could have caused any significant proteolysis since no starter culture was used in the making of these nonfat cheeses.

High temperature characteristics of cheese samples as reflected in the melt profiles (Fig 3.13), G' values at 85ºC (Table 3.5) and LT values (Table 3.6) did not show any differences between any of the cheese samples before or after reforming. Meltability of the cheese has been correlated with the LT values during heating (Ustunol et al., 1994;

Mounsey and O'Riordan, 1999; Lucey et al., 2005). Apparently incubating cheese at 30ºC did not have an impact on the melting properties of the cheese compared to lower incubation temperatures as seen from the LT values of the non-fat cheese base during heating and cooling (Fig 3.14). Although a hysteresis type of loop was observed between the LT values of heating and cooling, at the end of the cooling cycle, i.e., at 10ºC the LT values were the same. Heating the non-fat cheese base to 30ºC did not cause large structural changes in the cheese matrix as was observed from the relatively moderate increase in the power law exponent at that temperature range (Fig 3.12). When the cheese was cooled back, there were some recovery of the dynamic moduli values which eventually yielded the same LT values (Table 3.6). Kuo et al. (2001) studied the effect of different heating regimes on the meltability of Cheddar cheese. They found that holding the cheese at 40 or 50ºC before it is allowed to flow did not influence the meltability of the cheese.

3.6. CONCLUSIONS

Temperature of reforming influenced the rheological properties and the texture of the cheese as determined after 1 wk of storage of the reformed cheese at 4ºC after reforming process. Holding and processing the cheese at 30ºC made it soft and facilitated a smoother product during reforming the cheese through the extruder. After 1 wk of cold storage (~4ºC), no visible discontinuities were observed in any of the reformed cheese samples; however, cheeses reformed at higher temperatures were still softer in texture due to incomplete recovery of the bonds and interactions that were broken during the incubation and reforming process.

3.7. REFERENCES

Adhikari, B., Howes, T., Bhandari, B. R., and Truong, V. 2001. Stickiness in foods: a review of mechanisms and test methods. Intern. J. Food Properties 4:1–33.

Akkerman, J. C., Lewis, R. O., and Walstra, P. 1993. Fusion of curd grains. Neth. Milk Dairy J. 47:137–144.

Bourne, M. C. 2002. Food Texture and Viscosity: Concept and Measurement. 2nd edn. Academic Press, San Diego.

Brickley, C. A., Govindasamy-Lucey, S., Jaeggi, J. J., Johnson, M. E., McSweeney, P. L. H., and Lucey, J. A. 2008. Influence of emulsifying salts on the textural properties of nonfat process cheese made from direct acid cheese bases. J. Dairy Sci. 91:39-48.

Bryant, C.M., and McClements, D. J. 1998. Molecular basis of protein functionality with special consideration of cold-set gels derived from heat-denatured whey. Trends Food Sci. Technol. 9:143–151.

Chen, A. H., Larkin, J. W., Clark, C. J., and Irvine, W. E. 1979. Textural analysis of cheese. J. Dairy Sci. 62:901–907.

Childs, J. L., Daubert, C. R., Stefanski, L., and Foegeding, E. A. 2007. Factors regulating cheese shreddability. J. Dairy Sci. 90:2163-2174.

Choi, J., Horne, D. S. and Lucey, J. A. 2007. Effects of insoluble calcium concentration on rennet coagulation properties of milk. J. Dairy Sci. 90:2612-2623.

Code of Federal Regulations. 2006. Food and Drugs: CFR Part 101 Food Labeling. Department of Health and Human Services, Washington, DC.

Creamer, L. K., Zoerb, H. F., Olson, N. F., and Richardson, T. 1982. Surface hydrophobicity of α_{s1}-casein A and B and its implications in cheese structure. J. Dairy Sci. 65:902–906.

Dahlquist, C. A. 1969. Tack. Pages 143-151, in: Adhesion: Fundamentals and Practice. The Ministry of Technology (United Kingdom). Gordon and Breach Science Publishers, Inc., New York.

De Jong, L. 1978. Proteolytic breakdown in soft cheese and its relation to consistency of the micellar structure of Meshanger cheese. Neth. Milk Dairy J. 32:15–25.

De Kruif, C. G., and Grinberg, V. Y. 2002. Micellisation of β-casein. Colloids and Surfaces A: Physicochemical and Engineering Aspects 210:183–190.

Fox, P. F., Guinee, T. P., Cogan, T. M., and McSweeney, P. L. H. 2000. Fundamentals of Cheese Science. Aspen, Gaithersburg, MD.

Philips, L.G., Whitehead, D.M. and Kinsella, J. 1994. Structure-function Properties of Food Proteins. Academic Press, Inc., San Diego, CA.

Guinee, T. P., Auty, M. A. E., and Mullins, C. 1999. Observations on the microstructure and heat-induced changes in the viscoelasticity of commercial cheeses. Australian J. Dairy Tech. 54:84-89.

Guinee, T. P., Auty, M. A. E., Mullins, C., Corcoran, M. O. and Mulholland, E. O. 2000. Preliminary observations on effects of fat content and degree of fat emulsification on the structure-functional relationship of Cheddar-type cheese. J. Texture Studies. 31:645–663.

Gunasekaran, S. and Ak, M. M. 2000. Dynamic oscillatory shear testing of foods – Selected applications. Trends Food Sci. Tech. 11:115–127.

Gunasekaran, S. and Ak, M. M. 2003. Cheese Rheology and Texture. CRC Press, Boca Raton, Florida.

Horne, D. S., Banks, J. M., Leaver, J. and Law, A. J. R. 1994. Dynamic mechanical spectroscopy of Cheddar cheese. Pages 507-512 in Cheese Yield and Factors Affecting its Control, Proceedings IDF Seminar, Cork, Ireland- April 1993, IDF, Brussels, Belgium.

Horne, D. S. 1998. Casein interactions: Casting light on the black boxes, the structure in dairy products. Int. Dairy J. 8:171–177.

Joshi, N. S., Muthukumarappan, K., and Dave, R. I. 2004. Effect of calcium on microstructure and meltability of part skim mozzarella cheese. J. Dairy Sci. 87:1975-1985.

Kuo, M. I., Wang, Y. C., Gunasekaran, S., and Olson N. F. 2001. Effect of heat treatments on the meltability of cheeses. J. Dairy Sci. 84:1937-1943.

Lagoueyte, N., Lablee, J., Lagaude, A. and de la Fuente, B. T. 1994, Temperature affects microstructure of renneted milk gel. J. Food Sci. 59:956–959.

Lucey, J. A., Mishra, R., Hassan, A. and Johnson, M. E. 2005. Rheological and calcium equilibrium changes during the ripening of Cheddar cheese. Intern. Dairy J. 15:645-653

Lucey, J. A., Johnson, M. E. and Horne, D. S. 2003. Perspectives on the basis of the rheology and texture properties of cheese. J. Dairy Sci. 86:2725–2743.

Lucey, J. A., van Vliet, T., Grolle, K., Geurts, T. and Walstra, P. 1997. Properties of acid casein gels made by acidification with glucono-delta-lactone. 1: Rheological properties. Intern. Dairy J. 7:381-388.

Marshall, R. T. 1992. Standard methods for the examination of dairy products. 16th edn. Am. Public Health Assoc, Washington, DC.

Mishra, R., Govindasamy-Lucey, S., and Lucey, J. A. 2005. Rheological properties of rennet-induced gels during the coagulation and cutting process: impact of processing conditions. J. Texture Studies 36:190-212.

Mounsey, J. S., and O'Riordan, E. D. 1999. Empirical and dynamic rheological data correlation to characterize melt characteristics of imitation cheese. J. Food Sci. 64:701–703.

Muliawan, E. B., and Hatzikiriakos, S. G., 2007. Rheology of Mozzarella cheese. Intern. Dairy J. 17:1063–1072.

Muthukumarappan, K., Wang, Y-C, and Gunasekaran, S. 1999. Estimating softening point of cheeses. J. Dairy Sci. 82:2280-2286.

O'Callaghan, D. J. and Guinee, T. P. 2004. Rheology and texture of cheese. Pages 511-540 in Cheese: Chemistry, Physics and Microbiology. Vol. 1: General Aspects. 3rd edn., eds. Fox, P. F., McSweeney, P. L. H., Cogan, T. M., and Guinee, T. P. Elsevier Science and Technology, London.

O'Connell, J. E., Grinberg, V. Y., and de Kruif, C. G. 2003. Association behavior of β-casein. J. Colloid and Interface Sci. 258:33-39.

Pastorino, A. J., Dave, R. I., C. J. Oberg, and McMahon, D. J. 2002. Temperature effect on structure—opacity relationships of nonfat Mozzarella cheese. J. Dairy Sci. 85:2106–2113.

Peng, Y., Horne, D. S., and Lucey, J. A. 2010. Physical properties of acid milk gels prepared at 37ºC up to gelation but at different incubation temperatures for the remainder of fermentation. J. Dairy Sci. 93:1910-1917.

Prentice, J. H., Langley, K. R., and Marshall, R. J. 1993. Cheese rheology. Pages 303–340, in: Cheese: Chemistry, Physics and Microbiology. Vol. 1. P. F. Fox, ed. Elsevier, London.

Roefs, S. P. F. M., and van Vliet, T. 1990. Structure of acid casein gels. 2. Dynamic measurements and type of interaction forces. Colloids Surf. 50:161-175.

Rollema, H. S., and Brinkhuis, J. A. 1989. A 1H-NMR study of bovine casein micelles; influence of pH, temperature and calcium ions on micellar structure. J. Dairy Res. 56: 417-425.

Ross-Murphy, S. B. 1995. Rheological characterization of gels. J. Texture Studies 26:391-400

Subramanian, R., Muthukumarappan, K., and Gunasekaran, S. 2006. Linear viscoelastic properties of regular- and reduced-fat pasteurized process cheese during heating and cooling. Intern. J. Food Properties. 9:377-393.

Teo, C. T., Munro, P. A., Singh, H., and Hudson, R. C. 1996. Effects of pH and temperature on the water-holding capacity of casein curds and whey protein gels. J. Dairy Res. 63:83-95

Udayarajan, C.T., Horne, D. S., and Lucey, J. A. 2005. Use of Fourier transform mechanical spectroscopy to study the melting behavior of cheese. J. Texture Studies 36:489–515.

Udayarajan, C. T., Horne, D. S., and Lucey, J. A. 2007. Use of time–temperature superposition to study the rheological properties of cheese during heating and cooling. Int.. J. Food Sci. Tech. 42:686–698.

Ustunol, Z., Kawachi, K., and Steffe, J. 1994. Arnott test correlates with dynamic rheological properties for determining Cheddar cheese meltability. J. Food Sci. 59:970–971.

Venugopal, V., and Muthukumarappan, K. 2003. Rheological properties of Cheddar cheese during heating and cooling. Int. J. Food Properties 6:99–114.

Visser, 1991. Factors affecting the rheological and fracture properties of hard and semi-hard cheese. Bull. IDF No. 268, Intern. Dairy Federation, Brussels, Belgium.

Walstra, P. 1990. On the stability of casein micelles. J. Dairy Sci. 73:1965–1979.

Zhou, N., and Mulvaney, S. J. 1998. The effect of milk fat, the ratio of casein to water, and temperature on the viscoelastic properties of rennet casein gels. J. Dairy Sci. 81: 2561-2571

Zoon, P., van Vliet, T., and Walstra, P. 1988. Rheological properties of rennet-induced skim milk gels. 2. The effect of temperature. Neth. Milk Dairy J. 42:271-294.

Table 3.1 Cheese manufacture protocol used for the non-fat cheese bases used for reforming

Operation	Time (min)		Temperature/ pH, TA
Initial Skim Milk		TA	0.165
initial milk weight (kg)	272 kg	pH	6.64
Add Citric Acid to Drop pH	0 min	Temp	5 °C
Add diluted citric acid (10% w/w solution pH of ~1.6)		TA	0.37
		pH	5.54
Target: pH 5.60			
Add $CaCl_2$	20 min	TA	0.408
31 g/ 100 kg milk or 81 ml	48 g	pH	5.58
Add Coagulant - 33°C	25 min	Temp	32°C
Ch Hansen, Chymax Extra (dbl str)		TA	0.408
1.9 g / 100 kg milk	5.5 g	pH	5.58
Cutting	65 min	TA	0.320
1.3 cm knives		pH	5.63
Start Cooking / Begin Agitation	70 min	Temp	32°C
Reach Cooking Temp - 37°C	90 min	Temp	37°C
		TA	0.271
		whey-pH	5.55
		curd-pH	5.34
Complete Drain		TA	
Beginning	95 min	whey-pH	
End	105 min	curd-pH	
Add Flake Salt	120 min	TA	
		curd-pH	
220 g / 100 kg milk		g salt	613
Hooping (9 kg Wilson)	135 min	curd-pH	5.34
Pressing - In	155 min		
1 hour, 270 kPa - Out	215 min	curd-pH	5.33
Storage	Weight	20 kg	
Into cooler after 1 hr press			

Table 3.2 Chemical composition of milk and cheese base. Means are for three cheese making-reforming trials.

Milk	
Total solids, %	8.82 ± 0.1
Fat, %	0.09 ± 0.02
Casein, %	2.54 ± 0.03
Protein, %	3.26 ± 0.04
Casein:Fat ratio	28 ± 5
Total Ca, mg/100g milk	120 ± 2.3
Cheese	
Moisture, %	58.70 ± 0.49
Fat, %	1.22 ± 0.24
Protein, %	32.8 ± 0.49
pH	5.54 ± 0.09
Salt, %	1.34 ± 0.12
Total Ca, mg/100g cheese	439 ± 4.53

Table 3.3 Texture Profile Analysis results for cheese base and cheeses held and reformed at different temperatures (4, 18 or 30°C). 62% compression. Cheeses tested after storage at 4°C for 1 wk. Means are for three cheese making-reforming trials.

		Reforming temperature (°C)		
	Cheese base	4	18	30
Adhesiveness (g.mm)	-3.40E-03 ± 0.01 [a]	-5.10E-03 ± 0.01[a]	-5.40E-03 ± 0.01[a]	-3.80E-03 ± 0.01[a]
Springiness (mm)	11.63 ± 0.4 [a]	11.90 ± 0.2[a]	11.80 ± 0.1[a]	11.80 ± 0.2[a]
Cohesiveness	6.60E-01 ± 0.02 [a]	6.69E-01 ± 0.02[a]	6.70E-01 ± 0.02[a]	6.78E-01 ± 0.01[a]
Gumminess (g)	5.16E+03 ± 653[a]	4.90E+03 ± 608[ab]	4.62E+03 ± 531[b]	4.26E+03 ± 489[c]
Chewiness (g.mm)	6.03E+04 ± 8422 [a]	5.85E+04 ± 7509[ab]	5.44E+04 ± 5999[bc]	5.05E+04 ± 5013[c]

[a-b]Means with different letters within the same row are significantly different (P<0.05)

Table 3.4 Uniaxial compression results for cheese base and cheeses reformed at different temperatures (4, 18 or 30°C). 80% compression. Cheeses tested after storage at 4°C for 1 wk. Means are for three cheese making-reforming trials.

Cheese Type	Initial Slope (N/mm²/%)	Hardness (g)	Adhesiveness Force (g)	Adhesiveness Area (N/mm².%)
Cheese base	0.002 ± 0.00 [a]	1.38E+04 ± 7.E+02 [a]	-12.18 ± 10.2 [a]	-2.00E-04 ± 1.E-05 [a]
Cheese reformed at:				
4ºC	0.006 ± 0.01 [a]	1.47E+04 ± 4.E+03 [a]	-15.15 ± 18.8 [a]	-1.00E-04 ± 1.E-06 [a]
18ºC	0.006 ± 0.01 [a]	1.23E+04 ± 4.E+03 [a]	-1.91 ± 3.3 [a]	-2.00E-04 ± 2.E-06 [a]
30ºC	0.007 ± 0.01 [a]	1.32E+04 ± 2.E+03 [a]	-7.78 ± 6.1 [a]	-1.20E-04 ± 2.E-06 [a]

[a]Means with different letters within the same row are significantly different (P<0.05)

Table 3.5 Storage Modulus (G') values obtained from SAOS temperature sweep tests at measurement temperatures of 5 and 85ºC for cheese base and cheeses reformed at different temperatures (4, 18 or 30°C). Cheeses tested after storage at 4°C for 1 wk. Means are for three cheese making-reforming trials.

Cheese Type	G' at 5 ºC (Pa)	G' at 85 ºC (Pa)
Cheese base	3.24E+04 ± 1.E+03 [a]	4.8E+00 ± 2.57 [a]
Reforming temperature:		
4ºC	2.88E+04 ± 6.E+03 [ab]	3.8E+00 ± 1.80 [a]
18ºC	2.41E+04 ± 4.E+03 [bc]	3.8E+00 ± 1.00 [a]
30ºC	2.18E+04 ± 3.E+03 [c]	2.5E+00 ± 1.89 [a]

[a-c] Means with different letters within the same column are significantly different (P<0.05)

Table 3.6 Maximum loss tangent (LTmax) values, temperature at LTmax, and temperature where LT=1 and loss tangent (LT) values at measurement temperature of 85ºC obtained from SAOS temperature sweep tests of cheese base and cheeses reformed at different temperatures (4, 18 or 30°C). Cheeses tested after storage at 4°C for 1 wk. Means are for three cheese making-reforming trials.

Cheese Type	LTmax	Temperature at LTmax	Temperature at LT=1	LT at 85ºC
Cheese base	6.45 ± 0.28 [a]	70.34 ± 0.23 [a]	44.02 ± 0.25 [a]	3.46 ± 0.70 [a]
Reforming temperature:				
4ºC	6.45 ± 0.92 [a]	74.78 ± 0.03 [b]	44.40 ± 0.00 [a]	3.86 ± 0.79 [a]
8ºC	6.33 ± 0.89 [a]	74.90 ± 0.00 [b]	44.50 ± 0.00 [a]	4.27 ± 0.76 [a]
30ºC	6.71 ± 0.41 [a]	75.20 ± 0.52 [b]	43.80 ± 0.52 [a]	4.74 ± 0.43 [a]

[a-c] Means with different letters within the same column are significantly different (P<0.05)

Table 3.7 UW-MeltProfiler test results for the melting properties of cheese base and cheeses reformed at different temperatures. Cheeses tested after storage at 4°C for 1 wk. Means are for three cheese making-reforming trials.

Cheese Type	Degree of flow (%) at 60ºC	Softening point (ºC)
Cheese base	75 ± 3[a]	43.20 ± 1[a]
Reforming temperature:		
4ºC	75 ± 1[a]	43.16 ± 2[a]
18ºC	76 ± 1[a]	42.13 ± 1[a]
30ºC	77 ± 1[a]	40.86 ± 1[a]

[a]Means with different letters within the same column are significantly different (P<0.05)

Table 3.8 Dynamic moduli (G' and G") and loss tangent (LT) values of the cheese base at 5ºC, and after heating and cooling cycle from 5 to 35ºC and back to 5ºC. Means are for three cheese making-reforming trials.

	G' (Pa)	G" (Pa)	LT
Before heating	1.82E+04 ± 424[a]	5.21E+03 ± 530[a]	0.31 ± 0.01[a]
After heating and cooling	1.22E+04 ± 141[b]	3.87E+03 ± 163[b]	0.32 ± 0.01[a]

[a-b]Means with different letters within the same column are significantly different (P<0.05)

Figure 3.1 Double-screw vacuum filler (Vemag Robot500, Reiser, Verden, Germany).

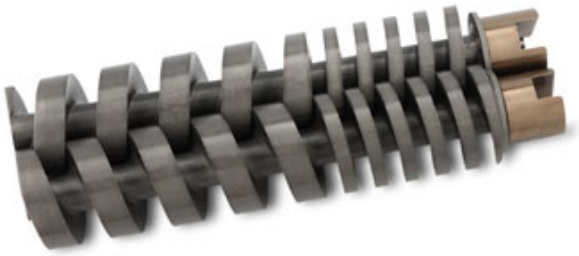


Figure 3.2 Double screws (Vemag Robot500, Reiser, Verden, Germany).

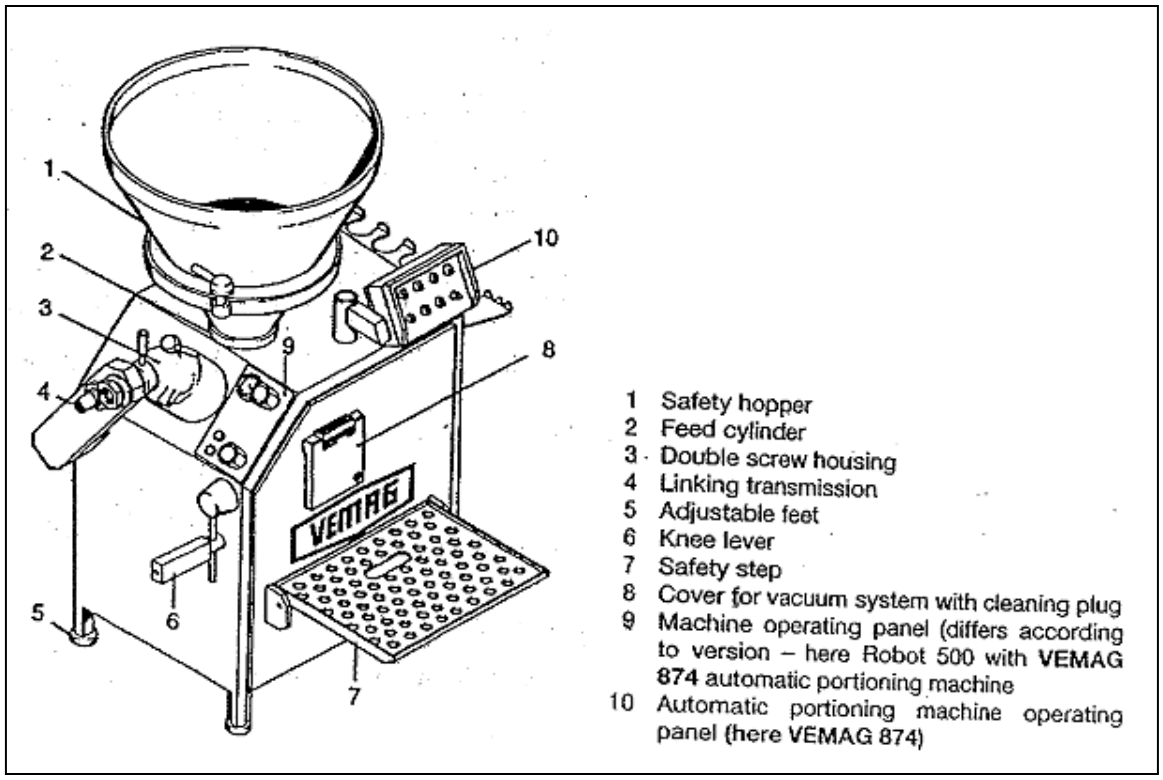


Figure 3.3 General view and parts of Vemag Robot 500. Instead of linking transmission (4), an extension tube was attached to the double screw housing (3) during the operation (Vemag Robot 500 manual, 2005)

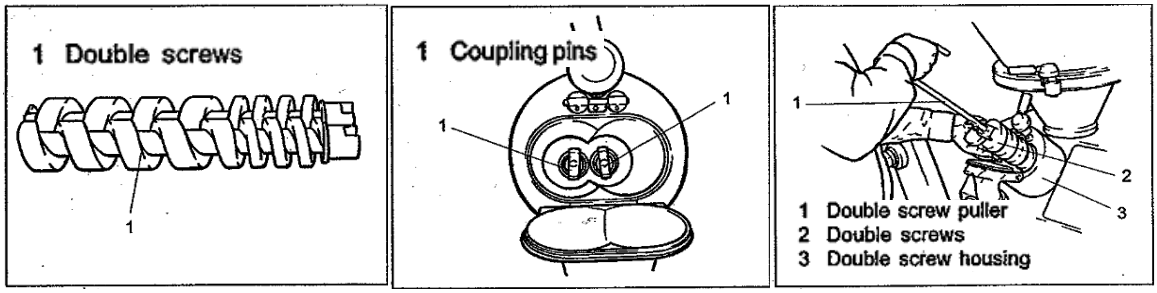


Figure 3.4 Alignment and fitting of double screws into double screw housing (Vemag Robot 500 manual, 2005)

(a)

(b)

(c)

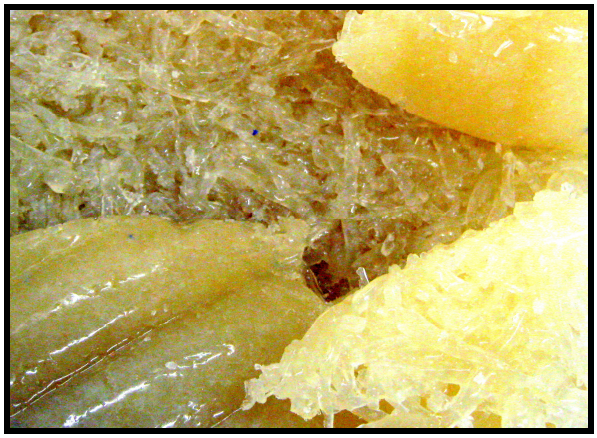


Figure 3.5 Pictures of the non-fat cheese shreds after incubation at (a) 4ºC, (b) 18ºC and (c) 30ºC for 6 h.

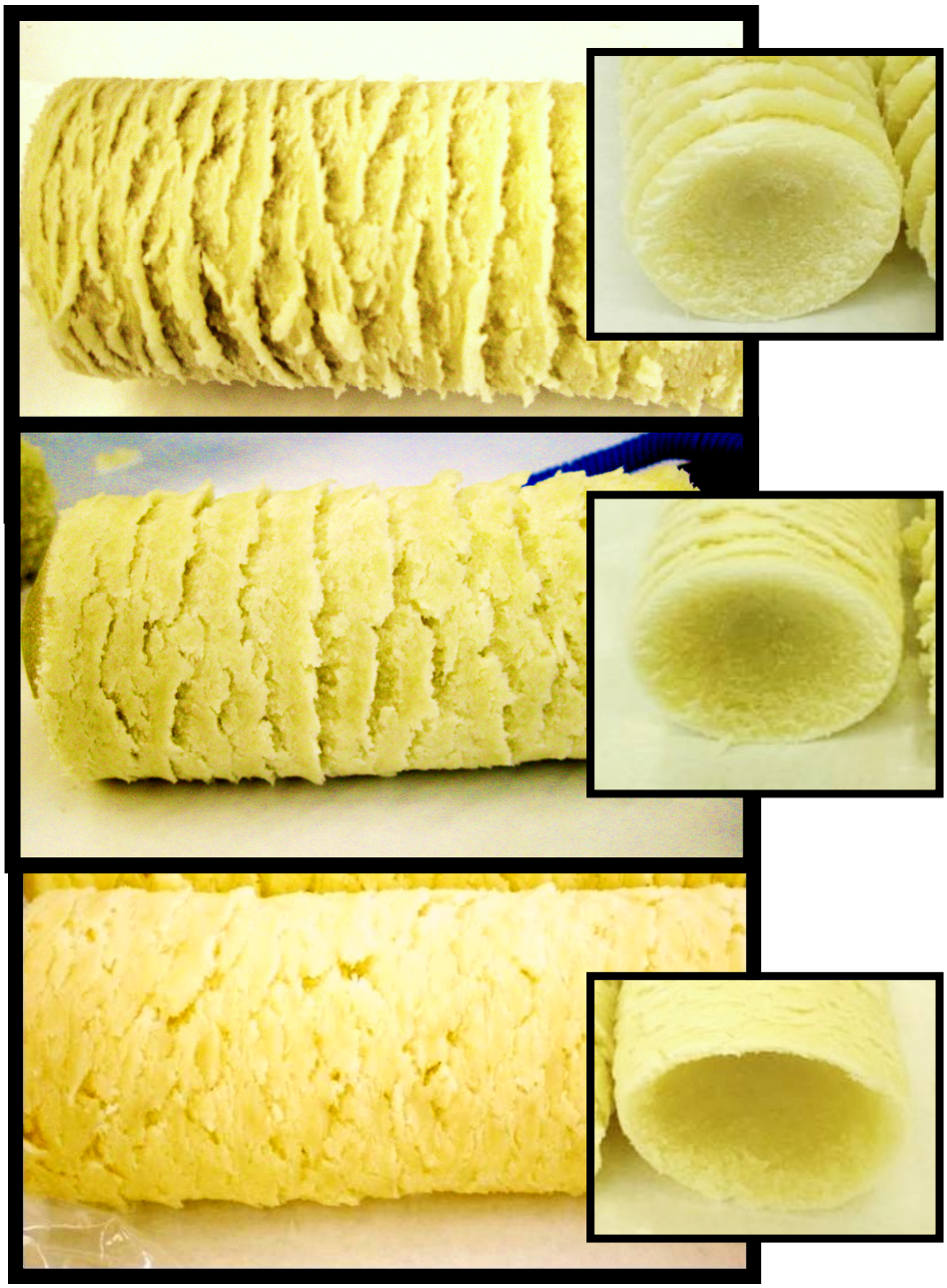


Figure 3.6 Pictures of the non-fat cheese samples immediately after reforming cheese that had been incubated at (a) 4ºC, (b) 18ºC and (c) 30ºC for 6 h.

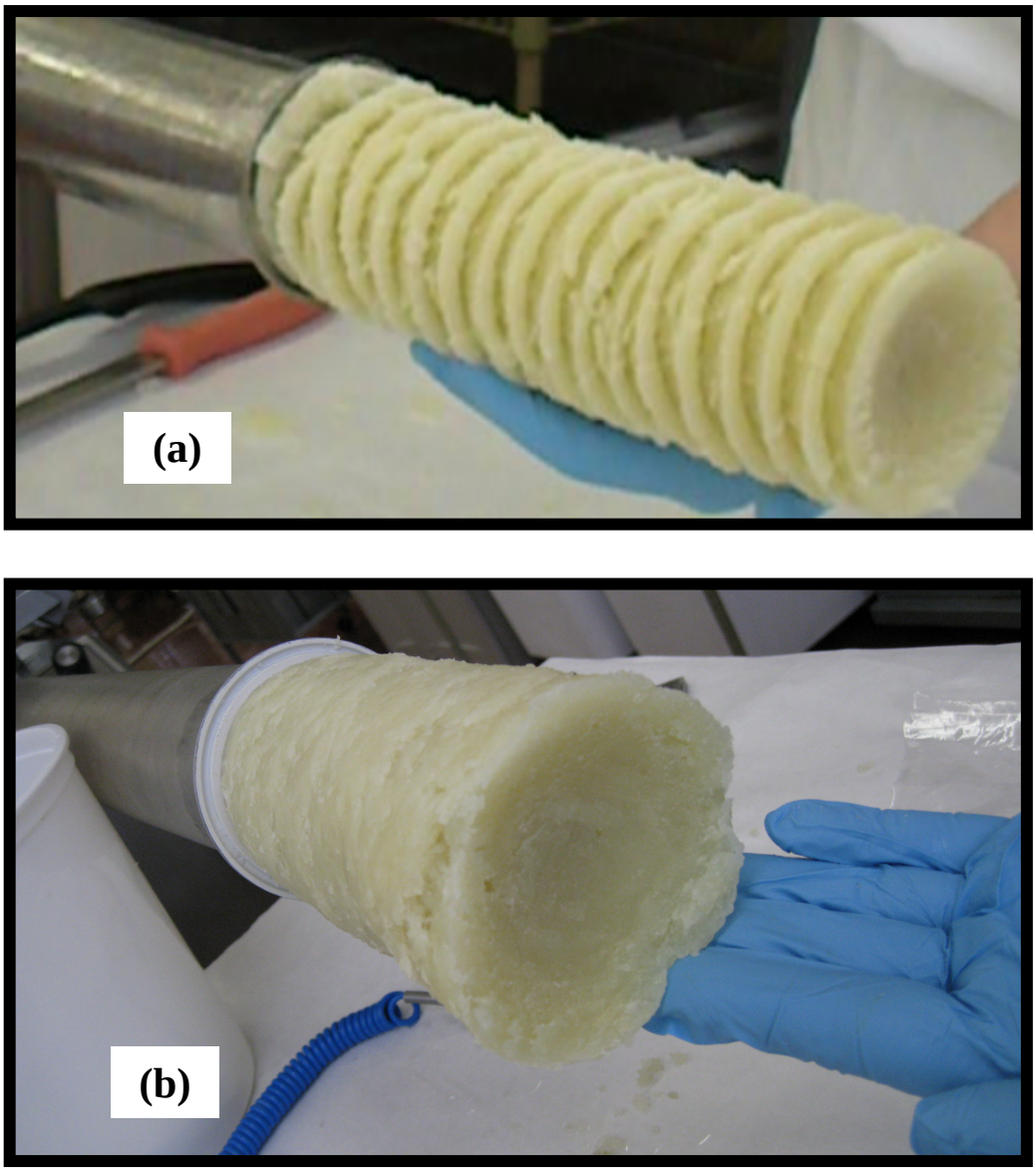


Figure 3.7 Non-fat cheese being reformed at 30ºC through an (a) uncoated extension tube and (b) teflon coated extension tube for the Vemag Robot 500

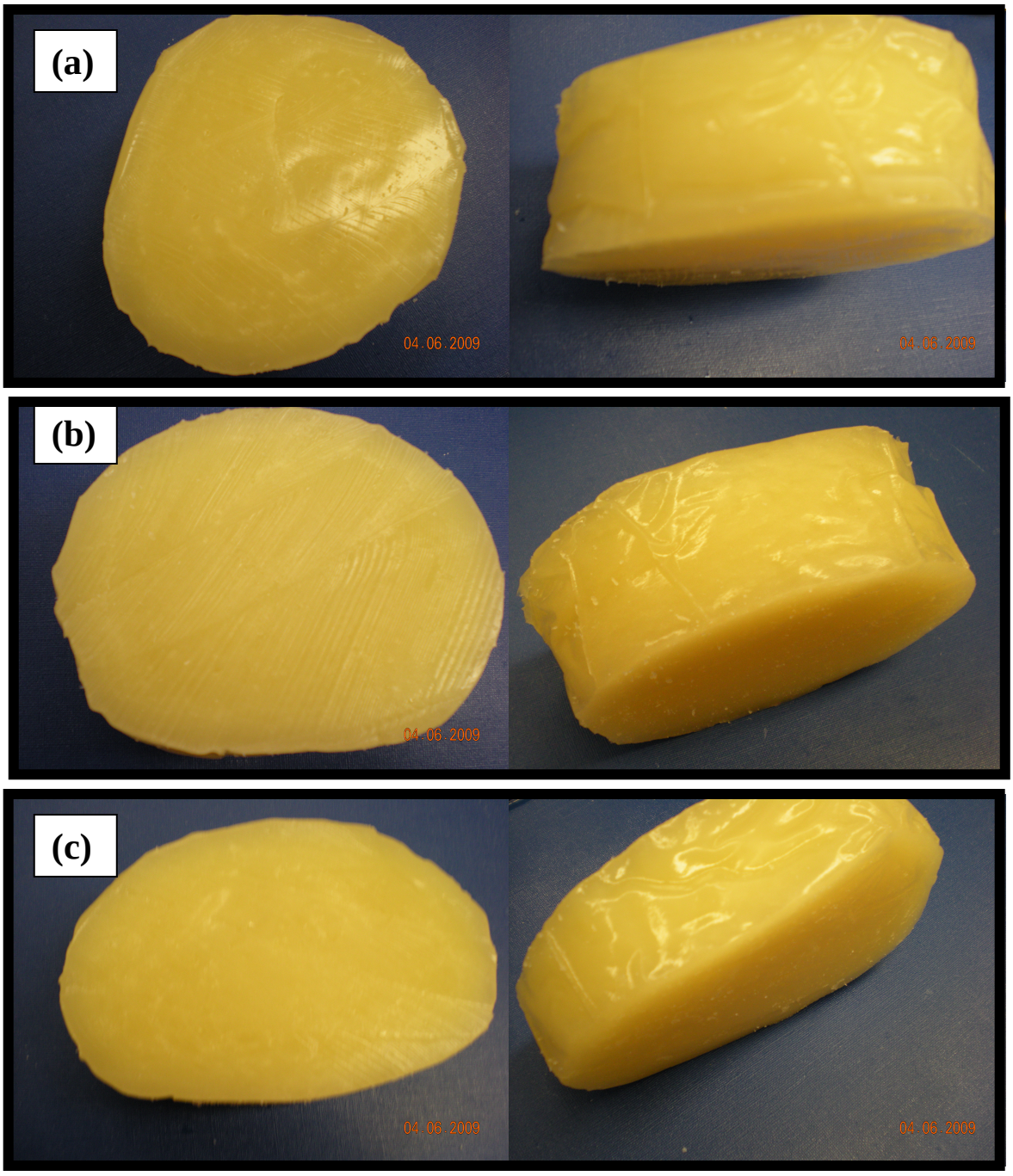


Figure 3.8 Non-fat cheese samples after storage at 4°C for 1 week. Cheeses were reformed at (a) 4°C, (b) 18°C and (c) 30°C

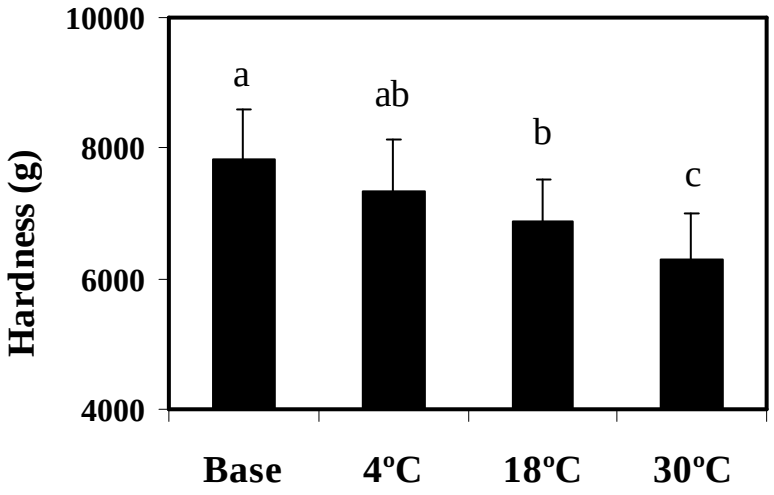


Figure 3.9 Hardness results of cheese base and cheese reformed at different temperatures (4, 18 or 30°C) as determined by the TPA test (62% compression). Cheeses tested after storage at 4°C for 1 wk. Bars with different letters indicate significant differences ($p<0.05$). Means are for three cheese making-reforming trials.

