INVESTIGATING THE EFFECT OF HIGH HYDROSTATIC PRESSURE PROCESSING ON TEXTURAL, RHEOLOGICAL, AND SENSORY PROPERTIES OF CHEESE

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Mustafa Ozturk

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The dissertation is approved by the following members of the Final Oral Committee:

John A. Lucey, Professor, Food Science

Srinivasan Damodaran, Professor, Food Science

Sundaram Gunasekaran, Professor, Biological Systems Engineering

Shinya Ikeda, Assistant Professor, Food Science

Scott A. Rankin, Professor, Food Science

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Mustafa Ozturk

Under the Supervision of Professor John A. Lucey
At the University of Wisconsin-Madison

ABSTRACT

Reducing the fat and salt (NaCl) content of cheese commonly causes quality defects in texture and flavor, primarily as a result of changes in microbial and enzymatic activity, and altered protein structure and interactions. High pressure processing has the potential to modify the protein network and control the microbial and enzymatic activity in cheese, which could improve the quality of reduced fat/salt cheeses. The main objective of this study was to understand how certain HHP conditions impacted flavor development, functionality, and shelf-life stability of the reduced fat and reduced sodium cheese. The impact of the magnitude of HHP (pressure), holding time during HHP, time of HHP application (after cheese manufacture) were investigated on reduced fat, and reduced salt cheeses by monitoring changes in the rheological, textural, microbial, microstructural and sensory properties. Cheese functionality during ripening was assessed using texture profile analysis (TPA) and dynamic low-amplitude oscillatory rheology. Quantitative descriptive analysis was conducted with ≥ 9 trained panelists to evaluate texture and flavor attributes using a 15 point scale. Softening of cheese structure, decrease in crossover temperature (loss tangent = 1) and increase in maximum loss tangent were observed with HHP treatment. Pressures ≥ 225 MPa decreased the microbial activity and increased cheese pH. Cheese proteolysis and the level of insoluble calcium (INSOL Ca) phosphate was unaffected by HHP treatment ≤ 500 MPa, while pressures ≥ 500 MPa decreased proteolysis and the level of INSOL Ca phosphate in cheese. HHP treatment ≥ 500 MPa decreased the formation of the key bitter peptide, β-casein (f1-189/192). HHP treatment at 600 MPa successfully maintained the unmelted (e.g., shredability, hardness) and melted (e.g., chewiness, strand length/thickness) functional properties of low-moisture, part-skim Mozzarella cheese and extended the shelf-life from 4-6 wk to 20 wk. Application of HHP treatment shortly after manufacture resulted in greater changes in cheese texture and rheology compared to HHP treatment after a few weeks of ripening.

In conclusion, post-manufacture HHP treatment of cheese could help in controlling cheese texture, physical characteristics, microflora, and sensory properties, as well as extended shelf-life.

SUMMARY

Fat and salt are important components of cheese and significant reduction in the fat and salt contents influence not only the flavor, but also causes major changes in cheese texture by affecting the microbial and enzymatic activity. Several methods have been investigated to improve the quality of reduced and low salt cheese. High hydrostatic pressure (HHP) processing is a promising technique to improve the quality of reduced fat and salt cheese post-manufacture and increase the shelf-life of cheeses. HHP can successfully control microbial and enzymatic activity, and modifies texture by influencing hydrophobic and electrostatic interactions in cheeses (Martinez-Rodriguez et al., 2012).

We investigated various pressures (48 to 402 MPa) and holding time (2.5 to 19.5 min) conditions to understand the impact of HHP on reduced fat (~7% fat) Cheddar cheese texture, rheology, microbiology and flavor development. Central composite experimental design was used to explore relationship between HHP treatment and holding time. The pressure had a greater impact on cheese texture and microflora compared to holding time. Cheese hardness, measured by texture profile analysis (TPA), was significantly decreased with HHP treatments ≥ 225 MPa. Rheological measurements were used to investigate the influence of HHP treatments on protein interactions. We found that pressures ≥ 225 MPa increased the maximum loss tangent (LTmax) values that observed during heating by decreasing hydrophobic interactions. Confocal laser scanning microscopy (CLSM) images indicated that fat was more dispersed in the protein network for cheeses that were HHP treated at ≥ 225 MPa. Cheese proteolysis, which was measured with water soluble nitrogen (W-SN) and trichloroacetic acid soluble nitrogen (TCA-SN), was unaffected by the HHP treatments investigated.

Reducing the salt content in Cheddar cheese by a significant amount adversely influences the texture and flavor, as enzymatic and microbial activities and the conformation of proteins are all influenced by salt content. The application of 405 MPa HHP treatment for 3 min (applied at 1 wk of ripening) on Cheddar cheeses that manufactured with four salt in moisture (S/M) levels: regular (5.3%), reduced (2.5%), low (1.9%), and no salt (~0.2%) were investigated. Lactococci starters were measured using aerobic incubation on de Man, Rogosa and Sharpe (MRS) agar at 32°C for 48 h. Cheese proteolysis was analyzed by TCA-SN and urea polyacrylamide gel electrophoresis (PAGE). Sensory properties of cheeses were monitored by using Quantitative Descriptive Analysis (QDA) with ≥ 9 trained panelists. For the cheeses with lower S/M content, HHP treatment caused greater reduction in the starter culture numbers. Neither level of S/M or HHP treatment influenced the level of TCA-SN. Accumulation of bitter β-casein (f1-189/192) peptide increased with reduction in S/M level while HHP treatment did not influence the levels of β-casein (f1-189/192) peptide. Sensory flavor properties of cheeses were not influenced by HHP treatment, while decrease in the level of NaCl increased acidity and bitterness attributes. At 2 wk ripening, HHP treatment increased LTmax values during heating, decreased crossover temperature (where LT = 1) and decreased TPA hardness; however, after 2 wk of ripening cheeses at the same S/M level exhibited similar rheological and textural properties.

Application of HHP treatment along with milk ultrafiltration (UF) retentate was studied on Cheddar cheeses manufactured by using a less proteolytic coagulant (fermentation-produced camel chymosin). Three types of low Na (0.8% NaCl) cheeses were manufactured: non-UF fortified, no HHP applied; UF fortified, no HHP applied; and UF fortified, HHP treated (500 MPa for 3 min applied at 1 d post manufacture). Pressure treatment significantly decreased the crossover point and increased LTmax values. Milk retentate fortified and HHP treated cheese exhibited lower levels of proteolysis after 1 mo of ripening. Lower levels of bitter β-CN (f1-189/192) peptide was observed for UF retentate fortified and HHP treated cheese. Milk UF retentate fortification and HHP treatment resulted in similar acidity and bitterness scores compared to control.

Influence of 500 and 600 MPa HHP treatment on the refrigerated (4°C) performance shelf-life of reduced Na, low-moisture, part-skim (LMPS) Mozzarella cheese was investigated. At 2 wk of storage, level of insoluble (INSOL) Ca decreased with increasing HHP treatment, causing lower crossover temperatures and higher LTmax values in cheeses at 2 wk of storage. Pressure treatment of 500 and 600 MPa significantly decreased TPA hardness until 8 wk of storage; however, by 20 wk storage 600 MPa HHP treated cheese exhibited higher hardness values. Pressure treatment of 600 MPa significantly decreased the α_{s1}-CN breakdown and the level of TCA-SN. Cheeses that were HHP treated at 600 MPa exhibited higher strand length, strand thickness, melted cheese firmness and chewiness, and lower blister quantity compared to control after 12 wk of storage.

In conclusion, this study demonstrated that HHP treatment influenced the textural, rheological, microbiological and sensory properties of rennet-milk gels. Pressure treatment created more-liquid like cheese structure by decreasing the level of INSOL Ca and weakening hydrophobic interactions. Time of HHP treatment significantly influences cheese texture and rheological properties. Cheeses HHP treated at earlier stages of ripening exhibited greater changes in texture and rheology. A negative relationship was observed between HHP treatment and starter bacteria numbers and the lethality impact of HHP on starter bacteria increased with decreasing NaCl levels. Unwanted changes in cheese texture and rheology during ripening can be minimized with HHP treatment.

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Chapter 1.

Literature Review

1.1 MILK

Milk is secreted by mammary gland of mammals to nourish its infants. The term “milk” in this thesis refers to bovine milk. There are many factors affecting the milk composition, such as, breed, feed, season, stage of lactation, health of the animal, geographical location, and cattle management. Average composition of milk is given in Table 1.1. Triglycerides are the primary component of milk fat, varying in chain length (2- 20) and saturation (0 – 4) (Walstra et al., 2006). The others lipids found in milk are phospholipids, free fatty acids, mono and di-glycerides and cholesterol. About 80% of the protein found in milk is caseins, which is a mixture of four proteins: α_{s1}-, α_{s2}-, β-, and κ-caseins. Caseins are phosphorylated in different extents and have little or no secondary structure. The remaining 20% is mostly serum proteins; β-lactoglobulin, α-lactalbumin, lactoferrin and immunoglobulins. Milk also contains large number of minor proteins, including a broad range of enzymes. Properties of structural components in milk are given in Table 1.2. The major carbohydrate in milk is lactose. Lactose is a disaccharide composed of glucose and galactose, and found only in milk. Primary mineral substances in milk are Ca, P, Mg, Na, and K; citrate is the principal organic acid (Walstra et al., 2006).

1.2 CASEINS

Caseins are the major proteins in bovine milk, they roughly consist of 80% of total protein content, and they are phosphorylated in nature (Fox and Brodkorb, 2008). Caseins contain a few cysteine and many proline residues, thus, they have only short lengths α-helix and little tertiary structure. The major types of caseins are: α_{s1}-, α_{s2}-, β-, and κ-caseins (Walstra et al., 2006). Caseins are found as micelles (large particles) in milk and the size of the micelles ranges from 50-600 nm (average 150 nm) (Fox and Brodkorb, 2008). The formation of casein micelles is controlled by the hydrophobic interactions and electrostatic repulsive interactions (Horne, 1998). According to dual-binding model (Horne, 1998), micelles grow by two linkages: hydrophobic interactions and colloidal calcium phosphate (CCP) crosslinks. κ-Casein terminates the growth of micelles because it does not take part in CCP linkages and it does not have a second hydrophobic region. Hydrophilic glycosylated C-terminals of κ-casein stick out of the micelle and stabilizes the micelle with steric hindrance and electrostatic repulsion. Casein micelles are destabilized by the enzymatic removal of these hydrophilic residues or decreasing the negative charge of micelles by approaching the isoelectric point (pH 4.6) of caseins (Horne, 1998).

1.2.1 α-Caseins

α_{s1}-Casein contains 199 amino acids and 17 proline residues. It has 8 phosphate group attached to the serine residue and have a high net negative charge. α_{s1}-Casein is very sensitive to Ca^{2+} ions and can be precipitated in ~7mM Ca^{2+} concentrations (Fox, 2003; Walstra et al., 2006).

α_{s2}-Casein contains 207 amino acids and 10 proline residues. α_{s2}-Caseins has several variants basically varying in amount of seryl-phosphate residues (10-14). High amount of seryl-phosphate residues in α_{s2}-casein makes it the most sensitive casein to Ca ions. α_{s2}-Casein also has 2 cysteine residues which form an –S-S- bridge (Fox, 2003; Walstra et al., 2006).

1.2.2 β-Caseins

β-Casein contains 209 amino acids and 35 proline residues. β-Casein is very flexible due to high number of proline found in its structure. β-Casein has 5 seryl-phosphate residues and no cysteine groups. β-Casein is less sensitive to Ca ions compared to α-caseins due to its lower number of seryl-phosphate residues. β-Casein has an amphiphilic nature: it has a hydrophilic head and hydrophobic tail (Fox, 2003; Walstra et al., 2006).

1.2.3 κ-Casein

κ-Casein contains 169 amino acids and 20 proline residues. κ-Casein has two cysteine and one seryl-phosphate residue. κ-casein is the least sensitive casein to Ca^{2+} precipitation. Unlike α-, β-caseins, it has charged residues on both C and N terminals. There is also a sugar group attached to κ-casein on the C-terminal, increasing the negative charge and the hydrophobic nature of the protein. κ-Casein has a vital role for the stability of casein micelle as it shields α- and β-caseins from precipitation by Ca ions and creates steric hindrance between micelles. During cheesemaking, casein micelles are destabilized by cleaving Phe_{105}-Met_{106} bond on κ-casein with the chymosin enzyme, which produces para κ-casein and glycomacropeptide and initiate rennet curd formation (Horne, 1998; Fox, 2003; Walstra et al., 2006).

1.3 COAGULANTS

Coagulants used in cheesemaking are proteolytic enzymes. Coagulants have been known and used for cheese manufacture for thousands of years. Traditionally, enzymes for cheesemaking used to be extracted from the stomach of ruminants. Currently there are microbial and plant origin coagulants commonly available for cheesemaking. The main enzyme used for milk clotting is called chymosin or rennet. However, the name “chymosin” is more preferred over “rennet” to prevent confusion with a similar proteolytic enzyme “renin”. Chr. Hansen produced the first commercially available standardized (purified) chymosin in 1874. Chymosin cleaves the Phe_{105}–Met_{106} bond on bovine κ-casein. This enzymatic action disturbs colloidal stability, leading to casein aggregation and liquid syneresis from the protein network. Coagulants that have been the most successful for cheesemaking are aspartic proteases (Harboe et al., 2010).

1.3.1 Bovine chymosin

Bovine chymosin has long been used as a milk coagulating enzyme for cheese manufacture. Specificity of bovine chymosin on the scissile bond, its low general proteolytic activity on milk proteins, and its mildly acidic pH optima makes it very preferable for cheese manufacturers (Kappeler et al., 2006).

Bovine chymosin is a single polypeptide chain containing 323 amino acid residues and two domains. Similar to other aspartic proteases, it is low in basic amino acids, and high in dicarboxylic and β-hydroxy amino acid residues. Catalytic site aspartates, Asp_{34} and Asp_{216}, are located on the corner of two extended loops (ψ-like structures) in N and C terminal domains residues. Interaction of two loops (residues 33-37 and residues 215-219) and a water molecule creates the network between side chains of aspartates residues (Chitpinityol and Crabbe, 1998).

A β-hairpin, located above the catalytic region, interacts with substrate upon binding (Sorensen et al., 2011).

1.3.2 Camel chymosin

Camel chymosin has 70 % more clotting activity on bovine milk casein compared to bovine chymosin (Keppaler et al., 2006). Moreover, its general proteolytic activity of bovine caseins was 5 times lower compared to bovine chymosin, which indicates higher specificity on the scissile (Phe_{105}-Met_{106}) bond. Unfortunately, no crystal structure is yet available for camel chymosin. Sorensen et al. (2011) reported that these two enzymes (camel and bovine chymosins) have 85 % sequence identity and 94 % sequence similarity. Knowing the high degree of sequence identity between bovine and camel chymosin, and high degree of structural homology between pepsin-like aspartic proteases, structure of camel chymosin should be very similar to bovine chymosin. Bovine and camel chymosin differs in 25 titratable groups for a total 48 amino acid residues. These different amino acid residues are not localized, they are spread out all over the polypeptide chain (Figure 1.1). Neighboring residues of catalytic Asp_{34} and Asp_{216} are conserved. There are only 6 different residues around the κ-casein binding region. Camel chymosin has -6 charge while bovine variant has -12. Camel chymosin contains a positively charged area leading to enzyme residue that interacts with Arg_{97} on the scissile region of κ-casein during hydrolysis, which might help the association of caseins, which carries net negative charge at pH values > 4.6 (Sorensen et al., 2011).

1.4 CHEESE

Fresh curd is basically concentrated milk that is manufactured by the coagulation of milk proteins. There is great variation in cheese manufacturing steps depending on the cheese type. Some common steps for cheese manufacture includes milk clotting, whey removal, acid production, salting, curd fusion and ripening (Walstra et al., 2006). The majority of cheeses are manufactured with the enzymatic coagulation of caseins. In this thesis, we have worked with Cheddar and Mozzarella cheeses, which are both manufactured with enzymatic coagulation of caseins.

1.4.1 Cheddar cheese

Cheddar cheese is thought to originate from the English town of Cheddar in Somerset. Cheddar is a hard cheese with an average composition of 34% fat, 35% moisture, and 25% casein (protein) (Carunchia Whetstine et al., 2006). Cheddar is ripened for several months (at temperatures between 4 to 12°C) to develop desired flavor and texture characteristics. Lactic culture and rennet is used in manufacture of Cheddar. Starter bacteria is primarily responsible for the development of characteristic flavor during ripening. During ripening, caseins are hydrolyzed with the activity of residual coagulant and proteolytic enzymes produced by starter bacteria, which help the cheese to develop desired flavor and smooth texture (Bassette and Acosta, 1999).

1.4.2 Mozzarella cheese

Mozzarella is a pasta filata, or stretched curd, cheese which was traditionally made with water buffalo milk. Even though Mozzarella originated in Italy, currently the United States is the largest producer (Kindstedt, 1993; Bassette and Acosta, 1999). In 2012, the US produced ~1.65 million tons of Mozzarella (Wisconsin Milk Marketing Board, Inc., Madison, WI, USA). Four different categories of Mozzarella cheese are defined in the standard of identity in the US based on their moisture and fat in dry matter content (Table 1.3). High moisture Mozzarella cheeses (Mozzarella and part-skim Mozzarella) are often used as table cheese (or in salads) due to their poor functional properties for pizza (e.g. shredding and melting) and low shelf-life. On the contrary, low moisture Mozzarella cheeses (low-moisture and low-moisture, part-skim) have firmer texture, good shredding and melting properties, and longer shelf-life; which makes them more suitable for use on pizza (Kindstedt, 1993).

Mozzarella cheese can be manufactured using either starter culture or an organic acid. Usually, a mixture of thermophilic lactic acid bacteria (*Lactococcus thermophilus, Lactobacillus delbrueckii ssp. bulgaricus,* and *Lactobacillus helveticus*) is used as starter culture. After gel formation, cutting, and draining of whey, the curds are formed into blocks and milled at pH ~5.3. Milled curds undergo cooking and stretching, which gives the final cheese its fibrous structure, melt and stretch properties. Hot curd is molded, briefly chilled in water and salted in brine. Mozzarella cheese is mostly stored at refrigeration temperature (4°C) (Kindstedt, 1993; Bassette and Acosta, 1999).

1.5 FAT AND SALT REDUCTION IN CHEESE

Cheese is a good source of protein, calcium and phosphorus; meanwhile many cheese varieties are high in fat and salt. Salt in this thesis refers to NaCl unless the type of salt is specified. High amount of fat and sodium in cheese are concern, especially for older consumers (Johnson et al., 2009). High dietary sodium intake has been correlated with high blood pressure and high blood pressure related cardiovascular problems, even though these studies need further verification for long term health outcomes (Taylor et al., 2011). Carunchia Whetstine et al.

(2006) reported that 41% of the US customers are careful about their fat intake. Manufacturing good quality low fat and salt Cheddar cheese is challenging because fat and salt content influences starter activity, flavor development, flavor release, textural and visual attributes of cheese (Bryant et al., 1995; Johnson et al., 2009).

1.5.1 Fat reduction in Cheddar cheese

Decreasing fat content directly, and indirectly, influences the sensory profile of Cheddar cheese. Direct influence of fat reduction on Cheddar cheese flavor is the lower production of aromatic compounds released by the breakdown of triglycerides and fatty acids. Fat reduction indirectly affects the flavor of Cheddar cheese by changing the enzymatic and microbial activity as a result of the resultant low salt in moisture (S/M) environment. Also the threshold levels for fat and water soluble aroma compounds are different in the low fat cheese environment. Regular and low fat Cheddar cheeses have significantly different flavor characteristics. Regular fat Cheddar cheese generally exhibits buttery, sweet, fruity and acidic flavor profile while low fat Cheddar cheeses commonly reported to have flat, bitter, meaty-brothy and non-characteristic flavors (Milo and Reineccius, 1997; Johnson et al., 2009; Govindasamy-Lucey et al., 2010).

Fat in cheese partly undergoes hydrolytic degradation by the lipases originated from milk and cheese microflora (e.g. starter bacteria, starter adjunct and non-starter lactic acid bacteria) (McSweeney, 2004). Oxidative degradation of fat is limited due to low redox potential of cheese. Liberated free fatty acids, especially short and intermediate chain fatty acids, directly contribute to Cheddar cheese flavor (McSweeney and Sousa, 2000). Free fatty acids can also be metabolized to fatty acid lactones, cyclic compounds formed by the intramolecular esterification of hydroxy fatty acids. The principal lactones in Cheddar cheese are γ- and δ-lactones which have 5- and 6-sided rings, respectively, and are stable and strongly flavored (McSweeney, 2004). Subramanian et al. (2009) reported that lactones are also one of the principal flavor compounds for Cheddar cheese. Free fatty acids degradation can also result in the creation of highly flavored esters, or thioesters, during ripening (McSweeney and Sousa, 2000). Esters contribute to a fruity flavor in Cheddar cheese (Carunchia Whetstine et al., 2006).

Bitterness is a common defect in low fat Cheddar cheeses. Salt in moisture (S/M) phase of the cheese decreases as the fat is replaced by water and protein. The decrease in S/M alters the activity of residual coagulant and starter bacteria, and possibly changes the conformation of caseins, leading to liberation and accumulation of bitter hydrophobic peptides (Govindasamy-Lucey et al., 2010). Drake and Swanson (1995) suggested that using lesser amount of starter culture or special starter strains, decreasing the ripening time of milk, and washing the curd help to prevent excessive acidity and improve low fat Cheddar cheese flavor. Adjunct starter cultures and less proteolytic coagulants have also been reported to improve the flavor of reduced and low fat Cheddar cheeses (Drake and Swanson, 1995; Govindasamy-Lucey et al., 2010). Adjunct starter bacteria can produce desirable flavorful peptides and decrease bitterness in cheeses (Drake and Swanson, 1995). *Lactobacillus* species are commonly used as starter adjuncts. Drake and Swanson (1995) reported that using *Lactobacillus helveticus* as a starter adjunct in the manufacture of low fat Cheddar cheese successfully decreased bitterness and increased the intensity of cheese flavor. Govindasamy-Lucey et al. (2010) reported that using less proteolytic camel chymosin successfully decreased bitterness in low fat Cheddar cheese.

Carunchia Whetstine et al. (2006) reported that low and reduced fat cheeses had different flavor threshold values in mouth during mastication. They reported that hydrophobic compounds had a higher sensory threshold and lower vapor pressure in oil than in water, suggesting when fat is present, hydrophobic compounds are bound to fat molecules by hydrophobic and van der Waals interactions, which decreased their perception.

Casein-casein interactions increase in the high protein environment of reduced and low fat Cheddar cheeses, leading to cheeses with firm and rubbery texture (Bryant et al., 1995). Bryant et al. (1995) reported that decreasing the fat content of cheese increased cheese firmness and springiness, and decreased adhesiveness and cohesiveness. Fat reduction also influences the viscoelastic behavior of the cheese. Ustunol et al. (1995) reported that storage modulus (G') and loss modulus (G'') values, measured by small amplitude oscillatory rheology, exhibited only slight change for reduced and low fat Cheddar cheeses during heating from 30°C to 70°C, while G' and G'' decreased steadily upon heating for regular fat cheeses (Ustunol et al., 1995). Loss tangent value (LT) is the ratio between the viscous properties and the elastic properties of the material ($LT= G'/G''$). Maximum loss tangent (LTmax) value, which is an indicator of how meltable the cheese is, were lower for reduced and low fat cheese samples compared to regular fat cheese. On the other hand, Lucey et al. (2003) reported that fat is liquid at temperatures $\geq$ 40°C, thus, the rheological properties of cheese above 40°C is primarily controlled by protein-protein interactions. Fat acts as an inert filler in cheese unless the milk is homogenized. The change in the rheological behaviors of cheese with fat reduction is primarily as a result of the change in the other components, such as, moisture and protein.

One way to improve the texture of reduced and low fat Cheddar cheese is modifying the make procedure in a way to increase the final moisture content of cheese, such as, decreasing cook temperature and time, washing the curd with cold water, making larger loaves during cheddaring, and shorter cheddaring time (Drake and Swanson, 1995). In general, reduced and low fat cheese firmness decreases as the moisture content increases, which could be as a result of lower concentration of protein due to high moisture content and/or water increases the lubricity between proteins. Homogenization of cheese milk decreased the firmness of low fat cheese (Drake and Swanson, 1995). More dispersed milkfat in low fat cheese matrix by the homogenization of cheese milk possibly interfered with the casein-casein interactions, resulting in decrease cheese firmness.

Fat substitutes and fat mimetics has been studied in cheese to help replace milkfat. Fat substitutes can be structured lipids or synthetic compounds, targeted to possess functional and physical properties of fats. For example, hydrophobic fat substitute, Olean^{TM} (Procter & Gamble, Cincinnati, OH, USA), successfully decreased low fat cheese firmness but caused inadequate clean up after chewing. Fat mimetics such as “Dairy Lo^{TM}” (Pfizer Inc., Groton, CT, USA), “Simplesse^{TM}” (Nutresweet Co., Deerfield, IL, USA) and “Novagel^{TM}” (FMC Corp., Philadelphia, PA, USA) also successfully improved the chew-down characteristics of low fat cheese. On the other hand, fat mimetics are hydrophilic molecules; thus, they fail to achieve the non-polar contribution of milkfat such as delivering the flavor and imitating the mouthfeel of milkfat.

1.5.2 Salt reduction in Cheddar cheese

Salt (NaCl) assist cheese manufacturers during and after cheesemaking to achieve desired final cheese characteristics and shelf-life, thus any significant reduction in the salt content of cheese is challenging. Salt directly contributes the Cheddar cheese flavor. It also indirectly influences the flavor by changing the microbial and enzymatic activity in cheese. Reduced and low NaCl Cheddar cheeses has often been reported to have soft, pasty and adhesive texture (Guinee and O’Kennedy, 2007; Johnson et al., 2009).

Schroeder et al. (1988) reported that the NaCl level was negatively correlated with the moisture content and level of proteolysis in Cheddar cheese. Cheddar cheese texture was significantly influenced by the NaCl reduction. As NaCl content decreased, sensory firmness of the cheese decreased and sensory adhesiveness and cohesiveness of the cheese increased. Low NaCl Cheddar cheeses also exhibited acidic and bitter flavor (Schroeder et al., 1988). Changes in conformation of β-casein with the NaCl content was also suggested (Fox and Walley, 1971).

Reducing the NaCl content of Cheddar cheese has been reported to cause bitter off-flavor development (Guinee, 2004). Increased microbial activity as a result of decreased osmotic pressure was indicated as a possible cause of bitterness in low NaCl Cheddar cheeses (Lawrence and Gilles, 1969; Lowrie and Lawrence, 1972). However, recent studies showed that manufacturing low NaCl Cheddar cheese with less proteolytic coagulant successfully decreased bitterness, indicating the activity of residual coagulant as a primary reason for the bitterness in low NaCl cheeses (Guinee and Fox, 2004; Grant, 2011). β-Casein (f-1/189-192) peptide is associated with bitterness in cheese. Previous studies reported that the level of β-casein (f-1/189-192) peptide increased as the level of NaCl decreased in cheese (Kelly et al., 1996; Grant, 2011).

Partial or complete replacement of NaCl with other salts, and using flavor enhancers, in Cheddar cheese manufacture has been previously investigated. Lindsay et al. (1982) reported that cheeses manufactured with 1:1 blend of NaCl/KCl were scored bitter and less acceptable by the sensory panelists compared to cheeses manufactured with NaCl alone. On the other hand, Grummer et al. (2013) reported that cheeses manufactured with 2:3 blend of NaCl/KCl exhibited comparable sensory characteristics compared to cheeses manufactured with NaCl alone. However, flavor enhancers increased, decreased or had no effect on the sensory properties of Cheddar cheeses manufactured with NaCl/KCl blend. Kosikowski (1983) reported that reduced

NaCl (~ 1% NaCl) cheese manufactured with milk ultrafiltration (UF) retentate fortified cheese milk exhibited good to excellent quality while control cheese (~ 1% NaCl, non-UF fortified) was acid, bitter and pasty.

1.6 HIGH HYDROSTATIC PRESSURE PROCESSING

High hydrostatic pressure (HHP) processing is a fairly old food processing technique that first applications go as back as late nineteenth century. However, HHP treated food products have been available on the market only for the past two decades. Application of HHP includes many food products, such as, guacamole, fruit jams, jellies, raw meat and meat products. High pressure improves food safety and extends the shelf life without significantly changing the product characteristics, which makes it an alternative technology to heat treatment, and advantageous for heat sensitive products. High pressure processing is a promising food processing technology due to the fact that HHP exhibits antimicrobial effects without impairing the nutritional quality. Functional properties of foods are also altered by HHP via its impact on weak interactions, such as, hydrophobic and electrostatic interactions. Thus, post manufacture flavor development and the texture of the dairy products can be successfully modified with HHP (San Martin-Gonzales et al., 2006).

The effect of HHP on chemical reactions and interactions between molecules are governed by Le Chatelier’s principle, thus when HHP treatment is applied to a food product, the system shifts to a state that occupies a lower volume than the original state to prevent equilibrium. As a result of the HHP driven negative volume change, processes and interactions that requires negative volume change are favored and vice versa. Covalent bonds are resistant to HHP treatments, at least up to 1 – 1.5 GPa, thus HHP related changes in biological systems are primarily as a result of the altered non-covalent interactions (Mozhaev et al., 1994; Mozhaev et al., 1996)

Ion formation decreases the volume of a system as a result of the coulombic effect of the ion created. Therefore, electrostatic interactions are not favored with HHP treatment (Mozhaev et al., 1994). Mozhaev et al. (1996) reported that solvation of a singly charged ion in water requires a volume volume change of -10 mL/mol. Second ionization of di- and tribasic acids requires a greater volume reduction as the electro-restriction is proportional to the square of the ionic charge. They also noted ionization of charged groups requires greater volume reduction in non-polar solvents. It is challenging to measure the effect of HHP on pH dependent processes as the dissociation constants of weak acids varies under pressure. Mozhaev et al. (1996) reported that buffers that have small ionization volumes (e.g. tris-HCl) were successful in minimizing the pH variation in HHP experiments compared to buffers with high ionization volume, such as phosphate.

Hydrogen bonds are nearly pressure insensitive due to negligible volume change during the formation and breakdown of these bonds. Van Eldik et al. (1989) reported that HHP processing stabilized hydrophobic interactions. Patterson (1999) related the high resistance of DNA to HHP processing due to the high numbers of hydrogen bonds found in the DNA structure. Kitchen et al. (1992) reported that HHP treatment of 1 GPa resulted in ~ 9% increase in the number of hydrogen bonding between protein and water. Even though the accessible surface area decreased with 1 GPa HHP treatment compared to 1 kPa (i.e., 1 atm), protein hydration increased with HHP as a result of the ionization of the charged groups.

In general, formation of hydrophobic interactions causes a positive volume change in the system thus, HHP treatment is unfavorable for hydrophobic interactions. Aromatic rings are an exception to this statement as stacking of aromatic rings exhibits negative volume change. Crisman and Randolph (2008) reported that the decrease in the hydrophobic interactions with HHP treatment was as a result of the increased hydration of the hydrophobic surfaces. Decrease in the hydrophobic interactions with HHP treatment was able to colloidally stabilize (suspended monomers in solution) prevent aggregation T4 lysozyme monomers against aggregation. Pressures of 100 to 300 MPa dissolves protein aggregates (created by hydrophobic association of monomers) and disfavors intermolecular (protein-protein) interactions and favors the protein to return to the native state. Similarly, Gross and Jaenicke (1993) reported that in general, protein dissociation is the initial step in oligomeric protein denaturation, while single chain proteins can directly undergo denaturation with HHP (at pressures ≥ 400 MPa, pH 7 and temperature 25oC). Payens and Heremans (1969) reported that HHP driven aggregation and dissociation depended also on the processing temperatures. They reported that at room temperatures purified β-casein molecules dissociate with pressures up to 125 MPa, but that further increase in pressure initiated aggregation of β-caseins. However, at low temperatures (temperature was not specified but likely < 7oC) HHP induced dissociation of β-caseins did not occur as β-caseins were already dissociated due to weak hydrophobic interactions, but a sharp increase was observed in the aggregation of β-caseins at pressures ≥ 275 MPa, which was suggested to due to conformational changes in β-caseins at high pressures. Payens and Heremans (1969) also reported that the HHP polymerization of β-caseins were completely reversible with pressure release.

HHP treatment causes elastic (reversible) and plastic (conformational, irreversible) changes in proteins. Elastic changes in proteins affect hydration, bond length and changes in the cavities in protein structure while secondary structure remains unaffected. Plastic changes are more related to protein unfolding or denaturation. α-Helical regions are more compressible under

HHP. Enzymatic activity is also influenced by HHP treatment as many enzymes have positive intrinsic compressibility. The compression of enzymes at high pressure conditions reduce their flexibility, which is crucial for enzyme action (Heremans and Smeller, 1998). Heremans and Smeller (1998) reported that low pressures (< 100 MPa) causes tightening of the protein structure, however, higher pressure has a loosening effect as a result of increased protein hydration.

1.6.1 High hydrostatic pressure treatment of milk

High hydrostatic pressure treatment of milk influences the size of casein micelles, denatures whey proteins, changes the mineral equilibrium, and alters rennet coagulation time (Lopez-Fandino, 2006). Whey proteins have different resistance to HHP. Huppertz et al. (2005) reported that α-lactalbumin was denatured at pressures ≥ 400 MPa while pressures ≥ 100 MPa was sufficient to denature β-lactoglobulin. The high baro-resistance of α-lactalbumin to HHP induced denaturation compared to β-lactoglobulin was explained by the more rigid structure of α-lactalbumin due to its high number of intra-molecular disulphide bonds, while β-lactoglobulin only has one free sulfhydryl group (Huppertz et al., 2005). Huppertz et al. (2004a) reported that the effect of HHP treatment on the size of the casein micelles were dependent on the magnitude of HHP, holding time, pH and temperature of milk during HHP application, and storage time after HHP. Casein micelle size was not influenced by HHP treatments ≤ 200 MPa; however, the application of 250 MPa increased micelle size by 25% at 20°C, which was explained by the integration of denatured β-lactoglobulin into casein micelles and formation of casein aggregates. The application of pressures from 300 to 800 MPa decreased the size of casein micelles. According the casein micelle model of Horne (1998), insoluble (INSOL) Ca in casein micelles neutralizes the charged phosphoserine residues, and excessive solubilization of INSOL Ca disrupts casein micelles. Huppertz et al. (2004a) suggested that solubilization of INSOL Ca due to HHP treatment might be responsible for the decrease in the size of casein micelles with the application of pressures ≥ 300 MPa. Casein micelles that were disrupted by HHP treatment (≤ 300 MPa) reassociated during decompressing, which was explained by the reformation of hydrophobic interactions with the pressure release (Huppertz et al., 2004a). Casein micelles that were treated ≥ 300 MPa HHP did not reassociate and remained smaller than non-pressurized control after pressure release. Excessive denaturation of β-lactoglobulin (up to 80%) with the HHP treatment ≥ 300 MPa was suggested to hinder the reformation of casein micelles after pressure release. Huppertz et al. (2005) also studied rennet coagulation properties of heat-treated milk (90°C for 10 min) under various HHP (0-600 MPa) conditions. They reported that heat-treated milk was unable to coagulate with rennet; however, HHP treatment at pressures between 250-600 MPa for 0-30 min resulted in heated milk that exhibited similar or shorter rennet coagulation time than unheated milk. The rennet gels produced from the heated milk that was HHP treated at 250–600 MPa for 30 min or 400 or 600 MPa for 0 min were stronger than unheated unpressurized milk.

Previous studies investigated the effect of HHP treatment on the level of INSOL Ca. Desobry-Banon et al. (1994) reported that HHP treatment up to 200 MPa decreased the level of INSOL Ca, but milk samples HHP treated ≥ 200 MPa exhibited similar INSOL Ca levels to control. Schrader et al. (1997) reported that HHP treatment of 400 MPa for 5 min decreased the level of INSOL Ca; however, solubilized Ca returned to the colloidal state after 72 h of storage. Similarly, Law et al. (1998) reported that INSOL Ca that was solubilized by the application of HHP returns back to INSOL phase after pressure release. Huppertz and de Kruif (2007) cross-linked the casein micelles and investigated the effect of HHP on INSOL Ca levels. They reported that there was a negative correlation between the level of INSOL Ca in milk with HHP treatment from 100 to 400 MPa, i.e., the level of INSOL Ca decreased with an increase in the magnitude of HHP.

1.6.2 High hydrostatic pressure treatment of cheese

The effect of various HHP treatments on the flavor development of different cheese varieties is given in Table 1.4. High pressure treatment of cheese has attracted attention of dairy scientists ever since the patent application of Yokohama et al. (1992), claiming that HHP treatment at 50 MPa for 3 d resulted in Cheddar cheeses comparable to 6 mo of conventionally aged Cheddar cheese. O'Reilly et al. (2000) studied the same HHP conditions used by Yokohama et al. (1992) for Cheddar cheese. They reported that the proteolysis increased, but the rate was much lower than reported by Yokohama et al. (1992). The application of 50 MPa HHP increased proteolysis in various cheeses, such as, Camembert (Kolakowski et al., 1998), caprine milk cheese (Saldo et al., 2002), Pere Joseph cheese (Messens et al., 2000a). However, proteolysis rates of Gouda (Messens et al., 1999; Reps et al., 1998) and Kurpiowski cheeses (Reps et al., 1998) were not affected by 50 MPa HHP treatment.

Several researchers studied the application of higher pressures for shorter time. Pressure treatment at 300 MPa for 10 min and at 400 MPa for 5 min increased the production of free amino acids in ewe's milk and goat milk cheeses, respectively (Saldo et al., 2002; Juan et al., 2007). The application of 400 MPa for 10 min did not influence the rate of proteolysis in Cheddar (Rynne et al., 2008). Likewise, proteolysis of Gouda cheese were unaffected by the HHP treatment at 50, 225, 400 MPa for 20, 60, or 100 min (Messens et al., 2000b). Saldo et al.

(2003) reported that HHP at 400 MPa for 5 min reduced flavor development by decreasing lipolysis in goat milk cheese.

High pressure treatment can influence enzymatic activity in cheese. The major proteolytic enzymes that contribute to the primary proteolysis in cheese are plasmin and chymosin. Malone et al. (2003) reported that pressures up to 800 MPa for 5 min at 25^{o}C did not influence plasmin activity. Huppertz et al. (2004b) reported that plasmin activity in HHP treated cheeses were dependent on the pressurization temperature. They reported that 800 MPa HHP treatment for 60 min at 8^{o}C did not influence plasmin activity, while the same HHP treatment at 20^{o}C and 30^{o}C decreased plasmin activity by 15 and 50%, respectively. Chymosin is more sensitive to HHP treatment. Pressures ≥ 400 MPa decreased chymosin activity (Martinez-Rodriguez et al., 2012). Intracellular starter culture enzymes are mostly responsible for the secondary proteolysis in cheese, and these enzymes are released into the cheese matrix as a result of starter lysis. Malone et al. (2002) studied the viability and lysis of various *Lactococcus lactis* subsp. *cremoris* strains. They reported that starter viability and lysis were strain dependent and HHP treatment increased starter lysis. Malone et al. (2003) reported that the activity of intracellular peptidases can be increased, or decreased, with HHP depending on the treatment conditions. They also reported that major proteolytic enzymes in cheese matrix (residual chymosin and starter proteases, such as, cell envelope protease, X-Prolyl-dipeptidyl aminopeptidase, and aminopeptidase N) were partially or completely inactivated with pressures ≥ 500 MPa. Juan et al. (2007) reported that aminopeptidase activity in ewes' milk cheese increased with HHP treatment from 200 to 500 MPa for 10 min at 12^{o}C. They also reported that HHP treated cheeses exhibited lower aminopeptidase activity compared to control at 1 d, however, HHP treated cheeses exhibited higher aminopeptidase activity compared to control at 15 d of ripening. They explained this phenomenon with the increase in membrane permeability with HHP treatment which possibly led to release of the endocellular material to the cheese medium.

High pressure treatment significantly influences the appearance, texture and rheology of cheese (Table 1.5). In general, HHP treatment decreases cheese firmness and caused more liquid like structure (Messens et al., 2000b; Saldo et al., 2000; O'Reilly et al., 2002). Messens et al. (2000b) reported that Gouda cheeses which were HHP treated ≥ 225 MPa exhibited more liquid like structure, and no mechanical openings. Johnston et al. (2002) reported that HHP treatment (100-800 MPa) decreased the hardness of half-fat Cheddar cheese. Saldo et al. (2000) reported that 50 MPa for 72 h or 400 MPa for 5 min HHP treatments resulted in goat milk cheese texture to become less crumbly and more elastic. Juan et al. (2008) also reported that HHP treatment of 300 MPa for 10 min performed at 1 d of ripening resulted in ewes' milk cheeses that became softer, less crumbly and more elastic. Juan et al. (2007) and Juan et al. (2008) reported that the application time of HHP was critical; cheeses HHP treated on 1 d of ripening exhibited significantly different characteristics (e.g. pH, level of free amino acids, texture, flavor) than control while cheeses HHP treated at 15 d of ripening was not significantly different from the control. The time required to reach optimum cooking (melting) performance of low-moisture Mozzarella cheese decreased with the application of 400 MPa for 20 min (O'Reilly et al., 2002). High pressure treatment decreased the melting time (heating at 280°C) and increased stretchability and fluidity of low-moisture Mozzarella cheeses.

Many researchers reported increased pH for various HHP treatments for cheeses like Gouda (Messens et al., 2000b), Cheddar (Rynne et al., 2008), Mozzarella (Johnston and Darcy, 2000), goat's milk (Saldo et al., 2003) and ewes' milk cheese (Juan et al., 2007). The time of HHP treatment, applied pressure and the holding time are the main factors influencing the pH values of cheeses. Casal and Gomez (1999) reported that HHP treatment decreased the acidifying activity of lactococci and lactobacilli strains even though no significant decrease was observed in the cell viability. Higher pH values for HHP treated cheeses could be the result of decreased numbers and/or reduced glycolytic activity of the starter bacteria before the complete fermentation of lactose, or by the buffering (pH increase) caused by INSOL Ca solubilization by the HHP process.

High pressure treatment can also be used to control cheese microflora, thereby the cheese shelf-life and safety. The effect of various HHP conditions on the survival of some bacteria species in various cheeses is given in Table 1.6. High pressure treatment causes structural changes in vegetative cells, such as, disruption of cell membrane, change in membrane permeability and proton efflux system, destruction of organelles, denaturation of membrane proteins, and inactivation of key enzymes, which eventually lead to cell damage or death (Martinez-Rodriguez et al., 2012). San Martin-Gonzales et al. (2006) reported that generally Gram-positive bacteria are more pressure resistant than Gram-negative bacteria. The lack of teichoic acid in the cell wall of Gram-negative bacteria was suggested as a reason for their HHP sensitivity (Martinez-Rodriguez et al., 2012). Yeasts and molds have pressure resistance that is between Gram-positive and Gram-negative bacteria. However, pressure resistance of microorganisms is strongly dependent on genus, species, strain, shape of the microorganism and pressurization medium (San Martin-Gonzales et al., 2006). Yeast and mold spores are more baro-sensitive compared to bacteria spores. Pressure treatment of 400 MPa at room temperature was reported to easily inactivate yeast and mold spores while bacterial spores can survive HHP treatment over 1000 MPa and temperatures exceeding at 100°C. High baro-resistance of bacterial spores is due to their low core water content, thick peptidoglycan layer, and high levels of dipicolinic acid and minerals (Martinez-Rodriguez et al., 2012).

In cheese medium, several components, such as, fat, protein, water, salt and carbohydrate content influence the survival of microorganisms. In general, carbohydrates decrease the barosensitivity of microorganisms more than salts. Water activity (a_w) and pH values of cheeses significantly influence microbial viability and outgrowth. In low a_w environment, cell membranes thicken and permeability decreases due to shrinkage of cells, causing more resistance to HHP treatment. However, low a_w environment is more unfavorable for injured cells. Low pH environment and HHP treatment have a synergistic effect on microbial reduction, i.e., decrease in the microbial numbers is higher in suboptimal pH environment. Low pH values also inhibit the growth the injured cells. Physiological state of microorganism also affects the pressure resistance: bacteria in exponential growth state are more susceptible to HHP treatment compared to bacteria in stationary phase. This phenomenon is explained with the synthesis of new peptides by the bacteria in the stationary phase that protects bacteria against adverse conditions (Martinez-Rodriguez et al., 2012).