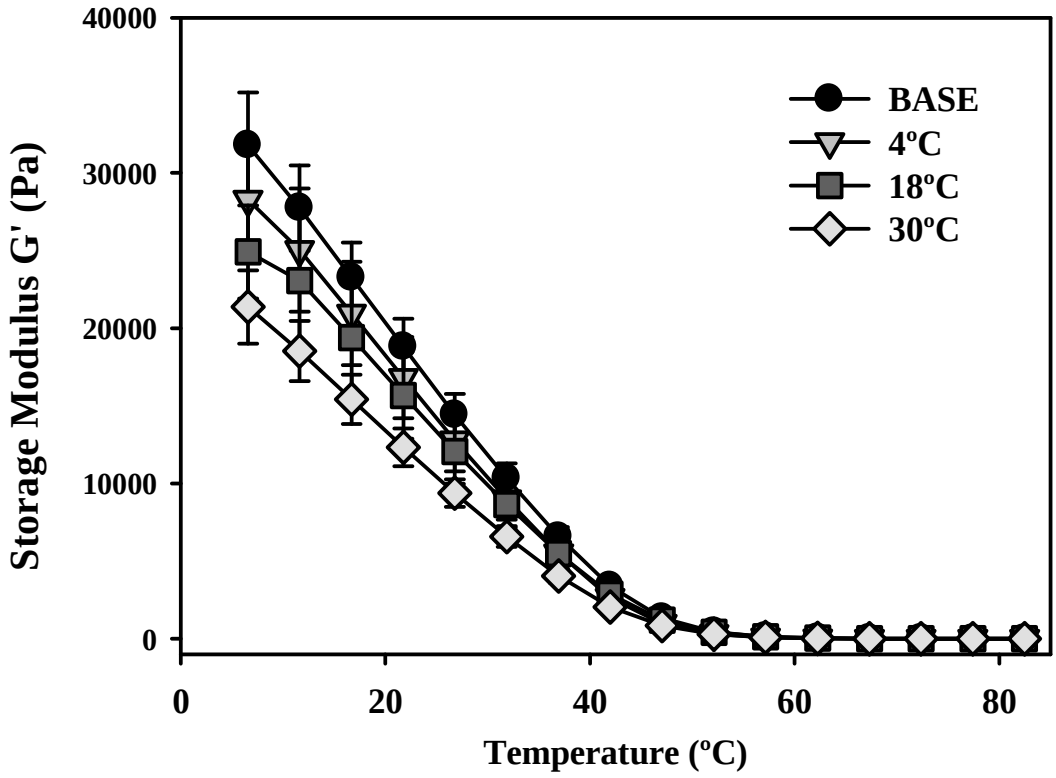


Figure 3.10 Storage modulus (G') as a function of temperature for cheese base and cheese reformed at different temperatures (4, 18 or 30°C) during heating from 5 to 85°C at 1°C/min. Cheeses tested after storage at 4°C for 1 wk. Means are for three cheese making-reforming trials.

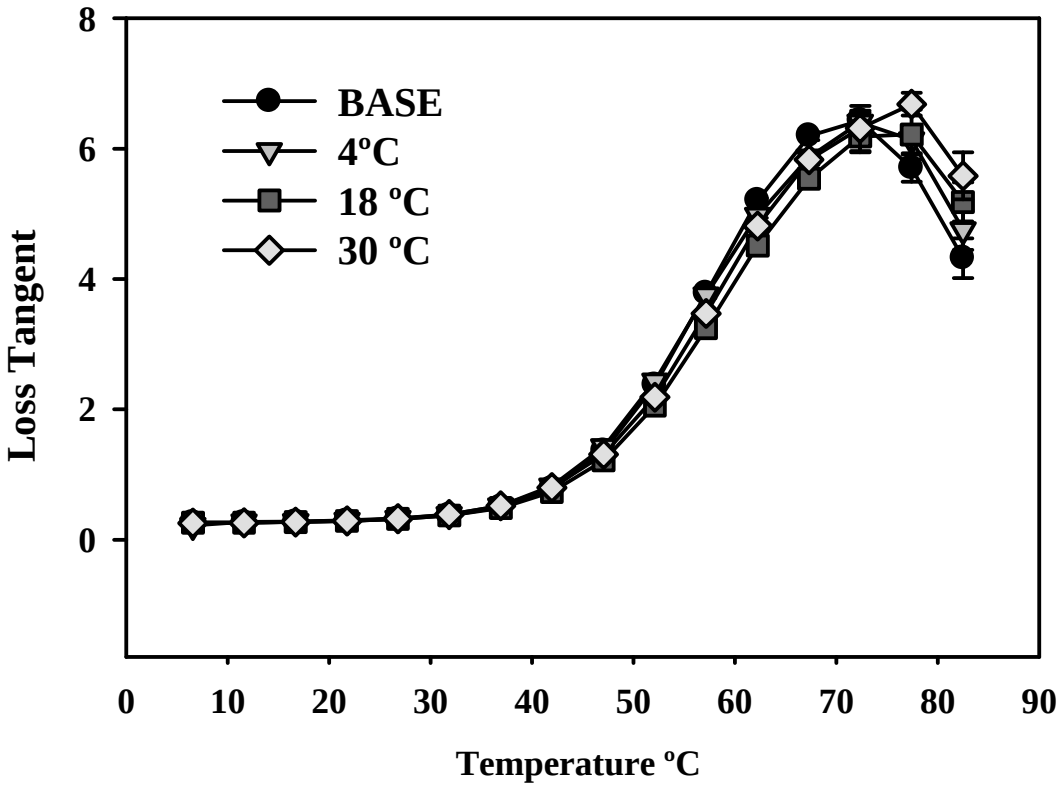


Figure 3.11 Loss tangent as a function of temperature for cheese base and cheese reformed at different temperatures (4, 18 or 30°C) during testing of cheese from 5 to 85ºC at 1ºC/min. Cheeses tested after storage at 4°C for 1 wk. Means are for three cheese making-reforming trials.

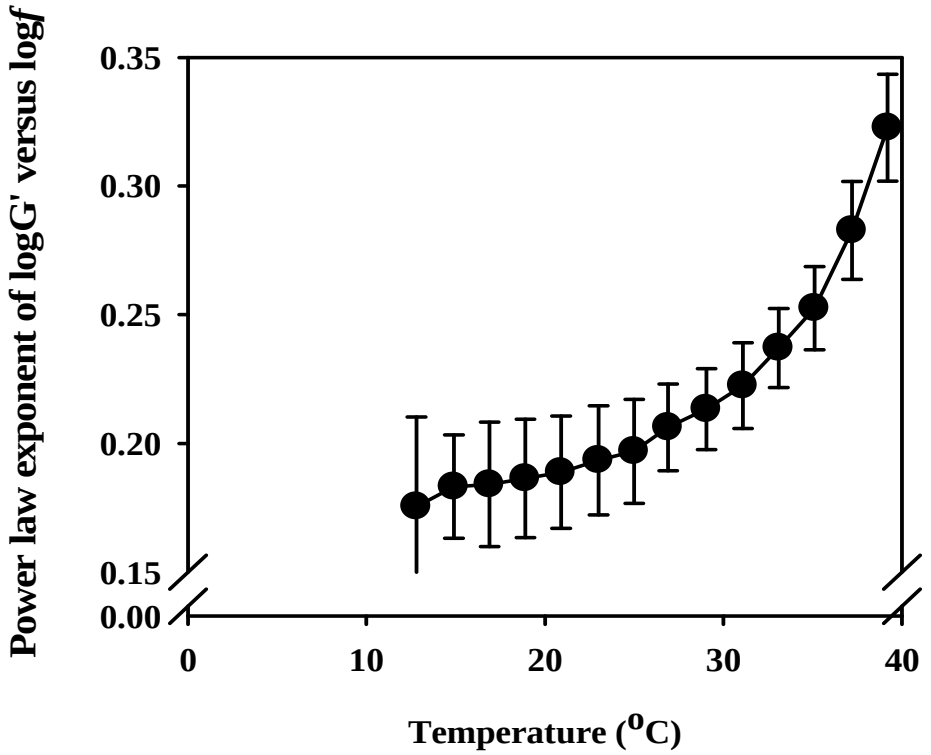


Figure 3.12 Power law exponent of the logG' vs log*f* curves plotted as a function of temperature for non-fat cheese base, temperature was raised from 5 to 40ºC at 1ºC/min. Means are for three cheese making trials.

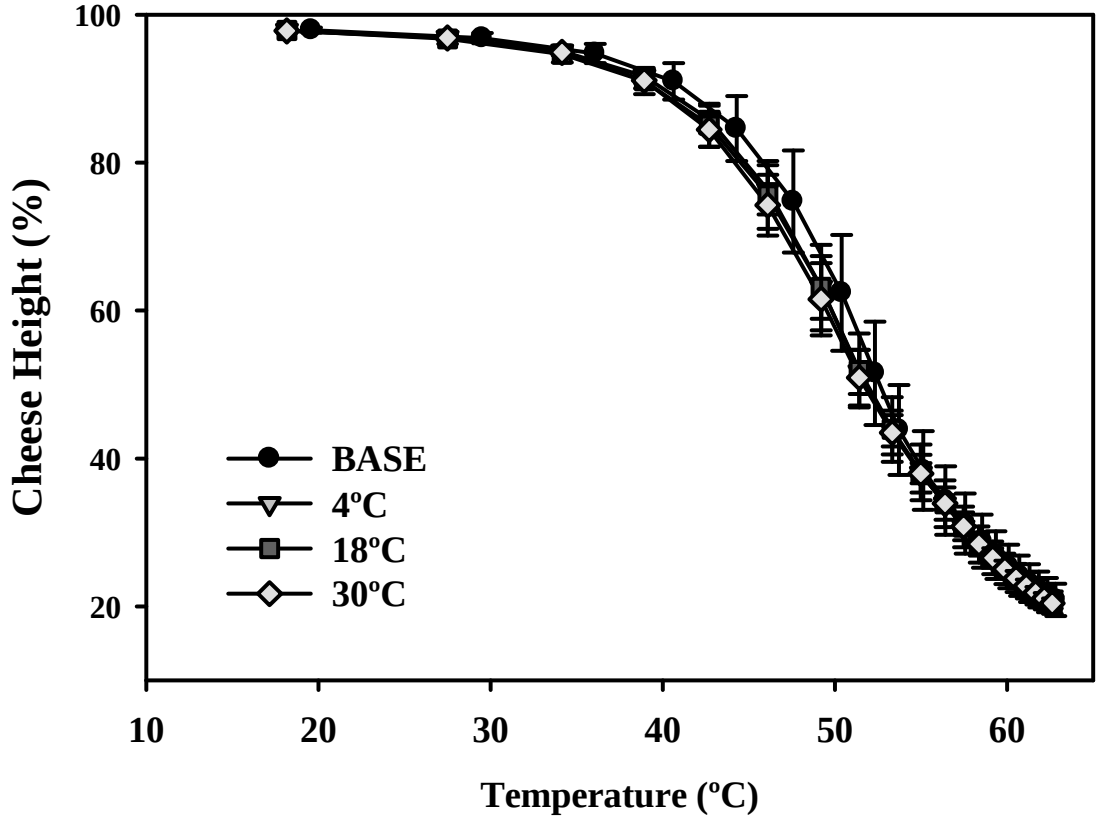


Figure 3.13 Decrease in the height of cheese base and cheese reformed at different temperatures (4, 18 or 30°C) during melt profile analysis. Cheeses tested after storage at 4°C for 1 wk.

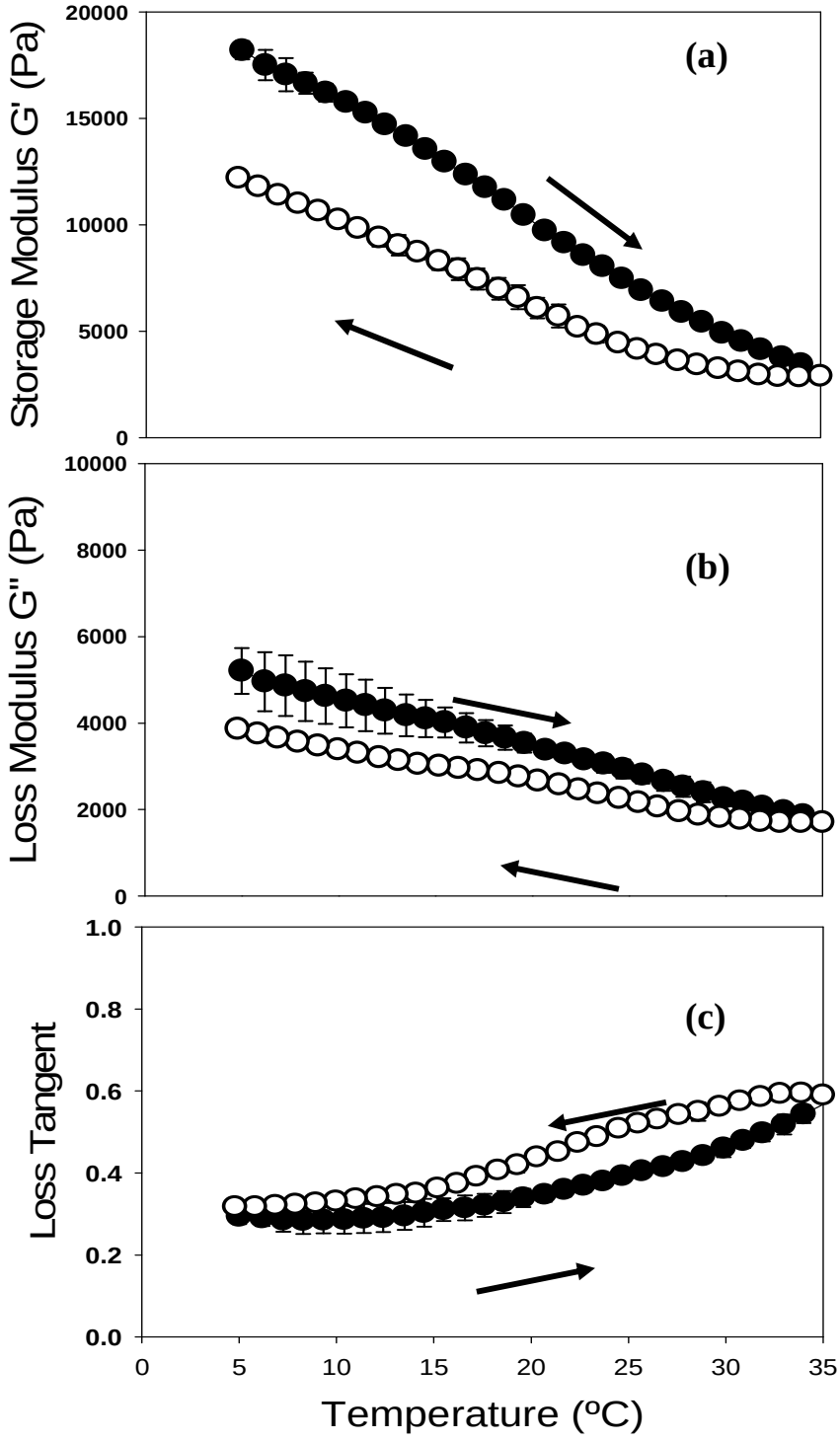


Figure 3.14 Changes in the storage modulus (G') (a), loss modulus (G'') (b) and loss tangent (c) during the cycle of heating from 5 to 35ºC (●) and cooling from 35 to 5 ºC (○).

Chapter 4

IMPACT OF pH ON THE TEXTURE AND RHEOLOGICAL PROPERTIES OF REFORMED LOW-FAT CHEESE

4.1. ABSTRACT

Impact of pH and soluble calcium content on the reformability of low-fat cheese was investigated. In this study we adjusted the pH values of cheese to solubilize the colloidal calcium phosphate (CCP), and improve the reformability of cheese. Low-fat cheese bases were produced to have 4 different pH values (6.2, 5.8, 5.5, 5.3). Milk was pre-acidified to pH 6.2 using citric acid and pH was adjusted by the addition of glucono-δ-lactone (GDL) at the milling step in order to alter the CCP solubilization levels while trying to keep the total calcium content constant. After 2 wk of storage at 4°C, cheese was ground with a food processor and then reformed into a cheese block using plastic beakers and a hydraulic press. Texture, rheology, microstructure, melting properties and visual appearance of cheese samples were examined 2 wk after reformation (stored at 4°C). Dynamic rheological properties were measured by small amplitude oscillatory rheology during heating of cheese from 5 to 85°C. Textural properties were determined with a Texture Analyzer. Melt properties were determined using UW-Melt-profiler. Compared to the base cheese, reforming cheese adjusted to pH 5.3 did not significantly change its

textural properties, dynamic moduli or degree of flow. Cheeses that had higher pH values exhibited a profound decrease in hardness and dynamic moduli after reforming, as well as, an increase in the degree of flow. The incomplete fusion of the grated cheese particles after repressing of cheese with higher pH values was the reason for the weaker structure in these samples. In low pH cheese, the loss of CCP crosslinks provided greater bond mobility between caseins, which allowed the cheese particles to fuse better and therefore provided more complete recovery in the texture properties of the cheese after reforming.

4.2. INTRODUCTION

It is well known that pH and insoluble calcium content have a major influence on the interactions between caseins (Lucey and Fox, 1993; Lucey et al., 2003; Lee et al., 2005; Choi et al., 2008). The rate and extent of the acid development is related to the calcium content of the cheese since the decrease in pH dissolves insoluble calcium from the casein micelles. In the cheese matrix, caseins interact through charge interactions (+/- bridges between phosphoseryl clusters and calcium phosphate nanoclusters; CCP) and hydrophobic interactions (hydrophobic segments on casein molecules). Charge interactions are controlled by the residual charge on the casein, which is directly related to pH, ionic strength and Ca^{++} binding. A decrease in pH solubilizes CCP crosslinks between caseins and also reduces the electrostatic repulsion due to the decrease in the net negative charge on caseins. When CCP is solubilized, negatively charged phosphoserine residues are exposed causing repulsion (Lucey et al., 2003). Cheese curds with a low pH tend to be crumbly (e.g., Cheshire cheese), whereas high pH curds tend to be more elastic (e.g., Emmental cheese) (Lucey and Fox, 1993). At high pH values (~6.5) cheese texture is firm and poorly meltable due to the excessive CCP crosslinks. At pH 5.2, protein

matrix has the maximum mobility, which provides for good curd fusion, melting and stretching characteristics. Below pH 5.0 hydrophobic interactions dominates as electrostatic repulsion greatly decreases. When the pH of the cheese approaches the pI of casein, the texture is brittle and crumbly due to the excessive (+/-) attraction between caseins (Lucey et al., 2003).

Reducing the insoluble calcium content of casein micelles increased the casein bond mobility and the flexibility of rennet gel networks made from Ca^{++}-depleted milks (Choi et al., 2007). Loosening of the interactions between, and within, casein particles by the solubilization of CCP can facilitate greater rearrangements with higher molecular mobility of casein micelles. We hypothesize that an increase in the relaxation of the bonds and flexibility of the caseins would promote rearrangements and association of the caseins in the reforming process of the cheese. In this study, our objective was to investigate the influence of the pH and calcium solubilization on the degree of reformability of the low-fat cheese as indicated by the recovery of the textural properties.

4.3. MATERIALS AND METHODS

4.3.1. Cheesemaking

Directly acidified low-fat cheese bases were made in small scale mini-cheese vats from pasteurized milk (73°C for 15 sec) obtained from the University of Wisconsin-Dairy Plant. After adjusting the fat content to 0.5% (by mixing skim milk that had 0.1% fat and whole milk with 3.7% fat), milk was pre-acidified to pH 6.2 with citric acid at 5ºC. Double strength chymosin (Chymax extra, Chr. Hansen's, Milwaukee, WI) was added to the milk at 33ºC. The coagulum had reached sufficient firmness after 1 h and was cut with 0.63 cm knives, allowed to heal for 5 min, and then gently (manually)

stirred for 5 min before cooking. Cooking temperatures were slightly altered to achieve similar moisture content (~59%) in all cheeses. A lower cooking temperature was used for the low pH cheeses and curd handling times kept shorter to be able to retain more moisture in those cheeses since lowering the pH cause more water expulsion from the curds. Whey was drained, and after the curd was matted, it was cut into blocks, turned upside down and stacked for 10 to 15 min until milling. Glucono-δ-lactone (GDL) was added to the milled curd at the amounts of 0, 0.1, 0.3 and 0.4% of milk (w/w) to obtain cheeses with pH 6.2, 5.8, 5.5 and 5.3, respectively. After salting (0.2% of the weight of the milk in the vat (w/w)) curd was pressed into hoops that were lined with cheese cloth and pressed for 2 h and vacuum sealed. Cheeses were then stored at 4°C for 2 wk prior to the grating-reforming treatment. Trials were replicated for 6 times. The manufacturing protocol of low-fat cheese base is given in Table 4.1.

4.3.2. Cheese reforming

A food processor (Cuisinart, USA) was used for grinding the cheese. The ground cheese were then filled into plastic beakers and pressed for 1 h with a laboratory hydraulic press (Carver Press, Wabash, IN). The repressed cheese samples were held at 4°C inside the plastic beakers for 1 d and then removed, vacuum sealed and stored for 1 wk at 4°C prior to analysis.

4.3.3. Compositional Analysis

Milk samples were analyzed for total solids, fat, protein, casein (Marshall, 1992) and total and soluble calcium (Hassan et al., 2004). The total solids, fat, protein and pH of cheese were determined (Marshall, 1992). The salt content of the cheese samples

measured using Corning Salt Analyzer (Marshall, 1992) and the total calcium content was analyzed by inductively-coupled Argon plasma emission spectroscopy (ICP). Soluble calcium content of cheese samples were determined using acid-base titration method (Hassan et al., 2004).

4.3.4. Textural analysis

A TA.XT2 Texture Analyzer (Stable Micro Systems, Go-dalming, Surrey, UK) with a TA-25 probe (50 mm diameter) and TA-90A flat plate was used for texture testing. Cylindrical cheese samples having 16 mm diameter and 17.5 mm height were cut and kept overnight at 4°C in sealed plastic bags prior to the analysis. Texture Profile Analysis where cheese was compressed by 62% of the original height, and uniaxial compression test when cheese was compressed by 80% of original height, was performed on cheese samples. Tests were performed at 4°C. Texture parameters were calculated as described by Bourne (2002).

4.3.5. Melt profile analysis

Melt Profile Analysis was performed using UW Melt-Profiler developed by Muthukumarappan et al. (1999). For melt analysis, cylindrical cheese samples having 30 mm diameter and 7 mm thickness were cut with cork borer cylinders and held overnight at 4°C in sealed plastic bags. Cheese samples were placed in an oven at 72°C immediately after taking out of the refrigerator at 4°C. A thermocouple was inserted in the center of the cheese disc and then placed between two aluminum plates having a dry film lubricant and a layer of oil sprayed on them. Decrease in cheese height during melting was measured over 15 min by a linear variable differential transformer, which

was connected to the top plate. Degree of flow (DOF) was calculated as the percentage decrease in the original cheese height when cheese temperature reached 55°C.

4.3.6. Rheological Analysis

Rheological properties were determined using a Paar Physica (UDS 200, Physica Messtechnik GmbH, Stuttgart, Germany) controlled stress rheometer, with a serrated parallel plate geometry. Cheese disks of 50 mm diameter and 3 mm thickness were cut out from a cylindrical stainless steel cork borer from the 3 mm thick cheese slices obtained using a meat slicer. Cheese discs were then sealed in plastic bags and held at 4°C overnight prior to the test. When loading samples in order to maintain a good contact between plate and cheese, the upper plate was lowered onto cheese not to exceed a normal force of 2 N and then samples were allowed to relax for about 15 min to a relatively constant normal force reading of 0.8 N before starting the test. A thin layer of vegetable oil was applied around the cheese sample to prevent the moisture loss. Rheological properties of cheese were evaluated with an applied strain of 0.2% and frequency of 0.1 Hz. Temperature sweeps were performed from 5 to 85°C at the rate of l°C/min. Storage modulus (G'), loss modulus (G") and loss tangent (LT) were the parameters determined from dynamic small amplitude oscillatory shear rheology tests.

4.3.7. Statistical Analysis

Statistical analysis was performed using SPSS (SPSS version 13.0). Analysis of variance (ANOVA) was performed to determine the effect of different pH values on the composition and textural properties of cheese base and reformed cheese samples at $p \leq 0.05$ significance level. Differences between means were analyzed using Tukey’s

method for multiple comparisons of means. A paired *t*-test was performed to determine the significance of the differences in textural properties of the cheese before and after reforming.

4.4. RESULTS

4.4.1. Milk and cheese composition

Composition of the cheese samples are given in Table 4.2. There were no significant differences between the moisture, fat, protein and salt content of the cheeses. Fat content of the cheese samples was 5%, which would classify it as low-fat according to the Code of Federal Regulations for the labeling of low and nonfat cheese, i.e., <6% fat, or 3 g or less fat for a 28-g serving (CFR, 2006). Buffering peak during the acid titration curve due to the solubilisation of CCP, diminished and finally disappeared with increasing GDL concentration (Fig. 4.1). As the level of added GDL increased, there was an increase in the buffering below pH ~4.5 during acid titration. At the beginning of the back titration with base, cheese having higher GDL levels showed higher initial buffering, which was likely due to buffering by gluconic acid (pKa = 3.6). The peak in base-titration curve at pH ~6 became smaller as the GDL concentration increased indicating less calcium phosphate precipitation due to the reduction in the insoluble calcium phosphate content of cheese. Total calcium concentrations did not differ between cheese samples, while insoluble calcium levels were significantly different and decreased with a decrease in pH. Reducing the cheese pH from 6.2 to 5.3 reduced the insoluble calcium amount by 38%. Previous studies have shown that as milk is acidified, the CCP dissolves from the casein micelles (e.g., McMahon et al., 2005; Guinee et al., 2002; Joshi et al., 2003; Sheehan and Guinee, 2004). When making cheese, acidification solubilizes

the calcium and if most acidification occurs before all the whey is drained, then this solubilized calcium can be lost along with the cheese whey. In our study, since the acidification was done after most of the whey was removed (i.e., prior to pressing), the total calcium content was similar for all cheese samples. There was a slight decrease in total calcium content as the pH was reduced, which was probably as a result of ongoing whey loss during the pressing step, however, that difference was not significant.

4.4.2. Visual attributes

Pictures taken before and after reforming for the cheese samples having different pH values showed that, cheese reformed at higher pH values (i.e., pH 6.2 and 5.8) did not visibly fuse together very well (Fig 4.2). Cheese reformed at pH 6.2 had a grainy texture; the boundaries between cheese particles that were created in ground cheese were still mostly visible. As the pH was decreased, the individual cheese particles in the reformed cheese became less apparent and disappeared completely. It was not possible to tell the difference in the visible appearance of cheese at pH 5.3 before and after reforming.

4.4.3. Texture properties

Hardness results for the cheeses before and after reforming are shown in Figure 4.3. There was no significant difference in TPA hardness between the reformed and untreated cheese except for the cheese at pH 6.2 (Table 4.3). However, uniaxial compression test, where a larger deformation was applied, showed that reforming reduced the hardness of the cheese significantly except for the cheese at pH 5.3 (Table 4.4). Conflicting trends for hardness between the TPA and uniaxial compression tests were also seen in our previous work on the impact of grating size on reforming cheese

(Akbulut et al., 2011; Chapter 2). All cheese samples fused well enough to form a single cheese piece that held together, however, discontinuities still existed in cheese structure reformed at higher pH values. The lower strain levels (62%) applied in TPA test was probably not enough to reveal the discontinuities or incomplete fusion (Akbulut et al., 2011).

A smaller difference between the hardness values obtained by uniaxial compression test before and after reforming was observed at higher GDL levels. No significant difference in hardness values compared to the cheese base was observed after reforming the cheese at pH 5.3 (Table 4.4). In higher pH cheese, hardness was greatly reduced in reformed cheese compared to the cheese base. The reduction in hardness was probably due to the cheese becoming more brittle since it did not fuse well and with deformation the cheese collapsed into the pieces (Fig. 4.5). The degree of recovery in the hardness of the cheese after reforming can be used as an indicator of the extent of cheese fusion. As the pH was reduced, most of the physically disrupted bonds and interactions between the proteins were restored back to almost its original level. The recovery in hardness of reformed cheese samples in respect to their original hardness, as determined by uniaxial compression test, was 93, 83, 74 and 68% in cheese with pH 5.3, 5.5, 5.8 and 6.2, respectively (Fig. 4.4).

Cohesiveness, gumminess and chewiness of the reformed cheeses did not differ from their corresponding cheese base except for the cheese reformed at pH 6.2, which had much lower cohesiveness, gumminess and chewiness as measured by TPA analysis (Table 4.3). The incomplete fusion of the cheese particles at pH 6.2 made the cheese

crumbly, therefore substantially reduced the cohesiveness and reduced the amount of force required to disintegrate it during compression.

4.4.4. Rheological properties

The recovery in the dynamic moduli values of the cheese after reforming followed a similar trend to the hardness at 80% compression; as the pH was reduced the degree of recovery of the dynamic moduli observed in the original cheese was greater (Fig 4.8). The recovery in dynamic moduli values (at 10ºC) of reformed cheese samples in respect to their original values was 85, 75, 69 and 66% for G' value and 87, 74, 70 and 68% for G" values in cheese with pH 5.3, 5.5, 5.8 and 6.2, respectively. Changes in the dynamic moduli and loss tangent during heating the cheese samples from 5 to 80ºC before and after reforming are shown in Figures 4.9 and 4.10 respectively.

4.4.5. Meltability

Melt profiles of the cheese samples are shown in Fig. 4.13. The degree of flow, a measure of meltability of the cheese, was significantly increased after reforming the cheese made at pH 6.2, 5.8 and 5.5 (Fig. 4.12 and Table 4.5). The change in the degree of flow (at 55ºC) of the cheese samples after reforming at different pH values is given in Fig 4.11. The difference in meltability between original and reformed cheeses became smaller as the pH of cheese reduced and at pH 5.3 the melt behavior was the same before and after reforming.

4.4.6. Microstructure of cheese after reforming

Decreasing the pH to 5.5 and 5.3 with GDL greatly improved the fusion of cheese, as observed from microscopy images (Fig. 4.14). Cheese at pH 5.3 visibly fused

almost completely, and little to no mechanical holes were obvious between the contact regions in micrographs (Fig. 4.14). On the other hand, at pH 6.2 the contact region of the cheese slices remained distinct (Fig. 4.14).

4.5. DISCUSSION

Reducing the pH improved cheese fusion and reformability. Texture properties of the low-fat cheese made at pH 5.3 were mostly restored after reforming (Tables 4.3 and 4.4). On the other hand, reforming the cheese made at high pH, i.e. pH 6.2, reduced its hardness (Fig 4.4) and storage modulus (Fig. 4.8) while making it more meltable (Fig. 4.12). It was apparent that, as the cheese base pH was decreased, reforming had a smaller impact on textural and rheological properties, which was presumably due to the greater extent of recovery in the bonds and interactions between proteins. Finally, at pH 5.3 no significant difference was observed in texture properties after reforming (Tables 4.3 and 4.4).

The para-casein network as the backbone of the cheese structure, and therefore it was assumed to play a major role in the reformation of cheese. Care was taken to ensure that the gross composition of cheese base was similar at different pH levels in order to eliminate any influence of compositional differences on the texture. Changes in reformability of the cheese samples at different pH levels were due to the differences in the type of protein interactions. It is well known that pH influences the casein interactions mainly because of its demineralization effect on casein micelles (Lucey et al., 2003). Influence of pH on the interactions between caseins is more indirect above pH 5.0 and is mediated through its effect on Ca^{++} solubility (McMahon et al., 2005). The proportion of insoluble calcium associated with casein particles (or the ratio of the Ca to

protein content) has been suggested as a useful structural parameter as it may regulate the textural properties of the cheese (Lucey et al., 2003).

Applying the Horne model for casein micelle formation/stability (Horne, 1998), the decrease in pH reduced the CCP crosslinks and increased electrostatic repulsion between the newly exposed phosphoserine groups of casein molecules. The net result was a weakening of cheese structure. The ratio of the insoluble to total calcium decreased from 86 to 53% as the pH was reduced from 6.2 to 5.3 (Table 4.2). Solubilization of the CCP crosslinks increased the bond mobility, which was the likely cause of the improved cheese fusion at low pH. Paulson et al. (1998) observed that, nonfat Mozzarella cheese with low calcium content was highly hydrated and sticky and adhered to the rubber gloves when hand stretching in hot water. When the CCP crosslinks in cheese protein matrix are solubilized, the proteins are more disassociated and available to interact with other surfaces. It was suggested that the dissociation of the casein aggregates due to the solubilization of calcium, exposed more hydrophobic sites, and charged sites as well as imparting a greater degree of flexibility (bonds are easily broken and reformed), thus making the proteins prone to interact with surfaces, such as, rubber or steel (McMahon et al., 2005).

Decrease in pH reduced the hardness, springiness, chewiness (Table 4.3) and storage modulus (Fig. 4.6) of the cheese base. In the light of previous studies (Watkinson et al., 2001; Guinee et al., 2002; Joshi et al., 2003; Pastorino et al., 2003a,b; Sheehan and Guinee, 2004), it was expected that reducing the pH from 6.2 to 5.3 would make the cheese base softer (Fig 4.3) as the Ca^{++} was solubilized and the bonds between the proteins became much weaker and required less energy to break. According to the

Dahlquist criterion, stickiness does not occur in hard materials; and it states that, for adhesion to occur, the storage modulus of an adhesive must be below 10^5 Pa (Dahlquist, 1969). It was only the cheese base at pH 5.3 that had a G' value $<10^5$ Pa (at 10ºC) and that sample exhibited the greatest extent of fusion with no significant change in textural and rheological properties after reforming. There was a substantial decrease in G' values (at 10ºC) as the pH reduced; however the G" values remained relatively similar at all pH values. Therefore the viscous character of the cheese base became more influential with a decrease in pH. Joshi et al. (2004) had also observed a similar trend in G' and G" values when they reduced the Ca^{++} content of cheese. The increase in LTmax values with the decrease in pH (Fig. 4.10) was also an indicator of increased viscous nature, or a more fluid-like character. The viscous component reflects the temporary character of the matrix with weak bonds having short relaxation times, meaning that they are mobile, they break and reform spontaneously by the application of the stress (Lucey et al., 2003; Horne and Banks, 2004). The relatively viscous nature of the cheese base at low pH was indicator of a more flexible protein matrix. This probably allowed more rearrangements, which helped to rebuild interactions and bonds at the contact surfaces of curd particles.

The significant decrease in the dynamic moduli and hardness that occurred when cheese at high pH (6.2) was reformed could be attributed to the presence of weaker interactions and incomplete recovery of the bonds between caseins. The continuity of the protein matrix is one of the factors that determine how stiff the cheese is. Hardness of the cheese increases as the strength and number of the protein-protein interactions in cheese increase (de Jong, 1978; Chen et al., 1979; Creamer and Olson, 1982; Prentice, 1992; Fox et al., 2000; Gunasekaran and Ak, 2003; Lucey et al., 2003). The presence of the

discontinuities in the cheese matrix due to the incomplete fusion reduced the amount of the force required to disintegrate cheese by creating a less chewy and gummy texture with a decrease in hardness (Table 4.3). Less energy was needed to disrupt the bonds within the casein network which could also allow proteins to flow when it was heated after reforming; thus reformed cheese samples with higher pH values showed higher degree of flow (Fig. 4.11).

In a cheese making process, for the curd fusion to occur, the flow of curd grains (deformation) that results in the creation of a large contact area and formation of bonds between those adjacent grains is essential (Akkerman et al., 1993; Lodaite et al., 2002). In cheese reforming process, motion of the particles is more restricted compared to the initial curd particles created when the rennet coagulum was cut; however, the type of interactions involved in the fusion of the cheese particles are similar. Curd fusion takes place as casein molecules at the surface of each particle associate with casein molecules on adjacent curd particles (Johnson and Law, 2010). Lodaite et al. (2002) found that, the fusion of the curd grains was impaired if the paracasein strands of the network become thicker and less flexible. Protein-protein interactions are enhanced and proteins are highly aggregated when there is a high insoluble Ca concentration in cheese (McMahon et al., 2005). At high pH, there is a higher proportion of insoluble calcium in cheese, which limits the flexibility of proteins due to the increased number of CCP crosslinks between caseins and that could hinder the fusion of the cheese particles at adjacent surfaces. Lowrie et al. (1982) studied the fusion of the cheese curds in Cheddar cheese making, and observed that curd granule and milled curd junctions were only a little affected by the use of different pressing systems; and pH was more important for cheese fusion. The

cheese base made at pH 6.2 had a firm texture and it fused poorly after reforming. It was not cohesive; a dramatic decrease in cohesiveness values was observed after reforming (Table 4.3). Micrographs of the cheese samples also showed that cheese at pH 6.2 did not fuse well (Fig 4.14). The texture of the cheese reformed at pH 6.2 can be described as very curdy. Curdiness is a condition in cheese that occurs at traditional cheese making process when cheese curds do not fuse sufficiently after filling them into hoops and pressing (Johnson and Law, 2010). When this cheese is chewed, it will readily break into the original curd particles. Curdiness is more prone to occur in cheeses at higher pH values (Johnson and Law, 2010).

4.6. CONCLUSIONS

This study demonstrated that changing the insoluble calcium levels by altering the pH influenced the reformability of the cheese greatly. Decrease in pH improved cheese fusion. Interactions and bonds between caseins were presumably restored back to the levels prior to grating/reforming as pH was reduced due to the increase in bond mobility with the solubilization of CCP crosslinks. The level of the fusion between cheese particles after reforming can be controlled by changing the pH. It is important to have a control over cheese fusion in reforming as this can help modify the final cheese texture depending upon consumer preferences. Reforming cheese with a lower pH did not alter the texture as much as high pH cheese since most bonds appeared to reform on storage of low pH cheese. The rubbery and firm texture of the low-fat cheese made at high pH was improved by reforming as the texture became shorter and softer.

4.7. REFERENCES

Akbulut, C., Govindasamy-Lucey, S., Lucey, J. A., Jaeggi, J. J., and Johnson, M. E. 2010. Impact of grating size on the texture and melting properties of reformed non-fat cheese. Milchwissenschaft 66:169-172.

Akkerman, J. C., Lewis, R. O., and Walstra, P. 1993. Fusion of curd grains. Neth. Milk Dairy J. 47:137–144.

Bourne, M. C. 2002. Food Texture and Viscosity: Concept and Measurement. 2nd edn. Academic Press, San Diego.

Chen, A.H., Larkin, J.W., Clark, C.J., and Irvine, W.E. 1979. Textural analysis of cheese. J. Dairy Sci. 62:901–907.

Choi, J., Horne, D. S., and Lucey, J. A. 2007. Effects of insoluble calcium concentration on rennet coagulation properties of milk. J. Dairy Sci. 90:2612-2623.

Choi, J., Horne, D. S., Johnson, M. E. and Lucey, J. A. 2008. Effects of the concentration of insoluble calcium phosphate associated with casein micelles on the functionality of directly acidified cheese. J. Dairy Sci. 91:513-522

Code of Federal Regulations. 2006. Food and Drugs: CFR Part 101 Food Labeling. Department of Health and Human Services, Washington, DC.

Creamer, L. K., Zoerb, H. F., Olson, N. F., and Richardson, T. 1982. Surface hydrophobicity of α_{s1}-casein A and B and its implications in cheese structure. J. Dairy Sci. 65:902–906.

De Jong, L. 1978. Proteolytic breakdown in soft cheese and its relation to consistency of the micellar structure of Meshanger cheese. Neth. Milk Dairy J. 32:15–25.

Dahlquist, C. A. 1969. Tack. Pages 143-151 in Adhesion: Fundamentals and Practice, The Ministry of Technology (United Kingdom), Gordon and Breach Science Publishers, Inc., New York.

Fox, P. F., Guinee, T. P., Cogan, T. M., and McSweeney, P. L. H. 2000. Fundamentals of Cheese Science. Aspen, Gaithersburg, MD.

Guinee, T. P., Feeney, E. P., Auty, M. A. E., and Fox, P. F. 2002. Effect of pH and calcium concentration on some textural and functional properties of Mozzarella cheese. J. Dairy Sci. 85:1655–1669.

Gunasekaran, S., and Ak, M. M. 2003. Cheese Rheology and Texture. CRC Press, Boca Raton, Florida.

Johnson, M. E., and Law, B. A. 2010. The origins, development and basic operations of cheesemaking technology. Pages 68-87 in Technology of Cheesemaking, 2nd edn, eds. B.A. Law, and A. Y. Tamime, Wiley Blackwell, West Sussex, UK.

Hassan, A., Johnson, M. E., and Lucey, J. A. 2004. Changes in the proportions of soluble and insoluble calcium during the ripening of Cheddar cheese. J. Dairy Sci. 87:854–862.

Horne, D. S., and Banks J. M. 2004. Rennet-induced coagulation of milk. Pages 47-70 in Cheese: Chemistry, Physics, and Microbiology. Vol. 1 General Aspects, 3rd edn, P. F. Fox, P. L. H. McSweeney, T. M. Cogan, and T. P. Guinee, ed. Elsevier Science and Technology, London.

Horne, D. S. 1998. Casein interactions: Casting light on the black boxes, the structure in dairy products. Int. Dairy J. 8:171–177

Joshi, N. S., Muthukumarappan, K., and Dave, R. I. 2003. Understanding the role of calcium in functionality of part-skim Mozzarella cheese. J. Dairy Sci. 86:1918–1926.

Joshi, N. S., Muthukumarappan, K., and Dave, R. I. 2004. Viscoelastic properties of part skim mozzarella cheese: effect of calcium, storage, and test temperature. Int. J. Food Properties 7:239–252.

Lee, M.-R., Johnson, M. E., and Lucey, J. A. 2005. Impact of modifications in acid development on the insoluble calcium content and rheological properties of Cheddar cheese. J. Dairy Sci. 88:3798-3809.

Lodaite, K., Östergren, K., Santos, O., Archambault, E., Paulsson, M., and Dejmek, P. 2002. Fusion of skim milk cheese curd grains: Development of a method to measure the fracture stress of the bonds between fused curd grains. Int. Dairy J. 12:455-461.

Lowrie, R. J., Kalab, M., and Nichols, D. 1982. Curd granule and milled curd junction patterns in Cheddar cheese made by traditional and mechanized processes. J. Dairy Sci. 65:1122-1129

Lucey, J. A., Johnson, M. E., and Horne, D. S. 2003. Perspectives on the basis of the rheology and texture properties of cheese. J. Dairy Sci. 86:2725–2743.

Lucey, J.A., and Fox, P.F. 1993. Importance of calcium and phosphate in cheese manufacture: A review. J. Dairy Sci. 76:1714–1724.

Marshall, R. T. 1992. Standard methods for the examination of dairy products. 16th edn. Am. Public Health Assoc, Washington, DC.

McMahon, D. J., Paulson, B., and Oberg, C. J. 2005. Influence of calcium, pH, and moisture on protein matrix structure and functionality in direct-acidified nonfat Mozzarella cheese. J. Dairy Sci. 88:3754-3763.

Muthukumarappan K, Wang, Y-C., and Gunasekaran, S. 1999. Modified Schreiber test for cheese meltability evaluation. J. Dairy Sci. 82:1068-1071.

Pastorino, A. J., Ricks, N. P., Hansen, C. L., and McMahon, D. J. 2003a. Effect of calcium and water injection on structure-function relationships of cheese. J. Dairy Sci. 86:105–113.

Pastorino, A. J., Hansen, C. L., and McMahon, D. J. 2003b. Effect of pH on the chemical composition and structure-function relationships of cheese. J. Dairy Sci. 86:2751–2760.

Paulson, B. M., McMahon, D. J., and Oberg, C. J. 1998. Influence of salt on appearance, functionality, and protein arrangements in nonfat Mozzarella cheese. J. Dairy Sci. 81:2053–2064.

Prentice, J. H., Langley, K. R., and Marshall, R. J. 1993. Cheese rheology. Pages 303–340, in: Cheese: Chemistry, Physics and Microbiology. Vol. 1. P. F. Fox, ed. Elsevier, London.

Sheehan, J. J., and Guinee, T. P. 2004. Effect of pH and calcium level on the biochemical, textural and functional properties of reduced-fat Mozzarella cheese. Int. Dairy J. 14:161–172.

Watkinson, P., Coker, C., Crawford, R., Dodds, C., Johnston, K., McKenna, A., and White, N. 2001. Effect of cheese pH and ripening time on model cheese textural properties and proteolysis. Int. Dairy J. 11:455–464.

Table 4.1 Cheese manufacturing protocol used for the low-fat cheese bases having final pH values of 6.2, 5.8, 5.5 and 5.3

Operation		Time (min)	Quantity		pH / Temperature	
Initial Milk – 0.40 to 0.50% butterfat						
initial milk weight (kg)			20 kg		pH	6.76
Add Citric Acid to Drop pH		0 min			Temp	5 °C
Add diluted citric acid (4:1)			75 ml diluted acid		pH	6.20
6.20 target						
Add Coagulant - 33°C		35 min			Temp	33°C
Ch Hansen, Chymax Extra (dbl str)						
0.02%			0.8 ml			
Cutting		95 min				
1.3 cm knives					pH	6.20
Start Cooking / Begin Agitation		110 min			Temp	33°C
Reach Cooking Temp		130 min	**Target pH 6.20**	**Temp**		**37°C**
			Target pH 5.80	**Temp**		**36°C**
			Target pH 5.50	**Temp**		**35°C**
			Target pH 5.30	**Temp**		**34°C**
Complete Drain						
	Beginning	140 min				
	End	145 min				
Cut, turn and stack 2 high		155 min				
Mill		170 min				
Add Salt						
0.4% of milk (2% of cheese)			40 g			
Add GDL			0 g	No GDL	pH 6.2 target	
			20g	0.1% of milk	pH 5.8 target	
			60 g	0.3% of milk	pH 5.5 target	
			80 g	0.4% of milk	pH 5.3 target	
Hooping		190 min				
Pressing	- In	195 min				
1 hour, ~300 kPa	- Out	315 min				

Table 4.2 Chemical composition of cheese bases made with different amounts of glucono-δ-lactone. Means are for replicates of 6 cheese making trials.

Cheese	
Moisture (%)	59 ± 0.4
Fat (%)	5 ± 0.6
Protein (%)	30 ± 2
Salt (%)	1.6 ± 0.3
Total Ca (mg/100g)	668 ± 24

	Glucono-δ-lactone (%)			
Cheese	0	0.1	0.3	0.4
pH				
1st day	6.18[a]	5.83[b]	5.52[c]	5.29[d]
1st week	6.15[a]	5.79[b]	5.32[c]	5.39[d]
2nd week	6.13[a]	5.79[b]	5.52[c]	5.31[d]
1st month	6.1[a]	5.76[b]	5.54[c]	5.31[d]
Insoluble Ca (as % of total Ca)	86[a]	69[b]	61[bc]	53[c]
Soluble Ca as (% of total Ca)	14[a]	31[b]	39[bc]	47[c]

[a-b]Means with different letters within the same row are significantly different ($P<0.05$)

Table 4.3 Comparison of texture profile analysis results (62% compression) before and after reforming the cheese at different pH values. Means are for replicates of 6 cheese making trials.

		Cheese					% Recovery
		Before reforming (Base)		After reforming (Reformed)			
Parameter	pH	$\overline{X}$	SD	$\overline{X}$	SD	*t*	
Hardness (g)	6.2	8.56E+03	628	5.59E+03	152	9.51*	65.5 [a]
	5.8	6.08E+03	564	5.74E+03	640	2.44	76.4 [ab]
	5.5	3.07E+03	991	2.47E+03	1337	1.62	94.4 [b]
	5.3	3.04E+03	1028	3.30E+03	521	-0.54	116.7 [b]
Adhesiveness (g.mm)	6.2	-2.90E+01	14	-1.00E+01	10	-3.09	34.5 [a]
	5.8	-2.80E+01	20	-1.70E+01	13	-0.76	60.7 [a]
	5.5	-1.17E+02	138	-6.90E+01	34	-0.79	59.0 [a]
	5.3	-2.40E+01	11	-2.70E+01	45	1.69	112.5 [a]
Springiness (mm)	6.2	1.30E+01	0.21	1.20E+01	0.32	1.92	95.4 [a]
	5.8	1.20E+01	0.2	1.20E+01	0.07	2.58	97.5 [a]
	5.5	1.10E+01	1.43	1.10E+01	0.38	-0.47	103.7 [a]
	5.3	1.10E+01	0.41	1.20E+01	0.24	-3.33	105.7 [a]
Cohesiveness	6.2	4.90E-01	0.08	1.10E-01	0.02	9.89*	22.4 [a]
	5.8	5.00E-01	0.09	4.80E-01	0.08	0.69	96.5 [b]
	5.5	4.60E-01	0.08	6.00E-01	0.04	-2.38	132.7 [c]
	5.3	5.50E-01	0.05	6.40E-01	0.02	-5.75	117.6 [bc]
Gumminess (g)	6.2	4.25E+03	948	6.20E+02	142	7.71*	14.6 [a]
	5.8	3.02E+03	423	2.74E+03	421	2.55	90.9 [b]
	5.5	1.39E+03	397	1.51E+03	865	-0.42	101.6 [b]
	5.3	1.70E+03	687	2.13E+03	390	-1.68	138.1 [c]
Chewiness (g.mm)	6.2	5.34E+04	1091	7.46E+03	1893	8.72*	13.9 [a]
	5.8	3.69E+04	4895	3.27E+04	5178	3.55	88.6 [b]
	5.5	1.54E+04	5667	1.71E+04	1003	-0.67	102.4 [b]
	5.3	1.95E+04	8367	2.56E+04	4552	-2.06	146.8 [c]

Notes: $\overline{X}$: mean, SD: standard deviation, *t*: t-value at the t-test of paired two samples for means, (*): $P<0.05$ (two-tailed), the difference between mean values before and after reforming is significant.

% Recovery values in same column with different letters show significant difference for corresponding texture parameter between different pH levels ($P<0.05$)

Table 4.4 Comparison of uniaxial compression test results (80% compression) before and after reforming the cheese at different pH values. Means are for replicates of 6 cheese making trials.

		Cheese					% Recovery
		Before reforming (Base)		After reforming (Reformed)			
Parameter	pH	$\overline{X}$	SD	$\overline{X}$	SD	*t*	
	6.2	8.86E+03	847	6.02E+03	868	27.1*	68.2 [a]
	5.8	8.22E+03	597	5.87E+03	895	9.45*	71.2 [a]
Hardness	5.5	6.74E+03	613	5.57E+03	607	18.7*	82.6 [b]
(g)	5.3	5.30E+03	793	4.88E+03	987	0.95	93.6 [c]

Notes: $\overline{X}$: mean, SD: standard deviation, *t*: t-value at the t-test of paired two samples for means, (*): *P*<0.05 (two-tailed), the difference between mean values before and after reforming is significant.
% Recovery values in same column with different letters show significant difference for corresponding texture parameter between different pH levels (P<0.05)

Table 4.5 Comparison of the degree of flow (DOF) measured by UW-Melt profiler before and after reforming the cheese at different pH values. Means are for replicates of 6 cheese making trials.

		Cheese					% Increase
		Before reforming (Base)		After reforming (Reformed)			
Parameter	pH	$\overline{X}$	SD	$\overline{X}$	SD	*t*	
	6.2	36.27	1.6	43.21	1.1	-13.7*	19.13 [a]
	5.8	50.21	1.2	59.75	1.3	-16.9*	19.00 [a]
	5.5	64.46	5.1	70.93	6.5	-4.15*	10.04 [b]
DOF at 55ºC	5.3	70.15	3.5	71.38	4.3	-1.21	1.75 [c]

Notes: $\overline{X}$: mean, SD: standard deviation, *t*: t-value at the t-test of paired two samples for means, (*): *P*<0.05 (two-tailed), the difference between mean values before and after reforming is significant.
% Recovery values in same column with different letters show significant difference for corresponding texture parameter between different pH levels (P<0.05)

Table 4.6 Comparison of the dynamic moduli values (storage modulus: G'; loss modulus: G") at 10ºC before and after reforming the cheese at different pH values. Means are for replicates of 6 cheese making trials.

		Cheese					
		Before reforming (Base)		After reforming (Reformed)			% Recovery
Parameter	pH	$\overline{X}$	SD	$\overline{X}$	SD	*t*	
G' (Pa)	6.2	3.77E+05	3.12E+04	2.50E+05	2.09E+04	20.0*	66.3[a]
	5.8	3.80E+05	3.09E+04	2.61E+05	2.83E+04	64.8*	68.7 [a]
	5.5	2.42E+05	2.66E+04	1.81E+05	1.06E+04	5.44	74.8 [a]
	5.3	1.56E+04	3.46E+03	1.31E+04	1.77E+03	2.03	84.0 [b]
G" (Pa)	6.2	8.39E+04	1.46E+04	5.96E+04	4.25E+03	3.19*	71.0 [a]
	5.8	9.08E+04	8.08E+03	6.34E+04	7.07E+03	38.3*	69.8 [a]
	5.5	6.15E+04	3.97E+03	4.56E+04	2.90E+03	21.0*	74.1 [a]
	5.3	3.97E+03	9.03E+02	3.44E+03	6.94E+02	0.65	86.6 [c]

Notes: $\overline{X}$: mean, SD: standard deviation, *t*: t-value at the t-test of paired two samples for means, (*): $P<0.05$ (two-tailed), the difference between mean values before and after reforming is significant.
% Recovery values in same column with different letters show significant difference for corresponding texture parameter between different pH levels ($P<0.05$)

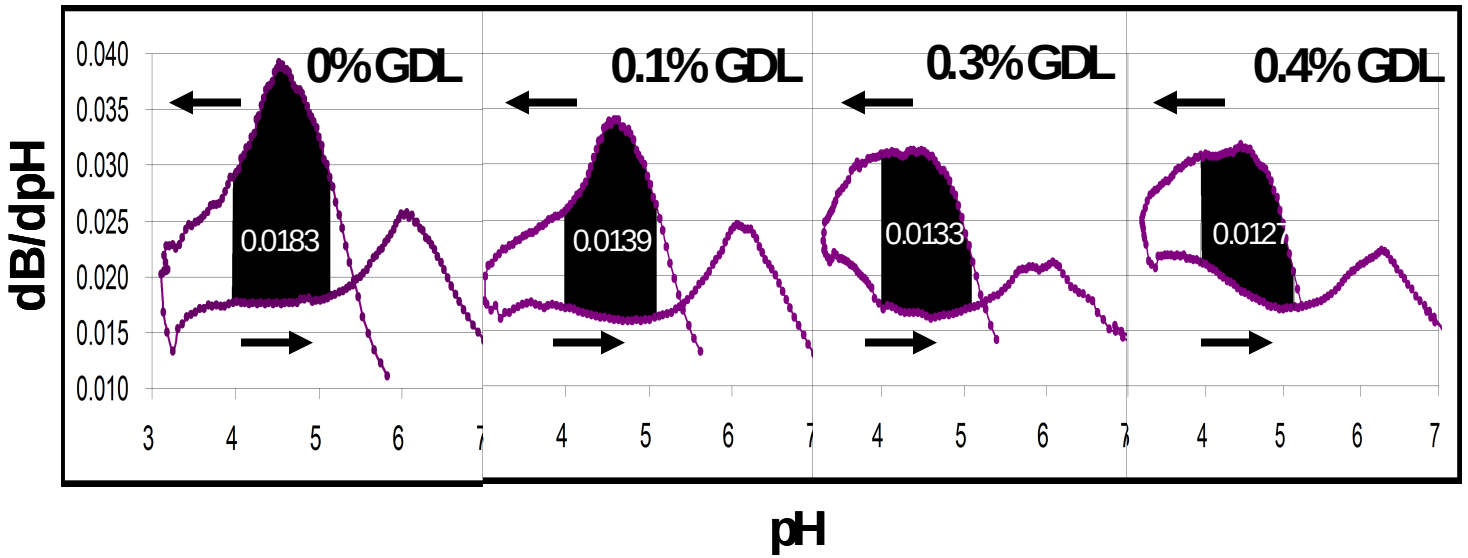


Figure 4.1 Buffering curves of cheeses made with different amounts of glucono-δ-lactone. Cheese homogenates were titrated from initial pH to pH 3.0 with 0.5 N HCl and then back titrated to pH 9.0 with 0.5 N NaOH. Area in black is the buffering area due to the solubilization of colloidal calcium phosphate with calculated areas given. Means are for replicates of 6 cheese making trials.

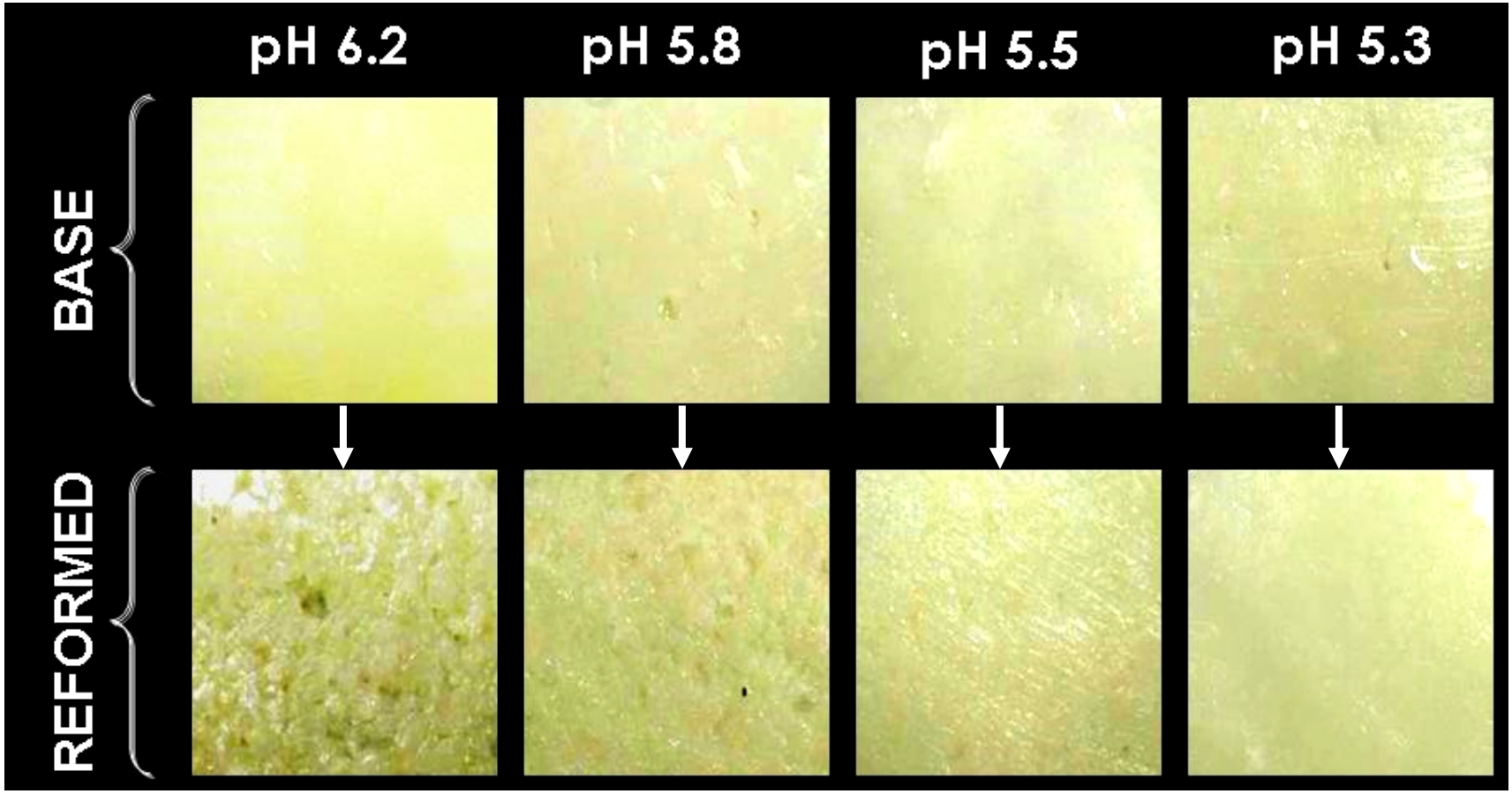


Figure 4.2 Photographs of cheese samples at different pH values (6.2, 5.8, 5.5 and 5.3) before (base) and after (reformed) reforming and 2 wk of storage at 4ºC.

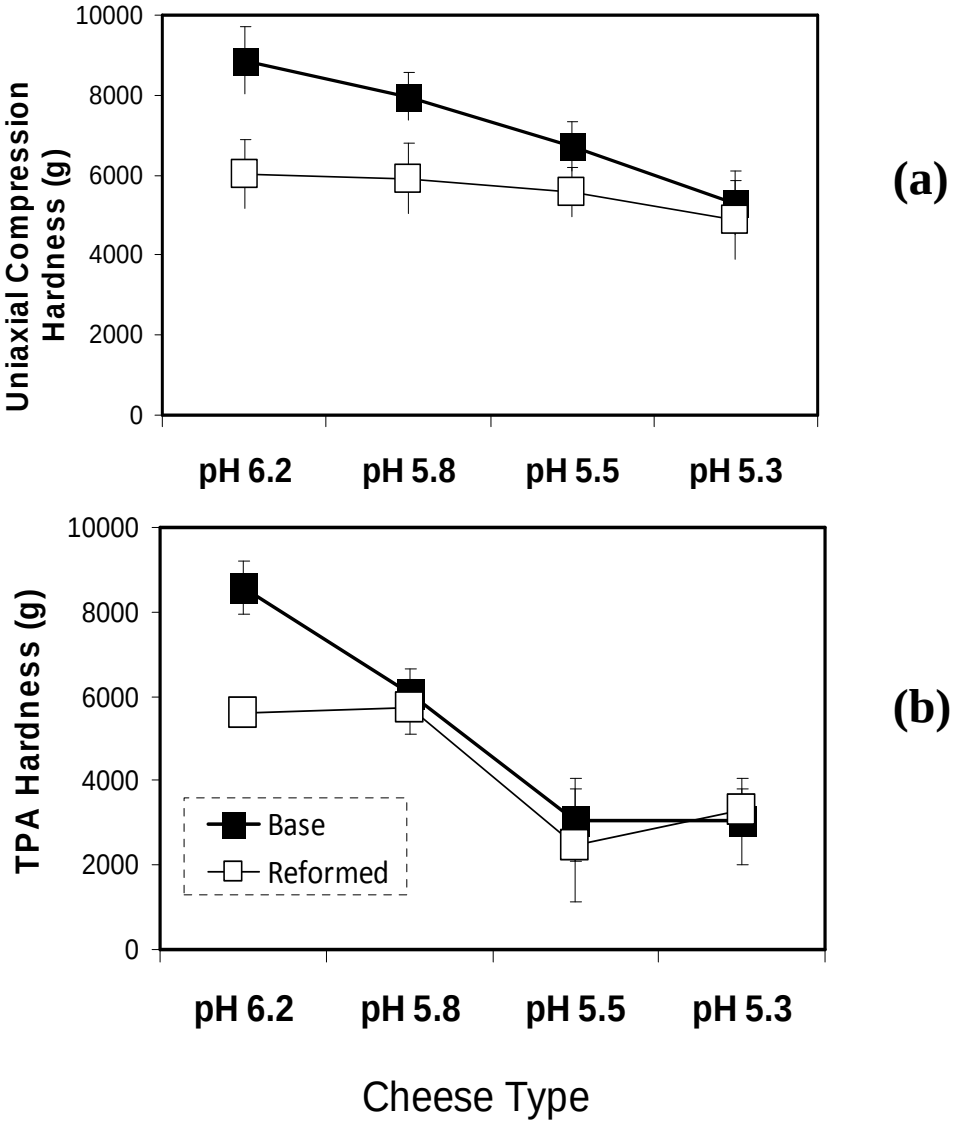


Figure 4.3 Hardness results for cheeses before reforming (base) and after reforming (reformed). Cheeses were made with different pH values and hardness was determined by texture profile analysis at 62% compression (a) and uniaxial compression test at 80% compression (b). Means are for replicates of 6 cheese making trials.

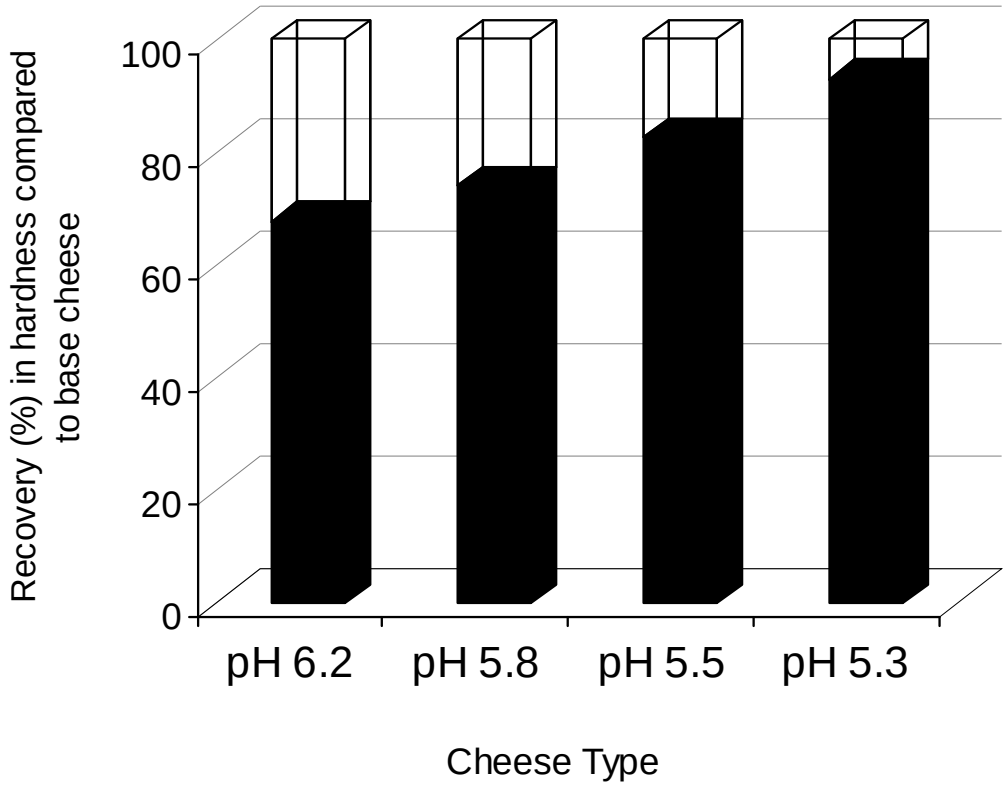


Figure 4.4 Recovery (%) in hardness of the cheese samples after reforming in respect to the hardness of the cheese base, as determined by uniaxial compression test at 80% compression. Means are for replicates of 6 cheese making trials.

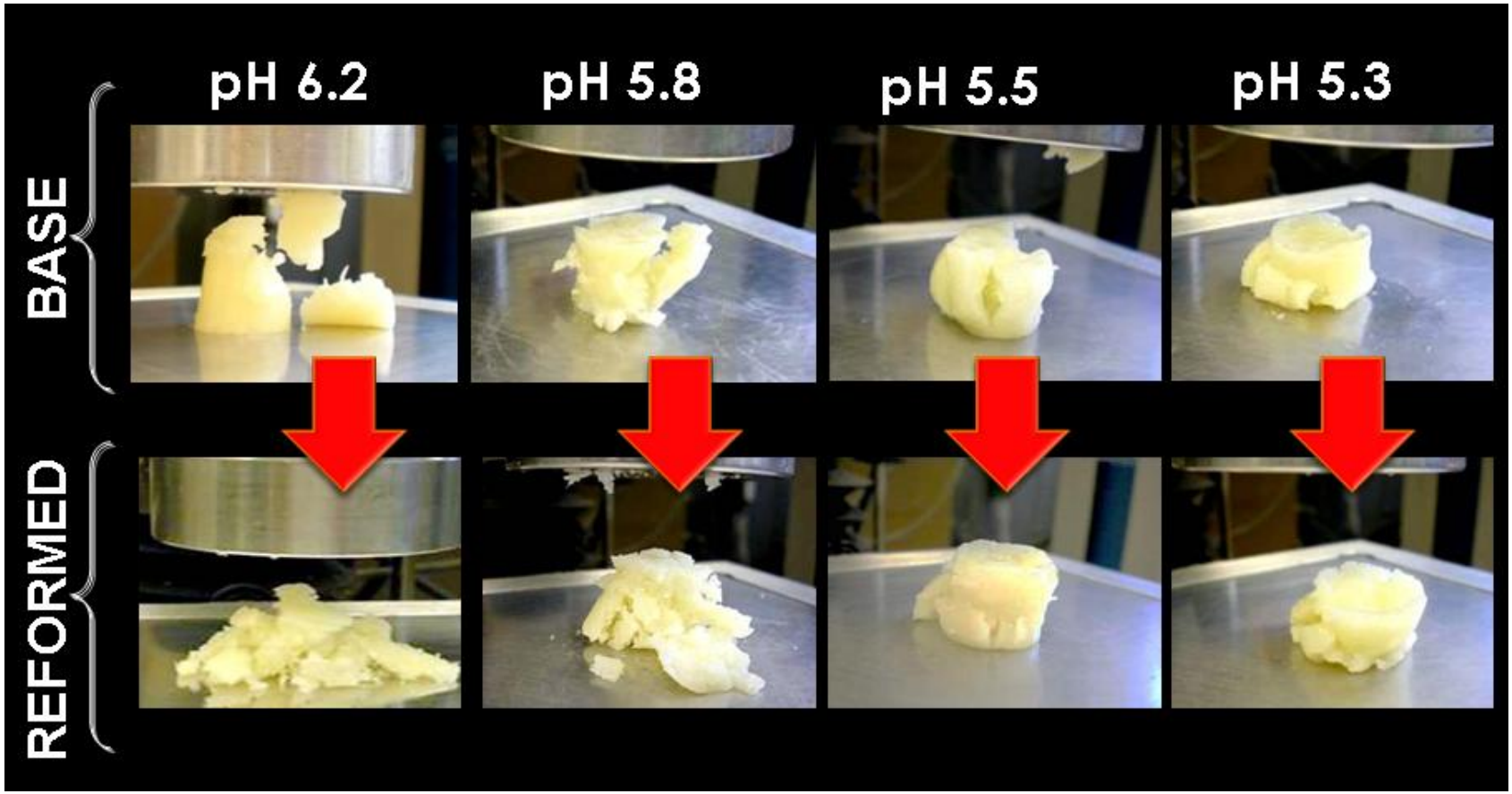


Figure 4.5 Representative pictures taken after the uniaxial compression test for the base and reformed cheese samples with pH values 6.2, 5.8, 5.5 and 5.3.