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Table 1.1. Approximate composition of bovine milk (Walstra et al., 2006)

Component (% w/w)	Average content in milk	Range
Water	87.1	85.3-88.7
Solids non-fat	8.9	7.9-10
Fat in dry matter	31.0	22-38
Lactose	4.6	3.8-5.3
Fat	4.0	2.5-5.5
Protein	3.3	2.3-4.4
Casein	2.6	1.8-3.5
Mineral substances	0.7	0.57-0.83
Organic Acids	0.17	0.12-0.21
Miscellaneous	0.15	-

Table 1.2. Properties of the structural components in bovine milk (Walstra et al., 2006)

	Milk			
		Plasma		
			Serum	
	Fat globules	Casein micelles	Globular proteins	Lipoprotein particles
Main components	Fat	Casein, water, salts	Serum protein	Lipids, proteins
Content (% dry matter)	4	2.8	0.6	0.01
Volume fraction	0.04	0.1	0.006	10^{-4}
Particle diameter	0.1-10 μl	20-300 nm	3-6 nm	10 nm
Number per ml	10^{10}	10^{14}	10^{17}	10^{14}
Surface area (cm^2/ml milk)	700	40,000	50,000	100
Density (20^{o}C, kg.m^{-3})	920	1,100	1,300	1100
Iso-electric pH	~3.8	~4.6	4-5	~4.0

Table 1.3. Compositional standards for Mozzarella cheese in the U.S. (Kindstedt, 1993).

Type	Moisture (%)	FDM[a] (%)
Mozzarella	> 52 but ≤ 60	≥ 45
Low-moisture	> 45 but ≤ 52	≥ 45
Part-skim	> 52 but ≤ 60	≥ 30 but < 45
Low-moisture, part-skim	> 45 but ≤ 52	≥ 30 but < 45

[a] Fat in dry matter.

Table 1.4. Effect of high pressure treatment on proteolysis and lipolysis in different cheeses (Martinez-Rodriguez et al., 2012).

Cheese variety	Time of application	Treatment conditions P[a]/t[b]/T[c]	Effects
Cheddar	After salting	50/72 h/25	Similar taste and FAA[d] content of a 6 mo-old commercial cheese obtained in 3 d
Cheddar	2, 7, 14, or 21 d	50/72 h/25	Faster α_{s1}-casein hydrolysis and accumulation of α_{s1}-I-casein. Increased pH 4.6 SN/TN[e] and FAA levels.
Cheddar	1 d	70–400/3.5–81.5 h/25	Maximum accumulation of α_{s1}-I-casein at 100 MPa and greatest increase in levels of pH 4.6 SN/TN below 150 MPa. Total FAA decreased as pressure increased.
Cheddar	1 or 4 mo	200–800/5/25	Ripening deceleration at pressure treatments ≥ 400 MPa.
Camembert	5 or 10 d	0.1–500/4 h/5	Most intense proteolysis at 50 MPa on d 10.
Blue-veined	42 d	400–600/10/20	Accelerated breakdown of β- and α_{s2}-casein and increased levels of PTA[f] SN/TN. Reduced lipolytic activity of *P. roqueforti*
Gouda	After brining, 5 or 10 d	50 or 500/20–100/14	No changes in pH 4.6 SN, PTA SN/TN, FAA content and SDS-PAGE profiles.
Edam	After salting, 4, 6, and 8 wk	200 or 400/30/25	No changes in different fractions of nitrogen compounds.
Garrotxa	1 d	400/5/14 followed by 50/72 h/14	Ripening period reduced from 28 to 14 d. Decelerated lipolysis due to lactic acid bacteria or lipolytic enzymes
Ewes' milk cheese	1 or 15 d	200–500/10/12	Lowest concentration of total FFA[g] at pressure treatments of 400 to 500 MPa applied on d 15 after 60 d of ripening compared to other treatments. Highest levels of FFAs were obtained at 300MPa applied on day 1 compared to other treatments.

[a] Pressure (MPa)
[b] Time in minutes unless it is specified
[c] Temperature (°C)
[d] Free amino acid
[e] SN/TN: Soluble nitrogen per total nitrogen
[f] Phosphotungstic acid
[g] Free fatty acid

Table 1.5. Effect of high pressure treatment on appearance, texture and rheological properties of different cheeses (Martinez-Rodriguez et al., 2012).

Parameter evaluated	Cheese variety	Moment of application	Treatment conditions $P^a/t^b/T^c$	Effects
Appearance	Mato	1 d	500/5, 10, or 15/10	L* and a* decreased, whereas b* increased compared to control cheese.
	Garrotxa	1 d	400/5/14	Lower lightness and higher chroma values than control cheese.
	Queso fresco	1 d	400/20/20	More yellowish after 1 d post-treatment than control cheese, but not after 8 d.
	Cheddar, Turkish white-brined	1 d	50–400/5, 10, or 15/22–25	Increasing pressure intensity and holding time did not affect L*, but a* decreased and b* increased compared to control cheese.
Texture and rheological properties	Cheddar	1 and 4 mo	200–800/5/25	Pressures up to 300 MPa applied to 1-mo-old cheese had no significant effect. At 800 MPa, cheese had similar fracture stress and Young's modulus as control cheese. Pressure applied to 4-mo-old cheese increased fracture work.
	Cheddar	1 d	400/10/25	Increased fracture strain and fracture stress values, lower fluidity, flowability, and stretchability increased up to 21 d, but to a lesser extent than in control cheese.
	Low-moisture Mozzarella	1 and 4 d	400/20/25	Reduced time required to attain satisfactory cooking performance (by 15 d). Increased fluidity, flowability, stretchability, and reduced melting time on heating at 280 °C.
	Reduced-fat Mozzarella	1 d	400/5/21	No significant effect on rheological properties
	Gouda	3 d	50, 225, or 400/1 h / 14	Less rigid and solid-like, more viscoelastic, and had less resistance to flow at longer times
	Ewes' milk cheese	1 or 15 d	200–500/10/12	Moderate pressures applied on day 1 increased firmness and cheese treated at higher pressures showed highest deformability, lowest fracturability, and rigidity.

[a] Pressure (MPa)

[b] Time (min)

[c] Temperature (°C)

Table 1.6. Effect of high pressure treatment on vegetative forms and microbial spores in different cheeses (Martinez-Rodriguez et al., 2012).

Microorganism evaluated	Cheese variety	Day of pressure treatment	Treatment conditions P[a]/t[b] /T[c]	Initial counts (log 10 cfu/g)	Inactivation (log 10 cfu/g)
Mesophilic and thermophilic LAB	Hispanico (raw milk cheese)	1	500/10/8	8.38 and 6.45	2.1 and 1.9
Lactic fermentation streptococci	Edam	56	400/120/25	9.41	5.06
Total aerobic mesophilic bacteria *Lactococcus* spp. *Lactobacillus* spp.	Turkish white-brined	1	1600/10/25	7.9, 8.3, and 7.6	5.2, 5.5, and 4.7
Lactococcus lactis (227 and 303)	Cheddar	1	400/20/25	6.24 and 9.75	2.8 and 3.1
Starter and nonstarter LAB	Swiss cheese slurry	1	500/30/25	7.7 and 4	1.7 and CI[d]
P. roqueforti IMI 297987 spores	Cheddar cheese slurry	1	500/20/20	6	CI
B. cereus ATCC 9139 spores	Washed-curd (raw milk cheese)	1	60/210/30–400/15/30	6	2

[a] Pressure (MPa)

[b] Time (min)

[c] Temperature (°C)

[d] CI: Complete inactivation

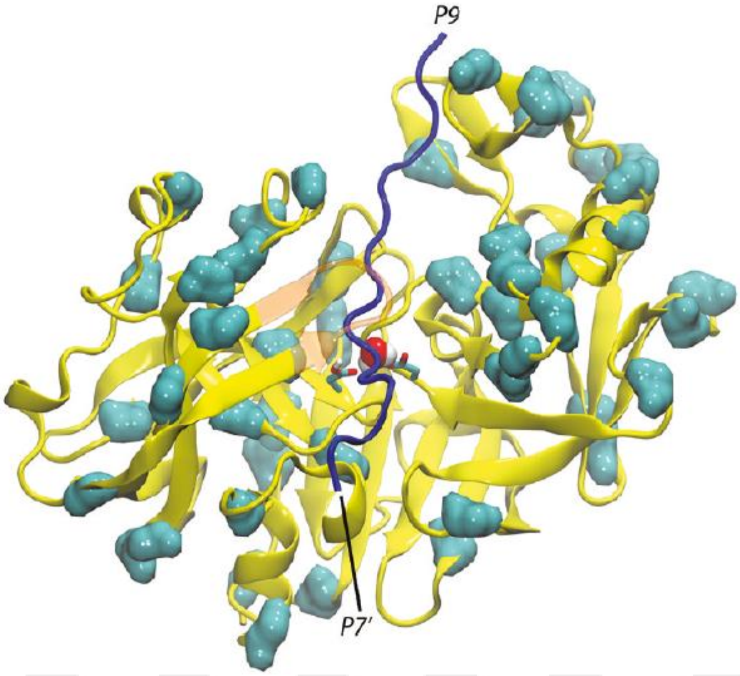

Figure 1.1. Bovine chymosin and 16 amino acid fragment of bovine κ-casein (97-112) complex at the active cleft, which is located between two sub-domains of the molecule and containing the catalytic Asp_{34} and Asp_{216} residues. Cyan areas are the different amino acid residues between bovine and camel chymosin. Molecular model illustrates that different amino acid residues are distributed over the entire molecule (Sorensen et al., 2011).

Chapter 2.

Impact of Various High Pressure Treatments on the Properties of Reduced Fat Cheddar Cheese

2.1 ABSTRACT

A major problem with reduced fat cheese is the difficulty in attaining the characteristic flavor and texture of typical full fat versions. Some previous studies have suggested that high hydrostatic pressure (HHP) can accelerate the ripening of full fat cheeses. Our objective was to investigate the impact of HHP on reduced fat (~7.3% fat) Cheddar cheese with the goal of improving its flavor and texture. We used a central composite rotatable design with response surface methodology to study the impact of pressure and holding time on the rheological, physical, chemical and microbial characteristics of reduced fat Cheddar cheese. A two–level factorial experimental design was chosen to study the effects of the independent variables (pressure and holding time). Pressures were varied from 50 to 400 MPa and holding times ranged from 2.5 to 19.5 min. High pressure was applied 1 wk after cheese manufacture, and analyses were performed at 2 wk, and 1, 3, and 6 mo. The insoluble calcium content as a percentage of total Ca in cheeses were not affected by pressure treatment. Pressure applications ≥ 225 MPa resulted in softer cheese texture during ripening. Pressures ≥ 225 MPa increased melt, and resulted in higher maximum loss tangent (LTmax) values at 2 wk. Pressure treatment had a greater impact on cheese microbial and textural properties than holding time. High pressure treated cheeses also had higher pH values than the control. We did not observe any significant difference in rates of proteolysis between treatments. In conclusion, holding times of around 5

min and pressures of ≥ 225 MPa could potentially be used to improve the excessively firm texture of reduced fat cheese.

2.2 INTRODUCTION

Cheese flavor development is a complex and time consuming process that is not fully understood. Proteolysis is one of the most important steps in cheese ripening (McSweeney and Sousa, 2000; Sousa et al., 2001). Several methods have been developed to improve cheese flavor development, such as, addition of adjunct cultures, using modified starters, increased storage temperatures, and the addition of exogenous enzymes (Law, 2001). Previous studies indicated that the application of high hydrostatic pressure (**HHP**) to cheese can have a positive impact on cheese ripening probably as a result of increased starter lysis and the breakage, and only partial reforming, of bonds in the protein matrix (Yokohama et al., 1992; Messens et al., 1997). Yokohama et al. (1992) claimed that HHP treatment of young cheese at 50 MPa for 72 h gave comparable proteolysis rates and flavor development to 6 mo old conventionally aged Cheddar cheese. Later, O'Reilly et al. (2000) and Saldo et al. (2002) applied similar conditions (50 MPa for 72 h) to Cheddar and goat milk cheeses, respectively, but the increase in the proteolysis was not as significant as the rates suggested by Yokohama et al. (1992). An increase in the proteolysis rate of Camembert cheese (Reps et al., 1998) was observed upon the application of 50 MPa for 4 h. Nevertheless, HHP treatment did not have any significant influence on the proteolysis of Gouda (Reps et al., 1998; Messens et al., 1999) cheese. Other authors have studied the use of higher pressures for shorter times. Saldo et al. (2003) treated goat milk cheese at 400 MPa for 5 min and reported that HHP treated cheese had twice the levels of total free amino acids compared to untreated cheese. However, this HHP treatment (400 MPa for 5 min) decreased lipolysis in this cheese, which was considered an unfavorable impact for goat cheese (Saldo et al., 2003).

Reducing the fat content of cheese significantly changes starter activity, starter lysis, flavor development, flavor release, and texture (Bryant et al., 1995; Carunchia Whetstine et al., 2006). High hydrostatic pressure treatment (100-500 MPa) of half fat (~15%) Cheddar cheese for 2 h resulted in softer cheese texture and increased meltability (Johnston et al., 2002). It was also reported that half fat Cheddar cheese treated at 200 MPa exhibited the closest textural properties to full fat control. On the other hand, the application of 400 MPa for 5 min to reduced fat Mozzarella cheese did not influence its rheological properties (Sheehan et al., 2005).

There have been conflicting results from the various HHP studies, one issue is the different process conditions used. We therefore wanted to explore a wide range of pressures and times to see if we could determine some suitable conditions to improve the quality of reduced fat Cheddar cheese. We are not aware of any studies on the use of HHP to improve the properties of cheese around this fat level (~7%). The objective of this study was to determine the impact of pressure and holding time on the texture and characteristics of reduced fat Cheddar cheese.

2.3 MATERIALS AND METHODS

Cheese Manufacture

A licensed Wisconsin cheese maker manufactured two independent batches of reduced fat, milled-curd Cheddar cheeses at the University of Wisconsin-Madison Dairy Plant over a period of 1 yr. Lowfat (0.45±0.15 fat) milk was pasteurized at 73°C for 19 s, cooled to 32.2°C, and preacidified to pH 6.50 with 25% citric acid solution. The milk was inoculated with a mesophilic mixed-strain starter culture consisting of *Lactococcus lactis* ssp. *cremoris*, *Lactococcus lactis* ssp. *lactis* (LL50: DSM Foods, JH Heerlen, the Netherlands), and starter adjunct *Lactobacillus helveticus* (LH-32: Chr. Hansen, Milwaukee, WI) at the rate of 381 and 9.5 g per 1672 kg of milk, respectively. Cheese milk was ripened at 33.3^{o}C for 30 min. Annatto (Cheese Color 2x: Chr. Hansen, Milwaukee, WI) was added as colorant right after starter culture addition at the rate 105 ml per 1672 kg of milk. Double strength chymosin (Chymax Extra (600 IMCU/ml): Chr. Hansen, Milwaukee, WI) was added at the rate of 142 g per 1672 kg of milk. The coagulum was cut with 1.9 cm knives and the curd was given a 10 min healing time before cooking. The temperature of the curd-whey mixture was raised from ∼32 to 34.5°C over 15 min. Curd was held at 34.5°C for 15 min before draining the whey. Curd slabs were cheddared, stacked 2 high, and milled at pH ∼5.9. Curd was rinsed with cold water (~ 20 L) for 15 sec after milling. Curd was salted at the rate of 6.3 kg per 1672 kg of milk. Curd was packed in 11 kg Wilson-style hoops and pressed at ambient temperature until the pH reached about 5.4 (~2 h), then cheese blocks were vacuum packaged and aged at 7°C.

High Hydrostatic Pressure Treatment

Cheeses were HHP treated one week after manufacture. The conditions used are given at Table 2.1. The commercial high pressure unit (Avure Ultra 215 L, Avure Technologies Inc., WA, USA) has a volume of 215 L and can process up to 150 kg of product per cycle depending on product size and packaging. The HHP unit can reach pressures of up to 600 MPa, and a complete compression/decompression cycle takes 4-5 min, excluding hold time. The machine reached 48 MPa in around 6 sec and ~ 400 MPa in around 100 sec. Water was used as the pressure transfer medium. The water temperature of the holding tank of the HHP unit was between 7 to 11^{o}C. An untreated cheese block was used as a control for each trial.

Sampling and Composition Analyses

In several cheese blocks, we observed acid spot defect occurring before the pressure treatment (possibly reflecting localized regions where citric acid was added). After realizing that the data we obtained from these regions were not representative of the remaining of the cheese block, we decided to avoid sampling from these regions. The data received from these regions were excluded in our calculations/results.

The cheese milk was analyzed for fat (Mojonnier method; AOAC, 2000), protein (total percentage N × 6.38, Kjeldahl method; AOAC, 2000), casein (AOAC, 2000), lactose (AOAC, 2000), total solids (Green and Park, 1980), total Ca (Park, 2000), and insoluble calcium (**INSOL Ca**) by the acid–base titration method (Lucey et al., 1993; Hassan et al., 2004). An unacidified milk was used to generate a rennet whey (Lucey et al., 1993), which was analyzed for total soluble Ca (Park, 2000). The cheeses were analyzed at 1 mo for moisture (Marshall, 1992), fat (AOAC, 2000), pH using a pH electrode (Sam Gray gold electrode; Nelson-Jameson, Marshfield, WI; Marshall, 1992), protein by the Kjeldahl method (AOAC, 2000), and salt by the chloride electrode method (model 926; Corning Glass Works, Medfield, MA; Johnson and Olson, 1985), lactose/galactose and lactic acid contents were analyzed enzymatically (Boehringer Mannheim, 1997; Severn et al., 1986), total Ca (Park, 2000). Proteolysis was monitored with water soluble nitrogen, and 12% trichloroacetic acid (**TCA**) soluble nitrogen (Kuchroo and Fox, 1982). The INSOL Ca content in cheeses were measured at time points of 2 wk, 1, 3 and 6 mo. Acid–base titration of the cheeses were performed as described by Lucey et al. (1993). The INSOL Ca contents in cheeses were calculated by the acid–base titration method as described by Hassan et al. (2004).

Rheological Measurements

Dynamic small amplitude oscillatory rheology was used to measure the rheological properties of the reduced fat Cheddar cheese. A Paar Physica UDS 200 controlled-stress rheometer (Anton Paar, Ashland, VA) with a 50-mm serrated parallel plate geometry was used as described by Lee et al. (2005). Cheese samples that were 50 mm diameter and 3 mm thickness were prepared, and heated from 5 to 85°C at a heating rate of 3°C/min. A frequency of 0.08 Hz and a strain of 0.5% were applied to measure the storage modulus (**G′**), loss modulus (**G″)**, and loss tangent (**LT**), which is the ratio between the viscous properties and the elastic properties of the material (LT= G′/G″). The maximum loss tangent (**LTmax**) value observed during heating was also recorded.

Microbiological Analysis

The numbers of lactococci starters were measured by incubation anaerobically on MRS agar at 32°C for 48 h (Wehr and Franks, 2004) and nonstarter lactobacilli were counted on Rogosa SL media grown anaerobically at 32°C for 48-72 h (Broadbent et al., 2003). The plates were incubated longer than usual because there were pinpoint colonies, which were hard to read. It might have been due to the processing conditions, causing stressed cells and impacting their growth.

Melt Profile Analysis

The UW-Meltprofiler was used, as described by Muthukumarappan et al. (1999), to evaluate melt and flow characteristics of cheese samples. Disc shaped cheese samples (7 mm thick and 30 mm in diameter) were prepared and stored at 6°C at least 3 h before testing. A thermocouple was inserted into the center of the cheese disk held between two lubricated aluminum plates and placed into an oven at 72°C (750F Forced Air Oven, Fisher Scientific, Pittsburgh, PA). Changes in cheese height and cheese temperature were measured for 15 min. Degree of flow (**DOF**) was calculated as the change in height when the cheese temperature reached 60°C as compared with the cheese height at the beginning of the test. Cheese samples were analyzed at 2 wk, 1, 3, and 6 mo of ripening.

Texture Profile Analysis and Uniaxial Compression

Texture profile analysis (**TPA**) and uniaxial compression were measured using a TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY). Samples were prepared with a cork borer with a diameter of 16 mm and a height of 17.5 mm. For the TPA test, cheeses were compressed by 20% using a 50-mm aluminum cylinder test probe with a cross-head speed of 0.8 mm/s. For uniaxial compression, cheeses were compressed by 80% with the same test probe and cross-head speed as for the TPA test. All tests were performed at 5°C and replicated at least 5 times.

Fluorescence Microscopy

Micrographs of the reduced fat Cheddar cheeses were obtained using a similar procedure as described by Sutheerawattananonda et al. (1997). Cheese samples were cut from the center of each cheese block, and sectioned using a sharp razor blade. Cheese sections were stained with

0.1% (wt/vol) Nile Blue A (Sigma-Aldrich, St. Louis, MO) or Acridine Orange (Sigma-Aldrich, St. Louis, MO) for 1 min, rinsed with milli-Q water. The stained sections were held at 4°C and analyzed within a day using a fluorescence microscope (Axioskop 2 plus, Carl Zeiss, Eching, Germany) fitted with a 40× objective (Achroplan 40×/0.75, Carl Zeiss).

Experimental Design and Statistical Analyses

A central composite rotatable design (Mullen and Ennis, 1979) and response surface methodology (RSM) (Montgomery, 2001) were used to investigate the effects of pressure and holding time on the rheological, physical, and chemical characteristics of reduced fat Cheddar cheese. A 2-level factorial experimental design was chosen to study the effects of the independent variables (pressure and holding time) with 2 star points ($\alpha = 1.414$) and 2 replicates of the center point (Table 2.1). Experimental conditions were repeated with two independent trials. The coded variables were related to the real units by Equations 1 and 2:

$$Coded\ Pressure = \frac{(Pressure - 225)}{125} \qquad [1]$$

$$Coded\ Time = \frac{(Holding\ Time - 11)}{6} \qquad [2]$$

Multiple (stepwise) regression and RSM were used to analyze the results (Statgraphics version 5.1 plus, Manugistics, Rockville, MD). Response variables were estimated by using second-order (polynomial) models, which provide a good description of the geometric slope of response surfaces. Stepwise regression was used to eliminate insignificant factors (F-value < 4) using backward selection, which begins with all of the variables in a model and removes them one at a time to simplify the model (Montgomery, 2001). The Tukey-Kramer test was used to compare means, and differences between means were considered significant at $P < 0.05$.

Pearson's correlation coefficients were estimated between various responses (i.e., INSOL Ca, TCA soluble N, water soluble N, TPA hardness, rheological parameters, and DOF).