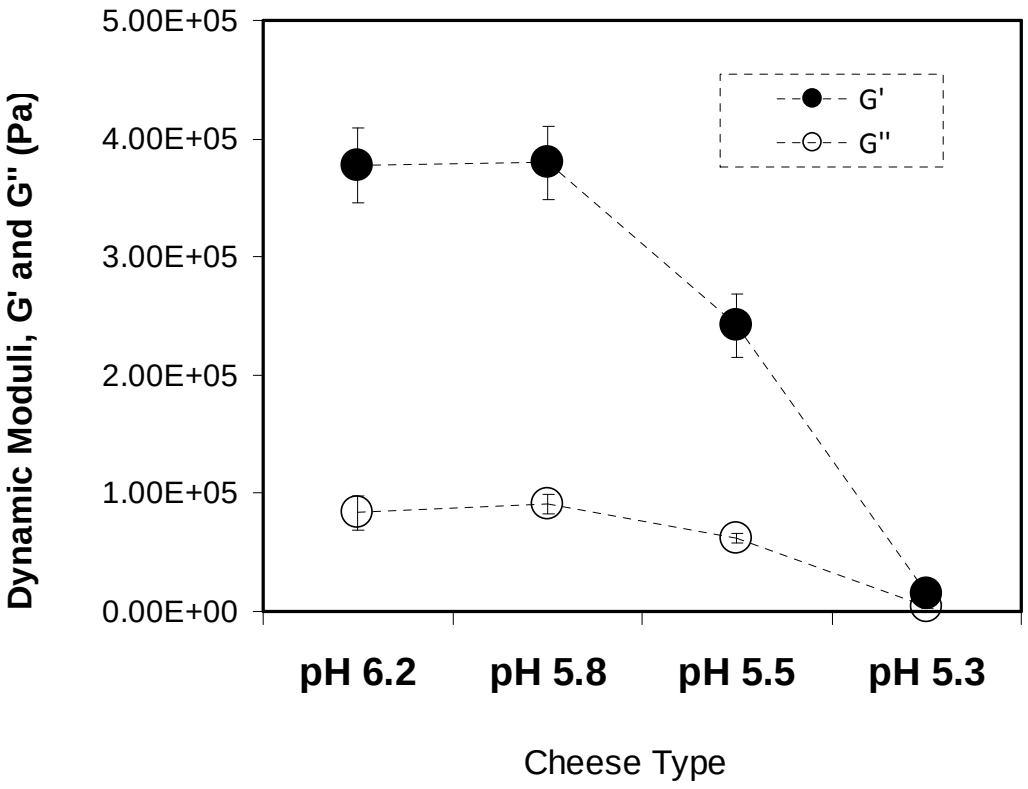


Figure 4.6 The dynamic moduli values (measured at 10ºC) of cheese base made at different pH values. Means are for replicates of 6 cheese making trials.

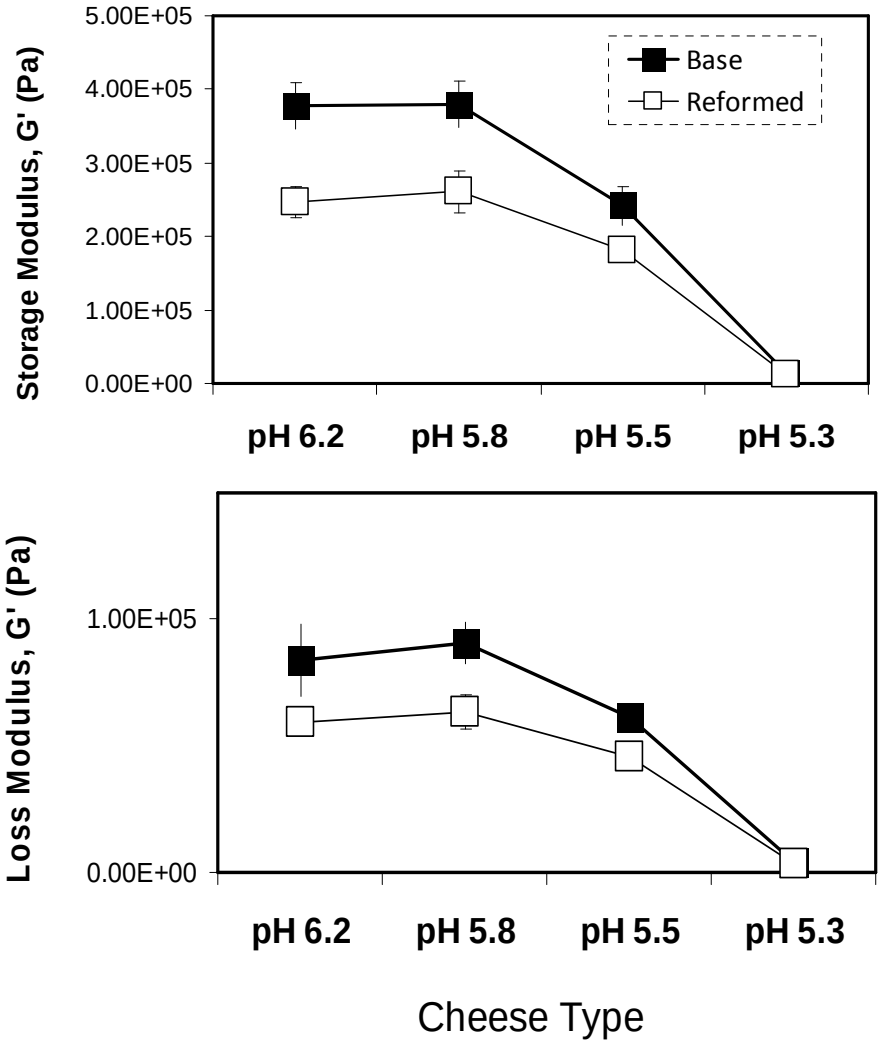


Figure 4.7 The dynamic moduli values measured at 10ºC of the cheese samples made at different pH values before and after reforming (stored at 4ºC for 2 wk). Means are for replicates of 6 cheese making trials.

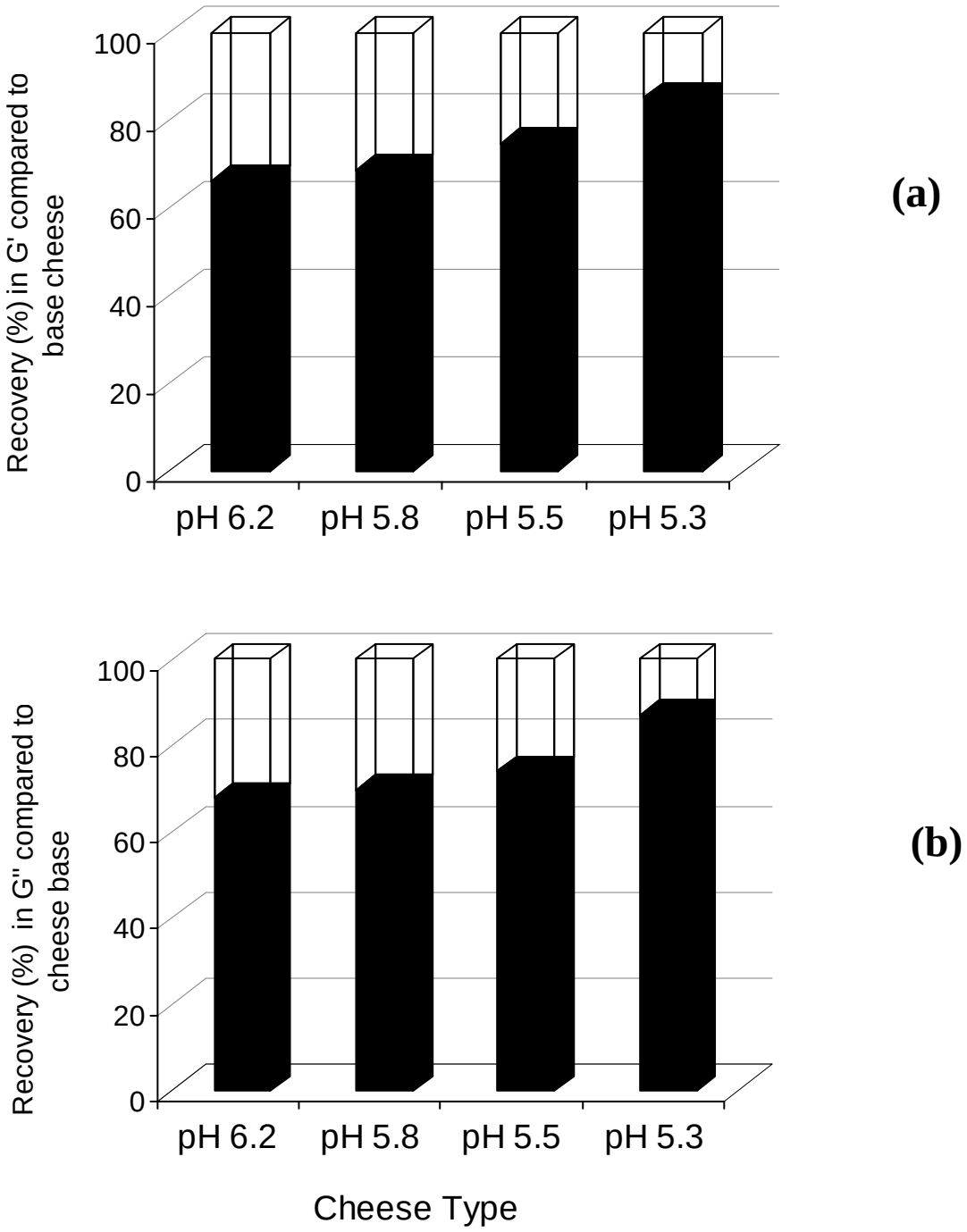


Figure 4.8 Recovery (%) in storage modulus, G' (a) and loss modulus, G'' (b) of the cheese samples after reforming (stored at 4ºC for 2 wk) in respect to cheese base. Means are for replicates of 6 cheese making trials.

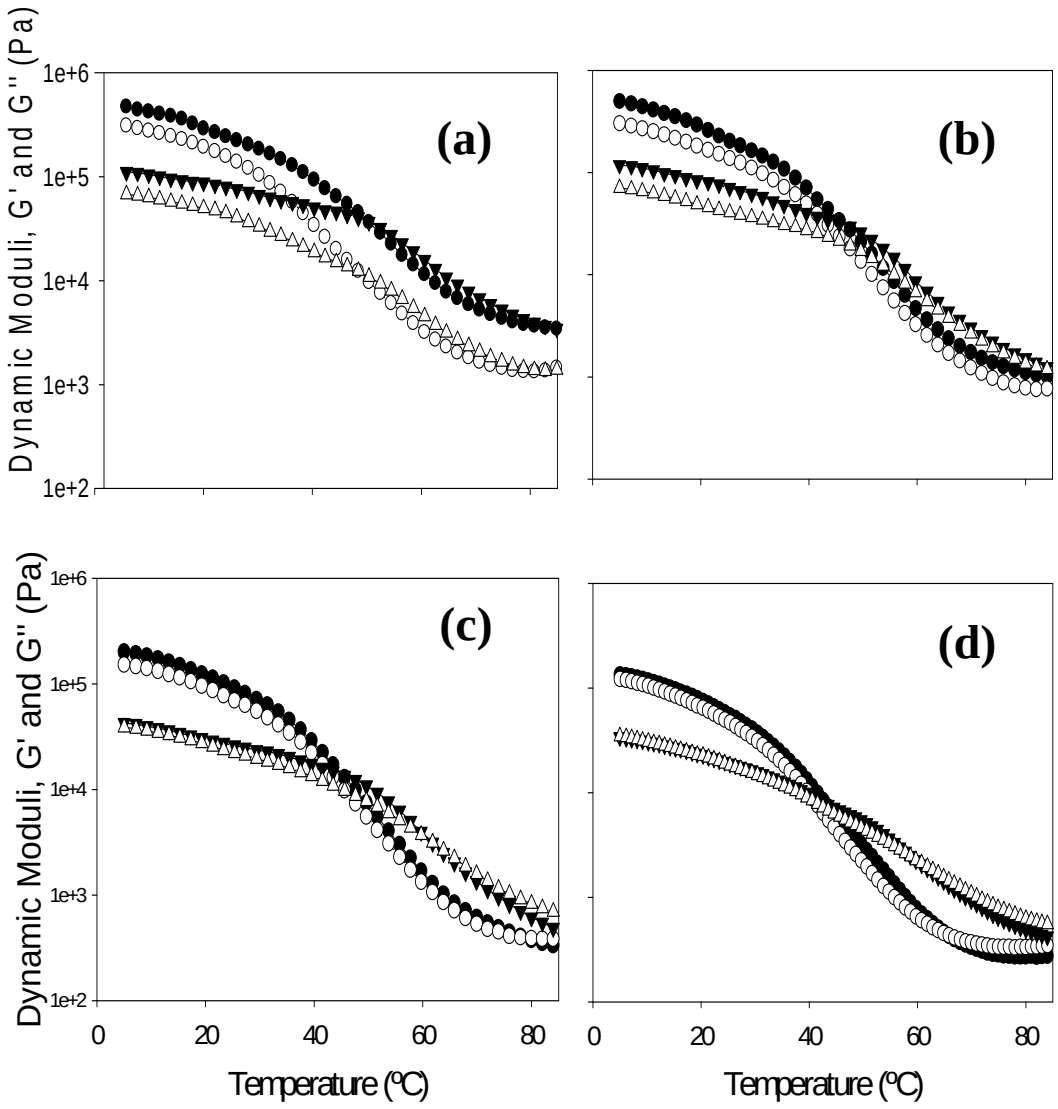


Figure 4.9 Representative dynamic moduli profiles (G':●,○ and G":▼,Δ) as a function of temperature during heating of cheese samples made at pH 6.2 (a), 5.8 (b), 5.5 (c) and pH 5.3 (d). Closed symbols represent cheese base while open symbols are for reformed cheese (stored at 4ºC for 2 wk).

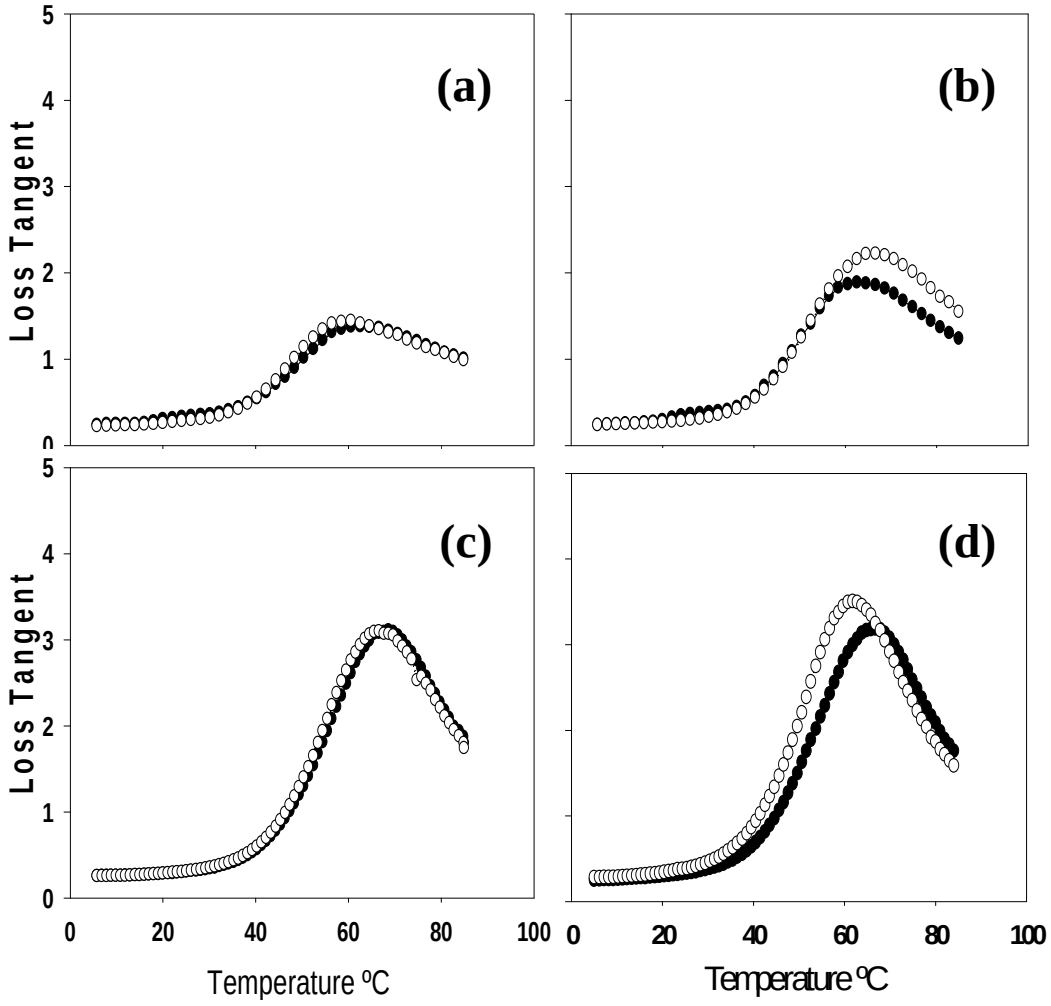


Figure 4.10 Representative loss tangent values as a function of temperature during heating of cheese samples made at pH 6.2 (a), 5.8 (b), 5.5 (c) and pH 5.3 (d). Closed symbols represent cheese base while open symbols are for reformed cheese (stored at 4ºC for 2 wk)

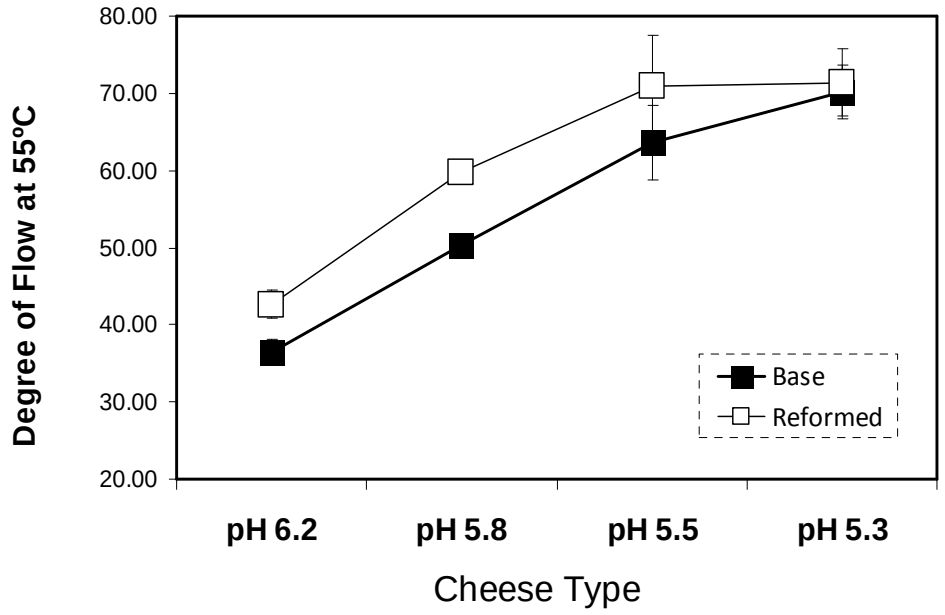


Figure 4.11 Degree of flow at 55ºC before and after the reforming of cheese made with different pH values. Means are for replicates of 6 cheese making trials.

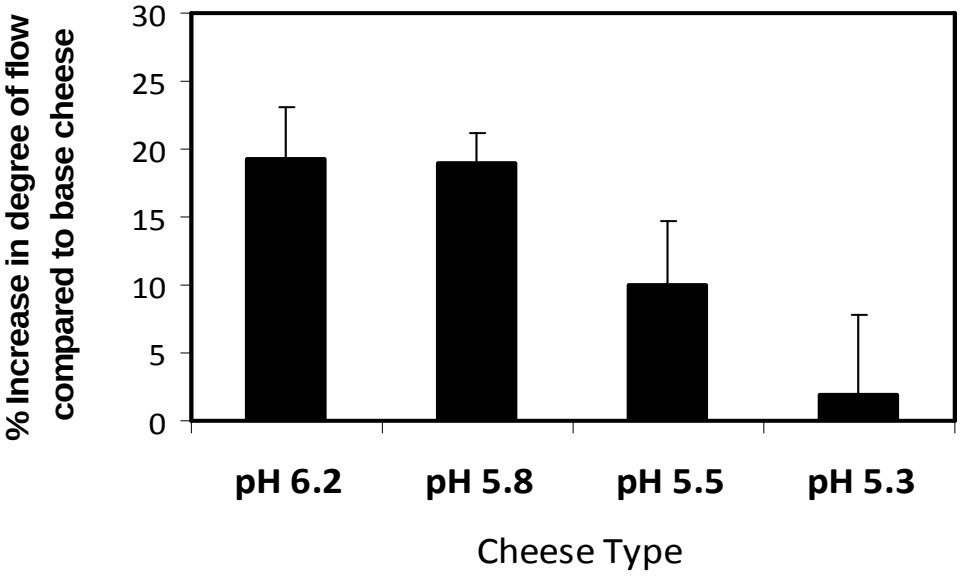


Figure 4.12 The percentage increase in the degree of flow of cheese samples after reforming at pH 6.2, 5.8, 5.5 and 5.3 as determined by UW-melt profile analyzer. Means are for replicates of 6 cheese making trials.

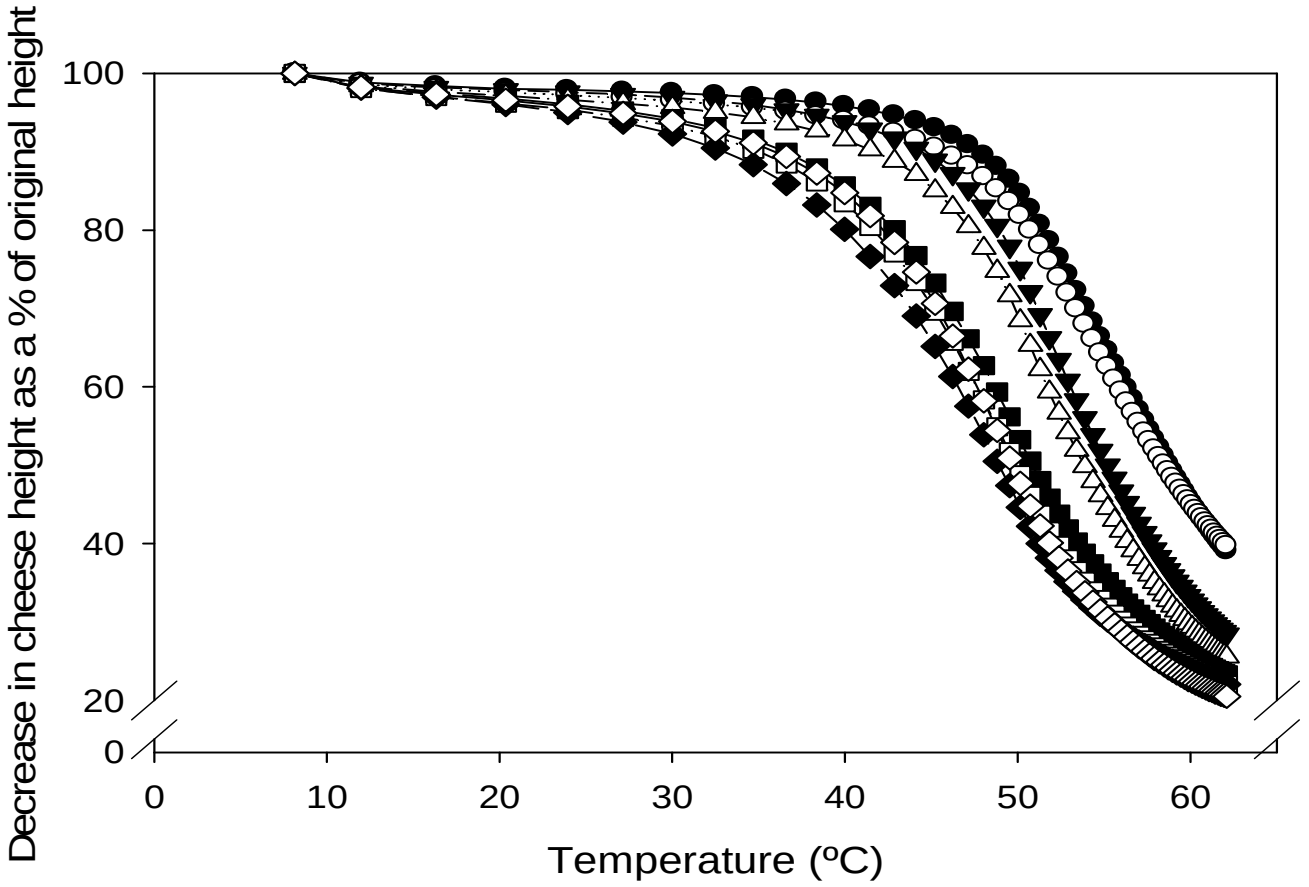


Figure 4.13 Melt profiles of cheese samples made at pH 6.2 (●,○), pH 5.8 (▼,Δ), pH 5.5 (■,□) and pH 5.5 (♦,◊) before and after reforming (stored at 4ºC for 2 wk). Filled symbols are for cheese base while the open symbols represent reformed cheese. Means are for replicates of 6 cheese making trials.

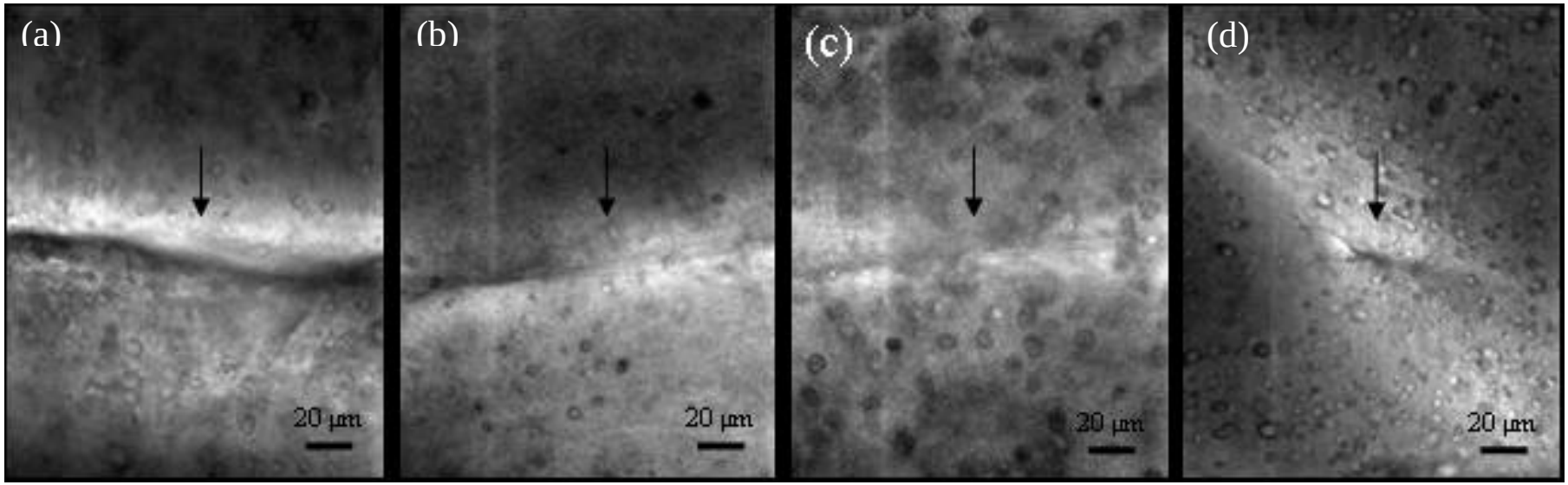


Figure 4.14 Fusion of cheese made at different pH values: (a) pH 6.2, (b) pH 5.8, (c) pH 5.5 and (d) pH 5.3. Fusion was observed between two slices of cheese after being stored in contact for 1 week at 4ºC. No pressure was applied (Arrows show the original contact region for the slices). Scale bar: 20 µm

Chapter 5

IMPACT OF EMULSIFIERS ON THE TEXTURE AND MELTING PROPERTIES OF REFORMED LOW-FAT AND FULL-FAT CHEDDAR CHEESE

5.1. ABSTRACT

In this study, the use of different types of emulsifiers during the process for reforming low-fat and full-fat Cheddar cheese was investigated. Eight different types of emulsifiers at the 4% (w/w) level were added to cheese, prior to reforming. These types included anionic emulsifiers: citric acid esters of monoglycerides (CITREM), diacetyl tartaric acid esters of monoglycerides (DATEM), sodium stearoyl lactylate (SSL), zwitterionic: lecithin and non-ionic: distilled monoglycerides (DM), lactic acid esters of monoglycerides (LACTEM), acetic acid esters of monoglycerides (ACETEM) and sorbitan tristearate (STS). Control reformed cheeses (i.e., made without any emulsifiers) were prepared for both low-fat and full-fat cheese. Textural and rheological analyses were performed on cheese bases and reformed cheeses that had been stored for 2 wk at 4°C. Dynamic rheological properties of cheese were measured using small amplitude oscillatory rheology during heating from 5 to 85°C. Textural properties were determined

with Texture Analyzer. Melt properties were determined using UW-Melt-profiler. Use of SSL reduced the hardness of low-fat cheese and made it very sticky and soft. The use of CITREM, DATEM and STS appeared to have a strengthening effect on cheese texture. DATEM and SSL exhibited significantly lower loss tangent maximum (suggesting poorer meltability) during heating as compared to control cheese. At low measurement temperatures, except for STS, non-ionic emulsifiers did not significantly alter the texture of full-fat cheese; however, at high temperatures cheese made with non-ionic emulsifiers differed from control as they had improved meltability. Use of non-ionic emulsifiers seemed to make low-fat cheese more prone to fracture during compression, except for STS. The results of this study showed that the level of cheese fusion, and thus the textural properties of the reformed cheese, can be modified with the use of emulsifiers.

5.2. INTRODUCTION

In the reforming process, cheese is broken down into pieces and then placed together in a container or mold and pressed to reform the shape. The reformation of cheese occurs due to the fusion of cheese particles via interactions between proteins on particle surfaces. At some point during storage individual cheese particles will disappear forming a continuous cheese network. The interactions between caseins in cheese are influenced by many factors, such as, temperature, pH, ionic strength and Ca binding (Lucey et al., 2003), and that in turn influences the reformability and fusion of the cheese protein, as seen in our previous studies on cheese reforming (Chapters 3 and 4). Adjusting the level of fusion between cheese particles during reforming provides an opportunity to manipulate the textural properties of cheese. For instance, reforming a firm cheese can soften its texture if all of the interactions and bonds do not completely recover. One of

the promising applications of this process could be for cheese with reduced and low-fat contents. Reforming low-fat cheese creates discontinuities in cheese matrix, which could help alleviate some of the textural problems (excessive firmness) caused by removal of fat.

In this study, we investigated the impact of adding different types of emulsifiers into cheese prior to the reforming process. Emulsifiers have been used in many food systems for various functions. Their major role is stabilizing water and oil mixtures. In addition to that they have been used for various applications such as dough conditioning, antistaling, whipping, dispersing, hydrating, inhibiting crystallization, antisticking, lubricating and release agent in several foods (Hasenhuettl and Hartel, 1997). Emulsifiers can interact with proteins, thereby influencing the reformability and texture of the cheese. Lucey et al. (2008) showed that the addition of mono-diglycerides to non-fat processed cheese improved its textural properties and meltability, which was attributed to possible interactions between emulsifiers and caseins. The lower molecular weight emulsifiers could be preferentially adsorbed to the caseins via hydrophobic regions, thus disrupting hydrophobic association of the caseins and weakening casein-casein interactions.

Emulsifiers are amphiphilic molecules having both hydrophilic head groups and hydrophobic tails. Depending on their ionic character, they can be divided into three classes; anionic, cationic and non-ionic. Chemical structure of the emulsifiers selected for our trials are given in Figures 5.1 to 5.4. Since cationic emulsifiers are not food grade, we did not use them in our study. Some properties of the emulsifiers used in our experiments are given in Table 5.1.

Caseins are also amphiphilic molecules having hydrophilic and hydrophobic residues. Therefore they could bind to the hydrophobic and hydrophilic sites on emulsifiers. Several possible interactions might occur in the cheese matrix due to the addition of emulsifiers (Hasenhuettl and Hartel, 1997). One possible interaction is casein-emulsifier interactions through the binding of hydrophobic and hydrophilic sites. Anionic emulsifiers could also interact with caseins by charge interactions. The second possible type of interaction is emulsifier-emulsifier interactions, where the monomer emulsifiers interact with each other to form micelles or other type of structures (e.g. mesophases). The third possible type of interaction is protein-protein interactions due to incompatibility or phase separation.

Functionality of emulsifiers can be divided into three main categories: (1) reducing surface tension at oil-in-water (O/W) interfaces and stabilizing the emulsion by forming phase equilibria between O/W emulsifiers at the interface, (2) interacting with starch and proteins in foods, which can modify texture and rheological properties, and (3) modifying crystallization of fats and oils (Krog and Lauridsen, 1976). Adsorption properties of the emulsifiers at interfaces determine their functionality and use for various applications in food industry. Destabilization of fat with emulsifiers in ice-cream production is an example to the competitive interfacial adsorption of protein and emulsifiers (Moonen and Hans, 2004). Another example for competitive adsorption of emulsifiers is the de-emulsification of fat and oiling off in process cheese with the addition of emulsifiers (Zehren and Nusbaum, 1992). Emulsifiers can displace the proteins from the interface depending on their concentration and type, and the amount of protein in the system. It has been found that non-ionic hydrophilic emulsifiers are more effective than hydrophobic

emulsifiers in displacing the proteins from interfaces (Chen and Dickinson, 1993). Cooperative interfacial adsorption of protein and emulsifiers can also take place. DATEM do not displace proteins much, but rather forms a mixture of protein-emulsifier film when the protein is bound to oil droplet surface by both hydrophobic and electrostatic forces (Dickinson et al., 1996).

Emulsifiers have been used in bakery products for longer self life, improved texture and better dough processing purposes. They provide mentioned improvements by functioning as starch complexing, protein strengthening and aeration agent in dough. Hydrophobic interactions between proteins and emulsifiers cause unfolding and denaturation, enhancing the interfacial absorption and emulsion stabilization. Anionic emulsifiers are commonly used as dough strengtheners (e.g. SSL, DATEM). The association of the hydrophobic groups of emulsifiers and gluten incorporates the negative charge into complex, which brings the pH up to isoelectric point of gluten, promoting their aggregation and strengthening of the dough. On the other hand, nonionic emulsifiers disrupt the hydrophobic portion of the protein and reduce dough viscosity and elasticity (Hasenhuettl and Hartel, 1997).