2.4 RESULTS AND DISCUSSION

Composition and Microbiology

The composition of the cheese was 52.2±0.9% moisture, 7.3±0.2% fat, 32.8±1% protein, and 1.84±0.24% salt. There was considerable variation among the pH values (5.01 – 5.28) and residual lactose contents (0.22 – 0.6%) observed in the experimental treatments at 1 mo. An increase in pressure resulted in higher pH values (Figure 2.1a), and residual lactose levels (Table 2.2), and lower levels of lactic acid (results not shown). Linear effect of pressure was the most significant experimental variable for pH and residual lactose, pressure positively affected both pH and residual lactose values (Table 2.2). Previous studies also indicated that HHP application to cheese resulted in higher pH values in Cheddar (Rynne et al., 2008), Gouda (Messens et al., 1999; Messens et al., 2000), Mozzarella (Johnston and Darcy, 2000; Sheehan et al., 2005), goat's milk (Saldo et al., 2000; Saldo et al., 2003), and ewe's milk cheese (Juan et al., 2007b). Two possible mechanisms could explain the high pH values in HHP treated cheeses; HHP could dissociate ionisable groups of protein and thus alter pH (Rynne et al., 2008), and/or inactivate the starter bacteria or their glycolytic enzymes before complete fermentation of lactose (Malone et al., 2003; Rynne et al., 2008). Residual lactose level was higher for the application of 225 MPa for 2.5 (0.42±0.04%) min compared to control (0.24±0.05%) even for this sample that did not exhibit significant microbial reduction (Figure 2.2) due to HHP. This indicates a possible impairment in the glycolytic pathway. Similar to our observation, Casal and Gomez (1999) reported that HHP affected the acidifying activity of lactococci and lactobacilli to a greater extent than their cell viability. However, we have limited information about the biological and enzymatic activity of the viable bacteria in our experimental cheese samples.

In our study, viable starter culture numbers decreased with HHP (Table 2.2). A highly significant (R^2 = 0.93) prediction model was obtained for starter culture numbers at 1 mo of ripening. Pressure was the significant term in this model, and pressure negatively affected starter bacteria numbers. Holding time seemed to have only very slight impact on bacterial reduction, which was in agreement with the results of Wick et al. (2004). At 2 wk of ripening, the use of pressures ≤ 225 MPa did not exhibit significant ($P > 0.05$) reduction in starter culture numbers compared to control cheese (Figure 2.2a). Previous studies reported that pressures ≥ 200 MPa was required to significantly decrease the viable lactic acid starter culture numbers (Casal and Gomez., 1999; O'Reilly et al., 2002a; Wick et al., 2004; Moschopoulou et al., 2010). The mechanisms by which HHP impacts microorganisms is not fully understood, but cellular membrane damage seems to play a key role in their inactivation (Malone et al., 2002; Black et al., 2011). Intracellular and cell envelope damage was visible in transmission electron microscopy images of *Lactococcus lactis* strains treated by HHP (Malone et al., 2002). Martinez-Rodriguez et al. (2012) reported that regardless of processing time, there was a minimum pressure that had to be overcome for microbial inactivation. Application of 225 and 350 MPa for ≥ 5 min could have been enough to damage starter bacteria cells, and possibly these cells were no longer viable at 1 mo. Application of 402 MPa for 11 min resulted in ~2 log reduction in the numbers of starter bacteria. It was reported that pressures of ~400 MPa resulted in a 1.5 to 3.5 log reduction depending on the cheese type and bacterial strain (Casal and Gomez, 1999; Saldo et al., 2000; O'Reilly et al., 2002a; Juan et al., 2007b; Rynne et al., 2008; Moschopoulou et al., 2010). Several studies have reported that an initial decrease in starter culture numbers immediately after HHP could be partially reversed (become viable again) during subsequent ripening (Wick et al., 2004; Moschopoulou et al., 2010; Martinez-Rodriguez et al., 2012). In our study, we did not observe any significant ($P > 0.05$) increase in starter culture numbers in HHP treated cheeses during ripening (Figure 2.2).

Application of HHP significantly affected the non-starter lactic acid bacteria (**NSLAB**) (including non-starter lactobacilli added as an adjunct) numbers at 1 mo of ripening (Figure 2.1b, Table 2.2). The prediction model had an R^2 value of 0.90. Pressure had a linear (positive) impact as well as quadratic (negative) impact on NSLAB numbers. The non-starter lactic acid bacteria appeared to be more resistant to HHP compared to the starter lactococci strains. Casal and Gomez (1999) reported a similar trend for the pressure resistance for lactobacilli. The viability of lactobacilli species inoculated in 10% skim milk was not affected by the application of pressures between 100-350 MPa. For pressures ≤ 225 MPa applied cheeses, NSLAB numbers did not show a significant ($P > 0.05$) increase from 2 wk to 1 mo while NSLAB numbers increased 1 log for the control cheese ($P < 0.05$) (Figure 2.2b). This result indicates that pressures 50 to 225 MPa initially slowed the development of NSLAB up to 1 mo of ripening. Intermediate pressure treatments (e.g., 350 MPa for 5 or 17 min) and control cheese exhibited similar NSLAB numbers at 2 wk, but at 1 mo of ripening NSLAB numbers were ~ 2 log lower for these samples compared to control. Both starter and NSLAB numbers exhibited a decrease from 2 wk to 1 mo of ripening with 350 MPa applications, indicating that 350 MPa did not cause immediate death of all pressure sensitive bacteria in cheese, but it created stressed/damaged cells that were unable to recover during ripening.

Acid-Base Buffering Curves

The INSOL Ca phosphate content plays a key role in the functional properties of cheese, such as, hardness and meltability (Hassan et al., 2004). We did not observe any significant ($P > 0.05$) difference in INSOL Ca levels between HHP treated cheeses and control cheese during ripening (Figure 2.3). There was a slight decrease in the INSOL Ca levels in cheeses with ripening, as was expected. The INSOL Ca content of cheese is reduced in low pH cheeses (Lee et al., 2005). High hydrostatic pressure application resulted in cheeses exhibiting higher pH values, but we did not observe a parallel increase in the INSOL Ca levels for HHP treated cheeses. Messens et al. (1998) investigated the serum phase of high pressure brined Gouda cheese. They reported that pressure treatment of 300 MPa for 4.5 min did not influence the level of soluble Ca in cheese serum. Several studies investigated the state of Ca in HHP treated milk by analyzing the supernatant obtained by ultracentrifugation. Desobry-Banon et al. (1994) reported that the application of HHP to milk resulted in a slight decrease in INSOL Ca up to 200 MPa, but a further increase in pressure resulted in milk with a similar INSOL Ca levels to the control. Schrader et al. (1997) observed a reversible (with 72 h storage) solubilization of INSOL Ca with the application of 400 MPa for 5 min. On the other hand, Law et al. (1998) did not observe any change in the state of Ca in goat’s milk with the application of pressures in the range 300-500 MPa. They suggested that pressure application solubilized INSOL Ca and pressure release shifted the calcium equilibrium back towards the INSOL phase.

Rheological Properties and Meltability

The LTmax values for all cheeses increased during ripening (Figure 2.4). The LTmax values of cheeses are used as an index of meltability. At 2 wk of ripening, the HHP applied cheeses exhibited higher LTmax values than the control (Figure 2.4). The DOF values of cheeses are shown in Figure 2.5. All cheeses exhibited an increase in the DOF (melt) during ripening. At 2 wk of ripening, several HHP treatments exhibited significantly ($P < 0.05$) higher DOF than the control. At 1 mo of ripening, all HHP treated and control cheeses exhibited similar DOF and LTmax values. Various types of casein interactions in cheese network determine its rheological and melting properties (Lucey et al., 2003). We did not observe any significant ($P > 0.05$) change in the state of Ca between HHP treated cheeses and control (Figure 2.3), indicating that other changes in casein-casein interactions contributed to the observed differences in rheological and melt properties shortly after HHP application. Messens et al. (2000) reported that the application of HHP to cheese created a more fluid-like structure immediately after pressure processing, and increased the LTmax values in Gouda cheese. In agreement with our study, differences in the rheological properties between HHP treated Gouda cheese and the control became smaller during ripening (42 d) (Messens et al., 2000). Messens et al. (2000) indicated that HHP decreased hydrophobic interactions between caseins immediately after pressure processing. O'Reilly et al. (2002b) and Johnston and Darcy (2000) indicated that HHP induced protein hydration was responsible for the increase in melt flow and LTmax after HHP treatment. By 1 mo of ripening, age related changes (e.g., proteolysis) had a greater influence on rheological properties during the rest of the ripening period.

Textural Properties

An increase in HHP decreased cheese hardness (Table 2.2; Figure 2.6). Significant prediction models were obtained for TPA hardness (obtained from 20% compression of cheese cylinders) during ripening. Pressure was the only significant term, and it negatively affected hardness values. We did not observe any significant difference ($P > 0.05$) between the control and cheeses treated at pressures ≤ 100 MPa. O'Reilly et al. (2000) reported that Cheddar cheeses became softer after HHP application of 50 MPa, but their holding times (3 d) were much longer than our study. Pressures ≥ 225 MPa treated cheeses remained softer than the control cheese during the entire ripening period (Figure 2.6). Uniaxial compression (obtained from 80% compression of cheese) profiles are shown in Figure 2.7. Application of HHP significantly increased the strain to fracture (Figure 2.1c, Table 2.2), indicating that these cheeses exhibited fracture at higher strains or did not fracture at all (Figure 2.7d). Similar observations were reported by Messens et al. (2000), who found that treatment of Gouda cheese at 225 and 400 MPa resulted in cheese that exhibited lower firmness and no visible fracture. Johnston et al. (2002) observed softening in half-fat (15% fat) Cheddar cheese with the application of HHP 100-500 MPa for 2 h. They attributed the HHP induced reduction in firmness to a change in the state of water and a decrease in serum filled pores in the protein network.

Fluorescence Microscopy

Confocal laser scanning microscopy (**CLSM)** images demonstrated that large fat droplets finely dispersed into the cheese matrix with HHP treatment (Figure 2.8). Large fat droplets became less visible or less distinct with an increase in pressure. The lowest pressure application (48 MPa for 11 min) had smaller fat droplets (Figure 2.8b) compared to control (Figure 2.8a). Fat droplets were hardly visible at medium (225 MPa for 11 min) (Figure 2.8c) or highest pressure applications (402 MPa for 11 min) (Figure 2.8d). The HHP treatment could have disrupted small fat droplets and dispersed the fat throughout the protein matrix, thereby individual large fat droplets became less visible with an increase in pressure. O'Reilly et al.

(2000) did not observe gross structural changes in Cheddar cheese by using CLSM with the application of 50 MPa for 70 h. Johnston and Darcy (2000) reported that fat globules and water channels in protein matrix of young Mozzarella cheese almost disappeared with HHP application of 200 MPa for 60 min. Other researchers also reported that pressures ≥ 350 MPa to Mozzarella cheese resulted in a more continuous and homogeneous protein matrix and reduced the size of fat-serum channels (O’Reilly et al., 2002b; Sheehan et al., 2005).

Proteolysis and Informal Sensory Evaluation

We did not observe any significant ($P > 0.05$) difference in water soluble nitrogen (Figure 2.9a) or TCA soluble nitrogen (Figure 2.9b) levels between HHP treated cheeses and control cheese during the 6 mo of ripening. O’Reilly et al. (2000) and Yokohama et al. (1992) reported an increased proteolysis rate in Cheddar cheese with the application of 50 MPa for 3 d. However, both of these groups applied significantly longer holding times (3 d) compared to our study. O’Reilly et al. (2003) studied the effect of various pressures (70-400 MPa) and holding times (3-81 h) on Cheddar cheese. They reported that the application of pressures < 150 MPa resulted in the highest levels of pH 4.6 soluble nitrogen and long (50-70 h) holding times further increased pH 4.6 soluble nitrogen levels. However, they did not explain why longer holding times increased proteolysis rates. Messens et al. (1999) did not observe changes in the proteolysis rates in Gouda cheese compared to untreated control when applying similar pressures and holding times (50-400 MPa, 20-100 min) as were performed in our study.

Informal sensory grading was conducted by 4 expert cheese graders, who indicated that HHP treatment altered the sensory properties of cheese. Several conditions; 100 MPa for 5 and 17 min, 225 MPa for 2.5 min, and 350 MPa for 5 min applied cheeses received promising sensory comments. Increase in pressure up to 350 MPa, increased the buttery and sweet notes up until 3 mo of ripening. Several mechanisms could be responsible for the possible changes in the sensory characteristics of experimental cheeses; more dispersed state of fat, impaired acid development and greater release of intracellular enzymes into protein matrix. The higher pH and residual lactose levels of HHP treated cheeses could have contributed to the sweet notes. Annato (cheese color) is associated with milk fat globular membrane (MFGM). Cheese juice samples exhibited great variation in color (Appendix I), indicating that fragments of MFGM dissociated to the moisture phase, which might have contributed the cheese flavor.

2.5 CONCLUSIONS

Application of HHP significantly affected the texture and microflora of reduced fat Cheddar cheese. In general, pressure exhibited greater impact on the textural and microbiological attributes compared to holding time. We did not observe any significant difference in proteolysis rates between HHP treated cheeses and control cheese at any time of ripening. Pressure applications ≥ 225 MPa resulted in softer cheese texture and increased melting thus, high firmness and lower melting characteristics of reduced fat cheeses could be improved by HHP treatment. High pressure did not alter the levels of INSOL Ca phosphate, it is still possibly that some INSOL Ca phosphate crosslinks between proteins may have solubilized during the HHP application but the overall levels of INSOL Ca phosphate in cheeses were similar after pressure release. Pressure processing resulted in smaller fat droplets and a more continuous protein network. Disruptions to protein-protein interactions (e.g. hydrophobic interactions), altered protein-water interactions (protein hydration), or physical disruption to the matrix were probably responsible for the altered cheese texture and rheological properties. High hydrostatic pressure appears to be promising to improve texture and flavor of reduced fat cheese.

2.6 ACKNOWLEDGEMENTS

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Table 2.1. Values of independent variables of each experiment in coded and actual values for the central composite experimental design

Sample No	**Coded Values**		**Actual Values**	
	Time	Pressure	Time (Min)	Pressure (MPa)
1	-1	1	5	350
2	1	-1	17	100
3	1	1	17	350
4	0	+α	11	402
5	+α	0	19.5	225
6	-1	-1	5	100
7	0	0	11	225
8	0	- α	11	48
9	- α	0	2.5	225
10	0	0	11	225

Where α =1.414.

Table 2.2. Second order polynomial models describing hardness at 2 wk, strain to fracture at 2 wk; residual lactose content as a percentage of total weight at 1 mo, pH at 1 mo, and starter bacteria numbers at 1 mo of age. Only prediction models with R^2(adjusted) > 0.5 are shown.

Dependent variable	Independent variable	Coefficient	R^2(adjusted)^1	*P*
pH	Constant	4.9	0.9	<0.05
	Pressure *	0.002		
	Time^2	0.0003		
Lactose content	Constant	0.007	0.67	<0.05
	Pressure	0.004		
Starter numbers (log)	Constant	8.39	0.93	<0.0001
	Pressure ***	-0.0078		
NSLAB numbers (log)	Constant	5.18	0.90	<0.05
	Pressure	0.0067		
	Pressure$^{2\,*}$	-3E-05		
Hardness (N)	Constant	14.23	0.79	<0.001
	Pressure **	-0.013		
Strain to fracture	Constant	58.42	0.94	<0.05
	Time × Pressure **	0.002		
	Pressure	0.018		

^{1}R^2 values were adjusted for the degree of freedom.

$P < 0.05$, $^{*}P < 0.005$, $^{**}P < 0.001$, and $^{***}P < 0.0001$

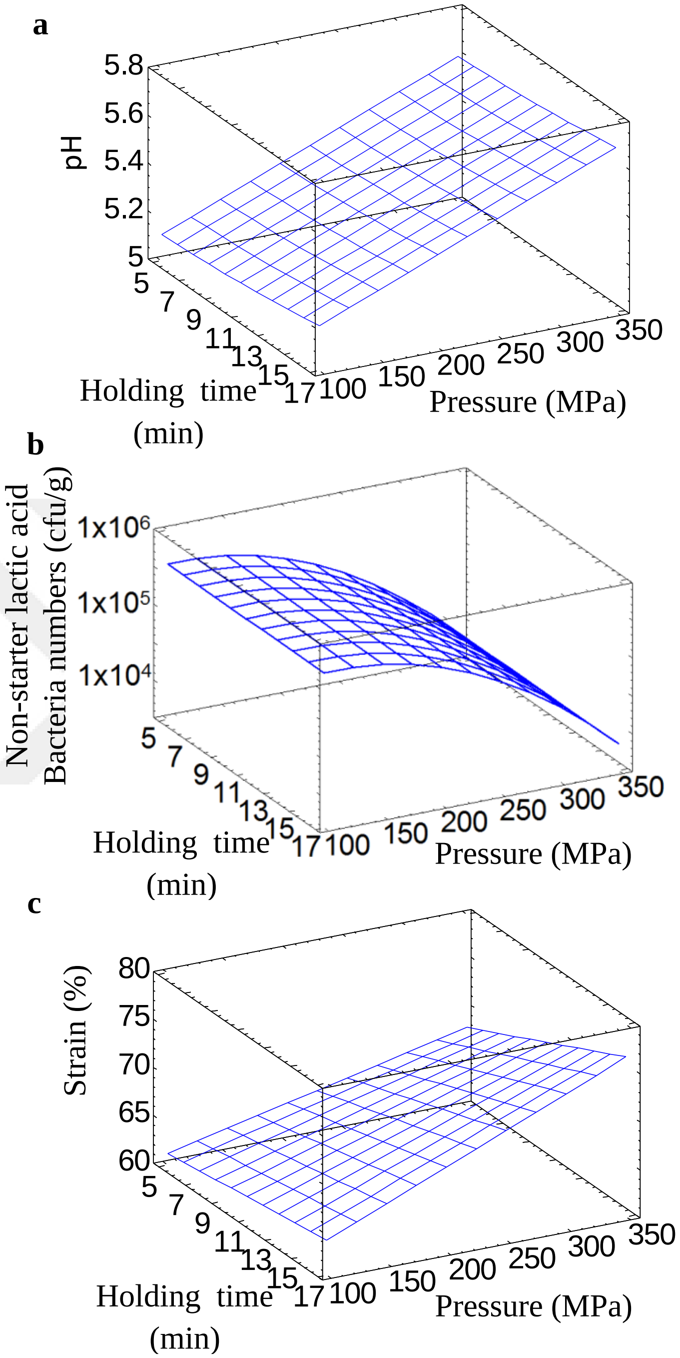

Figure 2.1. Response surface plots for the effect of pressure and holding times on: pH values at 1 mo (a), non-starter culture numbers (cfu/g) at 1 mo (b), and % fracture strain at 2 wk (c) of age.

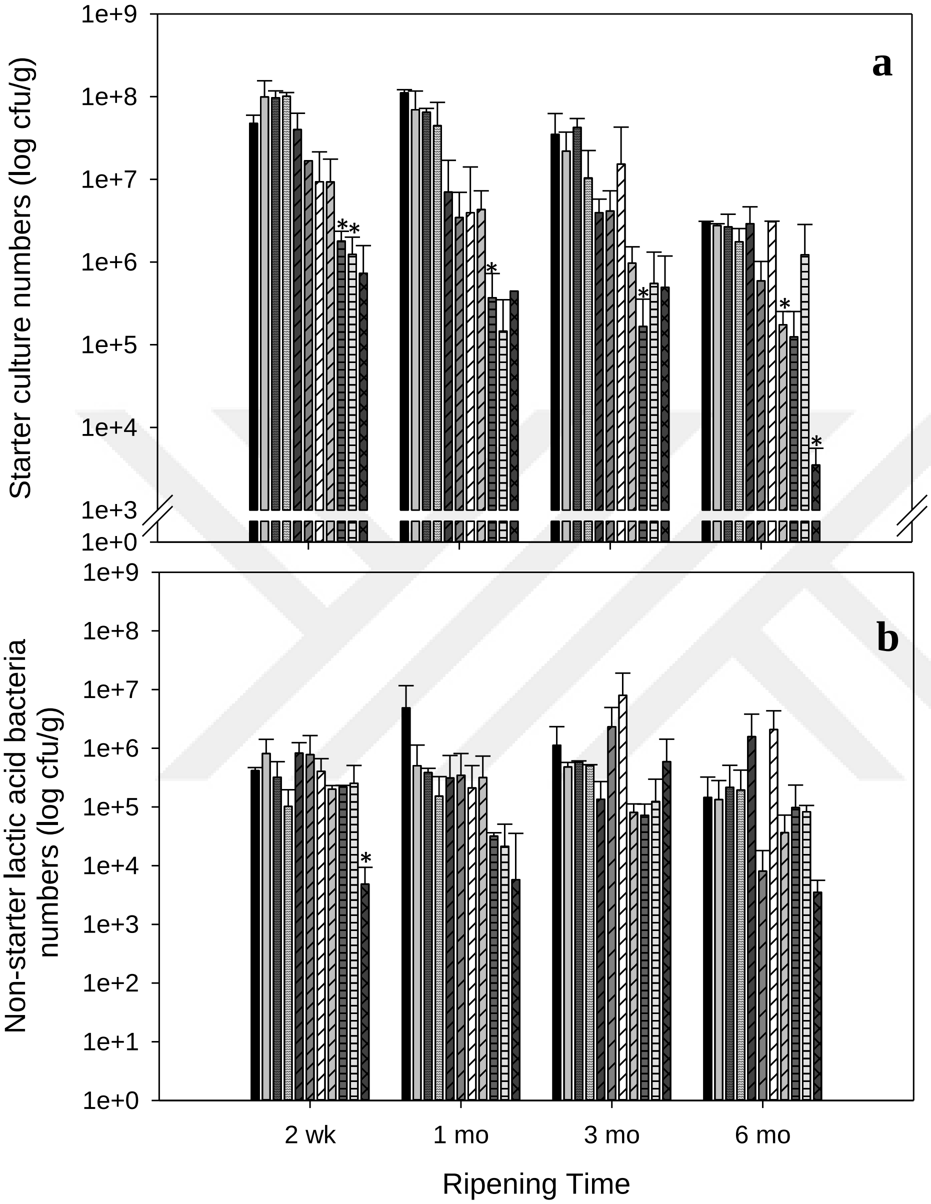

Figure 2.2. Starter culture numbers of reduced fat Cheddar cheeses for control (■), or cheeses treated at 11 min 48 MPa (□), 5 min 100 MPa (▦), 17 min 100 MPa (▦), 2.5 min 225 MPa (▨), 11 min 225 MPa (▨), 11 min 225 MPa-2 (▧), 19 min 225 MPa (▧) 5 min 350 MPa (▤), 17 min 350 MPa (▤), 11 min 402 MPa (▩) at 2 wk, 1, 3, and 6 mo of ripening at 7^{o}C.

*Significantly different from control ($P < 0.05$) at that indicated ripening time point.

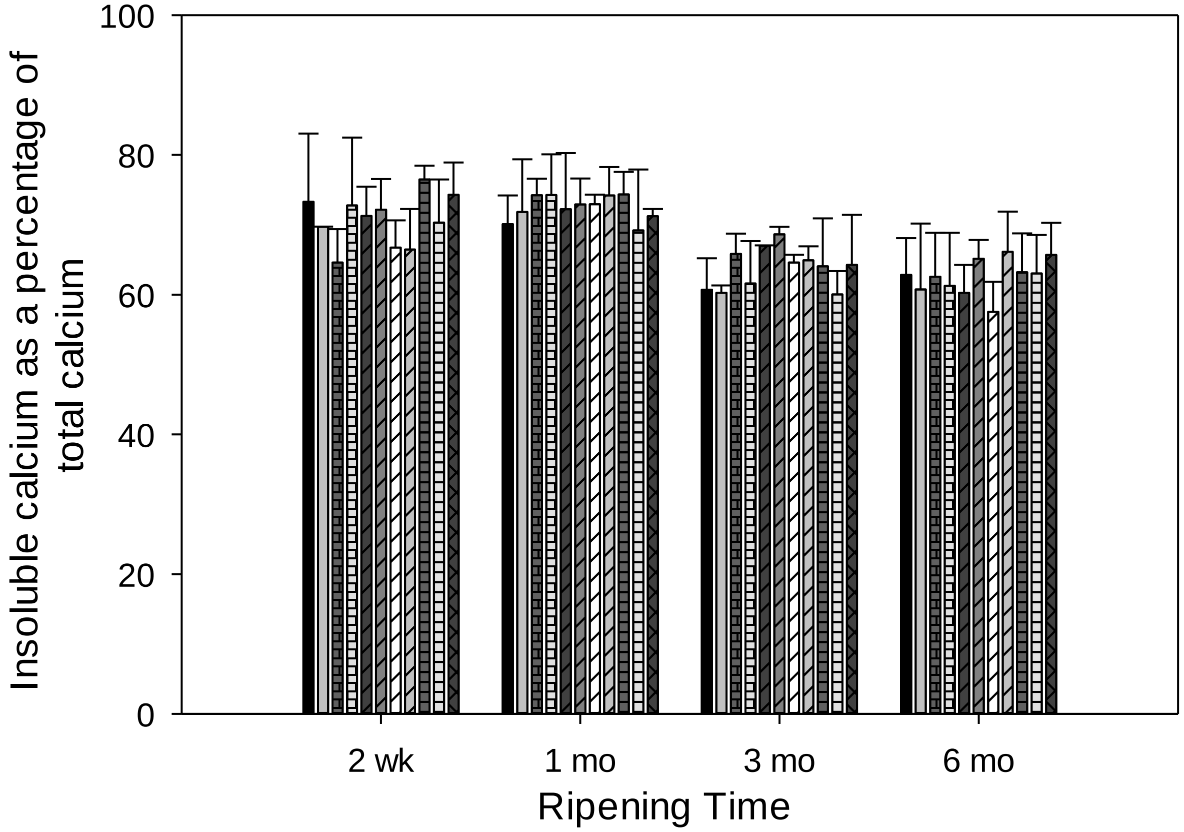

Figure 2.3. Insoluble calcium, expressed as a percentage of total calcium for reduced fat Cheddar cheeses for control (■), or cheeses treated at 11 min 48 MPa (░), 5 min 100 MPa (▦), 17 min 100 MPa (▦), 2.5 min 225 MPa (▨), 11 min 225 MPa (▨), 11 min 225 MPa-2 (▧), 19 min 225 MPa (▧) 5 min 350 MPa (▤), 17 min 350 MPa (▤), 11 min 402 MPa (▩) at 2 wk, 1, 3, and 6 mo of ripening at 7°C.

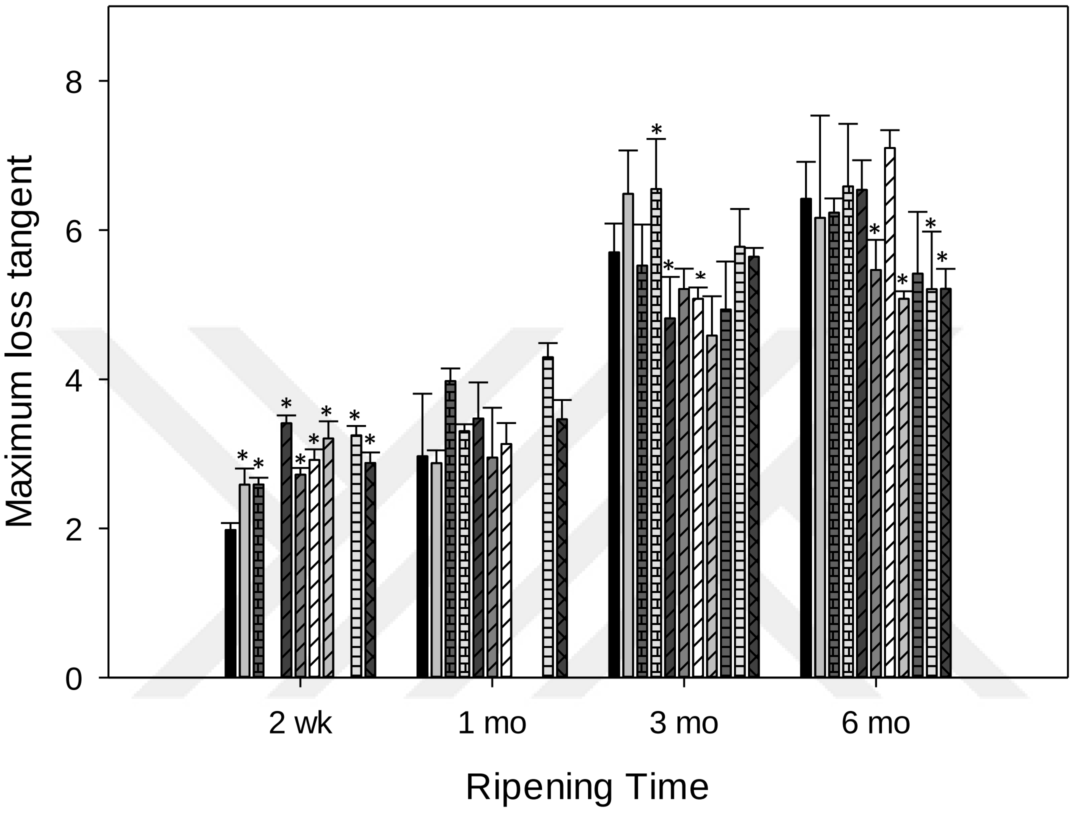

Figure 2.4. Maximum loss tangent values for reduced fat Cheddar cheeses from the small amplitude oscillatory rheology for control (■), or cheeses treated at 11 min 48 MPa (□), 5 min 100 MPa (▦), 17 min 100 MPa (▦), 2.5 min 225 MPa (▨), 11 min 225 MPa (▨), 11 min 225 MPa-2 (▧), 19 min 225 MPa (▨) 5 min 350 MPa (▤), 17 min 350 MPa (▤), 11 min 402 MPa (▩) at 2 wk, 1, 3, and 6 mo of ripening at 7^{o}C. (Some samples are not shown since we observed acid spots in these cheeses).

*Significantly different from control (*P* < 0.05) at that indicated ripening time point.

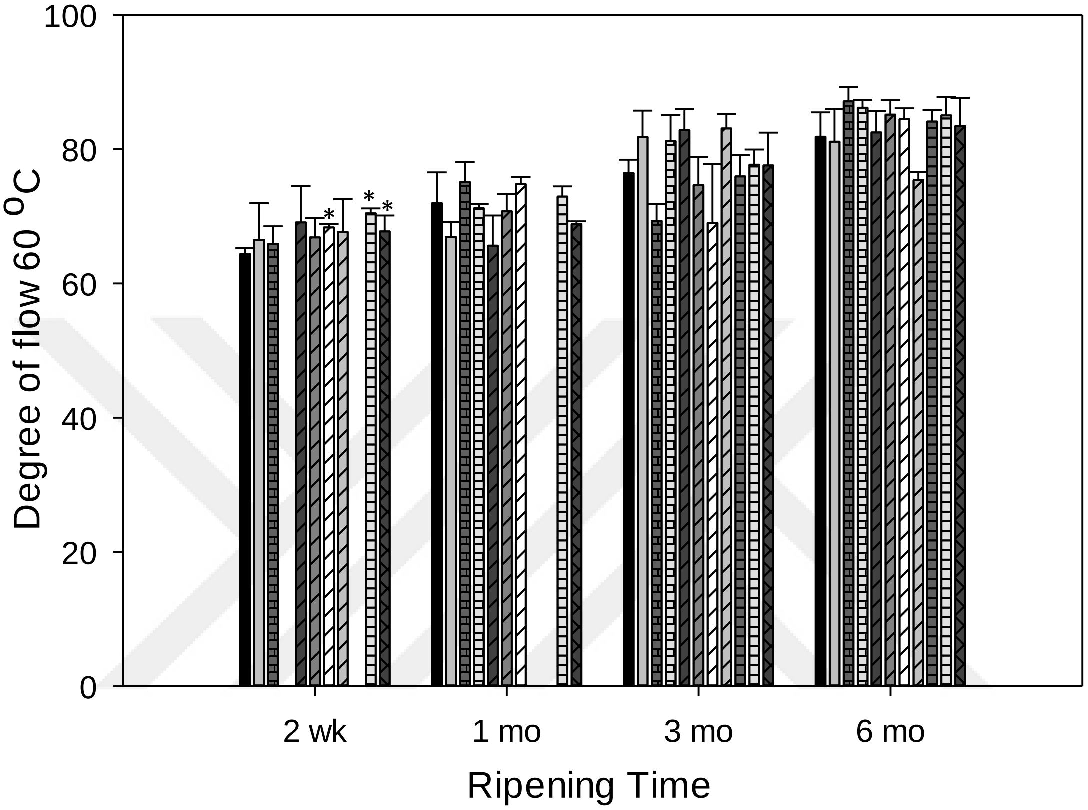

Figure 2.5. Degree of flow at 60^{o}C Changes in cheese height as a percentage of the original height from the UW - Meltprofiler for reduced fat Cheddar cheeses for control (■), or cheeses treated at 11 min 48 MPa (▒), 5 min 100 MPa (▩), 17 min 100 MPa (▦), 2.5 min 225 MPa (▨), 11 min 225 MPa (▨), 11 min 225 MPa-2 (▧), 19 min 225 MPa (▨) 5 min 350 MPa (▤), 17 min 350 MPa (▤), 11 min 402 MPa (▩) at 2 wk, 1, 3, and 6 mo of ripening at 7^{o}C. (Some samples are not shown since we observed acid spots in these cheeses).

*Significantly different from control ($P < 0.05$) at that indicated ripening time point.

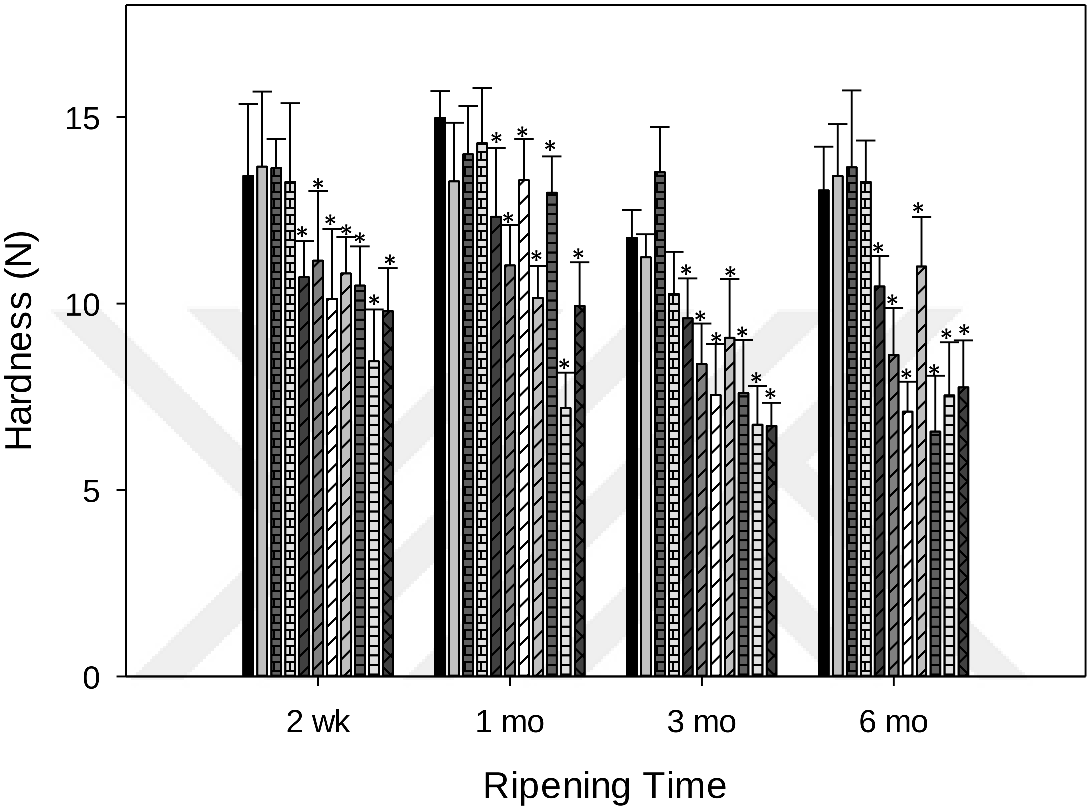

Figure 2.6. Hardness (N) from texture profile analysis testing (20% compression of cheese cylinders to original height) for reduced fat Cheddar cheeses for control (■), or cheeses treated at 11 min 48 MPa (▭), 5 min 100 MPa (▦), 17 min 100 MPa (▦), 2.5 min 225 MPa (▨), 11 min 225 MPa (▨), 11 min 225 MPa-2 (▨), 19 min 225 MPa (▨) 5 min 350 MPa (▤), 17 min 350 MPa (▤), 11 min 402 MPa (▩) at 2 wk, 1, 3, and 6 mo of ripening at 7^{o}C.

*Significantly different from control (*P* < 0.05) at that indicated ripening time point.

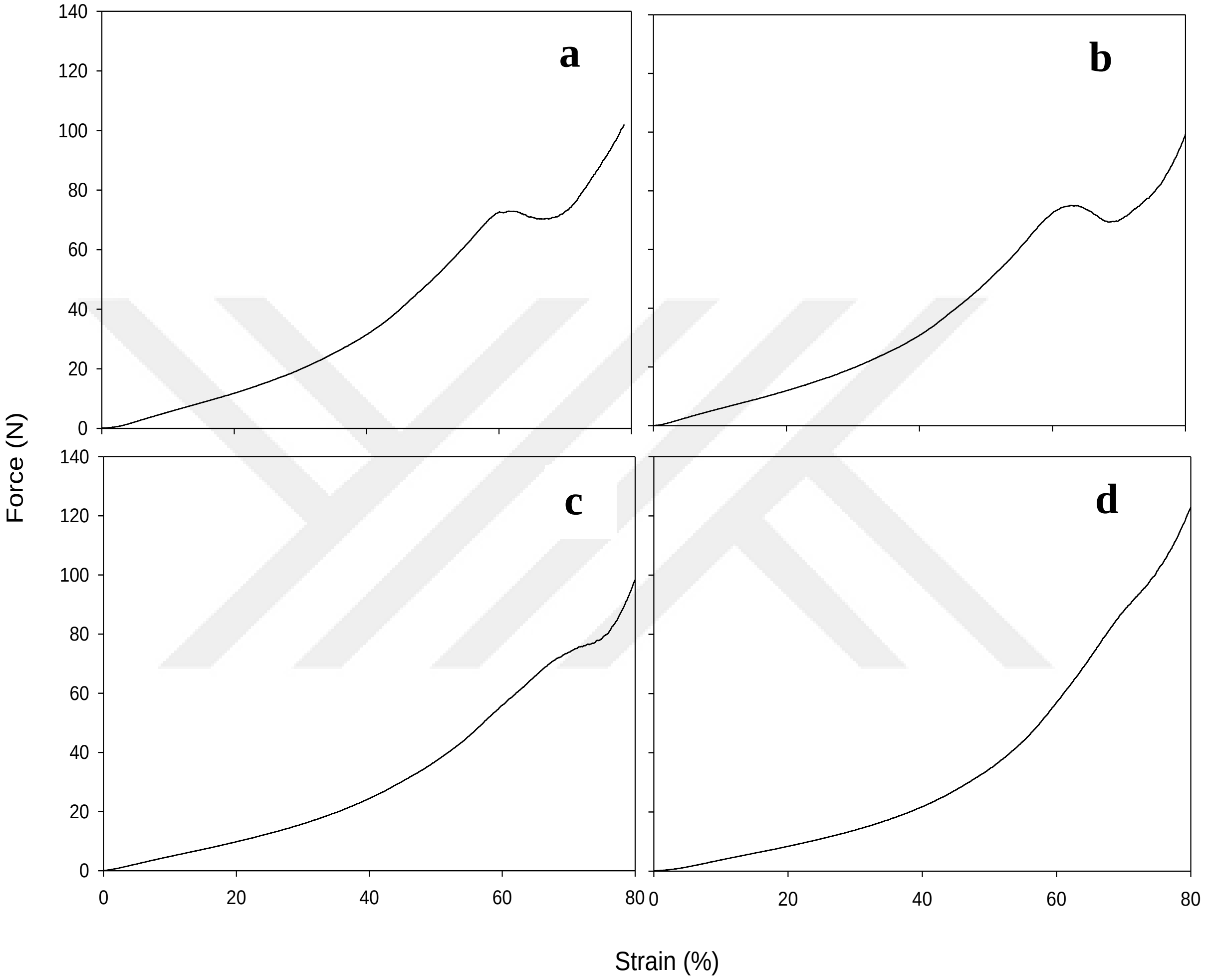

Figure 2.7. Uniaxial compression (compression by 80%) curves for control (a), cheeses treated at 11 min 48 MPa (b), 11min 225 MPa (c), or 11 min 402 MPa (d). Reduced fat Cheddar cheeses were tested at 2 wk of age.

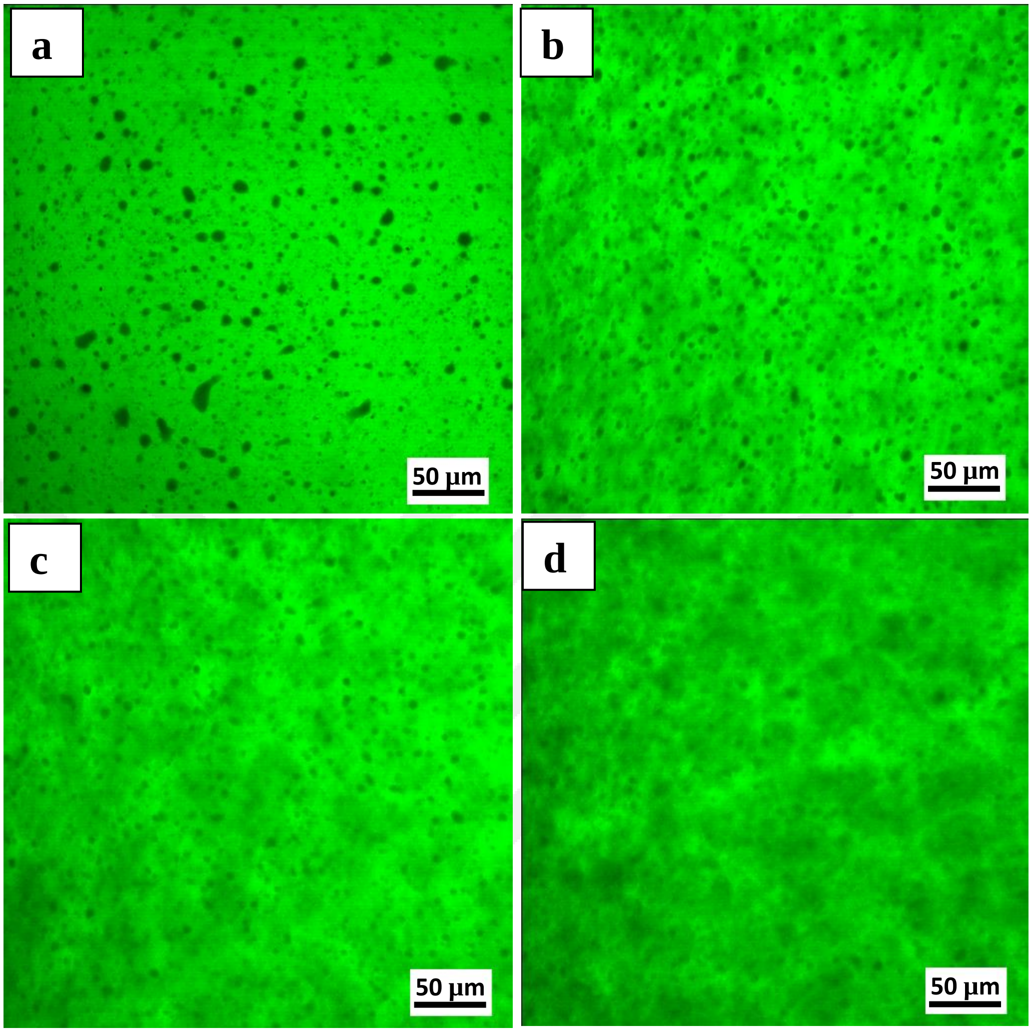

Figure 2.8. Fluorescence micrographs showing fat droplets as dark areas against a light (green) background (protein) for control (a), or cheeses treated at 11 min 48 MPa (b), 11 min 225 MPa (c), and 11 min 402 MPa (d). Reduced fat Cheddar cheese samples were at 3 wk of age.