Lee et al. (1996) suggested that anionic emulsifiers can increase the net negative charge of the proteins in cheese and therefore promote repulsion between caseins. The use of emulsifiers in reduced-fat systems can improve the rheological properties and they might function as fat extenders (Flack, 1996). Drake et al. (1999) reported that use of lecithin in reduced-fat process cheese improved its textural characteristics, which were partly attributed to the additional moisture retention in the cheese. Lecithin has been used as antisticking agent in process cheese (Zehren and Nusbaum, 1992).

The objective of this study was to investigate how the addition of different types of emulsifiers during reforming could influence the textural properties of cheese. Since the extent of reformation of cheese depends on the type and strength of the interactions between caseins, incorporation of molecules that would interrupt and modulate those interactions will change the structure of cheese produced after reforming. Emulsifiers were selected for incorporation into cheese on the basis of different likely interactions with proteins; by examining how different types of emulsifiers impact reformed cheese we can try to explain what type of interactions may have occurred between emulsifiers and casein.

5.3. MATERIALS AND METHODS

5.3.1. Cheesemaking

Full-fat Cheddar cheese (aged over 60 days) was obtained commercially (Tillamook Cheese, Tillamook, OR). Low-fat Cheddar cheese base was aged for 6 months at 4ºC and was made using the procedure shown in Table 5.2. Pasteurized milk having 0.4 to 0.5% fat content was pre-acidified to pH 6.5 with citric acid. Milk was inoculated with starter (DSM DELVO-TEC LL50) and adjunct cultures (Chr. Hansen's LH32). Annatto was added to color cheese (Chr. Hansen's Cheese Color 2X). Double strength chymosin (Chymax extra, Chr. Hansen's, Milwaukee, WI) was added to the milk at 33ºC. The coagulum reached sufficient firmness after 50 min and was cut with 0.95 cm knives, allowed to heal for 5 min, and then gently (manually) stirred for 5 min before cooking. Curds were then cooked to 34ºC while stirring for about 15 min. Whey was drained, and after the curd was matted, it was cut into blocks, turned upside down and stacked for about 30 min. When pH reached to 6.2, the curds were milled and rinsed with

cold water. After salting (0.3% (w/w) of the weight of the milk in the vat) curd was pressed into hoops that were lined with cheese cloth and pressed for 2 h at 0.34 MPa and vacuum sealed.

5.3.2. Cheese reforming

A food processor (Cuisinart, USA) was used for grinding the cheese. 8 types of emulsifiers were used: citric acid esters of monoglycerides (CITREM), diacetyl tartaric acid esters of monoglycerides (DATEM), sodium stearoyl lactylate (SSL), lecithin, distilled monoglycerides (DM), lactic acid esters of monoglycerides (LACTEM), acetic acid esters of monoglycerides (ACETEM) and sorbitan tristearate (STS). Soy lecithin was provided by ADM (Archer Daniels Midland Company, Decatur, IL) while all other emulsifiers were provided by Danisco (New Century, Kansas). Physical and chemical specifications of the emulsifiers are given in Table 5.1, as provided by the manufacturers. For the low-fat cheese, 250 g of cheese was loaded to the food processor after cutting them into cubes. Grinding and mixing the emulsifiers were done in three steps; after 20 sec of initial grinding step, 10 g of melted (~70-75ºC) emulsifier (4%, w/w) was added slowly using a dropper over 60 sec while continuing to grind the cheese and after a final 30 sec, grinding was completed. The ground cheese were then filled into polystyrene cups (89 x 89 x 25 mm weighing dish, Fisher Scientific) (Fig. 5.5) and pressed at ~1 MPa pressure for 1 h with a laboratory hydraulic press (Carver Press, Wabash, IN). The repressed cheese samples were then vacuum-packed and held for 2 wk until analysis. The reformation of the full-fat cheese was done similarly except for grinding time, and moulds used for reforming. The final 30s of grinding was omitted since full-fat cheese tend to clump together with the prolonged grinding times. Instead of plastic cups,

syringes were used to reform the cheese and they did not require any further pressing. A control reformed cheese was prepared for both full-fat and low-fat cheese where no emulsifiers were added during grinding and reforming.

5.3.3. Compositional Analysis

Milk samples were analyzed for total solids, fat, protein, and casein (Marshall, 1992). The total solids, fat, protein and pH of cheese were determined (Marshall, 1992). The salt content of the cheese samples measured using Corning Salt Analyzer (Marshall, 1992) and the total calcium content was analyzed by inductively-coupled Argon plasma emission spectroscopy (ICP) (Choi et al., 2007). Buffering index of cheese samples was determined using acid-base titration method as described by Hassan et al. (2004)

5.3.4. Textural analysis

A TA.XT2 Texture Analyzer (Stable Micro Systems, Godalming, Surrey, UK) with a TA-25 probe (50 mm diameter) and TA-90A flat plate was used for texture testing. Cylindrical cheese samples having 16 mm diameter and 17.5 mm height were cut and kept overnight at 4°C in sealed plastic bags prior to the analysis. Texture Profile Analysis at 20% compression and uniaxial compression test at 80% compression level was performed on cheese samples at 4°C. Texture parameters were calculated as described by Bourne (2002).

5.3.5. Melt profile analysis

Melt Profile Analysis was performed using the UW Melt-Profiler developed by Muthukumarappan et al. (1999). For melt analysis, cylindrical cheese samples having 30 mm diameter and 7 mm thickness were cut with cork borer cylinders and held overnight

at 4°C in sealed plastic bags. Cheese samples were placed in the melt profiler oven held at 72°C immediately after taking samples out of the refrigerator (at 4°C). A thermocouple was inserted in the center of the cheese disc and then placed between two aluminum plates having a dry film lubricant and a layer of oil sprayed on them. Decrease in cheese height during melting was measured over 15 min by a linear variable differential transformer, which was connected to the top plate. Degree of flow (DOF) was calculated as the percentage decrease in the original cheese height when cheese temperature reached 55°C.

5.3.6. Rheological Analysis

Rheological properties were determined using a Paar Physica (UDS 200, Physica Messtechnik GmbH, Stuttgart, Germany) controlled stress rheometer, with a serrated parallel plate geometry. Cheese disks of 50 mm diameter and 3 mm thickness were cut out with a cylindrical stainless steel cork borer from the 3 mm thick cheese slices obtained using a meat slicer. Cheese discs were then sealed in plastic bags and held at 4°C overnight prior to the testing. When loading samples, in order to maintain good contact between plate and cheese, the upper plate was lowered onto cheese not to exceed a normal force of 2 N and then sample was allowed to relax for about 15 min to a relatively constant normal force reading of ~0.8 N before starting the test. A thin layer of vegetable oil was applied around the cheese sample to prevent moisture loss. Rheological properties of cheese were evaluated with an applied strain of 0.2% and a frequency of 0.1 Hz. Temperature sweeps were performed from 5 to 85°C at the rate of l°C/min. Storage modulus (G'), and loss tangent (LT) were the parameters determined from dynamic small amplitude oscillatory shear rheology tests.

5.3.7. Statistical Analysis

Statistical analysis was performed using SPSS (SPSS version 13.0). Analysis of variance (ANOVA) was performed to determine the effect of different types of emulsifiers on the composition and textural properties of cheese base and reformed cheese samples at $p \leq 0.05$ significance level. Differences between means were analyzed using Tukey's method for multiple comparisons of means.

5.4. RESULTS

5.4.1. Milk and cheese composition

The chemical composition of cheese milk used for the manufacturing of low-fat cheese samples, and composition of the low-fat and full-fat cheese before, and after, reforming are given in Tables 5.3 and 5.4. Low-fat cheese samples had higher protein and total calcium than the full-fat cheese samples due to their higher protein: fat ratio. Addition of emulsifiers increased the fat content of all cheese samples by ~3% due to the contribution of lipids from EM (Table 5.3 and 5.4). There was a significant decrease in the pH of low-fat cheese with the addition of emulsifiers (Table 5.5). Cheese samples reformed with DATEM had the significantly lowest pH for both low-fat and full-fat cheese. Both for the low-fat and full-fat cheese, control had relatively higher buffering capacity (Figures 5.6 and 5.7). We were not able to determine the buffering capacity of lecithin added full-fat cheese due to the limited amount of sample.

5.4.2. Visual attributes

Pictures of low fat cheese samples reformed with the addition of different emulsifiers are given in Figures 5.8 and 5.9. All cheese samples reformed well enough to hold

together as a single mass. DATEM and DM formed white spots that were scattered throughout the low-fat cheese samples probably because of the white color of those emulsifiers. In addition they also could have been solidified before completely adsorbed in cheese.

5.4.3. Texture properties

The uniaxial compression profiles of both low-fat and full-fat cheese before, and after, reforming without the use of emulsifiers (control) are shown in Fig. 5.10. The texture analysis results are presented in Table 5.6 and Fig. 5.11 for full-fat cheese and in Tables 5.7 and 5.8 and Figures 5.12 and 5.13 for low-fat cheese samples before and after reforming with different types of emulsifiers.

Control

Full-fat and low-fat Cheddar cheese exhibited differences in their reforming behavior as reflected by their recovery in their texture properties after reforming. After reforming a dramatic decrease was observed in the hardness of full-fat cheese without emulsifiers as measured by uniaxial compression test (Fig 5.10). On the other hand, reforming the low-fat cheese in the absence of emulsifiers increased its hardness (Fig 5.10).

Non-ionic emulsifiers

Except for STS, the addition of non-ionic emulsifiers reduced the hardness of reformed low-fat cheese compared to the control (as determined at 80% compression). The addition of STS did not change the hardness value of low-fat reformed cheese measured at 80% compression. However, at lower compression levels, e.g. 20% compression, cheese reformed with STS and DM had higher hardness than the control, which was similar to the cheeses reformed with other non-ionic emulsifiers (Table 5.8).

Low-fat cheese made with DM had significantly higher initial slope than other treatments and visible fracture could be observed in the uniaxial compression curves (Fig. 5.8), which probably resulted in a low hardness value when measured at high compression levels. Since fracture occurred above 20% compression, the TPA results (Table 5.8) (performed at 20% compression) indicated that cheese made with DM were harder than the control.

For full-fat cheese, the addition of non-ionic emulsifiers did not change the hardness as determined at 80% compression except for STS (Table 5.6); STS increased the hardness of reformed full-fat cheese compared to the control.

The adhesiveness force obtained at 80% compression was smaller for low-fat cheeses made with DM and LACTEM, while low-fat cheeses made with ACETEM and STS had similar adhesiveness to the control (Table 5.7). Adhesiveness obtained at 20% compression was smaller than the control for all non-ionic emulsifiers in low-fat cheese (Table 5.8).

The full-fat cheese samples made with ACETEM and DM were more adhesive than the control while STS and LACTEM were similar to the control cheese (Table 5.6).

Anionic emulsifiers

CITREM did not change the hardness of low-fat cheese as measured at 80% compression while SSL and DATEM resulted in lower values than the control (Table 5.7). However, measurements at 20% compression showed that CITREM and DATEM increased the hardness significantly while use of SSL reduced it (Table 5.8). The reason for the low force response of cheese reformed with DATEM at 80% compression was

due to the occurrence of fracture (Figures 5.11 and 5.8). Cheese made with DATEM was actually the hardest sample but it was also very brittle.

Hardness of the full-fat cheese as measured at 80% compression increased with the use of CITREM, while SSL and DATEM were similar to control (Table 5.6).

DATEM and CITREM appeared to make the low-fat cheese stiffer (Table 5.8). However they exhibited quite different textures from each other. DATEM produced cheese that was stiff but very brittle and crumbly, in other terms cheese made with DATEM was short in texture. Cheese made with CITREM was also stiff but did not exhibit a brittle structure. Figure 5.14 shows pictures of the reformed low-fat cheese samples after compression by the uniaxial compression test. As seen in the deformed cheese pictures, DATEM collapsed into pieces due to its very brittle texture (Fig. 5.14). SSL was the softest cheese, and it showed a viscous deformation by not regaining much of its original shape – it was almost completely flattened out by the compression (Fig. 5.14).

Use of SSL dramatically increased the adhesiveness force (at 80% compression) of the low-fat cheese (Table 5.7). While cheese reformed with CITREM was similar to control, DATEM greatly reduced the adhesiveness (Table 5.7). Adhesiveness values as obtained at 20% compression (Table 5.8) were also in agreement with results obtained at 80% compression except for CITREM, which had a reduced adhesiveness as compared to control.

For the full-fat cheese, adhesiveness force was higher than the control for cheese reformed with DATEM, and the other anionic emulsifiers remained same as the control (Table 5.6).

The use of SSL and DATEM made low fat cheese less springy (Table 5.8), while CITREM cheese remained the same as the control. Low-fat cheese with DATEM was less cohesive then the control (Table 5.8). SSL reduced the gumminess and chewiness of low-fat cheese greatly as compared to the control (Table 5.8).

Zwitterion emulsifiers

Low-fat cheese reformed with lecithin showed similar hardness to the control at 80% compression (Table 5.7), while it was harder at 20% compression levels (Table 5.8).

Full-fat cheese reformed with lecithin was softer than the control at the 80% compression (Table 5.6). Adhesiveness force was higher for full-fat cheese with lecithin.

For low-fat reformed cheese made with lecithin was less adhesive (Table 5.8).

5.4.4. Rheological properties

Changes in G' and LTmax values during heating from 5 to 85C for both low-fat and full-fat cheese, before and after reforming, without the use of emulsifiers (control), are shown in Figures 5.15 and 5.16. The rheological properties are presented in Table 5.9 and Figures 5.17 and 5.18 for full-fat cheese and in Table 5.10 and Figures 5.19 and 5.20 for low-fat cheese samples.

Control

In reformed full-fat cheese samples, a shoulder was observed in the G' profile at around 20ºC, which was absent in the profile of cheese base before reforming (Fig 5.15). The difference between the G' of the low-fat control cheese before and after reforming was not as large as the difference observed for the full-fat control cheese before and after reforming (Tables 5.10 and 5.9, respectively). Compared to the base, reformed control low-fat cheese did not show any significant difference in the G' values at 8 or 40ºC,

while the G’ values at 8 or 40ºC of the reformed full-fat cheese were lower than the base cheese (Fig. 5.15). There was a decrease in LTmax value of both the control low and full-fat cheese after reforming (Fig. 5.16).

Non-ionic emulsifiers

The G' values at 8ºC of the full-fat cheese samples reformed with non-ionic emulsifiers was similar to the control except for cheese made with STS (Table 5.9). Full-fat cheese made with STS had higher G’ values at 8ºC compared to the control reformed cheese. At higher measurement temperatures, no differences were observed between the G' values of the full-fat cheeses reformed with non-ionic emulsifiers and the control.

In the case of low-fat cheese, the G' values at 8ºC were higher for cheese made with DM, and similar to the trend for full-fat cheese where the addition of STS gave higher G' values (Table 5.10). At a measurement temperature of 40ºC, the G' values for DM were higher than control cheese, while at 70ºC all non-ionic emulsifiers had higher G' values than the control low-fat cheese.

Full-fat cheese reformed with LACTEM and ACETEM exhibited higher LTmax values than the control cheese, while other non-ionic emulsifiers had similar LTmax values to the control (Fig. 5.18).

For the low-fat cheese, the control had higher LTmax values than the cheeses made with all non-ionic emulsifiers, except for STS, which exhibited a similar loss tangent curve to the control cheese (Fig. 5.20). At high temperatures (e.g. 70ºC) low-fat cheeses made with all the non-ionic emulsifiers exhibited higher G' values than the (Tables 5.9 and 5.10).

Anionic emulsifiers

The G' values at 8ºC of the full-fat cheese reformed with DATEM were lower than the control, and as the temperature was increased to 70ºC the G' values became higher than the control (Table 5.9). All anionic emulsifiers reduced the LTmax of reformed full-fat cheese compared to the control (Fig. 5.18).

For low-fat cheese, the use of SSL or CITREM increased the G' values of cheese at 8ºC; while at 40ºC all anionic emulsifiers exhibited higher G' values than the control cheese. The G' values at 70ºC of the low-fat cheese were higher than the control for all emulsifiers except for CITREM, which had larger G' values at 70ºC than the control cheese (Table 5.10). The LTmax values of the low-fat cheese reformed with SSL and DATEM were lower than the control, while the LTmax of the cheese with CITREM was similar to the control (Fig. 5.20).

Zwitterion emulsifiers

Full-fat cheese samples reformed with lecithin exhibited a lower LTmax than the control (Fig. 5.18). The G' values at 8 ºC were similar to the controls for both low-fat and full-fat cheese made with lecithin; however at 70ºC, the G' values became significantly higher (Tables 5.9 and 5.10). Loss tangent profile of reformed low-fat cheese was not influenced by the addition of lecithin (Fig. 5.20).

5.4.5. Melting characteristics

There were no differences in the DOF (at 55ºC) of reformed low-fat cheese made without emulsifiers compared to their respective bases, while a decrease in DOF was observed after reforming (control) of full-fat cheese (Fig. 5.21). Degree of flow (DOF) values at 55ºC obtained by UW melt-profile analyzer for low-fat and full-fat cheese

before and after reforming with and without the addition of emulsifiers are presented in Table 5.11.

Addition of emulsifiers to low-fat cheese did not change the meltability of the reformed cheese as determined by UW-Melt profiler (Fig. 5.22).

Addition of emulsifiers to reformed full-fat cheese improved meltability; non-ionic emulsifiers showed a greater degree of flow than the anionic ones and lecithin had the greatest meltability (Fig. 5.23).

5.5. DISCUSSION

5.5.1. Composition

There was a decrease in pH with the addition of emulsifiers to low-fat cheese whereas for full-fat cheese only DATEM reduced the pH (Table 5.5). However, low-fat cheese had a much higher protein and calcium content compared to full-fat cheese, which should have produced greater pH buffering (when acidic emulsifiers were added). Decrease in pH with the addition of DATEM was also observed by Salim (2009) when it was added to process cheese. DATEM has a low pH (2-3) due to its free carboxyl group (Krog, 1997). The pH of the DATEM we used was 2.1, so it reduced the pH of the cheese significantly. Titration results showed that, both for the low-fat and full-fat cheese, control had relatively higher buffering capacity than cheese samples reformed with emulsifiers (Figures 5.6 and 5.7), which could indicate that the addition of emulsifiers might have solublized some of the insoluble Ca associated with caseins.

5.5.2. Control

Reforming the control full-fat cheese created a larger disruption in cheese structure than reforming the control low-fat cheese as shown by greater fracture, lower hardness (Fig. 5.10), and lower G' values (Fig. 5.15). Only about 20% of the milk fat is in solid state at 20ºC (Shukla et al., 1994). Liquid fat in cheese could have helped to coat the surfaces of cheese particles of the ground cheese. This fat coating around cheese particles could prevent the fusion of the cheese particles at the contact surfaces of the cheese granules, as fusion of the cheese occurs through casein interactions.

For the low-fat cheese, the G' values at 8ºC (Table 5.10) and hardness values obtained at 20% compression did not change after reforming (Table 5.8). However, there was an increase in the hardness of low-fat cheese after reforming as determined at 80% compression (Table 5.7). The lower hardness of the cheese base at high compression could be due to its fracture (Fig. 5.10). Low-fat cheese was 6 m old. Several studies have shown an increase in brittleness with age due to proteolysis (Rosenberg et al., 1995; Watkinson et al., 2001; Lucey et al., 2005). Grinding the low-fat cheese and then reforming probably removed any localized brittleness by creating a smoother, more homogenous cheese. The reason for the formation of a more homogenous cheese texture after reforming aged cheese could be the better reorganization and fusion of the caseins that are already broken down into smaller fragments due to proteolysis. Results (Figures 5.10 and 5.15) showed that the bonds and interactions between caseins were restored to a greater extent in low-fat cheese compared to full-fat cheese. Low-fat cheese is a simpler system of mostly protein and water. Liquid fat droplets in full-fat cheese would be

disrupted and mixed throughout the cheese having a negative impact on fusion throughout the protein matrix.

There was a decrease in LTmax after reforming the controls for both low-fat and full-fat cheeses (Fig. 5.16) indicating a lack of mobility of the protein matrix at high temperatures compared to cheese base (Fig. 5.16). Apparently, grinding and reforming the aged cheese samples modified the texture of the cheese in a way that permits more protein aggregation at high temperatures with the increase in hydrophobic interactions. In process cheese, greater protein aggregation is called creaming and this process results in reduced meltability (Shirashoji et al, 2010).

5.5.3. Non-ionic emulsifiers

For most non-ionic emulsifiers there was no significant change in hardness when they were added to reformed full-fat cheese (Table 5.6). Interactions between non-ionic emulsifiers and proteins could be weak due to the absence of charged groups on this type of emulsifiers (Krog, 2004).

In the case of full-fat cheese, non-ionic emulsifiers would likely have dispersed in the fat phase due to their low HLB value, rather than interacting with proteins. Therefore, non-ionic emulsifiers were unlikely to have a very large impact on the cheese network formed after reforming.

However, in low-fat cheese due to the great reduction in fat content, emulsifiers might have more opportunity to interact with proteins, but since the interactions between those non-ionic emulsifiers and proteins were probably weaker than the interactions formed between proteins after reforming, low-fat cheese containing emulsifiers fractured

more readily at higher compression (Fig. 5.12) resulting in lower hardness values (Table 5.7).

STS exhibited a different trend than the other non-ionic emulsifiers. Cheese samples reformed with STS seemed to have a firmer structure than the control for both full-fat (Fig. 5.11 and Tables 5.6 and 5.9) and low-fat cheese (Fig. 5.13 and Tables 5.8 and 5.10). Apart from its ionic character, the molecular structure and the interaction properties of STS might play a role in its behavior in cheese. STS can self-associate and form dynamic networks (Rehage et al., 2002). Strong attractive interactions between the STS molecules lead to a two dimensional self-association process, eventually forming a viscoelastic network. This self-association behavior of STS is attributed to van der Waals attractions between the three long paraffin chains attached to the sorbitan, in addition to the hydrogen bonding and strong hydrophobic interactions (Rehage et al., 2002). This self-association ability of the STS might have contributed to the strengthening of the cheese structure formed after reforming by developing a strong viscoelastic network to help hold cheese particles together.

Compared to the control cheese there was an increase in meltability of the full-fat cheese reformed with the addition of all types of emulsifiers. Full-fat cheese exhibited the highest DOF with the addition of non-ionic emulsifiers (Fig. 5.23). In a study on milk based composite gels, when Tween 80 was added, gel strength did not change as much as the case when fat was stabilized by the proteinaceous emulsifiers (Xiong and Kinsella, 1991). It was suggested that Tween 80 displaced the proteins from the fat membrane making the fat phase unable to interact with protein matrix therefore fat globules behaved like plasticizers during shearing (Xiong and Kinsella, 1991). Although the full-fat cheese

was not made of homogenized milk, the interaction of emulsifiers with fat phase could still cause a greater weakening in cheese matrix at high temperatures. At the high temperatures interactions between the emulsifiers and with fat and proteins might have promoted the flow of the cheese either by promoting a plasticizing effect of fat or causing greater dispersion of proteins.

We did not observe any difference in meltability when emulsifiers were added to low-fat cheese compared to control (Table 5.11), however a significant decrease in LTmax values was observed with the addition of most emulsifiers (Fig 5.20).

In full-fat cheese non-ionic emulsifiers did not exhibit a decrease in the LTmax values as compared to control (Fig. 5.18). This could be due to the interaction of nonionic emulsifiers with the fat phase rather than the proteins.

The reason of the different trends between melt profile anlaysis results and loss tangent values could be due to differences in the time course and temperature of the two tests. The LTmax values occurred at the range of 63 to 70ºC during heating at the rate of 1 ºC/min while flow/melt monitored until the cheese samples reached around 63ºC within a 15 min of heating period in an oven at constant temperature (72ºC). The faster heating regime and the weight of the upper plate could have caused more flow of the cheese in the melt profile analysis test especially in the presence of liquefied fat (lubricated) while that same cheese exhibits a low LTmax. The slow temperature ramp under non-destructive conditions might have allowed greater extend of hydrophobic interactions in cheese samples with added emulsifiers, promoting greater hydrophobic aggregation of the proteins.

5.5.4. Anionic emulsifiers

Anionic emulsifiers were more effective in changing the overall texture properties of the cheese than other emulsifiers after reforming as indicated by the greater decrease in LTmax values with the use of anionic emulsifiers (Fig. 5.18 and 5.20) and from the texture profile analysis results (Table 5.8). Anionic emulsifiers probably had a stronger interaction with caseins via their charged groups. Anionic emulsifiers can interact with proteins through ionic bonds, hydrophobic interactions and hydrogen bonds (Giroux and Britten, 2004) therefore they can alter the structure, physico-chemical and rheological properties of cheese. A general model to describe how anionic emulsifiers interact with globular whey proteins was previously published (Jones, 1975, 1983; Oakes, 1974), and this model suggests that there could be three stages: 'specific binding', 'non-cooperative binding' and 'cooperative binding'. In the first stage, the emulsifier binds with specific sites on the surface of the protein. Ionic bonds may be formed between the negatively charged groups of the surfactants and the cationic amino acid residues of the protein. Hydrophobic interactions may take place between the aliphatic chains of the emulsifiers and the non-polar protein surface regions. Hydrogen bonds may potentially be formed between the oxygen groups of the emulsifiers and the nitrogen groups of the peptide linkages (Lundahl et al., 1986). In addition, the protein structure can be modified as a result of either electrostatic repulsion or the penetration of hydrophobic parts of the emulsifier to the hydrophilic regions of the protein, and finally saturation of all potential binding sites on the protein.

Caseins possess a net negative charge at the pH of cheese (pH 5.6). Association of anionic emulsifiers to caseins should increase the amount of negative charges in the

system (Lee et al., 1996). An increase in the number of negative charges would increase the electrostatic repulsion between the caseins. Antipova et al. (2001) showed that Na-caseinate exhibited a slight dissociation when treated with CITREM as indicated by the decreased value of the weight average molecular weight and increase in the hydrodynamic radius and they suggested that this dissociation might be due to the repulsion between similar charges and close-spaced charged groups of the added CITREM molecules. Addition of CITREM to cheese when reforming might have caused swelling of casein particles. Swelling of the casein particles would enlarge the contact area at contact surfaces during reforming and that might have lead to stronger interactions between caseins. That was presumably the reason for the higher hardness of both full-fat and low-fat cheeses reformed with CITREM as compared to control (Table 5.6 and 5.7).

The LTmax values of the cheese reformed with CITREM were similar to control for the low-fat cheese, while in full-fat cheese a decrease was observed, which could be due to lower protein content and presence of fat in full-fat system. CITREM exhibits self-association. CITREM can form stable emulsions with high orientation above melt point in liquid phase as all esters of dicarboxylic or tricarboxylic acids and monoglyserides show a high degree of long-range order in the melted state (Krog, 2005). They show this tendency towards thermotropic mesomorphism due to the strong molecular interactions between participating polar groups. This self-association behavior may help create a strong and viscoelastic network supporting a stronger cheese structure when added during reforming. DATEM on the other hand do not exhibit mesomorphism (Krog, 2004).

The addition of DATEM resulted in a firm reformed cheese structure as indicated by high hardness (Table 5.8) and G' values (Tables 5.9 and 5.10) values, however, it was

brittle unlike cheeses made with the CITREM. There was a decrease in pH from 5.3 to 5.0 in cheese samples reformed with DATEM, which might be the reason for the brittle texture and low LTmax values as the mobility of the protein bonds and interactions decrease, along with cheese texture becoming brittle at pH $\leq$ 5.0 (Lucey et al., 2003). DATEM can form hydrogen bridges with the amidic groups on gluten proteins (Greene, 1975). When the hydrophobic emulsifier moieties were oriented to the non-polar side chains of the proteins, DATEM can form an intermolecular matrix via hydrogens. This suggests that there might be some possibility of the formation of hydrogen bridges between DATEM molecules and caseins, hence re-enforcing the casein matrix structure and causing the observed increase in hardness (Table 5.8).

SSL reduced the hardness of low-fat cheese (Figures 5.12 and 5.13) and created a pasty cheese structure as indicated by the large increase in adhesiveness (Table 5.8). SSL is more hydrophilic (HLB value=17) than CITREM and DATEM. SSL can form strong interactions with proteins (Boutte and Skogerson, 2004). Lactylates interact strongly with proteins in at least two ways. The stearic acid moiety is believed to form hydrophobic bonds with non-polar regions on the protein. There may also be ion-pairing interactions between carboxylic portion of the lactylate and charged amino acid residues (Boutte and Skogerson, 2004). If the binding between lactylates and proteins is strong enough and the hydrophilic head group is large enough to sterically induce conformational changes, these emulsifiers can disrupt the native structure of protein (Nylander et al., 1997).We presume that this mechanism was responsible for why SSL had disruptive effect on low-fat cheese protein matrix, given its use weakened the cheese and the body became soft and pasty due to the weakening of the interactions between caseins.

In the presence of fat in cheese, SSL did not affect the hardness after reforming. This could be due to relatively lower protein content of the full-fat cheese.

5.5.5. Zwitterions

Lecithin has both acidic and basic groups and behaves as a zwitterion (Knightly, 1989). Low-fat cheese samples reformed with lecithin were harder and less adhesive than the control (Table 5.8). In contrast to low-fat cheese, use of lecithin in reforming full-fat cheese reduced its hardness and made it more adhesive than the control (Table 5.6). This could be due to lower protein content of full-fat cheese and interactions of lecithin with fat. Drake et al. (1996 and 1999) have also found that there was a decrease in the hardness of the reduced-fat cheese when lecithin was used. On the other hand, Lee et al. (1996) reported that process cheese samples made with lecithin had similar hardness, G' values and viscosity to the control. They related this to the fact that lecithin did not alter the net charge of the system. Differences between different studies could be due to wide variation in the composition of different type of lecithins (Bueschelberger, 2004).

A dramatic increase was observed in the meltability of full-fat cheese made with lecithin while reformed low-fat cheese with lecithin had similar meltability to the control (Table 5.11). Lecithin has been used for improving the texture of reduced fat cheese (Drake et al., 1999) due to its fat extending effect. Interaction of lecithin with fat could provide a greater lubrication affect when the cheese is heated.

The G' values of both low-fat and full-fat cheese were higher than the control at 70ºC, indicating a strengthening of the cheese matrix. LTmax values of both cheeses with lecithin were lower than the control (Table 5.9 and 5.10). This could be due to the increase in hydrophobic interactions as the cheese was heated.

5.6. CONCLUSIONS

Addition of emulsifiers influenced the reformability of the cheese and altered the textural and rheological properties depending on the type and molecular characteristics of the emulsifier. The emulsifiers added during reforming the cheese resulted in differences in the textural and rheological properties between full-fat and low-fat cheese especially for the melting properties and this was particularly true for the non-ionic emulsifiers.

In general, anionic emulsifiers were more effective in changing the texture properties of the cheese, probably due to their stronger interaction with caseins via their charged groups. The negative charges of the anionic emulsifiers could increase the electrostatic repulsion between caseins and thereby make the cheese stiffer. CITREM and DATEM increased the firmness of the reformed cheese. However, SSL, although an anionic emulsifier, formed the softest and stickiest cheese in the absence of fat presumably due to the high HLB value of SSL.

This study demonstrated that emulsifiers can alter cheese fusion during reforming and change the textural properties of the reformed cheese, depending on its type and molecular characteristics.

5.7. REFERENCES

Antipova, A. S., Semenova, M. G., Belyakova, L. E. and Il'in, M. M. 2001. On relationships between molecular structure, interaction and surface behavior in mixture: small-molecule surfactant+protein. Colloids and Surfaces B: Biointerfaces 21:217–230.

Bourne, M. C. 2002. Food Texture and Viscosity: Concept and Measurement. 2nd edn. Academic Press, San Diego.

Boutte, T., and Skogerson, L. 2004. Stearoyl-2-lactylates and oleoyl lactylates. Pages 206-224 in Emulsifiers in Food Technology, ed. R. J. Whitehurst. Blackwell Publishing Ltd, Oxford, UK.

Bueschelberger, H. G. 2004. Lecithins. Pages 1-39 in Emulsifiers in Food Technology, ed. R. J. Whitehurst. Blackwell Publishing Ltd, Oxford, UK.

Breene, W. M., Price, W. V., and C. A. Ernstrom. 1964. Changes in composition of Cheddar cheese curd during manufacture as a guide to cheese making by direct acidification. J. Dairy Sci. 47:840–848.

Chen, J. and Dickinson, E. 1993. Time-dependent competitive adsorption of milk proteins and surfactants in oil-in-water emulsions. J. Sci. Food Agric. 62:283-289.

Choi, J., Horne, D. S., and Lucey, J. A. 2007. Effects of insoluble calcium concentration on rennet coagulation properties of milk. J. Dairy Sci. 90:2612-2623.

Dickinson, E., Hong, S. T. and Yamamoto, Y. 1996. Rheology of heat-set emulsions gels containing β-lactoglobulin and small-molecule surfactants. Neth. Milk Dairy J. 50:199-207.

Drake, M. A., Truong, V. D., and Daubert, C. R. 1999. Rheological and sensory properties of reduced-fat processed cheeses containing lecithin. J. Food Sci. 64:744-747

Drake, M., Herrett, W., Boylston, T., and Swanson, B. 1996. Lecithin Improves Texture of Reduced Fat Cheeses. J. Food Sci. 61:639–642.

Flack, E. 1996. The role of emulsifiers in low-fat food products. Pages 213-235 in Handbook of Fat Replacers, eds. S. Roller, and S. A. Jones. CRC Press, Boca Raton, FL.

Greene, F. C. 1975. On the mechanism of the functionality of surfactant dough conditioners. Baker's Digest 49:3-26.

Giroux, H. J., and Britten, M. 2004. Heat treatment of whey proteins in the presence of anionic surfactants. Food Hydrocoll. 18:685-692.

Hasenhuettl, G. L., and Hartel, R. W. 1997. Food emulsifiers and their applications. 1st edn., Chapman and Hall, New York.

Hassan, A., Johnson, M. E., and Lucey, J. A. 2004. Changes in the proportions of soluble and insoluble calcium during the ripening of Cheddar cheese. J. Dairy Sci. 87:854–862.

Hicks, C.L., O'Leary, J., and Holbrook, J. 1985. Effect of lecithin on cheese yield. J. Dairy Sci. 68:1603–1607.

Jones, M. N. 1975. A theoretical approach to the binding of amphipathic molecules to globular proteins. Biochem. J. Cellular aspects 151:109–114.

Jones, M. N. 1983. Physico-chemical studies on the interaction between surfactants and globular proteins. Comunicaciones Presentadas a la Jornadas del Comite Espanol de la Detergencia 14:117–131.

Knightly, W. H. 1989. Lecithin in baking applications. Pages 174-107 in Lecithins: Sources, Manufacture and Uses, eds. B. F. Szuhaj, American Oil Chemists Society Champaign, Illinois.

Krog, N. J. and Lauridsen, J. B. 1976. Food Emulsifiers and their association with water. Pages 67-139 in Food Emulsions, ed. S. Friberg and K. Larsson, Marcel Dekker, Inc., New York.

Krog, N. J. 2004. Food emulsifiers: their chemical and physical properties. Pages 45-91 in Food Emulsifiers, eds. S. Friberg and K. Larson, Marcel Dekker Inc., New York.

Krog, N. J. and Sparso, F. V. 2005. Food emulsifiers: their chemical and physical properties. Pages 45-91 in Food Emulsions, eds. S. Friberg and K. Larson, J. Sjoblom, Marcel Dekker Inc., New York.

Lawrence, R. C., Creamer, L. K., and Gilles, J. 1987. Texture development during cheese ripening. J. Dairy Sci. 70:1748-1760.

Lee, S. K., H. Klostermeyer, Schrader, K. and Buchheim, W. 1996. Rheological properties and microstructure of model processed cheese containing low molecular weight emulsifiers. Nahrung 40:189-194

Lucey, J. A., M. E. Johnson, and D. S. Horne. 2003. Perspectives on the basis of the rheology and texture properties of cheese. J. Dairy Sci. 86:2725–2743.

Lucey, J. A., Mishra, R., Hassan, A., and Johnson, M. E. 2005. Rheological and calcium equilibrium changes during the ripening of Cheddar cheese. Int. Dairy J. 15:645-653.

Lucey, J. A., Brickley, C. A., Govindasamy-Lucey, S., Johnson, M. E., Jaeggi, J. J. and Salim, E. M. 2008. Low-fat and fat-free cheese with improved properties. U.S. Patent Application, No. P07144US.

Lundahl, P., Greijer, E., Sandberg, M., Cardell, S., and Eriksson, K. 1986. A model for ionic and hydrophobic interactions and hydrogen-bonding in sodium dodecyl sulfate–protein complexes. Bioch. Biophysica Acta 873:20–26.

Marshall, R. T. 1992. Standard methods for the examination of dairy products. 16th edn. Am. Public Health Assoc, Washington, DC.

Moonen, H. and B. Hans. 2004. Mono- and diglyserides. Pages 41-58 in Emulsifiers in Food Technology, ed. R. J. Whitehurst, Blackwell Publishing, Boston.

Muthukumarappan K, Wang, Y-C., and Gunasekaran, S. 1999. Modified Schreiber test for cheese meltability evaluation. J. Dairy Sci. 1:1068-1071.

Nylander, T., Arnebrant, M. Bos and Wilde. 2008. Protein/Emulsifier Interactions. Pages 89-156 in Food Emulsifiers and Their Applications, 2nd edn, eds. G. L. Hasenhuettl and R. W. Hartel, Springer, New York.

Oakes, J. 1974. Protein–surfactant interactions. J. Chem. Soc. Faraday Trans. 1, J. Physical Chem. 70:2200–2209.

Rehage, H., Achenbach, B., and Klaerner, F.-G. 2002. Ultrathin cross-linked networks at the interface between oil and water: structure, properties, and preparative perspectives. Langmuir 18:7115-7119.

Rosenberg, M., Wang, Z., Chuang, S. L., and Shoemaker, C. F. 1995. Viscoelastic property changes in Cheddar cheese during ripening. J. Food Sci., 60:640–644.

Salim, E. M. 2009. The impact of different types of emulsifiers on the functional properties of low-fat process cheese. M.S. Thesis, University of Wisconsin, Madison.

Shirashoji, N., Jaeggi, J. J. and Lucey J. A. 2010. Effect of sodium hexametaphosphate concentration and cooking time on the physicochemical properties of pasteurized process cheese. J. Dairy Sci. 93:2827–2837

Shukla, A., A. R. Bhaskar, S. S. H. Rizvi, and S. J. Mulvaney. 1994. Physiochemical and rheological properties of milk fat made from supercritically fractionated milk fat. J. Dairy Sci. 77:45–54.

Stauffer, C. E. 2005. Emulsifiers for the food industry. Pages 229-267 in Bailey's Industrial Oil and Fat Products, Vol. 4, Edible Oil and Fat Products: Products and Applications, 6th Edn, ed. F. Shahidi, John Wiley & Sons, Inc.

Watkinson, P., Coker, C., Crawford, R., Dodds, C., Johnston, K., McKenna, A., and White, N. 2001. Effect of cheese pH and ripening time on model cheese textural properties and proteolysis. Int. Dairy J. 11:455–464.

Xiong, Y. L., and Kinsella, J. E. 1991. Influence of fat globule membrane composition and fat type on the rheological properties of milk based composite gels. II. Results. Milchwissenschaft 46:207-211

Zehren, V. L., and Nusbaum, D. D. 1992. History of cheesemaking. In Process Cheese, Cheese Reporter Publishing Co., Inc., Madison, WI.

Table 5.1 Physical and chemical properties of emulsifiers (as provided by manufacturers: Danisco, New Century, KS; ADM Inc., Archer Daniels Midland Company, Decatur, IL)

Emulsifiers	Physical/ chemical specifications	
Anionic		
Citric Acid Esters of Monoglycerides (GRINDSTED® CITREM N 12 VEG KOSHER)	Citric acid	min. 12%
	Acid value	10-25
	Saponification value	220-250
	Iodine value	max. 3
	pH of aqueous dispersion (5%)	5-6
	Dropping point	approx. 64ºC
	Form	coarse powder
Di-Acetyl Tartaric Ester of Monoglycerides (PANODAN® FDP K)	Saponification value	380-425
	Acid value	62-76
	Iodine value	max. 3
	Dropping point	approx. 56ºC
	Form	powder
Sodium Stearoyl Lactylate (GRINDSTED® SSL P 55 VEG)	Ester value	150-190
	Acid value	60-80
	Iodine value	max. 2
	Lactic acid content	31-34%
	Sodium content	3.5-5.0%
	Melting point	approx. 50ºC
	Form	beads

Non-ionic

Acetic Acid Esters of Monoglycerides (GRINDSTED® ACETEM 50-00 P KOSHER)	Degree of acetylation Iodine value Acid value Saponification value Dropping point Form	0.5 max. 2 max. 2 approx. 285 approx. 40ºC plastic
Distilled Monoglycerides (GRINDSTED® PS 211 K-A)	Monoester content Free glycerol F.F.A. Iodine value Dropping point Form	min. 72% max. 1.5% max. 2% 25-30 approx. 60ºC beads
Lactic Acid Esters of Monoglycerides (GRINDSTED® LACTEM P 22 K-A)	Lactic acid content Iodine value Saponification value Acid value Free glycerol Melting point Form	20-25% max.2 270-300 max. 4 max. 1% approx. 44ºC beads
Sorbitan Tristearate (GRINDSTED® STS 30 KOSHER)	Iodine value Saponification value Hydroxyl value Acid value Melting point Form	max. 2 176-188 66-80 max. 7 approx. 55ºC beads

Zwitterion		
Lecithin (soy lecithin) (BEAKIN™ LV3 Lecithin)	Moisture (%) Color, Gardner Acetone insolubles (%) Acid value (mg KOH/g) Hexane insolubles (%) Viscosity (Stokes @77ºC) Effective HLB Form	0.22 12 32.94 16.1 0.025 2.8 approx. 2 transparent liquid

Note: Lecithin was obtained from ADM while all other emulsifiers were obtained from Danisco

Table 5.2 Manufacturing protocol for the low-fat cheddar cheese

Operation	**Time (min)**		**°C, pH, TA**
Milk - 0.40 to 0.50 % Butterfat	1678 kg	TA	0.16
pasteurized at 73°C for 19 s	0.45% fat	pH	6.65
Preacidify Milk	-30 min	Temp	6°C
pH target **6.50**		pH	6.5
Dilute citric acid 4:1 with water	~2900 g	g dilute acid	
Add Starter Culture	0 min	Temp	---
DSM DELVO-TEC LL50 DSF		TA	0.18
	381 g	pH	6.50
Add Culture Adjunct 0.44 g/100 kg milk	9.5 g		
Chr. Hansen's LH32			
Add Double Strength Annatto	0 min		
Chr. Hansen's Cheese Color 2X	105 ml		
Add Coagulant	30 min	Temp	33°C
Chr Hansen Chymax Extra	142 g	pH	6.45
Cut @ 50 min	80 min	TA	0.13
1.3 cm knives		pH	6.40
Start Cook	90 min	Temp	33°C
Reach Cook Temp of 34°C	105 min	Temp	34°C
		whey-pH	6.30
		curd-pH	6.10
Drain @ 5 min	120 min		
Cut, Turn, and Stack 2 High	135 min	TA	---
pH target **6.20**		curd-pH	6.05
Mill	165 min	TA	---
pH target **5.90**		curd-pH	5.90
Cold Water Rinse	170 min		
Rise curd for 15 seconds, no hold			
Add Sodium Chloride	190 min	curd-pH	---
1702 g/453 kg milk	6295 g	g NaCl	
Hoop	210 min	curd-pH	5.65
Press	in @ 220		
2 hr in the horizontal press @0.03 MPa	out @ 340	curd-pH	5.35
Leave @ Room Temp Until pH 5.35		curd-pH	5.35

Table 5.3 Chemical composition of the cheese milk and low-fat cheese before and after reforming

	Milk	
Total solids, %	9.26	
Fat, %	0.34	
Casein, %	2.68	
Protein, %	3.44	
Casein:Fat ratio	7.94	
	Cheese	
	Before reforming	After reforming
Moisture, %	53.47 ± 0.63	54.2 ± 0.84
Fat, %	4.39 ± 0.53	7.08 ± 0.58
Protein, %	33.83 ± 1.18	31.15 ± 0.50
Salt, %	1.98 ± 0.16	-
Total Ca, mg/100g cheese	923 ± 42	-

Table 5.4 Chemical composition of the full-fat cheese before and after reforming

	Before reforming	After reforming
Moisture, %	34.71 ± 0.58	34.00 ± 0.89
Fat, %	32.09 ± 0.7	35.71 ± 1.5
Protein, %	23.4 ± 0.19	21.08 ± 0.51
Salt, %	1.63 ± 0.06	-
Total Ca, mg/100g cheese	658 ± 19	-

Table 5.5 pH values of the low-fat and full-fat cheese after reforming

Treatment	pH	
	Low-fat cheese	Full-fat cheese
CONTROL	5.34 ± 0.02 [c]	5.34 ± 0.04 [c]
Non-ionic		
ACETEM	5.21 ± 0.05 [b]	5.30 ± 0.04 [c]
LACTEM	5.21 ± 0.06 [b]	5.28 ± 0.01 [bc]
DM	5.20 ± 0.05 [b]	5.23 ± 0.01 [b]
STS	5.21 ± 0.10 [b]	5.37 ± 0.04 [c]
Anionic		
DATEM	5.08 ± 0.03 [a]	5.04 ± 0.01 [a]
CITREM	5.15 ± 0.01 [ab]	5.30 ± 0.02 [c]
SSL	5.19 ± 0.06 [b]	5.33 ± 0.05 [c]
Zwitterion		
LECITHIN	5.20 ± 0.00 [b]	5.29 ± 0.02 [bc]

[a-c] Means with different superscript letters within a column are significantly different ($P<0.05$).

Table 5.6 Uniaxial compression test results of full fat cheese before reforming (base) and after reforming with no emulsifier (control) in comparison to reformed cheese made with the addition of different types of emulsifiers. Means are for two replicates.

Treatment	Initial slope (g/%)	Hardness (g)	Compression area (g.%)	Adhesiveness force (g)	Adhesiveness area (g.%)
BASE	1.91E+02 ± 1 ^{abc}	9.06E+03 ± 5.E+01 ^{f}	3.63E+05 ± 5.E+03 ^{f}	-3.0E+03 ± 1.E+01 ^{a}	-9.1E+03 ± 14^{a}
CONTROL	2.01E+02 ± 4 ^{abcd}	3.75E+03 ± 1.E+02 ^{bc}	1.57E+05 ± 4.E+03 ^{b}	-8.3E+02 ± 2.E+01 ^{e}	-2.2E+03 ± 66^{d}
Non-ionic					
ACETEM	1.58E+02 ± 1^{a}	3.71E+03 ± 8.E+01 ^{bc}	1.49E+05 ± 1.E+03 ^{b}	-1.7E+03 ± 7.E+01 ^{b}	-5.9E+03 ± 721^{b}
STS	3.55E+02 ± 104 ^{d}	4.77E+03 ± 7.E+01 ^{e}	1.95E+05 ± 3.E+03 ^{e}	-8.4E+02 ± 5.E+01 ^{e}	-1.5E+03 ± 137^{d}
DM	2.72E+02 ± 22 ^{bcde}	3.90E+03 ± 2.E+02 ^{bc}	1.69E+05 ± 1.E+04 ^{c}	-1.4E+03 ± 2.E+02 ^{c}	-4.3E+03 ± 1180^{c}
LACTEM	3.64E+02 ± 35 ^{d}	4.00E+03 ± 2.E+00 ^{c}	1.81E+05 ± 4.E+03 ^{d}	-1.0E+03 ± 3.E+01 ^{de}	-2.0E+03 ± 7^{d}
Anionic					
SSL	2.95E+02 ± 36 ^{de}	3.57E+03 ± 9.E+01 ^{b}	1.57E+05 ± 3.E+03 ^{b}	-8.8E+02 ± 5.E+00 ^{e}	-1.9E+03 ± 5^{d}
CITREM	2.83E+02 ± 25 ^{cde}	4.37E+03 ± 3.E+02 ^{d}	1.73E+05 ± 3.E+03 ^{cd}	-9.5E+02 ± 1.E+02 ^{de}	-2.0E+03 ± 491^{d}
DATEM	2.09E+02 ± 16 ^{cd}	3.93E+03 ± 5.E+01 ^{cd}	1.55E+05 ± 1.E+03 ^{b}	-1.2E+03 ± 4.E+01 ^{cd}	-3.1E+03 ± 75 ^{c}
Zwitterion					
LECITHIN	1.73E+02 ± 23 ^{ab}	3.04E+03 ± 4.E+01 ^{a}	1.24E+05 ± 4.E+02 ^{a}	-1.1E+03 ± 3.E+01 ^{d}	-2.5E+03 ± 87 ^{d}

$^{a-c}$ Means with different superscript letters within a column are significantly different ($P<0.05$).

Table 5.7 Uniaxial compression test results of low fat cheese before reforming (base) and after reforming with no emulsifier (control) in comparison to reformed cheese made with the addition of different types of emulsifiers. Means are for three replicates.

Treatment	Initial slope (g/%)	Hardness (g)	Compression area (g.%)	Adhesiveness force (g)	Adhesiveness area (g.%)
BASE	6.20E+02 ± 124 ^{b}	9.36E+03 ± 5.E+02 ^{de}	5.78E+04 ± 4.E+03 ^{d}	-3.3E+02 ± 3.E+02 ^{e}	-2.2E+02 ± 60 ^{de}
CONTROL	6.54E+02 ± 59 ^{bc}	1.08E+04 ± 3.E+02 ^{gh}	7.26E+04 ± 9.E+02 ^{f}	-2.0E+03 ± 3.E+02 ^{bc}	-6.4E+02 ± 119 ^{b}
Non-ionic					
ACETEM	7.38E+02 ± 44 ^{c}	9.99E+03 ± 1.E+02 ^{ef}	6.25E+04 ± 7.E+02 ^{e}	-1.8E+03 ± 3.E+02 ^{c}	-5.7E+02 ± 98 ^{b}
STS	6.28E+02 ± 28 ^{b}	1.07E+04 ± 2.E+02 ^{ef}	6.59E+04 ± 2.E+03 ^{e}	-1.9E+03 ± 3.E+01 ^{bc}	-6.0E+02 ± 85 ^{b}
DM	9.07E+02 ± 46 ^{d}	7.54E+03 ± 4.E+02 ^{c}	4.63E+04 ± 2.E+03 ^{c}	-9.6E+02 ± 1.E+02 ^{d}	-3.6E+02 ± 53 ^{cd}
LACTEM	6.60E+02 ± 16 ^{bc}	9.05E+03 ± 8.E+02 ^{d}	6.22E+04 ± 3.E+03 ^{e}	-1.3E+03 ± 1.E+02 ^{d}	-4.7E+02 ± 8 ^{bc}
Anionic					
SSL	4.80E+02 ± 12 ^{a}	5.57E+03 ± 2.E+02 ^{a}	2.78E+04 ± 3.E+03 ^{a}	-3.5E+03 ± 4.E+02 ^{a}	-1.6E+03 ± 247 ^{a}
CITREM	7.51E+02 ± 51 ^{c}	1.13E+04 ± 3.E+02 ^{h}	7.11E+04 ± 9.E+02 ^{f}	-2.0E+03 ± 7.E+01 ^{bc}	-6.4E+02 ± 32 ^{b}
DATEM	9.44E+02 ± 26 ^{d}	6.38E+03 ± 5.E+02 ^{b}	3.46E+04 ± 1.E+03 ^{b}	-8.0E+00 ± 1.E+01 ^{e}	-1.6E+02 ± 29 ^{e}
Zwitterion					
LECITHIN	7.46E+02 ± 67 ^{c}	1.03E+04 ± 4.E+02 ^{fg}	6.54E+04 ± 5.E+03 ^{e}	-2.2E+03 ± 1.E+02 ^{b}	-6.6E+02 ± 45 ^{b}

$^{a\text{-}c}$ Means with different superscript letters within a column are significantly different ($P<0.05$).

Table 5.8 Texture profile analysis results of low fat cheese before reforming (base) and after reforming with no emulsifier (control) in comparison to reformed cheese made with the addition of different types of emulsifiers. Means are for three replicates.

Treatment	Hardness (g)	Adhesiveness (g.s)	Springiness (s)	Cohesiveness	Gumminess (g)	Chewiness (g.s)
BASE	1.88E+03 ± 66 ^{bc}	-26.00 ± 12 ^{cd}	4.31 ± 0.17 ^{e}	0.54 ± 0.01 ^{b}	1.03E+03 ± 34 ^{b}	4.41E+03 ± 155 ^{b}
CONTROL	1.90E+03 ± 112 ^{bc}	-53.30 ± 8 ^{b}	3.83 ± 0.12 ^{cde}	0.55 ± 0.00 ^{b}	1.04E+03 ± 60 ^{b}	3.98E+03 ± 347 ^{b}
Non-ionic						
ACETEM	1.74E+03 ± 157 ^{b}	-26.80 ± 7 ^{cd}	3.8 ± 0.10 ^{cde}	0.54 ± 0.01 ^{b}	9.38E+02 ± 77 ^{b}	3.56E+03 ± 325 ^{b}
STS	2.16E+03 ± 20 ^{d}	-30.30 ± 3 ^{cd}	3.78 ± 0.17 ^{cde}	0.55 ± 0.01 ^{b}	1.19E+03 ± 24 ^{b}	4.49E+03 ± 297 ^{b}
DM	2.17E+03 ± 166 ^{d}	-23.90 ± 4 ^{cd}	2.83 ± 0.18 ^{abc}	0.41 ± 0.02 ^{a}	8.85E+02 ± 77 ^{ab}	2.51E+03 ± 321 ^{ab}
LACTEM	2.01E+03 ± 162 ^{cd}	-37.00 ± 2 ^{c}	3.22 ± 0.66 ^{bcd}	0.45 ± 0.07 ^{ab}	9.00E+02 ± 194 ^{ab}	2.98E+03 ± 1276 ^{ab}
Anionic						
SSL	1.23E+03 ± 51 ^{a}	-81.50 ± 20 ^{a}	2.08 ± 0.48 ^{a}	0.47 ± 0.01 ^{ab}	5.74E+02 ± 31 ^{a}	1.20E+03 ± 281 ^{a}
CITREM	2.48E+03 ± 162 ^{e}	-35.30 ± 6 ^{c}	3.6 ± 0.68 ^{cde}	0.49 ± 0.08 ^{ab}	1.23E+03 ± 265 ^{b}	4.56E+03 ± 1681 ^{b}
DATEM	2.57E+03 ± 65 ^{e}	-17.70 ± 6 ^{d}	2.32 ± 1.13 ^{ab}	0.39 ± 0.16 ^{a}	1.01E+03 ± 432 ^{b}	2.70E+03 ± 2537 ^{ab}
Zwitterion						
LECITHIN	2.17E+03 ± 77 ^{d}	-28.50 ± 7 ^{cd}	3.88 ± 0.06 ^{de}	0.54 ± 0.00 ^{b}	1.17E+03 ± 36 ^{b}	4.53E+03 ± 156 ^{b}

$^{a-c}$ Means with different superscript letters within a column are significantly different ($P<0.05$).

Table 5.9 Small deformation oscillation rheology results for full fat cheese before reforming (base) and after reforming with no emulsifier (control) in comparison to reformed cheese made with the addition of different types of emulsifiers. Means are for two replicates.

	G' values at 8ºC	G' values at 40ºC	G' values at 70ºC	Temperature at LTmax (ºC)	LTmax	Temperature at LT=1 (ºC)
BASE	3.15E+05 ± 2.6E+04 ^{f}	5.06E+03 ± 4.70E+02 ^{d}	28.5 ± 10.0 ^{cd}	67.8 ± 7.0 ^{bcd}	3.0 ± 0.0E+00 ^{e}	47 ± 0.0E+00 ^{a}
CONTROL	1.79E+05 ± 5.7E+03 ^{bcd}	2.18E+03 ± 9.20E+01 ^{bc}	12.0 ± 0.6 ^{ab}	63.8 ± 1.5 ^{a}	2.8 ± 0.0E+00 ^{d}	47 ± 7.0E-01 ^{a}
Non-ionic						
ACETEM	2.06E+05 ± 2.5E+04 ^{cde}	1.71E+03 ± 3.40E+02 ^{abc}	15.3 ± 4.5 ^{ab}	65.8 ± 1.4 ^{ab}	3.0 ± 1.0E-01 ^{e}	47 ± 7.0E-01 ^{a}
STS	2.36E+05 ± 2.1E+04 ^{e}	1.42E+03 ± 3.70E+02 ^{ab}	14.0 ± 2.1 ^{ab}	68.3 ± 0.7 ^{bcd}	2.8 ± 1.0E-01 ^{d}	49 ± 7.0E-01 ^{bc}
DM	1.59E+05 ± 6.0E+03 ^{abc}	1.55E+03 ± 1.50E+02 ^{abc}	9.5 ± 2.1 ^{a}	66.3 ± 0.7 ^{abc}	2.9 ± 1.0E-01 ^{de}	48 ± 7.0E-01 ^{ab}
LACTEM	2.18E+05 ± 2.1E+04 ^{de}	1.77E+03 ± 3.80E+02 ^{abc}	10.1 ± 1.6 ^{ab}	65.8 ± 0.0 ^{ab}	3.0 ± 0.0E+00 ^{e}	47 ± 1.4E+00 ^{ab}
Anionic						
SSL	1.83E+05 ± 9.6E+03 ^{bcd}	1.69E+03 ± 7.00E+02 ^{abc}	20.6 ± 10.0 ^{bc}	69.9 ± 2.1 ^{d}	2.3 ± 0.0E+00 ^{b}	50 ± 7.0E-01 ^{c}
CITREM	1.49E+05 ± 4.4E+04 ^{ab}	1.15E+03 ± 4.20E-01 ^{a}	15.8 ± 2.9 ^{ab}	69.1 ± 0.0 ^{cd}	2.6 ± 1.0E-01 ^{c}	50 ± 7.0E-01 ^{c}
DATEM	1.28E+05 ± 9.2E+03 ^{ab}	2.26E+03 ± 2.80E+01 ^{c}	36.0 ± 2.5 ^{d}	68.1 ± 2.1 ^{bcd}	1.9 ± 0.0E+00 ^{a}	50 ± 0.0E+00 ^{c}
Zwitterion						
LECITHIN	1.44E+05 ± 8.5E+03 ^{bcd}	1.23E+03 ± 9.20E+01 ^{a}	26.9 ± 3.7 ^{cd}	69.2 ± 0.4 ^{cd}	1.9 ± 1.0E-01 ^{a}	52 ± 0.0E+00 ^{d}

$^{a-c}$ Means with different superscript letters within a column are significantly different ($P<0.05$).

Table 5.10 Small deformation oscillation rheology results for low fat cheese before reforming (base) and after reforming with no emulsifier (control) in comparison to reformed cheese made with the addition of different types of emulsifiers. Means are for three replicates.

	G' values at 8ºC	G' values at 40ºC	G' values at 70ºC	Temperature at LTmax (ºC)	LTmax	Temperature at LT=1 (ºC)
BASE	6.30E+04 ± 2.7E+03 ^{ab}	5.21E+03 ± 3.8E+02 ^{ab}	4.3 ± 2.0 ^{a}	63.8 ± 1.4 ^{abc}	7 ± 1.4E+00 ^{e}	46 ± 8.0E-01 ^{a}
CONTROL	5.61E+04 ± 5.7E+02 ^{a}	4.33E+03 ± 4.6E+02 ^{ab}	7.4 ± 0.3 ^{b}	64.8 ± 0.0 ^{bc}	5.7 ± 2.0E-01 ^{d}	47 ± 7.0E-01 ^{abc}
Non-ionic						
ACETEM	6.72E+04 ± 1.6E+03 ^{ab}	5.38E+03 ± 4.0E+00 ^{ab}	12 ± 2.2 ^{cd}	63.1 ± 0.4 ^{ab}	4.5 ± 1.0E-01 ^{bc}	48 ± 0.0E+00 ^{bc}
STS	7.93E+04 ± 4.1E+03 ^{c}	5.53E+03 ± 1.2E+02 ^{ab}	14.5 ± 1.8 ^{d}	64.4 ± 0.4 ^{abc}	4.9 ± 2.0E-01 ^{cd}	46 ± 0.0E+00 ^{a}
DM	1.05E+05 ± 2.2E+04 ^{d}	1.00E+04 ± 1.8E+03 ^{d}	11.1 ± 0.7 ^{c}	63.8 ± 1.4 ^{abc}	3.8 ± 3.0E-01 ^{abc}	48 ± 7.0E-01 ^{cd}
LACTEM	6.43E+04 ± 5.3E+03 ^{ab}	4.67E+03 ± 3.0E+02 ^{ab}	15.1 ± 0.2 ^{d}	62.6 ± 0.4 ^{a}	3.7 ± 1.0E-01 ^{ab}	47 ± 0.0E+00 ^{ab}
Anionic						
SSL	8.18E+04 ± 9.6E+03 ^{c}	5.87E+03 ± 5.7E+02 ^{c}	20.2 ± 1.6 ^{e}	66.6 ± 0.7 ^{de}	3.3 ± 1.0E-01 ^{a}	48 ± 0.0E+00 ^{cd}
CITREM	8.18E+04 ± 6.0E+03 ^{c}	5.73E+03 ± 2.7E+02 ^{c}	6.8 ± 0.5 ^{a}	63.3 ± 0.4 ^{ab}	4.8 ± 1.0E-01 ^{cd}	46 ± 0.0E+00 ^{a}
DATEM	6.30E+04 ± 6.9E+03 ^{ab}	6.02E+03 ± 7.0E+02 ^{c}	23.2 ± 1.9 ^{e}	65.3 ± 0.7 ^{cd}	2.7 ± 0.0E+00 ^{a}	49 ± 7.0E-01 ^{d}
Zwitterion						
LECITHIN	5.39E+04 ± 3.1E+03 a	4.00E+03 ± 2.5E+01 ^{d}	14.4 ± 0.1 ^{d}	67.6 ± 0.4 ^{e}	4.6 ± 1.0E-01 ^{bc}	47 ± 0.0E+00 ^{ab}

$^{a-c}$ Means with different superscript letters within a column are significantly different ($P<0.05$).

Table 5.11 Melt profile analysis results for low fat cheese before reforming (base) and after reforming with no emulsifier (control) in comparison to reformed cheese made with the addition of different types of emulsifiers. Means are for three replicates.

Treatment	Degree of Flow(%) at 55ºC	
	Full-fat cheese	Low-fat cheese
BASE	75.12 ± 4.09 [bc]	66.7 ± 5.0 [a]
CONTROL	65.46 ± 3.08 [a]	69.1 ± 1.4 [ab]
Non-ionic		
ACETEM	82.65 ± 1.42 [c]	70.6 ± 2.5 [ab]
STS	79.18 ± 1.64 [bc]	67.0 ± 1.4 [a]
DM	82.75 ± 0.13 [c]	67.9 ± 5.6 [a]
LACTEM	83.44 ± 0.53 [c]	74.1 ± 3.2 [b]
Anionic		
SSL	75.74 ± 2.05 [b]	69.2 ± 0.6 [ab]
CITREM	77.43 ± 0.69 [b]	67.1 ± 1.2 [a]
DATEM	74.53 ± 0.65 [b]	69.0 ± 0.6 [ab]
Zwitterion		
LECITHIN	83.81 ± 0.25 [c]	71.5 ± 2.1 [ab]

[a-c] Means with different superscript letters within a column are significantly different ($P<0.05$).

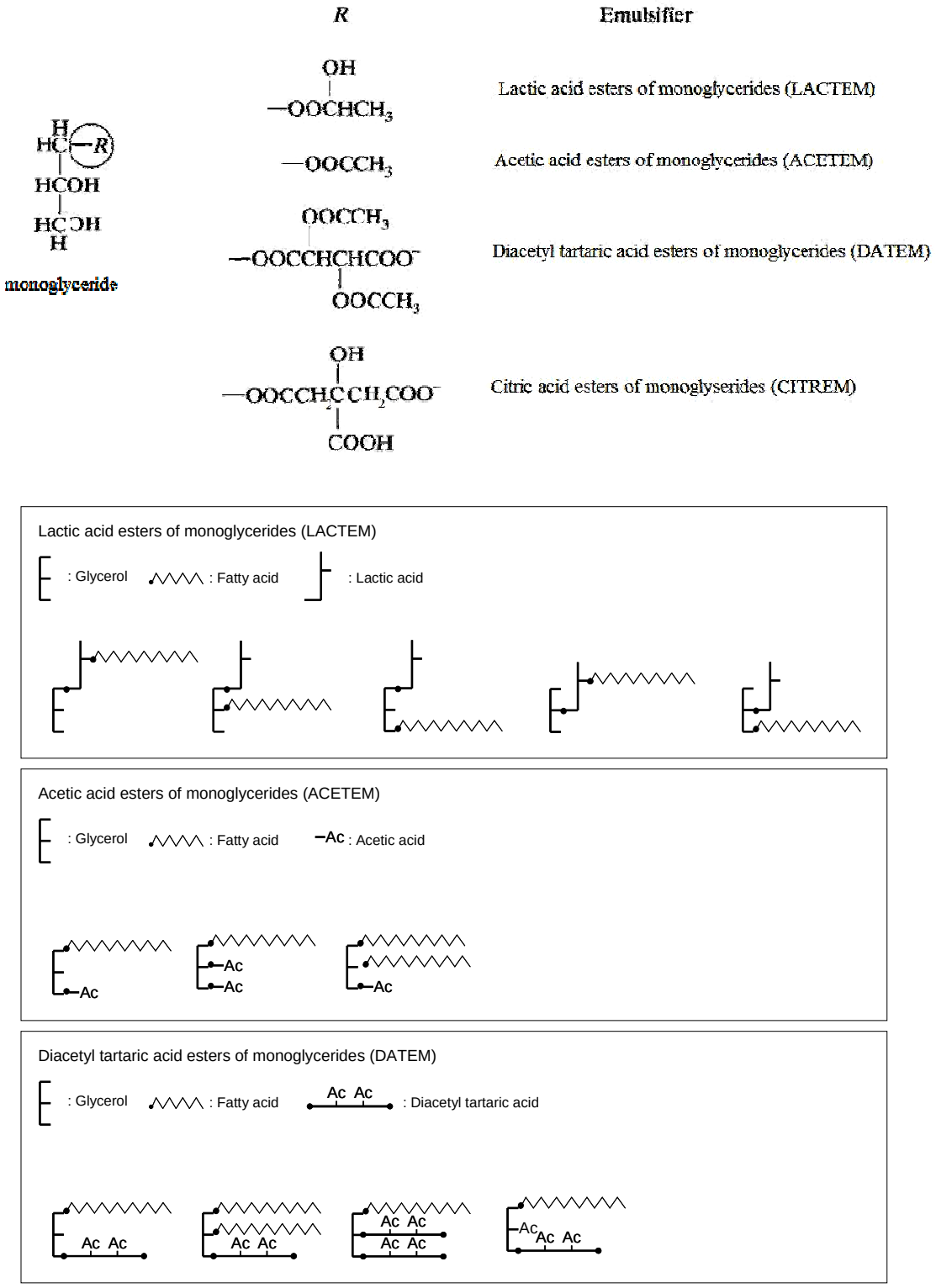


Figure 5.1 Chemical structure of monoglyseride and its esters (Krog, 1997; Stauffer, 2005)

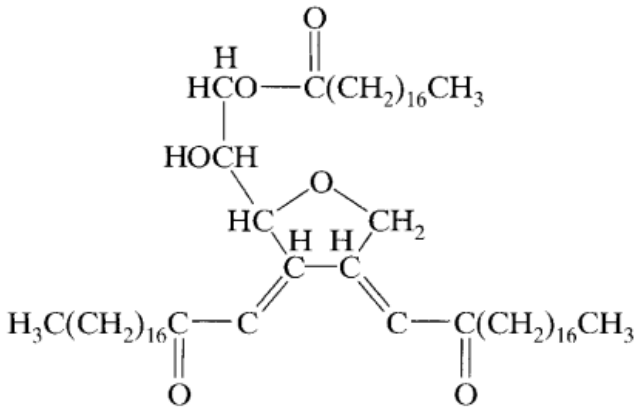


Figure 5.2 Chemical structure of sorbitan tristearate (STS) (Stauffer, 2005)

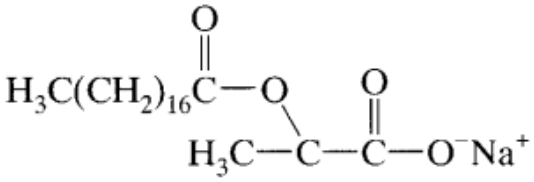


Figure 5.3 Chemical structure of sodium stearoyl lactylate (SSL) (Stauffer, 2005)

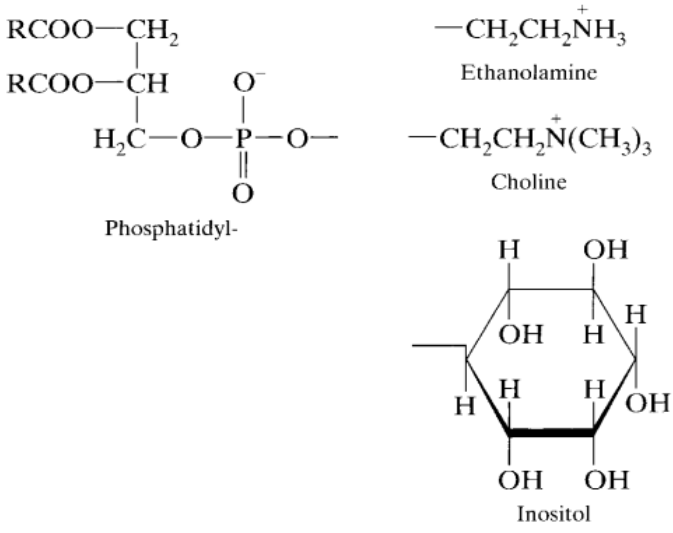


Figure 5.4 Chemical structure of lecithin (Stauffer, 2005)

Figure 5.5 Cheese sample after reforming; vacuum packed into polystyrene cup

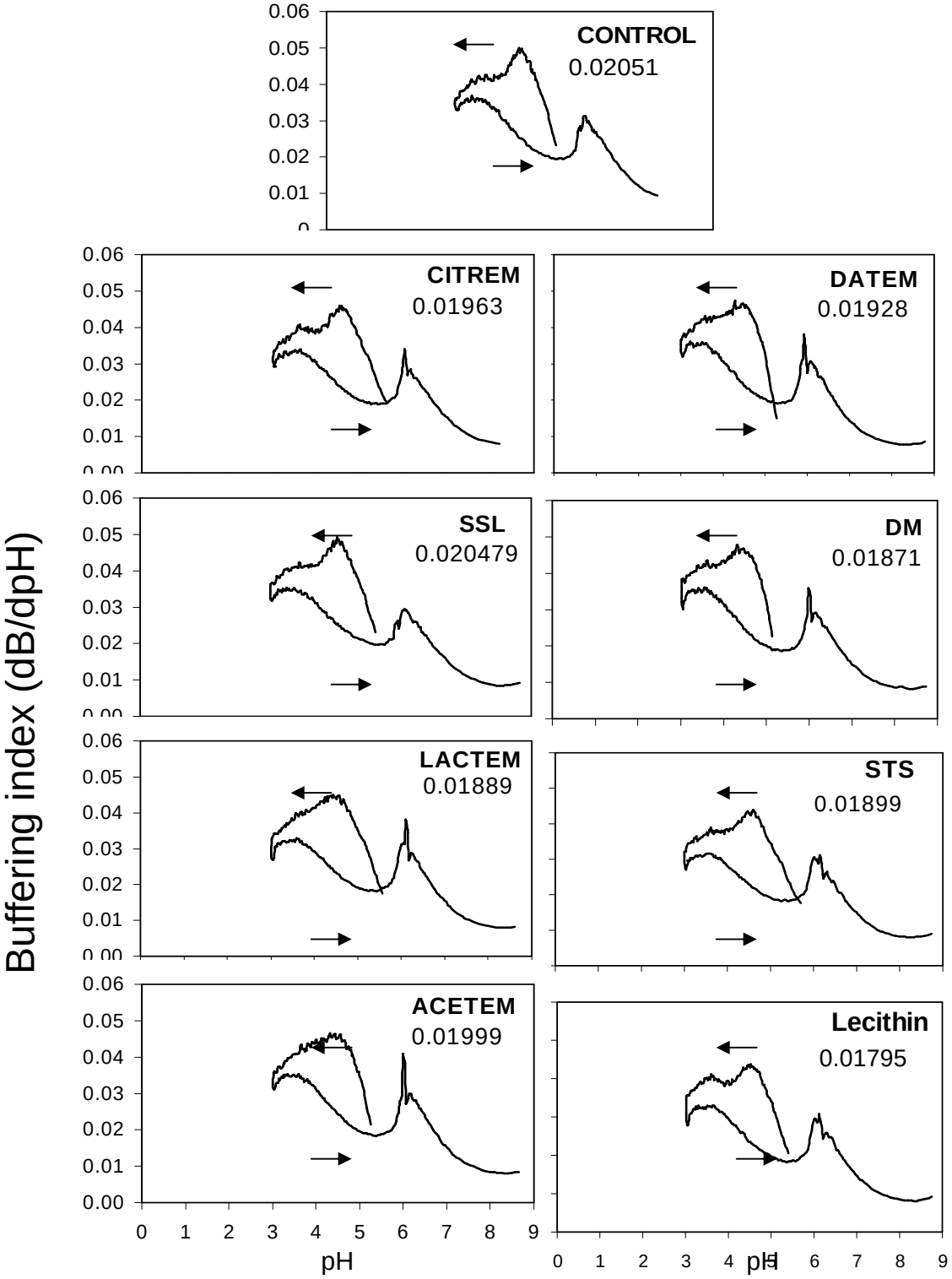


Figure 5.6 Buffering curves of reformed low-fat cheeses made with different types of emulsifiers. Cheese homogenates were titrated from initial pH to pH 3.0 with 0.5 N HCl and then back titrated to pH 9.0 with 0.5 N NaOH. Buffering areas due to the solubilization of colloidal calcium phosphate are given on each figure. Arrows indicate the direction of the titration.