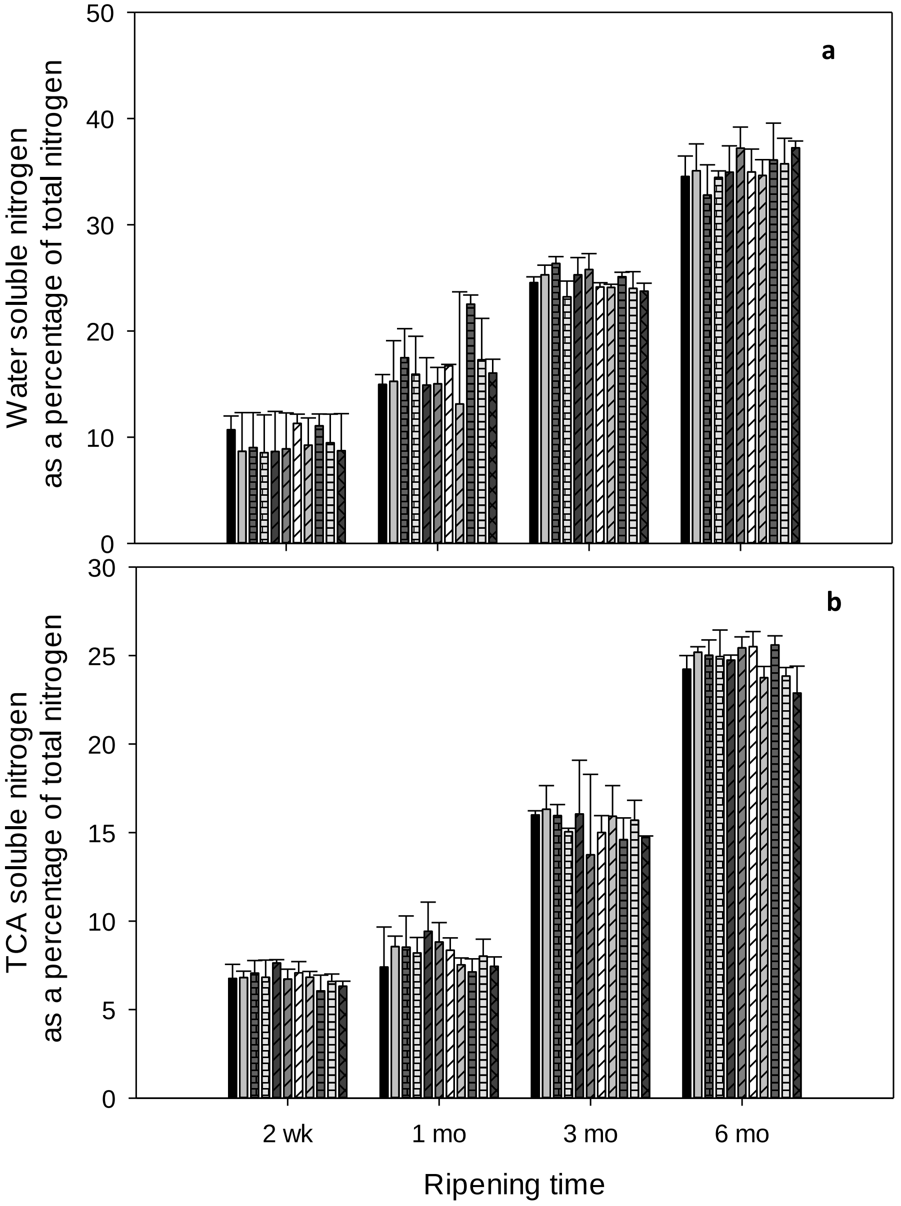

Figure 2.9. Water soluble nitrogen (a) and trichloroacetic acid soluble nitrogen (b) levels, expressed as a percentage of total nitrogen for reduced fat Cheddar cheeses for control (■), or cheeses treated at 11 min 48 MPa (▭), 5 min 100 MPa (▦), 17 min 100 MPa (▦), 2.5 min 225 MPa (▨), 11 min 225 MPa (▨), 11 min 225 MPa-2 (▨), 19 min 225 MPa (▨) 5 min 350 MPa (▤), 17 min 350 MPa (▤), 11 min 402 MPa (▩) at 2 wk, 1, 3, and 6 mo of ripening at 7°C.

Chapter 3.

The Influence of High Hydrostatic Pressure on Regular, Reduced, Low and No Salt Added Cheddar Cheese

3.1 ABSTRACT

Cheddar cheeses were made with four salt in moisture (S/M) levels: regular (5.3%), reduced (2.5%), low (1.9%), and no salt (~0.2%). High hydrostatic pressure (HHP) (405 MPa for 3 min) treated 5.3, 2.5, 1.9 and 0.2% S/M level cheeses exhibited approximately 1, 2.5, 3, and 4 log reduction in numbers of starter bacteria, respectively. At 2 wk of ripening, significant ($P > 0.05$) differences were observed in the maximum loss tangent (LTmax) values, and melting temperature with HHP treatment. Hardness tended to decrease with HHP and reduction in S/M. Neither HHP nor S/M concentration had a significant ($P > 0.05$) effect on trichloroacetic acid (TCA) soluble nitrogen levels during ripening. Urea polyacrylamide gel electrophoresis analysis did not indicate any differences in the levels of the bitter peptide β-casein (f1-189/192) with HHP. Non-treated and HHP treated cheeses had similar sensory flavor profiles (acidity, saltiness, bitterness) during ripening.

3.2 INTRODUCTION

NaCl is an important component used in cheese manufacture; it is added to cheeses at a level ranging from ~0.7 to 6% (w/w), depending on the cheese type (Guinee, 2004). There is a current trend in the food industry to reduce NaCl in processed foods. However, there are many challenges associated with NaCl reduction in cheese, such as, texture, flavor and shelf life issues.

The dairy industry has to overcome these challenges to consistently produce high quality reduced and low NaCl cheese (Agarwal et al., 2011).

NaCl plays several key functions in cheese: provides flavor, controls microbial growth and enzymatic activity, influences moisture content by affecting curd syneresis, and causes physical changes to cheese proteins (Guinee and Fox, 2004). Reduction in NaCl can impair flavor and textural properties of cheeses. No NaCl added and low NaCl cheeses exhibit soft, pasty and adhesive texture while high NaCl cheeses typically exhibit short, firm and crumbly texture (Guinee and O'Kennedy, 2007). Schroeder et al. (1988) evaluated the effect of various NaCl levels on the sensory, microbiological and chemical properties of Cheddar cheese. Their study indicated that the NaCl level and cheese moisture content were inversely related, i.e., decreasing the NaCl level resulted in a higher moisture content. No salt added and low salt cheeses showed high levels of proteolysis. Sensory evaluation of cheeses indicated that reduction in NaCl levels decreased hardness, and increased adhesiveness, cohesiveness, acid flavor, and bitterness (Schroeder et al., 1988).

NaCl plays a key role in controlling microbial activity in cheeses. The primary effect of NaCl as a preventative agent is due to its impact on reducing water activity (Guinee and Fox, 2004). Salt increases the osmotic pressure of the cheese aqueous phase, and above a certain level reduces the growth of bacteria by causing dehydration of cells (Guinee and Fox, 2004). The impact of NaCl on microbial growth is bacterial species and strain dependent.

Bitterness development is a major challenge when reducing the NaCl content of cheese. Excessive bitterness development in cheese has been consistently reported with NaCl reduction (Guinee, 2004). Several researchers suggested excessive microbial numbers for the main cause of bitterness in cheese (Lawrence and Gilles, 1969; Lowrie and Lawrence, 1972), although not all researchers agree (Guinee and Fox, 2004; Visser et al., 1983). Lowrie and Lawrence (1972) claimed that higher microbial numbers, or their activity, was the primary reason for bitterness development in cheese. An inverse relationship was reported between S/M level and intact α_{s1}- and β-caseins (Thomas and Pearce, 1981). Kelly et al. (1996) reported that lower S/M cheeses also exhibited higher levels of water soluble nitrogen (WSN). They also reported that NaCl did not alter the type of peptides formed, but mainly impacted the quantity of peptides produced. Lower NaCl concentration resulted in higher degradation of β-casein. A degradation product of residual chymosin, β-casein (f-1/192) fraction was produced in lower levels in higher NaCl cheeses, indicating that NaCl affects chymosin activity (Kelly et al., 1996).

Previous studies indicated that application of high hydrostatic pressure (HHP) to cheese impacts proteolysis, acid development, microflora and protein structure (Martinez-Rodriguez et al., 2012). Cheeses treated with HHP yielded higher pH values in Cheddar (Rynne et al., 2008), Mozzarella (Johnston and Darcy, 2000; Sheehan et al., 2005), Gouda (Messens et al., 1999; Messens et al., 2000a), goat’s milk (Saldo et al., 2003), blue veined (Voigt et al., 2009), and ewe’s milk cheese (Juan et al., 2007). It is well known that HHP causes a reduction in microbial numbers. Many researchers reported that no significant changes occurred in viable starter culture numbers with application of pressures $\leq$ 200 MPa (Martinez-Rodriguez et al., 2012). Application of 400 MPa resulted in 1.5 to 3.5 log reduction in starter numbers depending on cheese type, bacterial strain and length of HHP treatment. O’Reilly et al. (2002) studied the effect of HHP on various *Lactococcus lactis* strains in 0.1 M citrate buffer (pH 5.3) and 0.1 M citrate buffer (pH 5.3) containing 4.5% NaCl. They concluded that addition of salt into citrate buffer did not affect the stability of lactococcal strains to HHP. Several researchers studied the effect of HHP on enzymatic activity (Martinez-Rodriguez et al., 2012). Malone et al. (2003) reported that plasmin was not affected by any HHP treatment within the pressures studied (100 to 800 MPa). Pressures ≤ 400 MPa did not affect chymosin activity, but application of 400 MPa HHP inactivated cell envelope protease, X-prolyl-dipeptidyl aminopeptidase, and aminopeptidase N activity. On the other hand, aminopeptidase A remained unaffected by any pressure applied (100 to 800 MPa treatments), and aminopeptidase C activity was positively affected by pressures up to 700 MPa. Trujillo et al. (2000) reported that HHP treatment of 450 and 500 MPa decreased chymosin activity, while 400 MPa pressure application resulted in only a slight decrease in chymosin activity.

The objective of this study was to determine the impact of the application of 405 MPa for 3 min on microbial, textural and flavor characteristics of regular, reduced, low and no salt added Cheddar cheeses. A previous study conducted in our laboratory showed that HHP treatment of around 405 MPa caused about 2 log reduction in starter and NSLAB without impairing flavor development in lowfat Cheddar cheese (unpublished). Therefore, we expected that 405 MPa HHP could be useful to improve the quality of Cheddar cheeses of different salt contents by decreasing excessive starter numbers/activity in cheeses with reduced salt levels.

3.3 MATERIALS AND METHODS

Cheese Manufacture

Cheddar cheese was manufactured by licensed Wisconsin cheese makers at the University of Wisconsin-Madison dairy processing plant. Four cheese vats, that can hold 300 kg of milk, were utilized to separately manufacture 5.3, 2.5, 1.9 and 0.2% S/M cheeses. Four independent batches of milled-curd Cheddar cheeses were produced within a three month period.

A previous study carried out in our laboratory indicated that moisture content of cheese increased with a decrease in the NaCl content, thus we applied certain modifications to the cheese manufacturing procedure to try to keep the moisture levels constant over all the salt levels (Table 3.1) (Grant, 2011). Cheese milk contained 3.54 ± 0.21 % fat, and was pasteurized at ~72°C for 19 s. All vats were filled with 227 kg milk.

Direct vat-set culture consisting of *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* blend (MA11, Danisco, Inc., Madison, WI, USA) was used as starter culture, and was added at the rate of 10 g for 227 kg of milk. Cheese milk was ripened at the temperature and times outlined in Table 3.1. Following ripening, coagulant (Chymax Extra, Chr. Hansen, Milwaukee, WI, USA) was added at the rate of 20 g for 227 kg of milk and coagula was cut on firmness (~25 min) using 6.35 mm knives. Curds were cooked and stirred at 39°C until the curd reached pH 6.12 ± 0.05, then the whey was drained. Cheese slabs were cheddared, stacked two high (except for 1.9 and 0.2% S/M cheeses) and milled at a pH of 5.53 ± 0.02 (0.2% S/M cheese was double milled). NaCl was added in 3 incremental saltings for the 5.3, 2.5 and 1.9% S/M cheeses. Cheeses for each salt level were divided into two ~11 kg cheese blocks, then hooped and pressed at 414 kPa for 4 h. Cheeses were ripened for 3 months (mo) at 7°C and analyzed at 2 week (wk), 1 and 3 mo.

High Pressure Treatment

One wk after manufacture, one block from each NaCl level was HHP treated at 405 MPa for 3 min at 9±2ºC. All the four blocks (from each of the different NaCl levels) underwent one single HHP treatment in a high pressure unit (Avure Ultra 215 L, Avure Technologies Inc., WA, USA) that had a volume of 215 L and can process up to 150 kg of product in a single run. The high pressure unit reached 405 MPa in around 100 s. Water was used as the pressure transfer medium. The temperature of the water in holding tank was between 7 to 11°C. The decompression took about 20 s. An untreated cheese block at the same NaCl level was kept as a control for each corresponding HHP block.

Cheese Composition

The cheese milk was analyzed for casein (AOAC, 2000), protein (total percentage N × 6.38, Kjeldahl method; AOAC, 2000), non-protein nitrogen (AOAC, 2000), fat (Mojonnier method; AOAC, 2000), lactose (AOAC, 2000), total solids (Green and Park, 1980), total Ca (Park, 2000), and insoluble Ca (INSOL Ca) by the acid–base titration method (Lucey et al., 1993; Hassan et al., 2004). The rennet whey was prepared as described in Lucey et al. (1993) and analyzed for soluble Ca content (Park, 2000). The cheese composition was analyzed at 2 wk of age for protein by the Kjeldahl method (AOAC, 2000), fat (AOAC, 2000), moisture (Marshall, 1992), pH by the quinhydrone method (Marshall, 1992), salt by the chloride electrode method (model 926, Corning Glass Works, Medfield, MA; Johnson and Olson, 1985), and total Ca and Na (Park, 2000). The INSOL Ca content in cheeses were measured at 2 wk, 1 and 3 mo. The INSOL Ca content in cheeses was calculated by the acid–base titration method as described by Hassan et al. (2004). All analyses were done in triplicate.

Proteolysis

Proteolysis was monitored during ripening by measuring the amount of 12% TCA-soluble nitrogen at 2 wk, 1 and 3 mo (AOAC, 2000). Analysis was performed in duplicate.

Urea PAGE gels

To understand the effect of NaCl levels and HHP on the breakdown of α- and β-caseins during ripening, Urea-polyacrylamide gel electrophoresis (PAGE) was carried out. Urea-PAGE gels (12.5% acrylamide, 4% cross-linking agent- bisacrylamide, pH 8.9) were prepared by the method of Andrews (1983), as modified by Shalabi and Fox (1987). Cheese samples were standardized by protein content and dissolved in the sample buffer at 55°C. Gels were stained with Coomassie Brilliant Blue G250 (Bio-Safe^{TM} Coomassie G-250 Stain, Bio-Rad Laboratories Inc., Hercules, CA, USA) according to the method of Blakesley and Boezi (1977), and destained in deionized water. Pictures of the gels were taken and images were analyzed with densitometric analysis using image analysis software (Gel Expert 3.5; Nucleo Tech, San Carlos, CA, USA).

Rheological Measurements

Dynamic small amplitude oscillatory rheology was used to measure the rheological properties of cheese samples. A controlled-stress rheometer (Paar Physica UDS 200, Anton Paar, Ashland, VA, USA) with 50-mm serrated parallel plate geometry was used as described by Lee et al. (2005). Cheeses cylinders were prepared (50 mm in diameter and 3 mm in thickness), and heated from 5 to 85°C at a heating rate of 1°C min^{-1}. A frequency of 0.08 Hz and a strain of 0.5% were applied to measure the storage modulus (G′), loss modulus (G″), and loss tangent (LT) which is the ratio between the viscous and the elastic properties of the material (LT= G′/G″). The temperature where the LT=1, maximum LT (LTmax) and the temperature where LT was maximum were also determined.

Microbiological Analysis

Lactococci starters were measured using aerobic incubation on de Man, Rogosa and Sharpe (MRS) agar at 32°C for 48 h and nonstarter lactobacilli were counted on Rogosa SL media incubated anaerobically at 32°C for 48 h (Frank and Yousef, 2004).

Texture Profile Analysis and Uniaxial Compression

Texture profile analysis (TPA) and uniaxial compression were measured using a TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA). Samples were prepared by a cork borer to a diameter of 16 mm and a height of 17.5 mm. For the TPA test, cheeses were compressed in a double bite test by 20% using a 50-mm aluminum cylinder test probe with a cross-head speed of 0.8 mm s^{-1}. For uniaxial compression, cheeses were compressed by 80% with the same test probe and cross-head speed as for the TPA test. All tests were performed at 5°C and replicated at least 5 times.

Descriptive Sensory Analysis

Quantitative descriptive analysis (Herbert, 1992) was conducted to evaluate cheese flavor with 8-12 trained panelists at 1 and 3 mo of ripening. Cheeses cubes (2 × 2 × 2 cm) were evaluated at 12°C temperature. Cheeses were identified with random 3 digit numbers. Sensory panels were conducted in duplicate on two different days. A 15-point flavor intensity scale was used to evaluate the following attributes: acid, bitter, salt, sweet, buttery, oxidized, metallic, and sulfur.

Experimental Design and Statistical Analysis

Cheeses were manufactured in four replicate cheese making trials. In each trial, 8 treatments were used: regular, reduced, low and no NaCl added cheeses made with both pressure treated and non-treated. Analysis of variance (ANOVA) testing was carried out by using Stata (version 11.1; StataCorp, College Station, TX, USA). Data obtained from analyzes were compared with Tukey-Kramer multiple comparisons test, and significance was determined at $P < 0.05$. Pearson's correlation coefficients were estimated between various responses.

3.4 RESULTS AND DISCUSSION

Composition and Microbiology

The regular salt cheeses (5.3% S/M) had slightly lower moisture content compared to the reduced, low and no salt added cheeses, which were in the same moisture range (Table 3.2). As expected, NaCl, total sodium, and S/M levels were significantly different for each S/M treatment. The other compositional parameters were not significantly different between treatments.

The 5.3% S/M level cheeses had significantly higher pH compared to the 2.5, 1.9, and 0.2% S/M cheeses, which were not significantly different from one another (Figure 3.1). A slight increase in cheese pH was observed with ripening probably due to solubilization of INSOL Ca (Hassan et al., 2004). Pressure application did not significantly affect pH values of cheeses within a S/M level, although it caused a slight increase in some cases. Previous research reported that use of HHP resulted in cheeses having higher pH values during ripening (Johnston and Darcy, 2000; Juan et al., 2007; Messens et al., 1999; Saldo et al., 2000; Rynne et al., 2008; Voigt et al., 2009) likely due to a decrease in starter culture numbers and/or impaired glycolytic pathways (Malone et al., 2003; Rynne et al., 2008). In our study, the cheeses were HHP treated 1 wk after cheese manufacture which allowed for the fermentation of all residual lactose into lactic acid (results not shown). The residual lactose was depleted at 2 wk, and all cheeses contained less than 0.01% lactose of total weight.

There was no significant difference in a_w values between HHP treated and non-treated cheeses, within the same S/M level (Figure 3.2). The concentration of solutes in the aqueous phase determine the water activity of food; therefore, the NaCl level is the major contributor to the a_w values of cheese (Schroeder et al., 1988). As expected, decreasing NaCl resulted in increased water activity and there was a significant ($P < 0.05$) negative correlation ($r \geq 0.97$) between a_w and NaCl content.

Starter (Figure 3.3a) and NSLAB (Figure 3.3b) numbers decreased with HHP. The impact of HHP on bacterial numbers increased with salt reduction. High pressure of the 5.3, 2.5, 1.9 and 0.2% S/M level cheeses resulted in approximately 1, 2.5, 3, and 4 log reduction in numbers of starter bacteria, respectively, compared to non-treated samples, at the same salt level. It was previously reported that the addition of NaCl (4.5%) to a citrate buffer did not affect the stability of lactococci strains to HHP (O'Reilly et al., 2002); however, cheese is a more complex environment. Changes in osmotic pressure of the environment with change in salt content could be a likely explanation for the changes in HHP efficiency. At lower water activity, bacterial cell tend to increase the intracellular osmotic pressure with several mechanisms. One mechanism is removing water from cytoplasm which leads to cell shrinkage. This could make bacteria more durable to HHP. The combination of low pH (<5.0) and reduced ionic strength could have been contributory factors responsible for the greater reduction in bacterial numbers by HHP in the lower salt cheeses. At the same salt level, HHP treatment caused a 3 log decrease in the NSLAB compared to the non-treated cheese. The NSLAB numbers increased in all cheeses during ripening (as expected). There was at least 1 log lower number of NSLAB during the first 3 mo of ripening in the pressure treated cheeses compared to non-treated cheeses.

Acid-base Buffering Curves

The INSOL Ca phosphate levels in the experimental cheeses are shown in Figure 3.4. The 2.5, 1.9, and 0.2% S/M level cheeses reached a relative stable level of INSOL Ca within 2 wk, while it took 3 mo for 5.3% S/M cheeses to reach stable levels, about 55% as previously reported for mature Cheddar cheese (Hassan et al. 2004) . At 2 wk of ripening, the 5.3% S/M cheeses also had higher INSOL Ca levels compared to the 2.5, 1.9, and 0.2% S/M level cheeses. This difference could be due to lower pH values of 2.5, 1.9, and 0.2% S/M level cheeses (Hassan et al., 2004). The pH values of cheeses were significantly positively correlated ($P < 0.05$) with INSOL Ca levels with r values of 0.89, 0.96, and 0.77 at 2 wk, 1 mo, and 3 mo, respectively. Pressure application did not significantly affect the INSOL Ca levels at any salt level.

Rheological Properties

The crossover point (or melting point), the temperature where the cheese changed from a solid to viscous-like material (Gunasekaran and Ak, 2003), decreased in all cheeses with a reduction in salt content (Figure 3.5a-d). Similarly, Grant (2011) also found that the melting point of cheese significantly decreased with reduction in salt level at 3 d and 3 wk of ripening, after which there was no difference in the melting point for all the cheeses, irrespective of the salt content. At 2 wk of ripening, HHP treatment significantly ($P < 0.05$) affected melting temperatures compared to the non-treated cheeses; the melting temperatures of the HHP treated cheeses were slightly lower than the non-treated cheeses within the same S/M cheeses. However, after 1 mo of ripening, no significant difference was observed between HHP treated and non-treated cheeses within the same S/M level.

The loss tangent values at temperatures ≤ 30 ºC remained constant during ripening with a value of ~0.3 and the loss tangent increased at ≥30 ºC to a maximum at ~60–75 ºC and then decreased (results not shown). The LTmax value is an indicator of cheese meltability, with a higher LTmax indicating a greater likelihood of a more meltable cheese. Reducing salt levels in Cheddar cheese resulted in lower LTmax values (Figure 3.5e-h). The LTmax values were highest in the 5.3% S/M cheeses compared to the reduced, low, and no salt added cheeses regardless of HHP treatment. For the 5.3% S/M cheeses, the LTmax values increased with ripening time while the LTmax values decreased for the 2.5, 1.9 and 0.2% S/M cheeses. Electrostatic repulsion between caseins decreases as the pH approaches the isoelectric point of casein (pH 4.6), causing decreased melt in cheese due to increased plus-minus charge interactions in the matrix (Lucey et al., 2003). Lee et al. (2005) reported that low pH values (≤ 4.9) hindered cheese meltability, thus the lower LTmax values for 2.5, 1.9 and 0.2% S/M cheeses could be due to their lower pH values. At 2 wk of ripening, application of HHP significantly ($P < 0.05$) increased LTmax values compared to non-treated cheeses within the same S/M level (Figure 3.5e-h). Cheeses within the same S/M level exhibited similar LTmax values with 1 mo of ripening. Messens et al (2000b) reported significant increases in LT values with HHP for Gouda cheese. Similar to our findings, Messens et al. (2000b) also found that with longer ripening (42 day), the rheological properties of the HHP treated and untreated Gouda cheeses became similar.