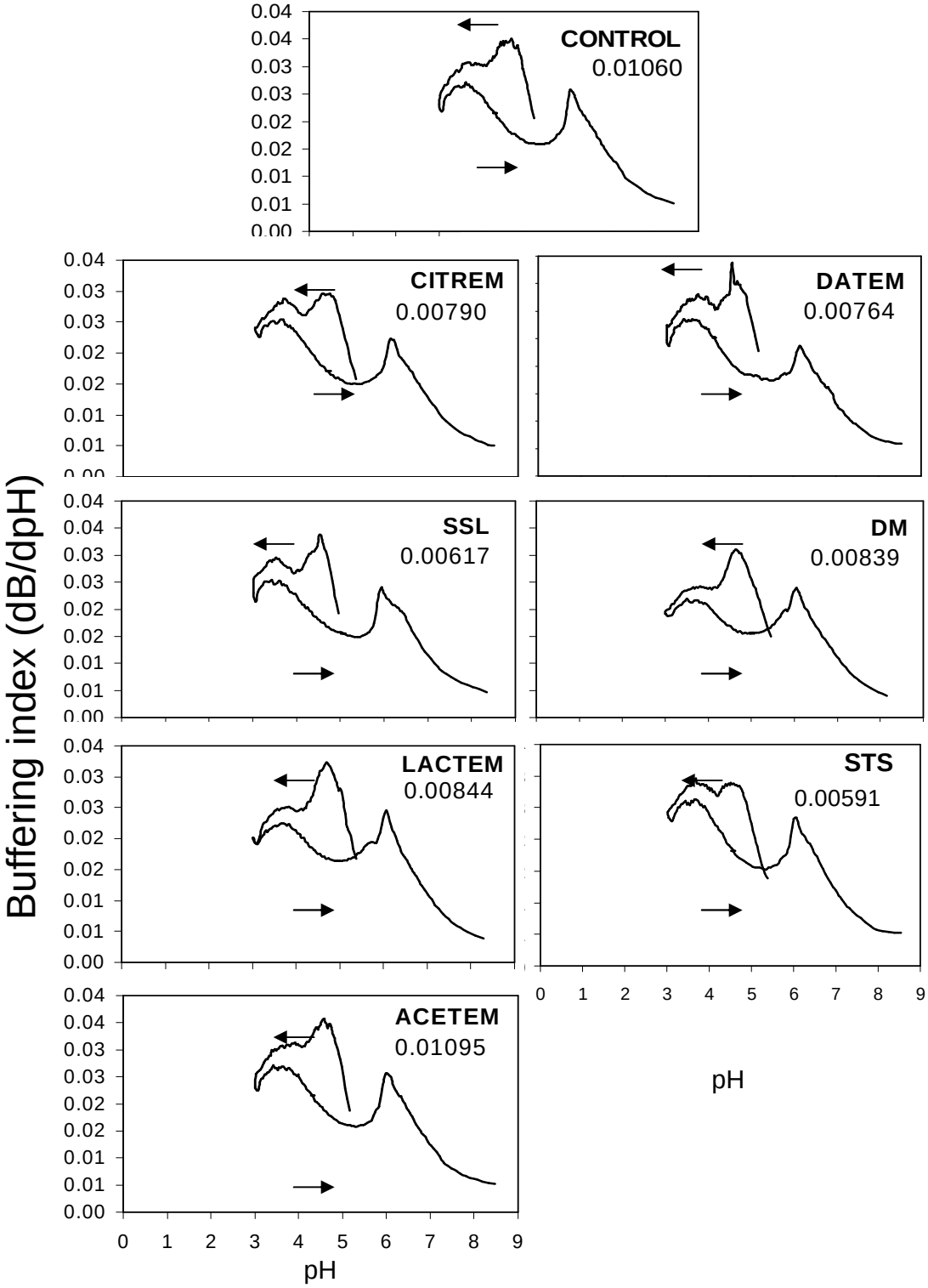


Figure 5.7 Buffering curves of reformed full-fat cheeses made with different types of emulsifiers. Cheese homogenates were titrated from initial pH to pH 3.0 with 0.5 N HCl and then back titrated to pH 9.0 with 0.5 N NaOH. Buffering areas due to the solubilization of colloidal calcium phosphate are given on each figure. Arrows indicate the direction of the titration.

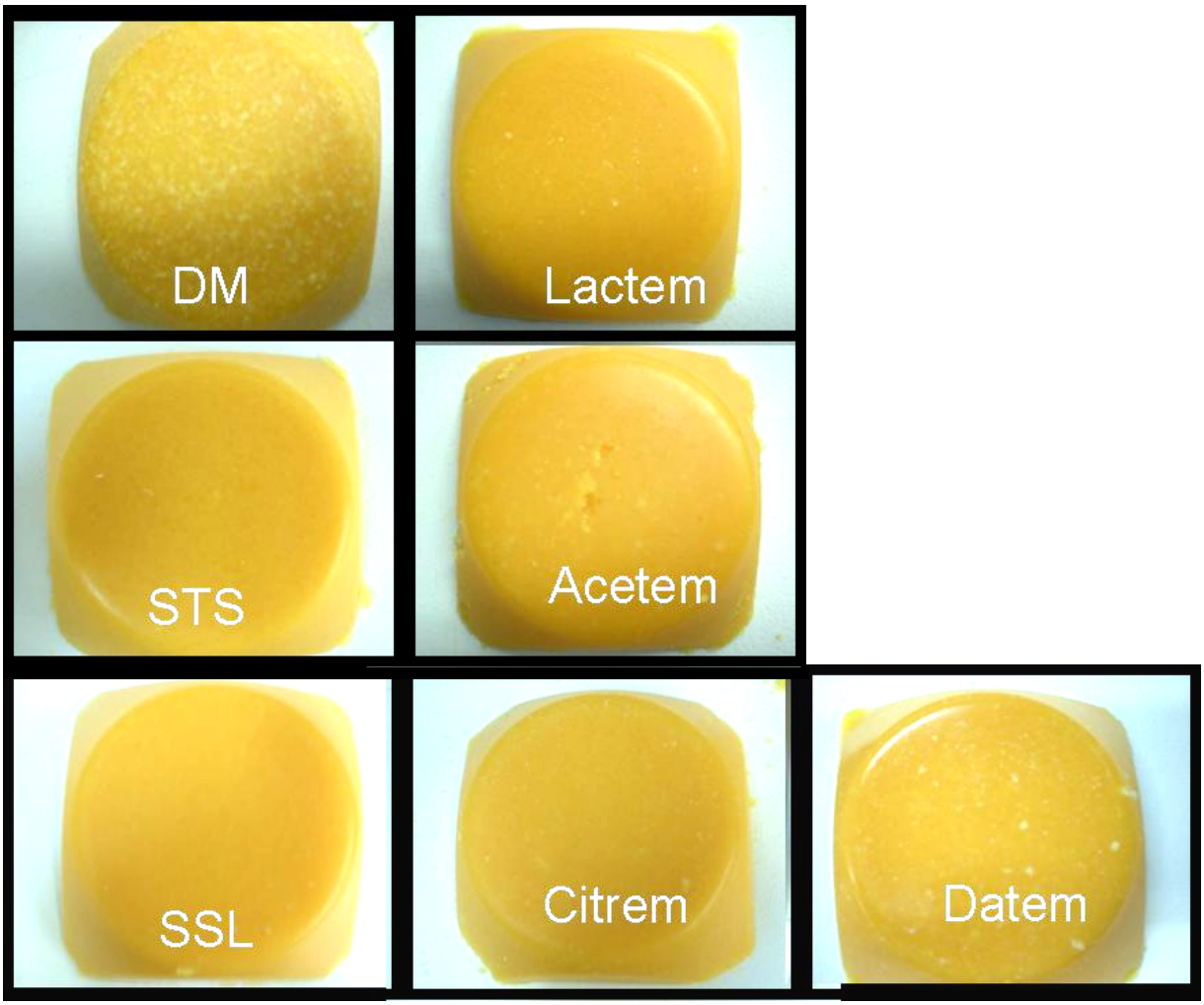


Figure 5.8 Pictures of low-fat cheese reformed with the addition of emulsifiers after 2 wk of storage at 4ºC

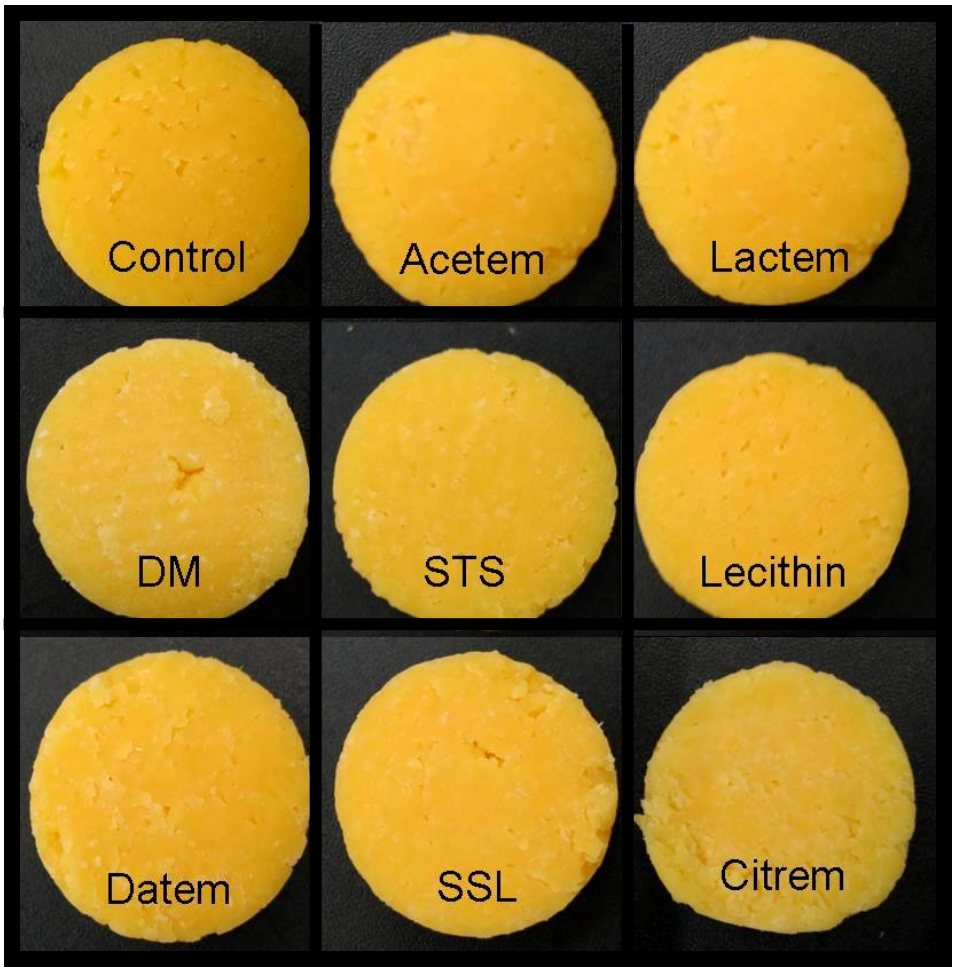


Figure 5.9 Pictures of full-fat cheese reformed with the addition of emulsifiers after 2 wk of storage at 4ºC

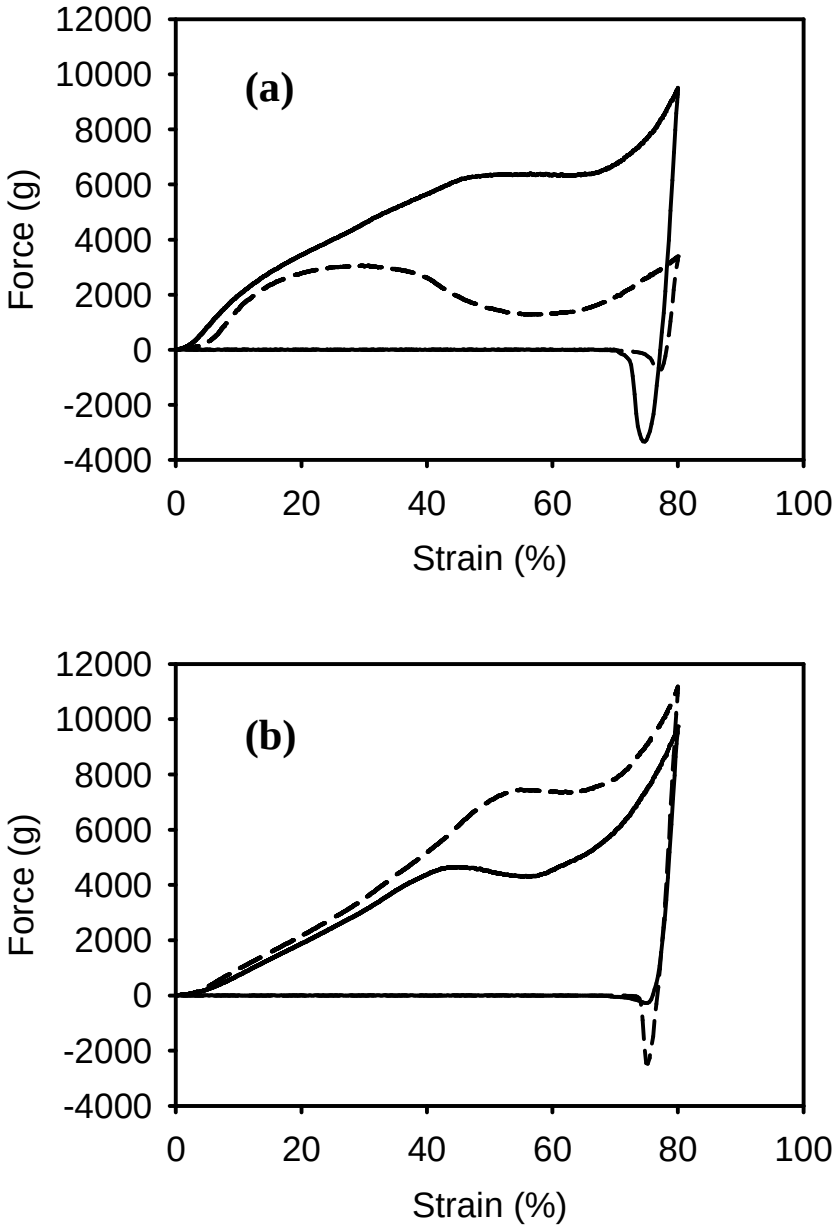


Figure 5.10 Representative uniaxial compression test profiles of full-fat (a) and low-fat (b) cheese samples before reforming (solid line) and after reforming without emulsifier (dashed line)

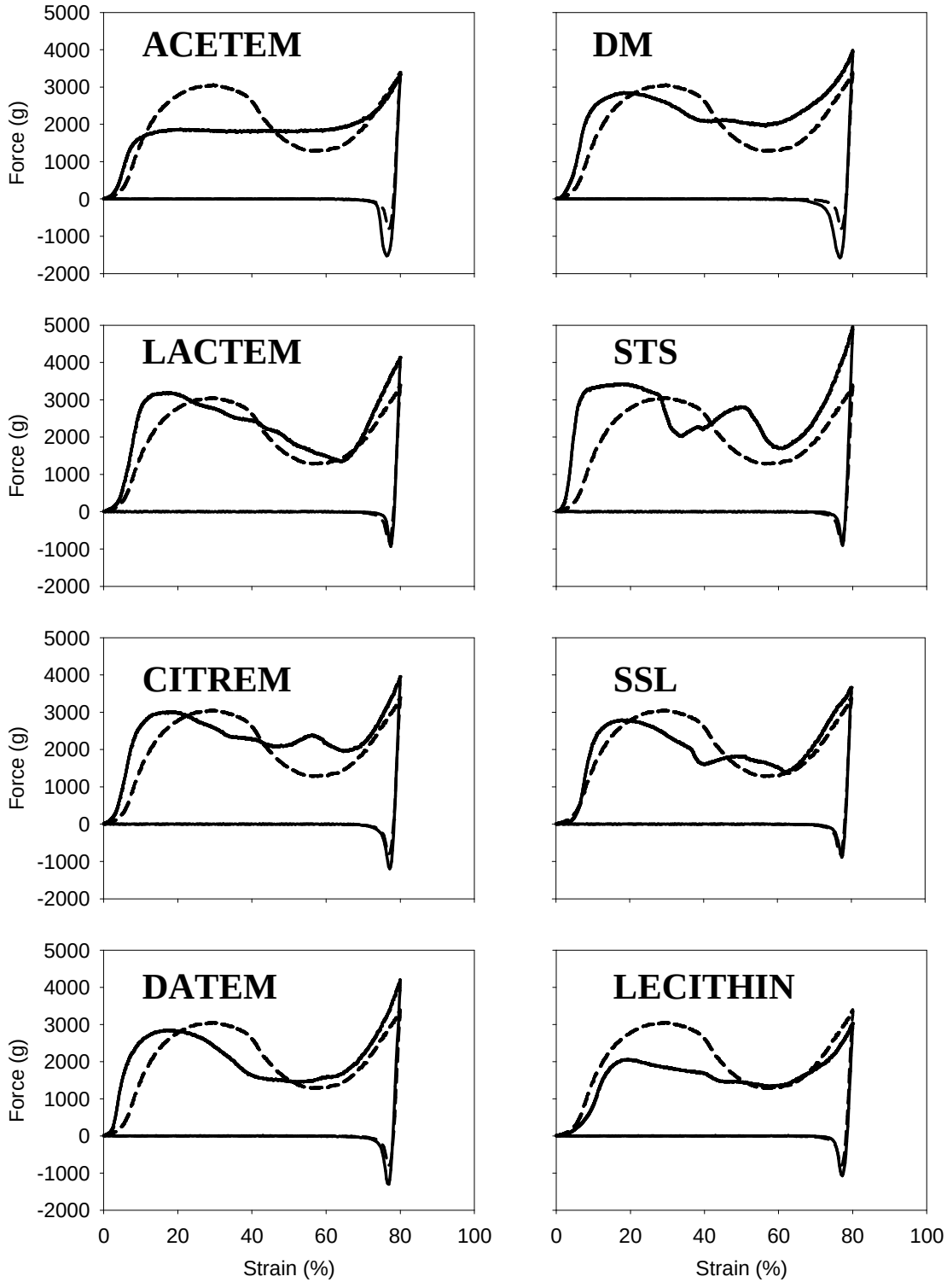


Figure 5.11 Representative uniaxial compression test profiles of full-fat cheese samples reformed with various emulsifiers (solid line) in comparison to cheese reformed without added emulsifier (dashed line)

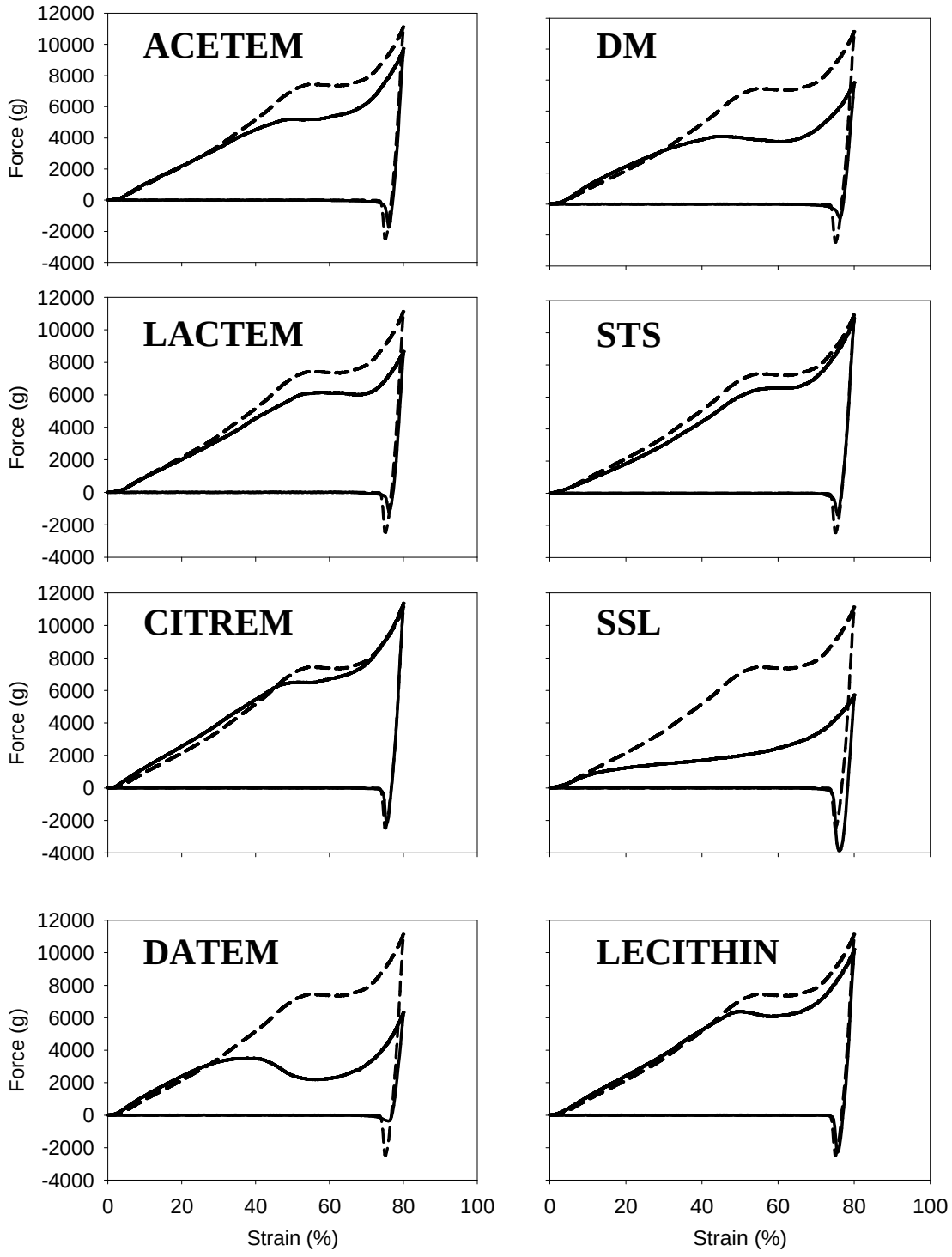


Figure 5.12 Representative uniaxial compression test profiles of low-fat cheese samples reformed with various emulsifiers (solid line) in comparison to cheese reformed without emulsifier (dashed line)

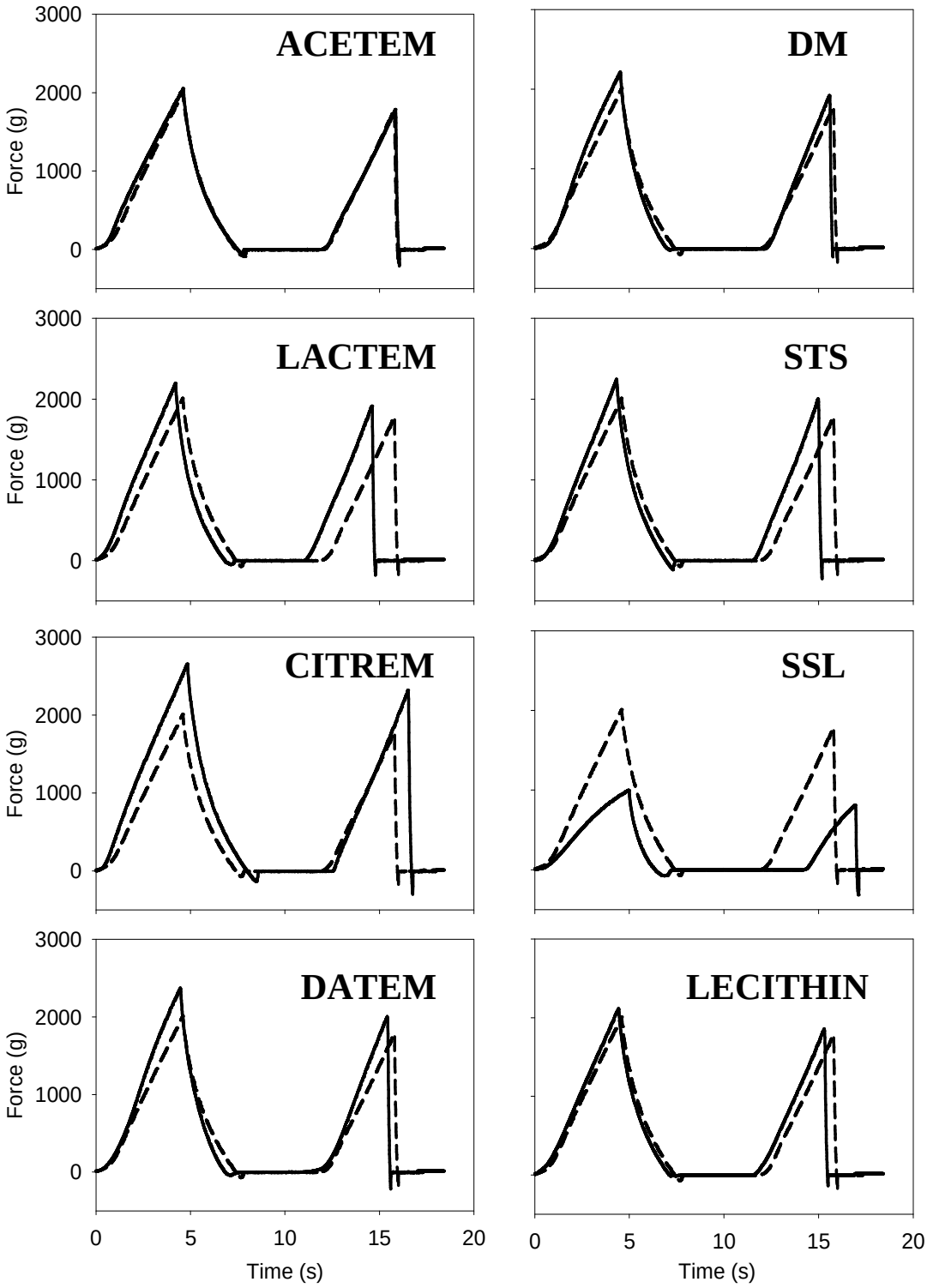


Figure 5.13 Representative Texture Profile Analysis (TPA) profiles of low-fat cheese samples reformed with various emulsifiers (solid line) in comparison to cheese reformed without emulsifier (dashed line)

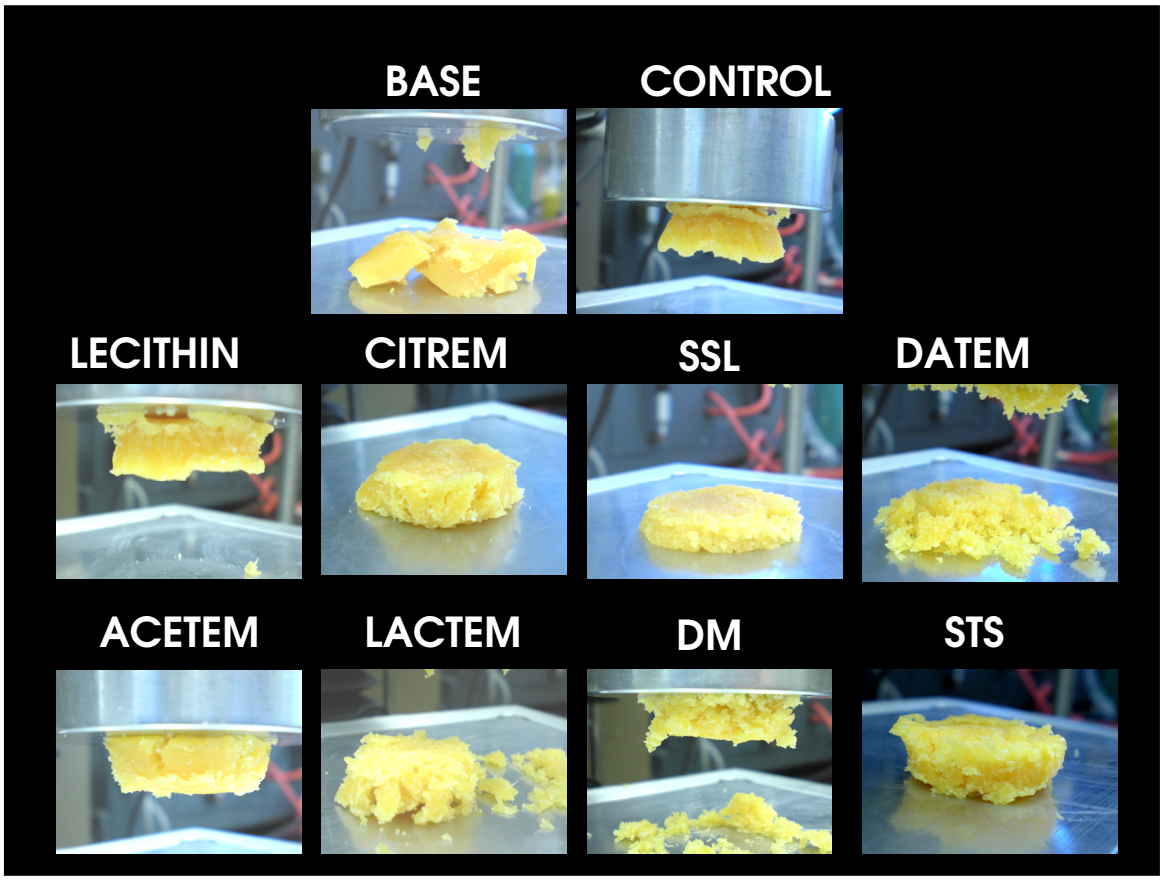


Figure 5.14 Pictures taken at the end of the uniaxial compression test (80% compression) of low-fat cheese base, control and low-fat cheese reformed in the presence of 4% (w/w) of different emulsifiers.

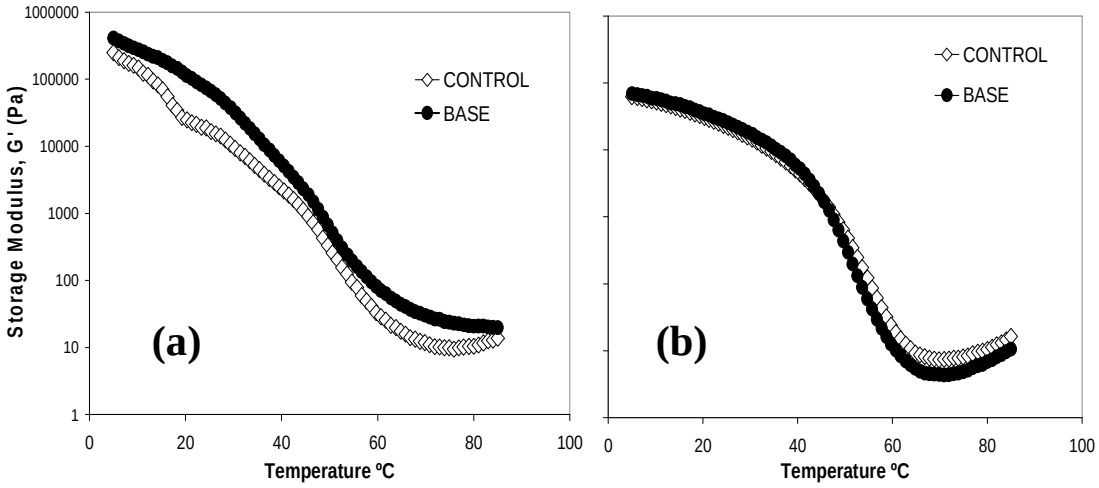


Figure 5.15 The storage modulus (G') profiles during heating from 5 to 85ºC at 1ºC/min for full-fat (a) and low-fat (b) cheese samples before reforming (base) and after reforming without emulsifier (control).