Pressure treatment resulted in significant differences in the temperature of the LTmax (results not shown). The 2.5, 1.9 and 0.2% S/M level cheeses treated with HHP exhibited significantly lower temperatures for LTmax compared to the control at 2 wk; however, by 1 mo of ripening there were no significant differences.

Textural Properties

At 2 wk of ripening, HHP treated cheeses had significantly lower hardness (as measured by texture profile analysis, TPA) compared to their corresponding non-treated cheese. Differences in hardness values between HHP treated and non-treated cheeses became smaller with ripening. At 3 mo of ripening, at the same S/M level, no significant difference was observed between HHP treated and non-treated cheeses (Figure 3.6).

Hardness decreased with a reduction in S/M level. The 5.3% S/M level cheeses were significantly harder compared to the 2.5, 1.9, and 0.2% S/M level cheeses up to 3 mo of ripening. The slightly lower moisture content (~36%) in the 5.3% S/M cheese compared with the 2.5, 1.9, and 0.2% S/M cheeses (moisture contents ~38%) (Table 3.2) may have contributed to the higher hardness values. Changes in the amount of INSOL Ca could also affect cheese hardness (Lucey et al., 2003). The 5.3% S/M cheeses had high INSOL Ca levels and were also harder than the other S/M cheeses. The TPA hardness values of cheeses were significantly ($P < 0.05$) positively correlated with INSOL Ca levels with r values of 0.96, 0.94, and 0.90 at 2 wk, 1, and 3 mo, respectively. Although, the 2.5, 1.9 and 0.2% S/M cheeses had similar INSOL Ca levels, they differed in hardness. Previous studies also reported a softening of cheese with a reduction in S/M (Grant, 2011; Mistry et al., 2003). The salt content in the aqueous phase could impact casein interactions, for example, the low S/M levels in the cheese aqueous phase causes greater casein hydration (Guerts et al., 1972), thereby weakening the matrix. The concentrations of soluble Ca and NaCl in cheese have a significant effect on cheese texture (Geurts et al., 1972; Lucey and Fox, 1993).

Uniaxial compression (obtained from 80% compression of cheese) profiles showed that cheese fracture decreased with salt reduction and HHP treatment (results not shown), which could be due to softening of the cheese network. Visible fracture was only observed for the 5.3% S/M non-treated cheese.

Proteolysis

Proteolysis increased in all cheeses with ripening (Figure 3.7). At a single time point, we did not observe any significant (*P>0.05*) difference in TCA soluble nitrogen levels with either salt reduction or HHP treatment. An increase in proteolysis in Cheddar cheese with HHP was previously reported (O'Reilly et al., 2000; Yokohama et al., 1992); however, holding time used during the HHP treatment in those studies were significantly longer (3 d) compared to that in this study (3 min). Schroeder et al. (1988) reported that reduction in NaCl increased soluble nitrogen levels, determined by measuring free tyrosine and tryptophan concentrations. Kelly et al. (1996) reported that salt concentration slightly affected the peptides produced, but it altered significantly the peptide concentrations. Urea-PAGE gel analysis of the 3 mo old cheeses is shown in Figure 3.8. The β-casein (f1-189/192) peptide is a strongly hydrophobic residue that contributes to bitterness in cheese (Visser et al., 1983). Within the same S/M levels, we did not observe any difference between the intensity of β-casein (f1-189/192) band in HHP treated or non-treated cheeses. Decreasing S/M levels resulted in an increase in the intensity of this band. Kelly et al. (1996) reported that β-casein degradation was influenced by NaCl concentration, and lower quantities of β-casein (f1-192) fraction were produced at higher S/M concentrations. Guinee and Fox (2004) reported that NaCl concentration in cheese greatly influences enzymatic activity.

Sensory Analysis

Many flavors (sweet, oxidized, metallic, sulfur) were only detected at very low levels, at 1 and 3 mo are given in Appendix II. As expected, salt flavor intensity decreased with a decrease in NaCl levels (Table 3.3). The application of HHP did not alter the sensory properties of cheeses at the same S/M level, although interestingly HHP did significantly decrease both starter and NSLAB numbers (Figure 3.3a, Figure 3.3b). Casal and Gomes (1999) investigated the peptidolytic activity of lactococci and lactobacilli strains in 10% reconstituted bovine skim milk. They reported that HHP (300 and ≥ 350 MPa for 20 min) treated lactococci and lactobacilli strains hydrolyzed the bitter β-casein (f1-193/209) peptide at a higher rate compared to control, indicating a potential debittering aspect resulting from HHP treatment. We did not observe any impact of HHP on bitterness in our cheeses. Pressure treated and non-treated cheeses at the same NaCl level, showed similar sensory profiles with regards to acid, salt, and bitterness (Table 3.3). Reducing the S/M concentration increased bitter, acid, and metallic flavor intensities (results not shown) in cheeses regardless of HHP treatment. Ripening resulted in an increase in bitter intensity scores in all cheeses and significant differences were observed between different S/M level cheeses after 3 mo of ripening. This was also in agreement with our observations of increased production of the β-casein (f1-189/192) peptide with a reduction in S/M levels (Figure 3.8).

3.5 CONCLUSIONS

Textural, rheological and microbial properties of reduced and low salt Cheddar cheese were affected by HHP. Application of HHP resulted in lower melt temperatures and softer cheeses at initial stage of ripening. However, the effects of HHP induced textural and rheological changes were dependent on the age of the cheeses. After one month of ripening, there were no textural or rheological differences between the HHP treated or non-treated cheeses.

Pressure application significantly reduced the starter and NSLAB numbers in all S/M cheeses, but there was a larger reduction in bacterial numbers in low S/M cheeses. Although there was a reduction in the starter and NSLAB numbers with HHP treatment, there was no difference in our measured indices of proteolysis. However, low S/M cheeses became bitter during ripening, even in HHP treated cheese where the starter numbers were significantly reduced. Urea-PAGE analysis indicated that pressure application did not affect the production of β-casein (f1-189/192) peptide at any S/M levels during ripening. There are two possible explanations as to why proteolysis and bitterness development were unaffected by HHP treatment even though there was a reduction in the starter and NSLAB numbers. Firstly, pressure application of 405 MPa may have not been sufficient to inactivate/activate chymosin or plasmin activity. Secondly, HHP may have contributed to starter or NSLAB lysis, enhancing the release of microbial enzymes that promote biochemical reactions, resulting in bitter peptide formation. There were no changes in pH or proteolysis in the HHP treated or non-treated cheeses. It is possible that the changes in the rheological properties observed during the intial stage of ripening could be due to weakening of hydrophobic interactions by HHP (Messens et al., 2000) and during ripening the similar proteolysis helped to minimize differences between cheeses.

In conclusion, application of 405 MPa for 3 min to 2.5, 1.9, and 0.2% S/M level Cheddar cheeses, at 1 wk of age, did not improve cheese characteristics. Pressures ≥ 400 MPa or earlier application of HHP (i.e., 1 d after manufacture) could be helpful to improve low salt cheese characteristics via impairing the coagulant activity and/or excessive lactic acid production preventing the low pH.

3.6 ACKNOWLEDGMENTS

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Table 3.1. Modified Cheddar cheese make procedures used to reduce moisture variation between the different S/M level cheeses

	Treatments			
	5.3% S/M	2.5% S/M	1.9% S/M	0.2% S/M
Ripening Temperatures (°C)	32.2	33.3	33.9	33.9
Ripening Times (min)	60	60	60	60
Cutting the gel	3 slash passes	10 slash passes	15 slash passes	15 slash passes
Stacking procedure during Cheddaring	Stack (2 high)	Stack (2 high)	Single layer (no stacking)	Single layer (no stacking)
Milling	Single Mill	Single Mill	Single Mill	Double Mill
Wait time between salt applications (min)	5	20	20	N/A[1]
Wait time between salting and hooping (min)	10	30	40	60

[1] Not applicable – No salt was added to this treatment

Table 3.2. Composition of two-week old Cheddar cheeses manufactured with various S/M levels (n = 4)

	Treatments			
	5.3 % S/M	2.5% S/M	1.9% S/M	0.2 % S/M
Moisture, %	35.75^{a}	38.38^{b}	38.17^{b}	37.96^{b}
S/M, %	5.32^{a}	2.50^{b}	1.93^{c}	0.25^{d}
Fat, %	34.21^{a}	33.18^{a}	33.25^{a}	33.5^{a}
Protein, %	24.67^{a}	24.02^{a}	24.18^{a}	24.81^{a}
NaCl[1], %	1.90^{a}	0.96^{b}	0.74^{c}	0.09^{d}
Na[2] (mg/100g)	518^{a}	271^{b}	197^{b}	66^{c}
Ca (mg/100g)	655^{a}	611^{a}	634^{a}	652^{a}
MNFS[3] %	54.35^{a}	57.44^{b}	57.19^{b}	57.08^{b}
FDM[4] %	53.25^{a}	53.84^{a}	53.78^{a}	54.00^{a}

[1] Determined using salt analyzer

[2] Determined using ICP method (Park, 2000)

[3] Moisture in nonfat substance

[4] Fat in dry matter

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

Table 3.3. Sensory flavor intensities (on a 0-15 point scale) for high hydrostatic pressure treated and control Cheddar cheeses made with various S/M levels (n = 4).

		5.3% S/M		2.5% S/M		1.9% S/M		0.2% S/M	
		Control	HHP	Control	HHP	Control	HHP	Control	HHP
Acid	1 mo	5.2^{c}	5.0^{c}	6.5^{b}	6.7^{b}	6.6^{b}	6.5^{b}	7.3^{a}	7.5^{a}
	3 mo	4.9^{a}	4.7^{a}	6.2^{b}	6.2^{b}	6.3^{b}	6.3^{b}	6.8^{b}	6.7^{b}
Bitter	1 mo	0.3^{c}	0.4^{c}	1.0^{ab}	0.9^{b}	1.0^{ab}	1.1^{ab}	1.3^{a}	1.2^{ab}
	3 mo	1.6^{ab}	1.3^{a}	3.6^{abc}	3.0^{abc}	4.2^{b}	4.9^{c}	5.3^{c}	5.4^{c}
Salt	1 mo	5.4^{a}	5.2^{a}	3.5^{b}	3.4^{b}	2.4^{c}	2.3^{c}	0.5^{d}	0.4^{d}
	3 mo	6.1^{a}	5.7^{a}	3.6^{b}	3.5^{b}	2.5^{bc}	2.0^{cd}	0.8^{d}	0.7^{d}

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

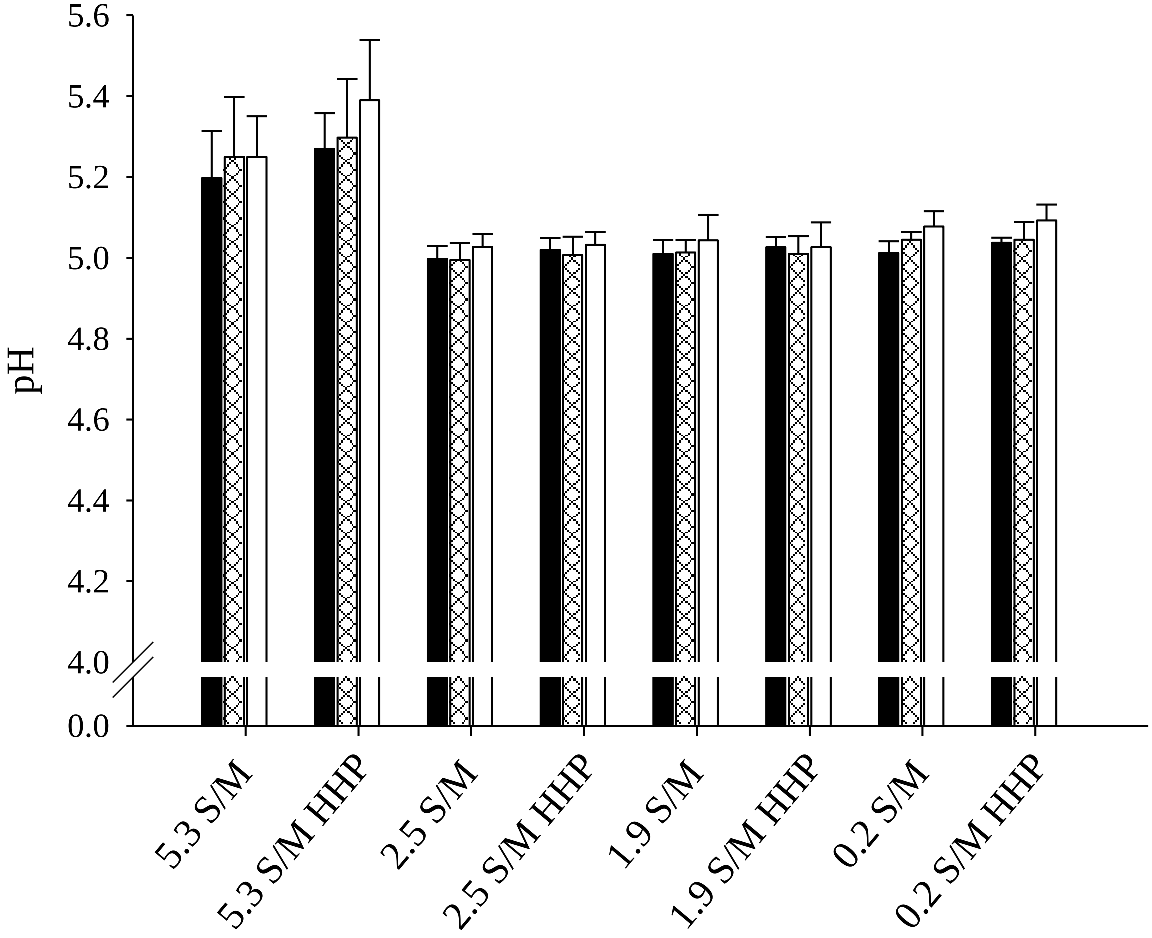

Figure 3.1. The pH values for 5.3, 2.3, 1.9 and 0.2% S/M, non-treated and HHP treated Cheddar cheeses at 2 wk (■), 1 (×), and 3 (□) mo of ripening at 7°C. Vertical bars represent standard deviations.

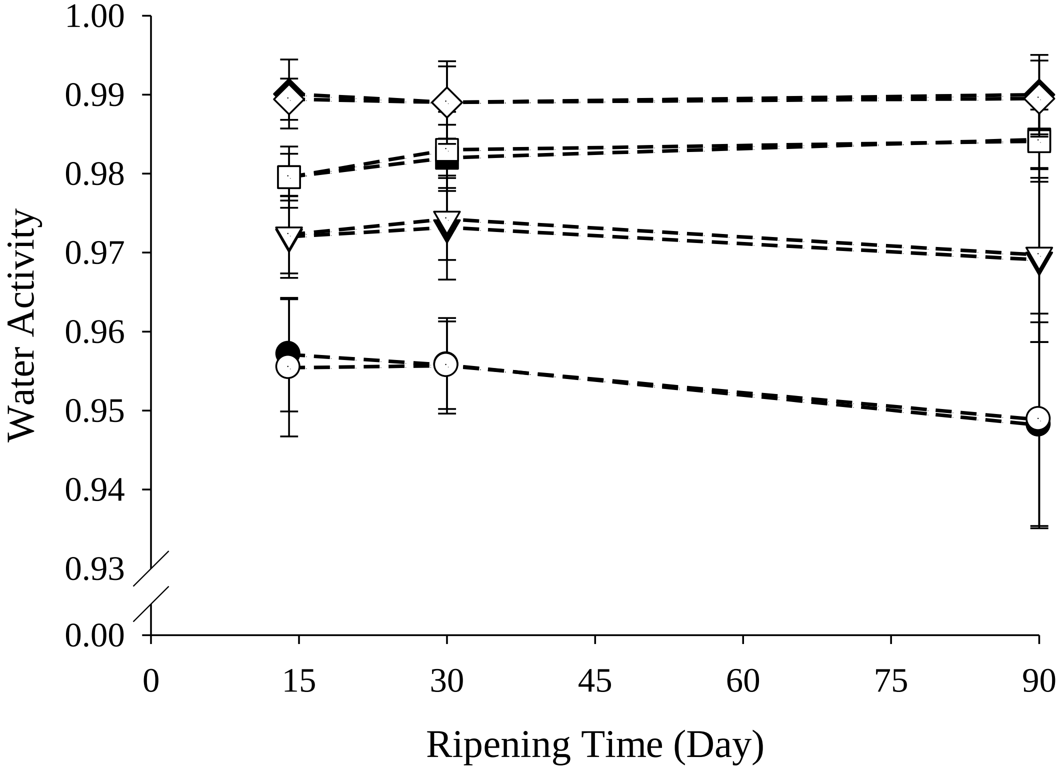

Figure 3.2. Water activity of the 5.3 (●), 2.5 (▼), 1.9 (■), and 0.2% S/M (♦), non-treated and HHP treated Cheddar cheeses during 3 mo of ripening (closed symbols denote non-treated, open symbols denote HHP treated cheese). Vertical bars represent standard deviations.

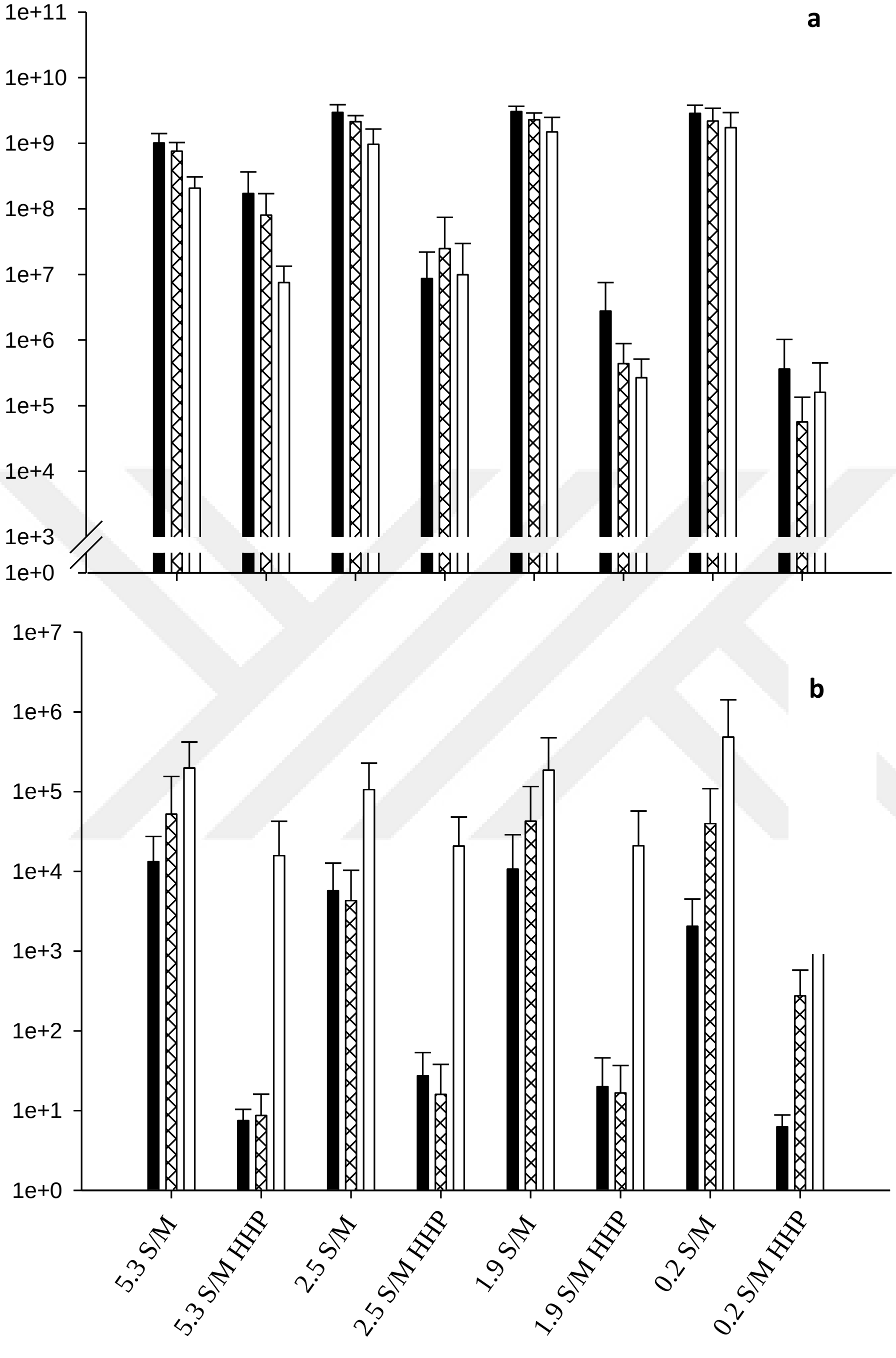

Figure 3.3. Starter culture numbers (a) and nonstarter lactic acid bacteria numbers (b) of non-treated and HHP treated Cheddar cheeses at 2 wk (■), 1 (×), and 3 mo (□) of ripening at 7°C. Vertical bars represent standard deviations.