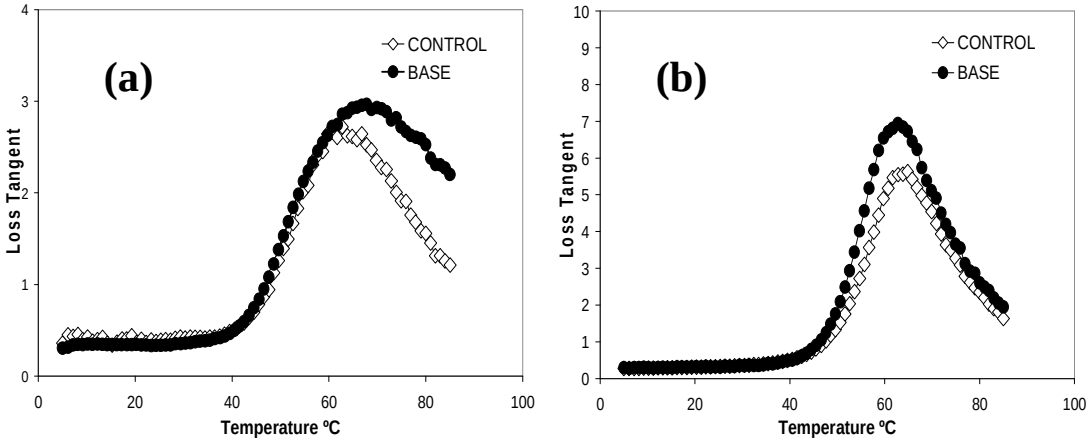


Figure 5.16 The loss tangent profiles during heating from 5 to 85ºC at 1ºC/min for full-fat (a) and low-fat (b) cheese samples before reforming (base) and after reforming without emulsifier (control)

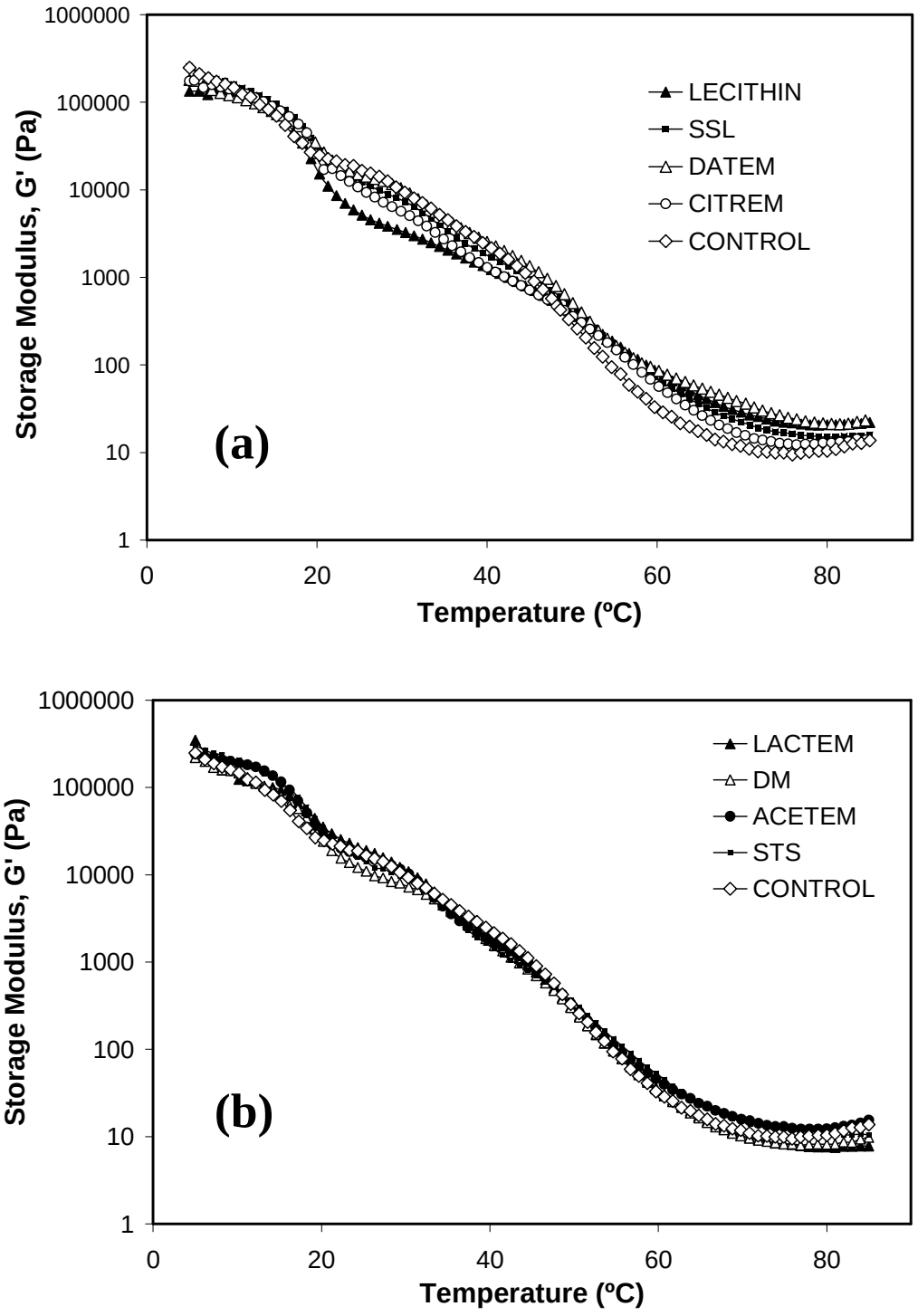


Figure 5.17 The storage modulus (G') values during heating of full-fat cheese from 5 to 85ºC at 1ºC/min; cheese before reforming (base) and after reforming with no emulsifier (control) in comparison to reformed cheese made with the addition of anionic (a) and nonionic (b) emulsifiers. Data points are means of two replicates.

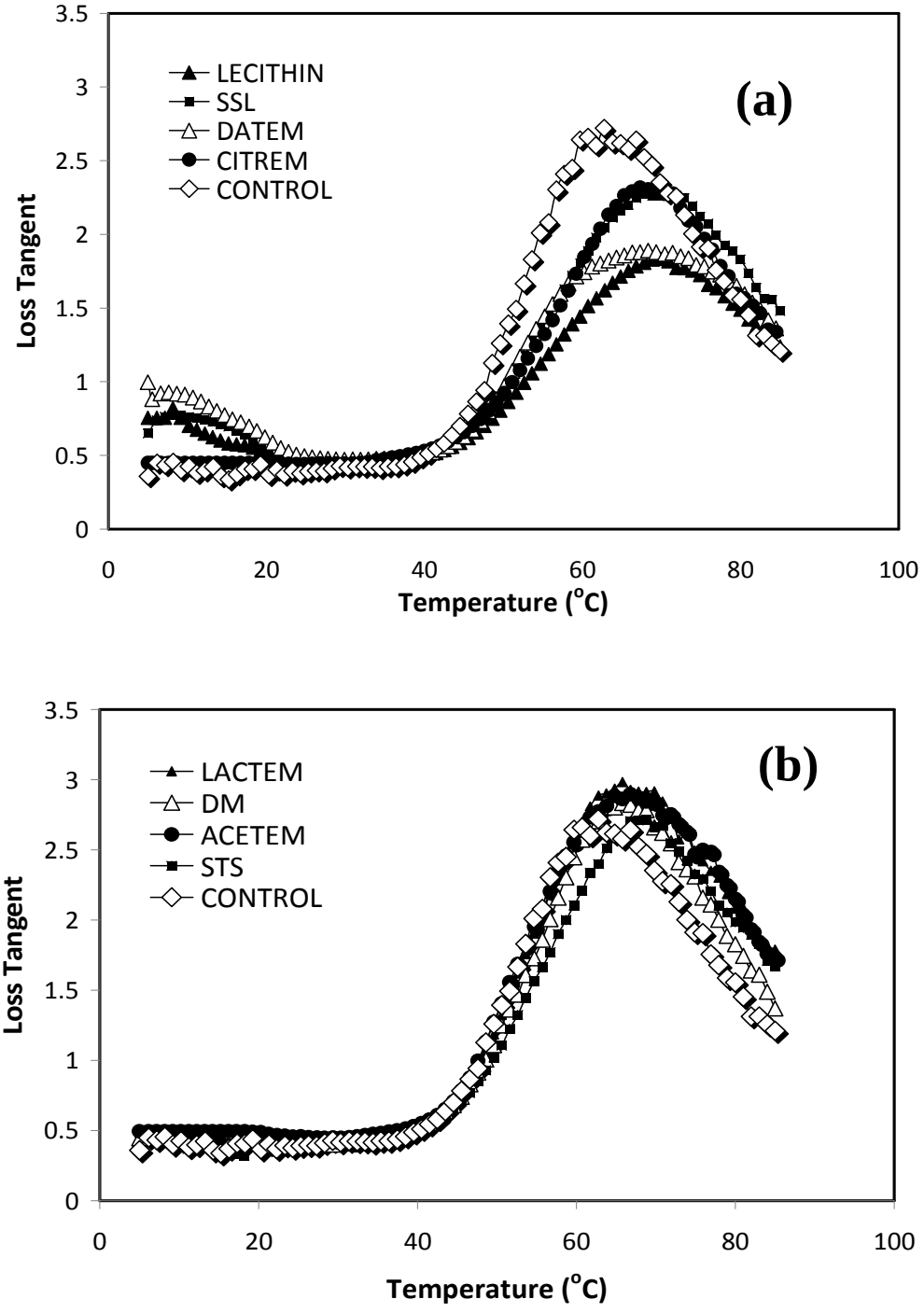


Figure 5.18 The loss tangent values during heating from 5 to 85ºC at 1ºC/min for full-fat cheese; cheese before reforming (base) and after reforming with no emulsifier (control) in comparison to reformed cheese made with the addition of anionic (a) and nonionic (b) emulsifiers when reforming. Data points are means of two replicates.

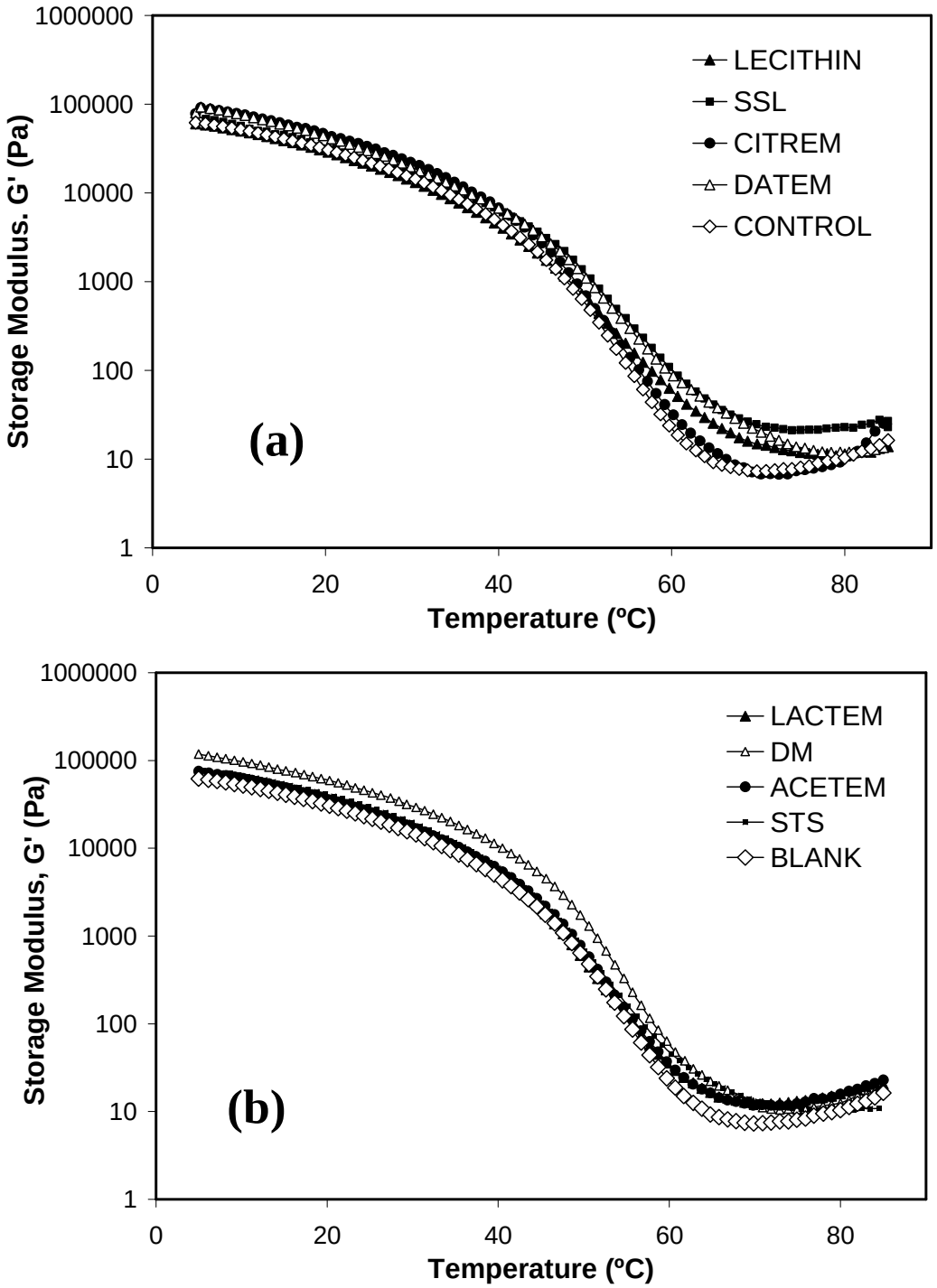


Figure 5.19 The storage modulus (G') values during heating low-fat cheese from 5 to 85ºC at 1ºC/min for low-fat cheese; cheese before reforming (base) and after reforming with no emulsifier (control) in comparison to reformed cheese made with the addition of anionic (a) and nonionic (b) emulsifiers. Data points are means of three replicates.

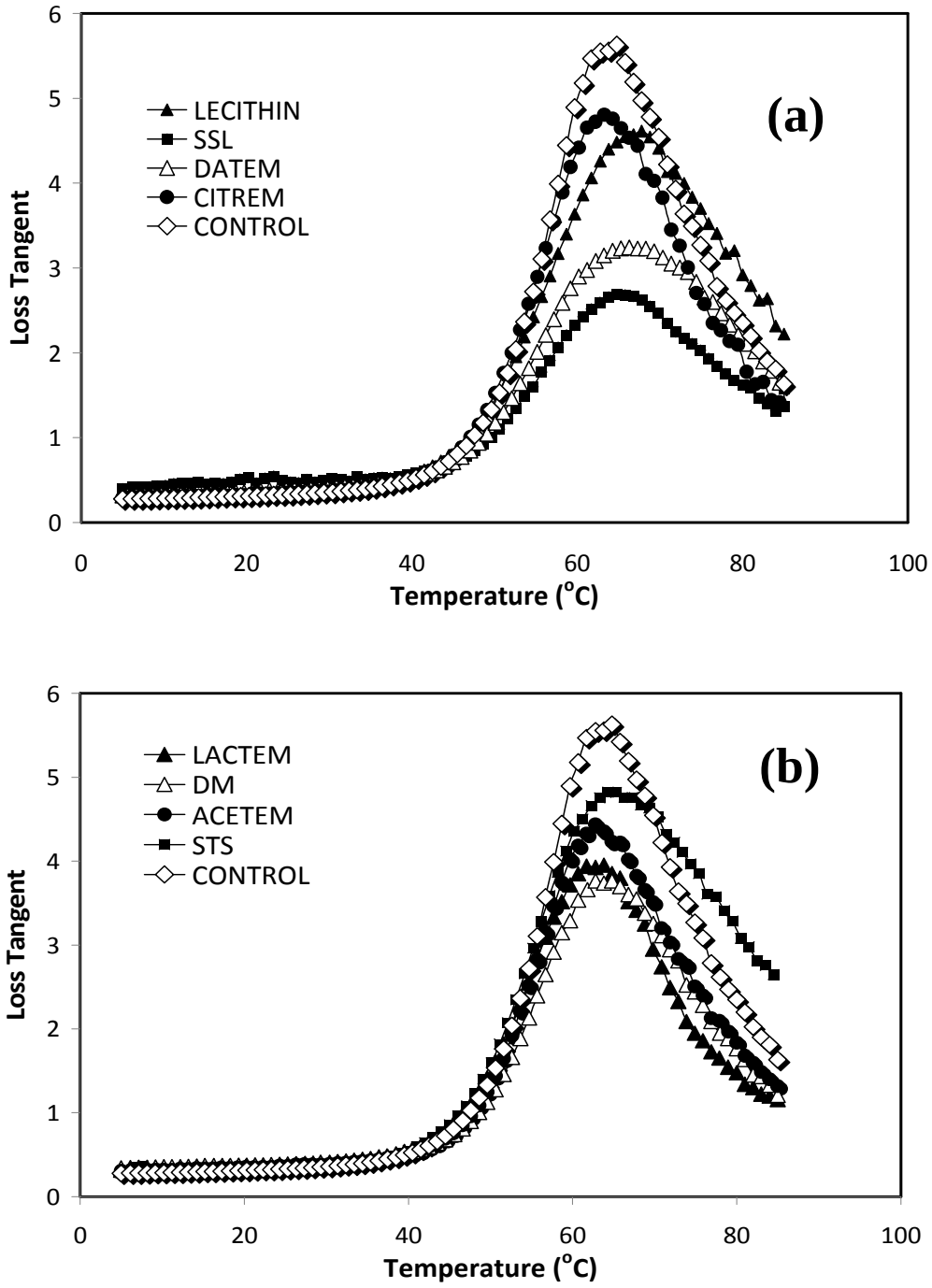


Figure 5.20 The loss tangent values during heating from 5 to 85ºC at 1ºC/min for low-fat cheese; cheese before reforming (base) and after reforming with no emulsifier (control) in comparison to reformed cheeses made with the addition of anionic (a) and nonionic (b) emulsifiers when reforming. Data points are means of three replicates.

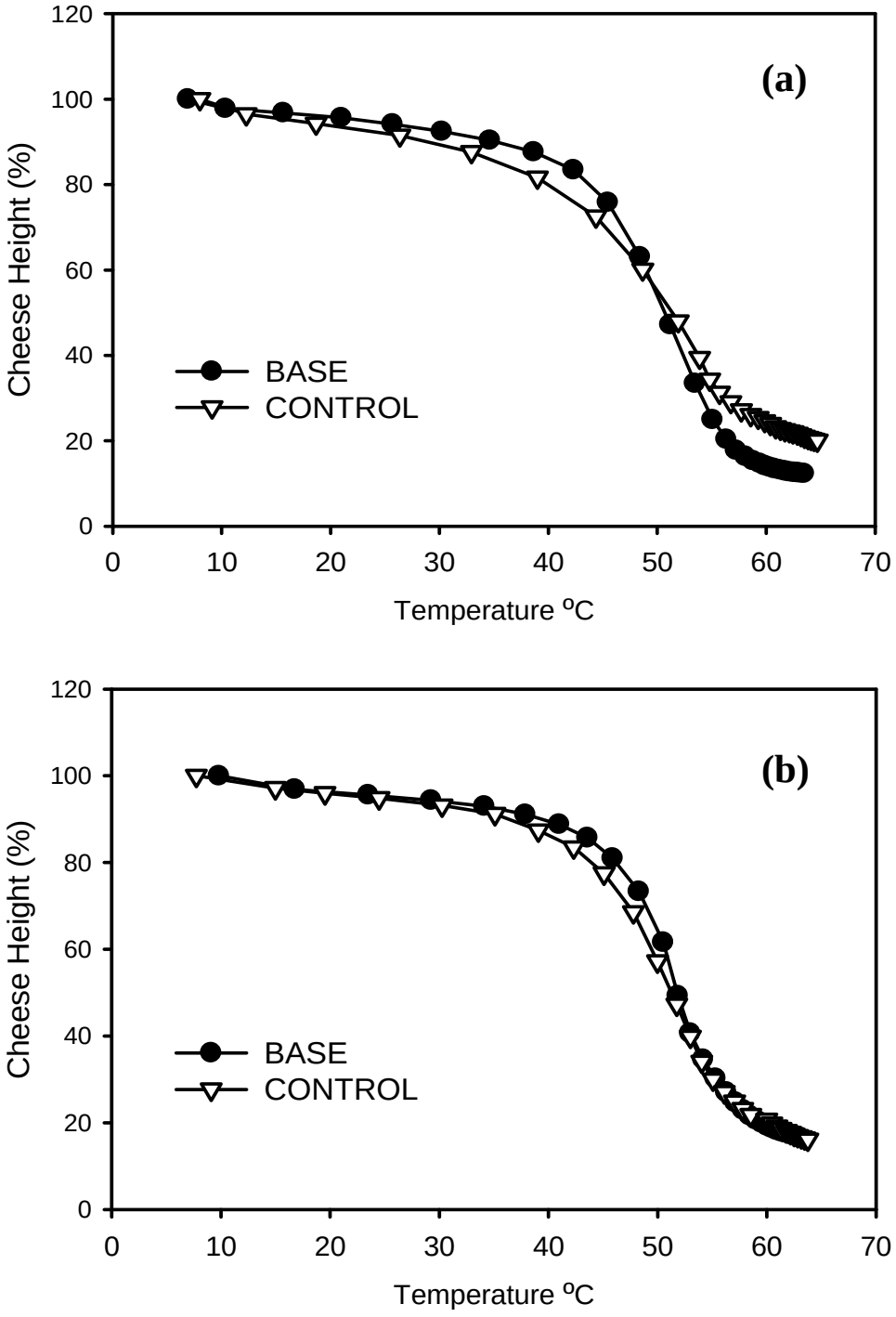


Figure 5.21 Melt profiles of full-fat (a) and low-fat (b) cheese samples before reforming (base) and after reforming without emulsifier (control)

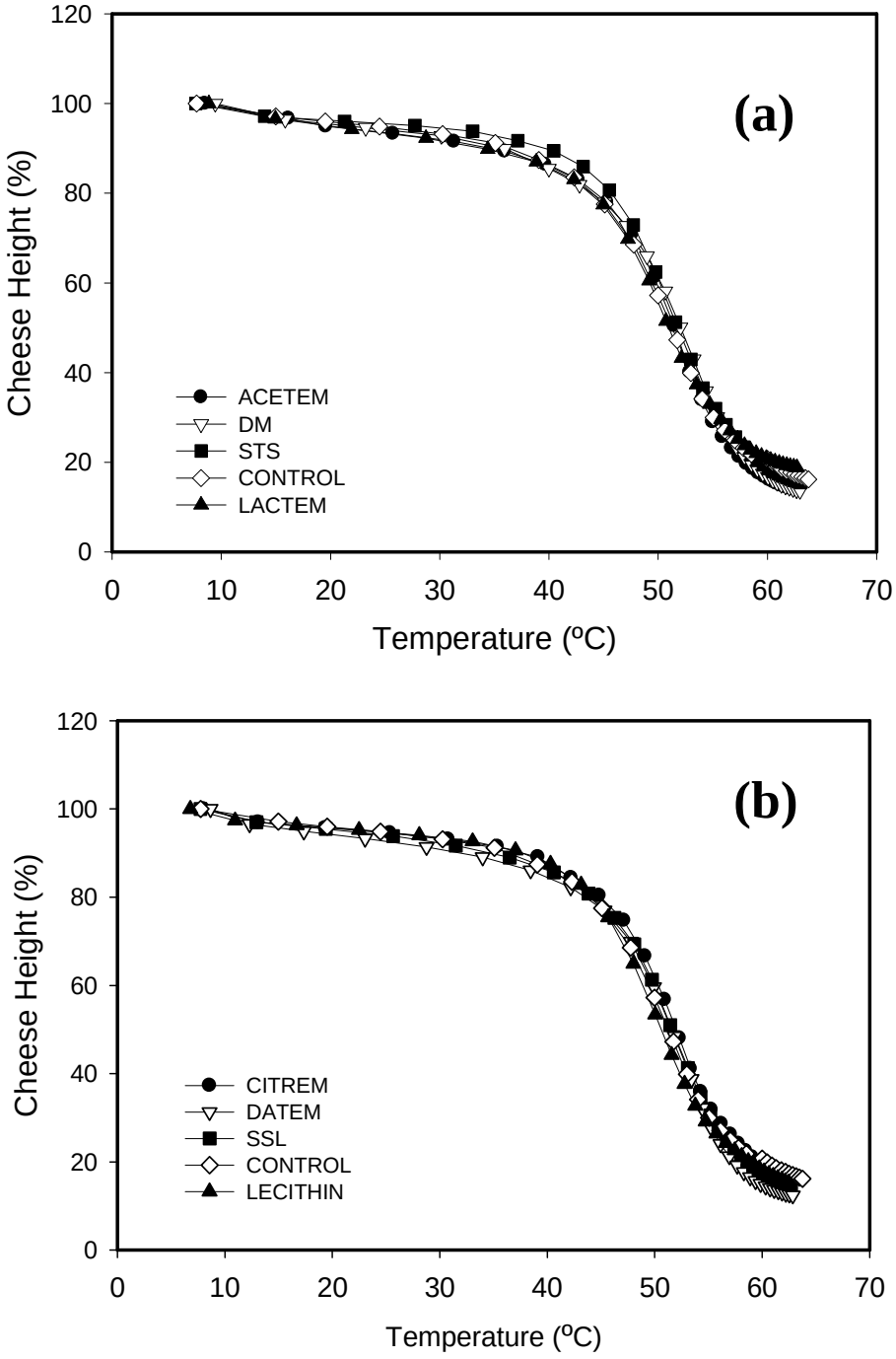


Figure 5.22 Changes in the height of low fat cheese samples during heating after reforming with no emulsifier (control) in comparison to reformed cheese made with the addition of nonionic (a) and anionic (b) emulsifiers. Data points are means of three replicates.

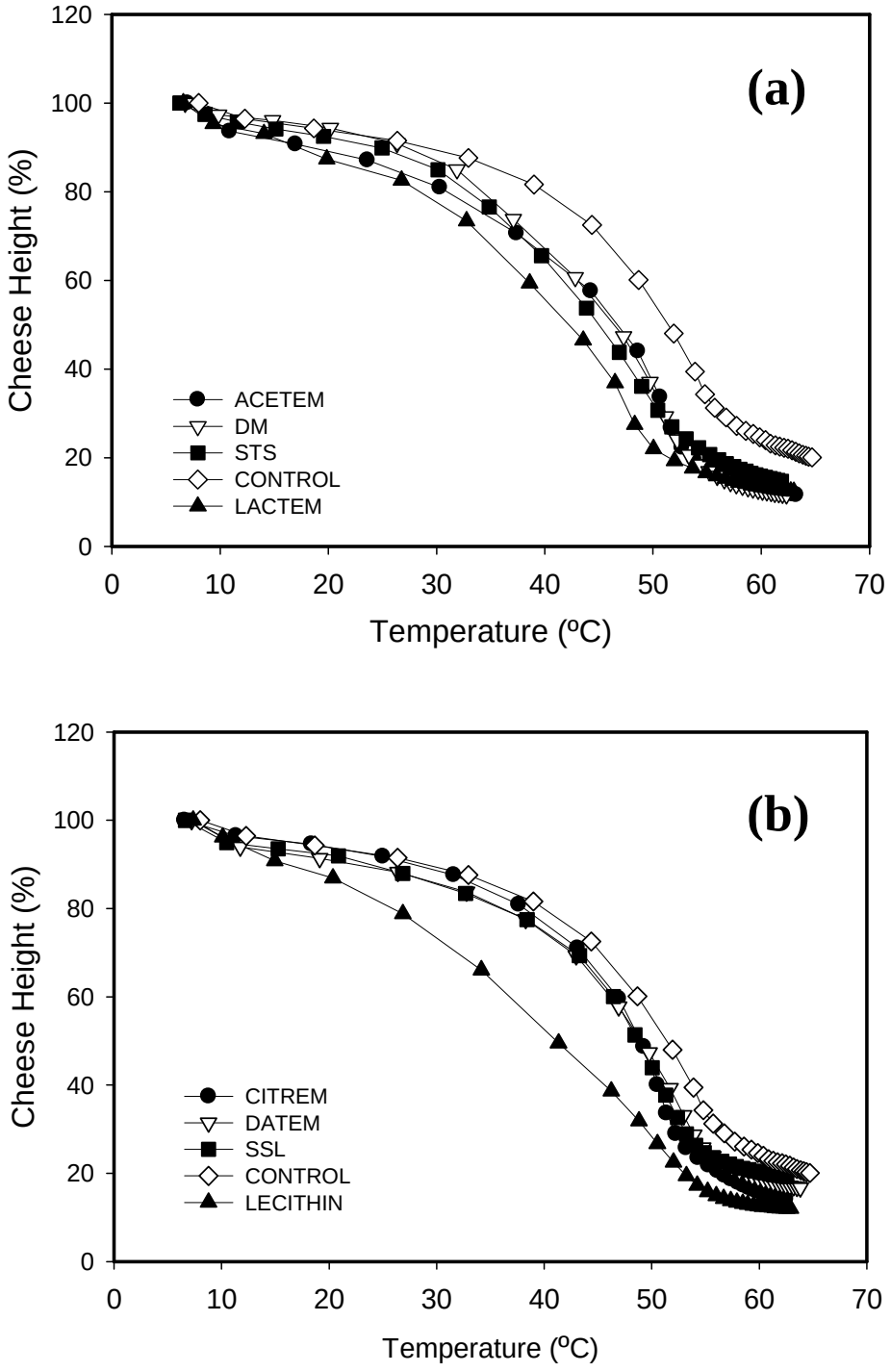


Figure 5.23 Changes in the height of full fat cheese samples during heating after reforming with no emulsifier (control) in comparison to reformed cheese made with the addition of nonionic (a) and anionic (b) emulsifiers. Data points are means of three replicates.

Chapter 6

SUMMARY, CONCLUSIONS AND FUTURE DIRECTIONS

6.1. SUMMARY AND CONCLUSIONS

The reforming process of the cheese involves the re-fusion of cheese particles initially through the interactions between proteins on the exposed surfaces of these pieces that were created during grinding. As the bonds and interactions between the caseins on the contact surfaces of the cheese particles are re-established, fusion of the cheese will be observed, and after some point those individual cheese particles will disappear forming a continuous cheese network. The extent of recovery of the bonds and interactions present in the cheese prior to grinding however depends on the mobility of bonds/interactions in the system and relaxation behavior of the bonds, which are influenced by many factors including temperature, pH, ionic strength, amount of CCP crosslinks, protein hydration and proteolysis.

Cheese reforming is a process that has many applications in dairy industry helps milled curd to form cheese blocks and it has allowed the incorporation of ingredients; like spices, herbs, fruits or different types/color of cheeses. There has not been a study on the impact of reforming process on the textural properties of the cheese. In our study, we found that reforming cheese weakened the protein network through the physical

disruption of bonds and interactions. Disrupting the protein network is helpful for improving the excessive firm and rubbery texture of reduced fat cheeses. In full fat cheese, disruption in the continuity of the protein network is provided by fat globules that are dispersed throughout it. Removing the fat results in a less interrupted protein network which leads to an increase in firmness along with a coarse and rubbery texture (Guinee et al., 2001). In our studies on reformation of non-fat and low-fat cheese, we observed that reformed cheese samples (2 w after reforming) had lower hardness values then the cheese base when reformation was done on cheese stored at ≥18ºC and where the pH was >5.5. In those cheese samples decrease in hardness occurred by fracturing of the cheese. This reduction in hardness was not as a result of softening in the structure with the loss in elastic character, but rather because of the creation of weak spots and the disruption of the cheese matrix which helped to propagate fracture. Nevertheless, this shorter texture can be seen as an improvement in the chew-down characteristics of low-fat cheese in comparison to its normal rubbery or chewy texture. The disruption in cheese structure due to grinding made it more meltable with a higher degree of flow in the reformed cheese. Incorporation of certain type of emulsifiers during reforming or an increase in the reforming temperature created a softer cheese structure that was not brittle or short. Use of sodium steoryl lactylate (SSL) did reduce the hardness of the low-fat cheese in that way. The interactions between certain type of emulsifiers and proteins can create a higher disruptive effect than what it is obtained by grinding the cheese into pieces physically and then reforming it.

Reformation of the full-fat cheese showed some distinct differences from the low-fat cheese due to the presence of fat and lower protein content. The lower G’ values at

20ºC of the reformed full fat cheese compared to the cheese base was possibly because fat coated the surfaces of cheese particles during grinding and that helped prevent re-establishing all of the interactions between proteins. This reduction in the G' values at ≤20ºC was not observed in low or non-fat cheese samples.

Grating disrupted the cheese matrix, weakened the continuous nature of the network and broke many physical bonds between casein particles. Many of these weak interactions reformed during cold storage of the cheese, however not all interactions were recreated. Therefore, grating the non-fat cheese into smaller shreds lowered the hardness compared to large ones. Cheese that had been grated into smaller shreds showed a higher flow rate, which indicated it was easier to flow during heating.

An increase in cheese temperature softens the texture indicating a weakening in the interactions with the increase in the mobility of the system (Lucey et al., 2003). Cheese reformed at higher temperatures (i.e., 30ºC) exhibited a softer texture as indicated by the low hardness and storage modulus values (at 5ºC) even after 1 wk of cold storage following the reforming. Heating the cheese reduce the total number and/or strength of the bonds and interactions in protein matrix (Lucey et al., 2003). Although the higher temperatures increased the mobility of the bonds leading a faster cheese fusion, the net impact was a softening of the cheese structure presumably due to loosening of the para-casein matrix with this decrease in the strength and loss of interparticle bonds.

Reducing the pH improved the cheese fusion as shown by micrographs and increasing recovery of the original textural properties. Reforming low-fat cheese that had a high pH, i.e. 6.2, reduced its hardness and storage modulus while making it more meltable, which was due to incomplete recovery of the bonds between caseins after the

reforming process. One of the major reasons for the pH impact on casein interactions was due to demineralization of casein particles at low pH (Lucey et al., 2003). The ratio of the insoluble to soluble calcium phosphate was decreased from 86% to 53% as the pH reduced from 6.2 to 5.3. Improvement in cheese fusion at low pH (i.e., pH 5.3) was possibly as a result of the increased bond mobility and flexibility caused by the solubilization of CCP crosslinks.

Addition of emulsifiers influenced the reformability of the cheese and altered the textural and rheological properties depending on the type and molecular characteristics of the emulsifier. The interaction properties of emulsifiers with proteins and their HLB values seem to play an important role on their behavior in cheese. Anionic emulsifiers were more effective in changing the texture properties of the cheese, probably due to their stronger interactions with caseins through their charged groups. There were differences between full-fat and low-fat cheese especially with the use of more hydrophobic non-ionic emulsifiers, which was probably due to their higher affinity to interact with fat rather than proteins.

6.2. FUTURE DIRECTIONS

The reformation of cheese has not been the subject of much scientific study. There are patents for the extrusion of the cheese blocks or grated/ground cheese into the form of slices, shreds or cheese blocks (Mueller, 2005; Holmes and Rivero, 2007; Reeve and Justiz, 2008; Holmes et al., 2011). However, the factors that promote cheese fusion and conditions that retard fusion have not been studied before. In our study, we investigated the impact of some of the important factors, such as, pH, temperature and size of grating on reformability and textural properties of the cheese after reforming.

The following studies could be of interest for future researchers:

A better understanding of the mechanisms of reformation of protein interactions and bonds in the reforming process requires more a detailed study of the specific types of protein interactions involved. It is not clear what types of the bonds are broken when the cheese was physically broken down into pieces. One assumption could be that weak bonds/interactions would break first in the case of fracture. Covalent bonds can also be disrupted if they are physically cut through or if under rigorous shear forces. The mechanical disruption caused by grinding, shredding and extruding the cheese caused various bonds to break. We did not analyze the system to identify any of the protein bonds and interactions involved in our study. Also, an investigation of the type of the interactions and bonds that are formed when cut surfaces are brought back into close contact, will provide more insights on the mechanisms of cheese fusion.

Model studies could be used to probe the interaction mechanisms of the different types of emulsifiers with rennet casein systems. These studies could examine micelle formation and the critical micelle concentration, changes in the weight average molecular weight of the proteins and the thermodynamics of the protein-protein interactions, adsorption behavior of caseins and emulsifiers at interfaces.

The degree of disruption needed to “permanently” reduce hardness could be explored by passing cheese through the Vemag type apparatus

various times to see how the extent of shear impacts refusion and restoration of bonds.

Fat influenced the reformability of cheese as evidenced by the differences between full-fat and low-fat cheese studies. It could be explored adding fat separately, e.g. liquid oil to shredded cheese, to investigate if liquid fat could prevent refusion. Also different types of oils/fats could be used to see the nature of the fat influenced fusion.

Surface coatings could also be investigated as it is known that so called anti-caking agents prevent shreds from clumping. The exact mechanism involved has not been explored.

6.3. REFERENCES

Guinee, T. P., Auty, M. A. E., and M. A. Fenelon. 2000. The effect of fat content on the rheology, microstructure and heat-induced functional characteristics of Cheddar cheese. Int. Dairy J. 10:277-288.

Holmes, T.L., and Rivero, O. 2007. Method and System for Making Sliced Cheese. US Patent Application No: 20070178212.

Holmes, T.L., Rivero, O., and Rivero, A. 2011. Method and System for Making Extruded Portions of Cheese. US Patent Application No: 20110014311.

Lucey, J. A., Johnson, M. E., and Horne, D. S. 2003. Perspectives on the basis of the rheology and texture properties of cheese. J. Dairy Sci. 86:2725–2743.

Mueller, A. P. 2005. Cheese extruding machine and process for producing pieces of cheese. US Patent No: US 6,942,888 B2.

Reeve, J., and Justiz, J. 2008. Reduced fat Cheese Having Enhanced Organoleptic Properties. US Patent No: 2008/0124428 A1.