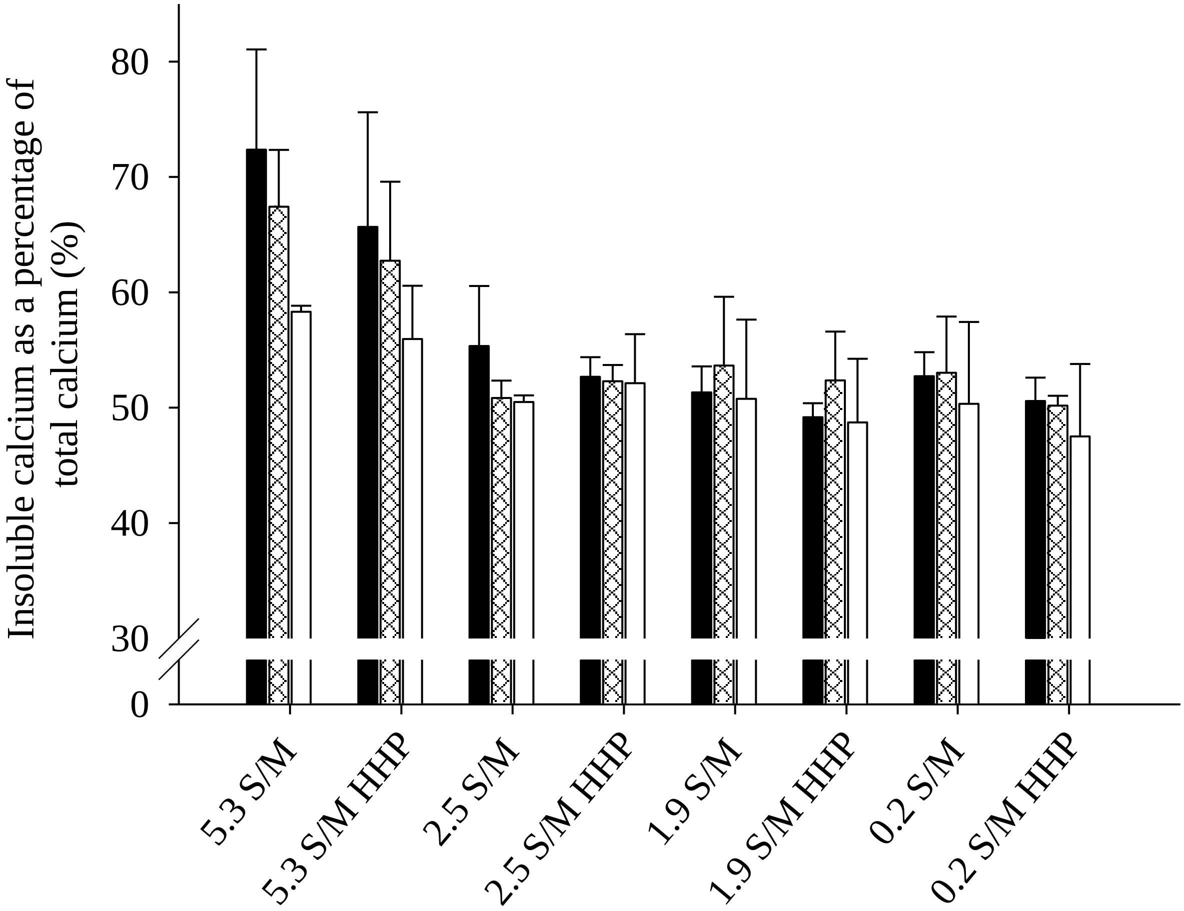

Figure 3.4. Changes in the percentage of insoluble Ca as a percentage of total cheese Ca in non-treated and HHP treated Cheddar cheeses at 2 wk (■), 1 (×), and 3 (□) mo of ripening at 7°C measured by the acid-base titration method. Vertical bars represent standard deviations.

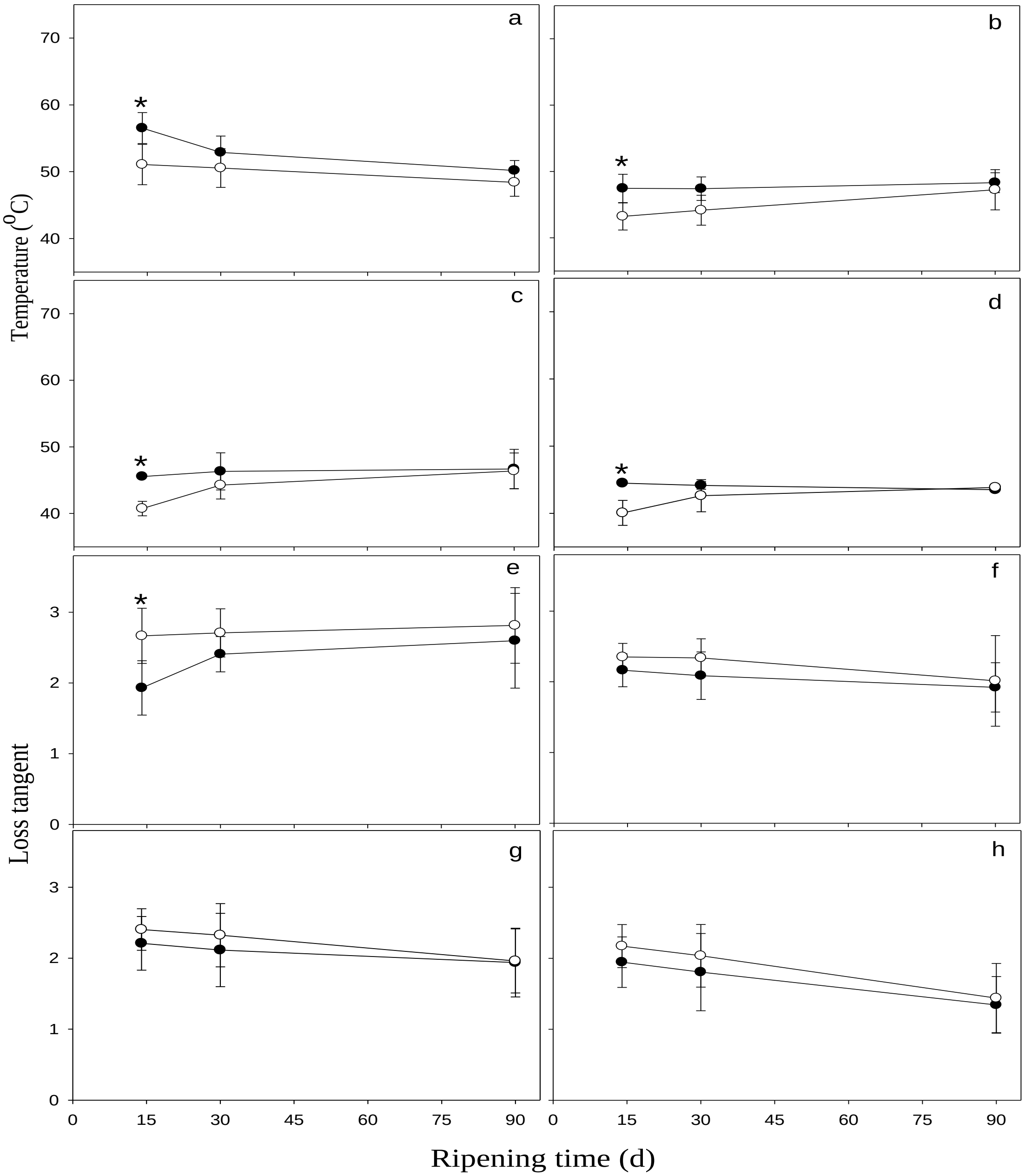

Figure 3.5. Temperature of the crossover point (where LT = 1), and the maximum loss tangent values for the 5.3 (a), (e); 2.5 (b), (f); 1.9 (c), (g); and 0.2% S/M (d), (h) Cheddar cheeses, respectively, from the small amplitude oscillatory rheology test carried out during first 3 mo of ripening (closed symbols denote non-treated, open symbols denote HHP treated cheese). Vertical bars represent standard deviations.

* refers to cheeses which are significantly different from control (*P* < 0.05) within the same S/M level at a single ripening time point.

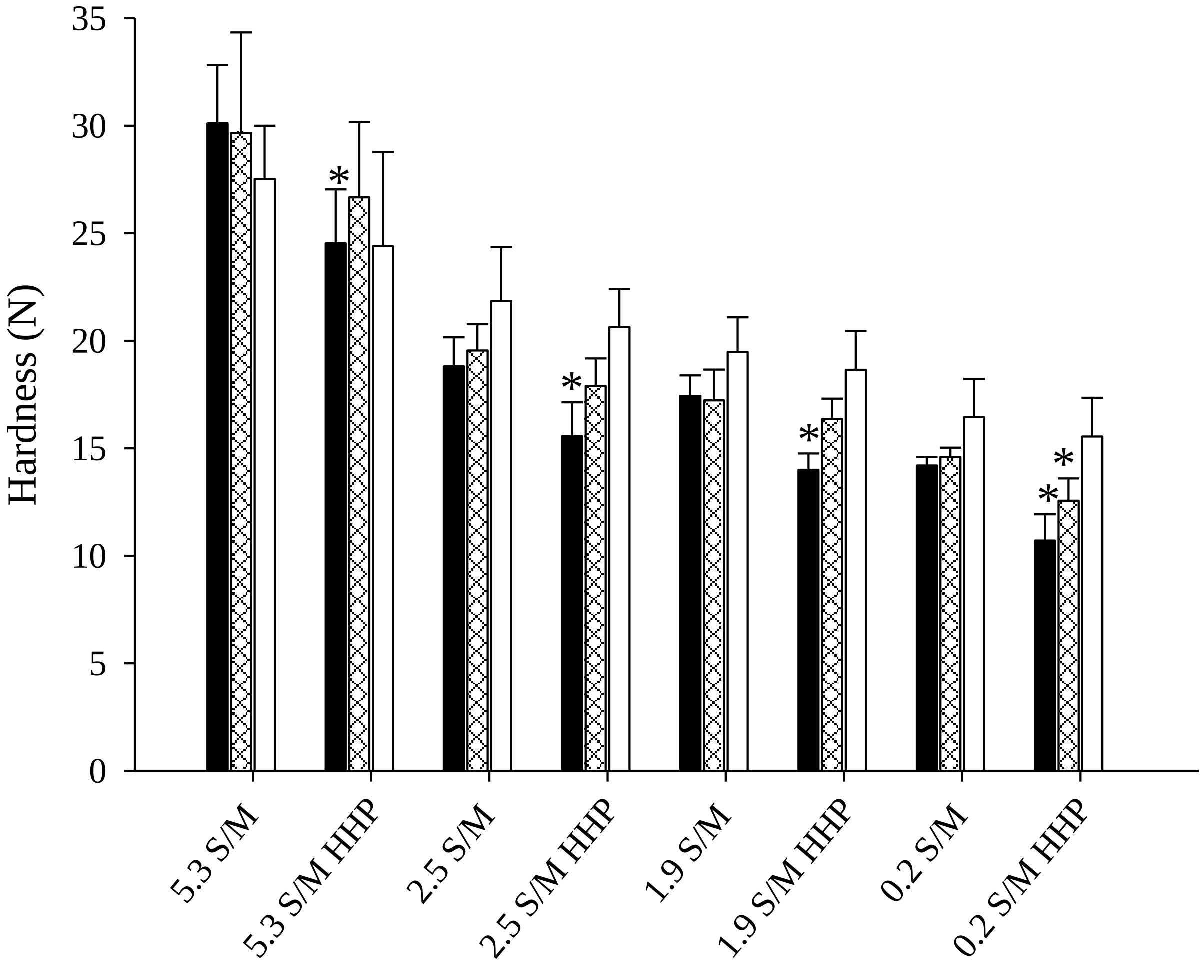

Figure 3.6. Hardness from texture profile analysis testing (20% compression) for 5.3, 2.5, 1.9, and 0.2% S/M, non-treated and HHP treated Cheddar cheeses at 2 wk (■), 1 (×), and 3 (□) mo of ripening at 7°C. Vertical bars represent standard deviations.

* refers to cheeses which are significantly different from control ($P < 0.05$) within the same S/M level at a single ripening time point.

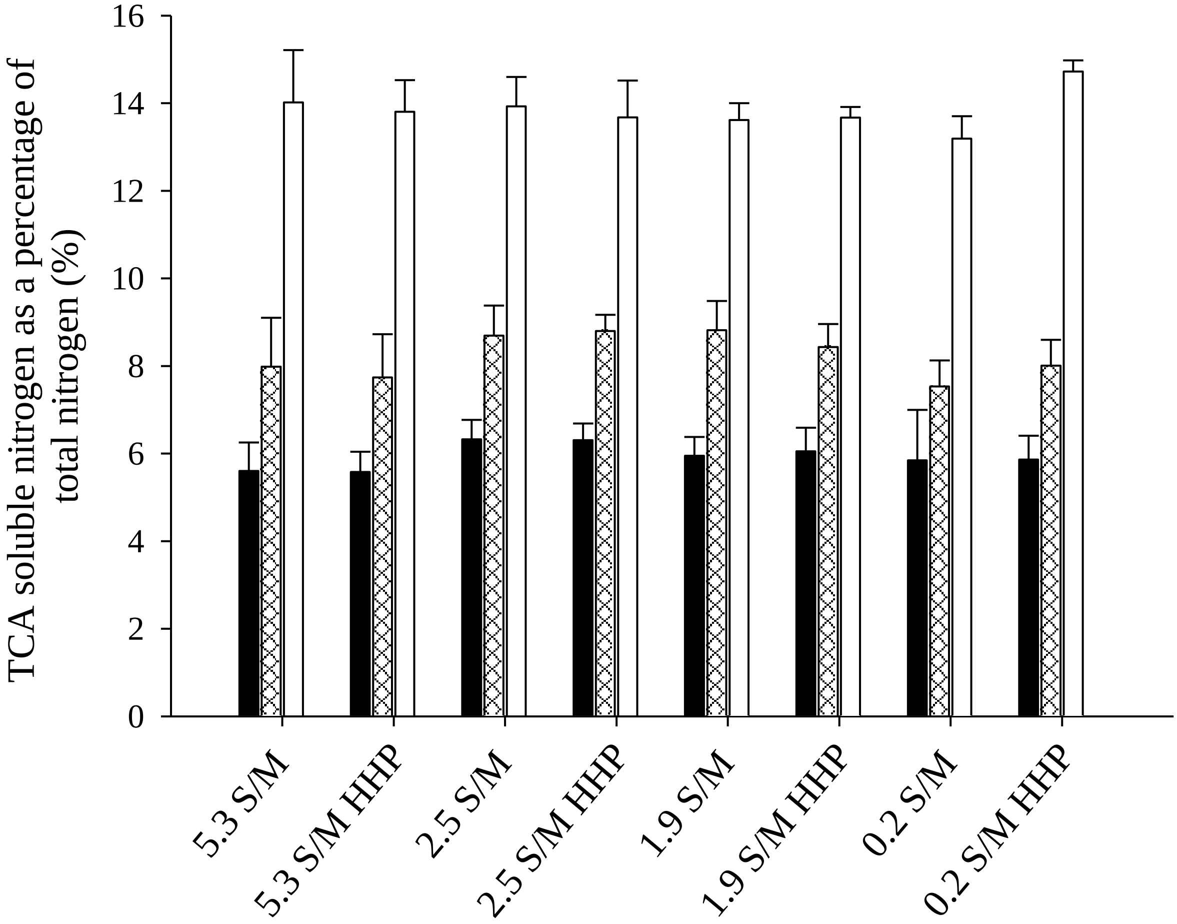

Figure 3.7. TCA soluble nitrogen as a percentage of total nitrogen for non-treated and HHP treated Cheddar cheeses at 2 wk (■), 1 (×), and 3 (□) mo of ripening at 7°C. Vertical bars represent standard deviations.

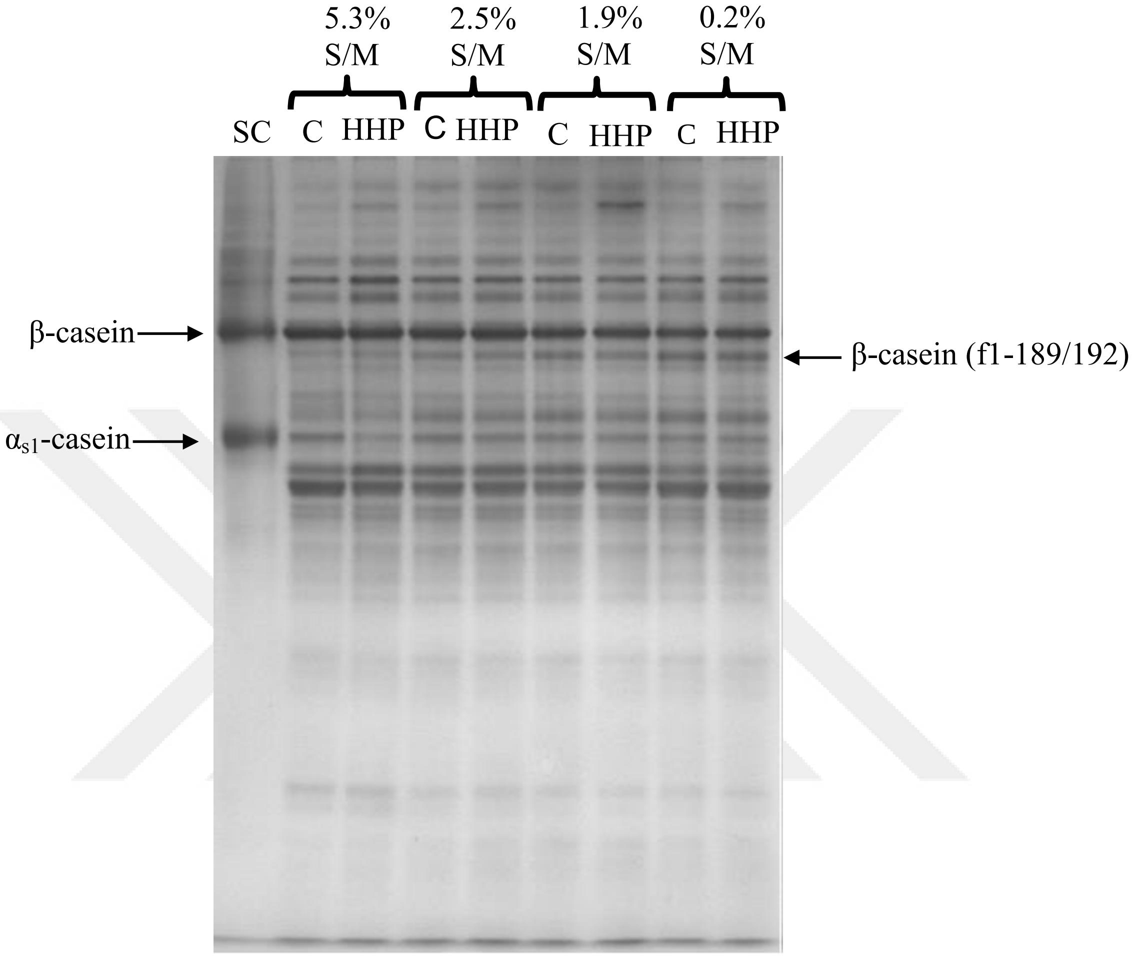

Figure 3.8. Urea-PAGE electrophoretograms showing the breakdown of caseins in 5.3, 2.5, 1.9, and 0.2% S/M, non-treated; C) and HHP treated Cheddar cheeses at 3 mo of ripening at 7°C. Lane with SC refers to sodium caseinate (standards).

Chapter 4.

Low Sodium Cheddar Cheese: Impact of Fortification of Cheese Milk with Ultrafiltration Retentate and High Hydrostatic Pressure Treatment of Cheese

4.1 ABSTRACT

Low sodium cheeses often exhibit an acidic flavor due to rapid acid production by starter cultures, at the initial stage of ripening, due to the low salt in moisture (S/M) levels. We proposed that excessive initial (post-manufacture) starter-induced acidity could be prevented by decreasing microbial activity using the application of high hydrostatic pressure (HHP) treatment (to reduce residual starter numbers), and by the fortification of cheese milk with ultrafiltration (UF) retentates (to increase curd buffering). Camel chymosin was also used as a coagulant to help reduce proteolysis and lessen bitterness development (a common defect in low sodium cheeses). Three types of low Na (0.8% NaCl) cheeses were manufactured: non-UF fortified, no HHP applied (L-Na); UF fortified (17.2 ± 0.6% TS), no HHP applied (L-Na-UF); and UF fortified, HHP treated (L-Na-UF-HHP) (500 MPa for 3 min applied at 1 d post manufacture). Regular salt (2% NaCl) non-UF fortified, no HHP applied (R-Na) cheese was also manufactured for comparison purposes. Analysis was performed at 4 d, 2 wk, 1, 3 and 6 mo after cheese manufacture. Cheese functionality during ripening was assessed using texture profile analysis (TPA) and dynamic low-amplitude oscillatory rheology. Quantitative descriptive analysis was conducted with 9 trained panelists to evaluate texture and flavor attributes using a 15 point scale. At 4 d and 2 wk of ripening, pressure treated low Na cheese had ~2 and ~4.5 log lower starter culture numbers, respectively, than all other cheeses. Retentate fortification of cheese milk and

HHP treatment resulted in low Na cheeses with similar insoluble calcium and pH values compared to R-Na cheese during ripening. L-Na-UF cheese exhibited significantly higher hardness values (measured by TPA) compared to L-Na cheese until 1 mo; however, by 1 mo all low Na cheeses exhibited similar (*P* > 0.05) hardness values, which were significantly lower than R-Na cheese. Pressure treatment significantly (*P* < 0.05) increased maximum loss tangent (meltability) for rheology testing and decreased melt temperature. Sensory results indicated that there was only very slight bitterness (< 2.5 out of 15 point scale) detected in all cheeses during the 6 mo of ripening (possibly due to the use of camel chymosin as coagulant). The L-Na-UF-HHP cheese did not significantly (*P* > 0.05) differ in bitterness and acidity from R-Na cheese during ripening. Pressures treatment of cheese at 500 MPa and cheese milk retentate fortification could be used to improve the quality of low Na cheese.

Key Words: High pressure processing, milk retentate, low sodium cheese

4.2 INTRODUCTION

Concerns over a possible relationship between dietary sodium intake and cardiovascular disease have led to an effort to reduce salt in processed foods (US Department Health and Human Services, 2005), although long term health impacts of high salt consumption have yet to be demonstrated (Taylor et al., 2011). Reducing salt in cheese is challenging because salt assists manufacturers to control various important cheese parameters including: final moisture content, microbial activity, survival of the starter bacteria, and residual enzymatic activity in cheese (Johnson et al., 2009). Salt content of cheese directly influences flavor and texture with reduced salt cheeses often reported to be bitter, acidic and pasty (Guinee, 2004).

A number of previous studies have investigated the impact of reducing the sodium content, and/or partial/complete replacement of sodium with other salts, on the quality of Cheddar cheese. Lindsay et al. (1982) studied Cheddar cheese manufactured with either NaCl or 1:1 blend of NaCl/KCl, at various final salt levels (1.25, 1.5 and 1.75%). Cheeses made with NaCl/KCl blends received lower overall acceptability scores, and exhibited higher bitterness and lipolysis compared to the cheeses made with NaCl alone (at the same total salt level). Grummer et al. (2013) investigated the partial (60%) replacement of NaCl with KCl with/without flavor enhancers. They reported that low Na cheeses made with NaCl/KCl blends were well liked by the sensory panelists and they were comparable to the control cheese (which contained only NaCl). However, the type of flavor enhancers used either positively, or negatively, affected the quality of reduced NaCl cheese. Kosikowski (1983) used a different approach rather than replacing Na; he investigated fortification of cheese milk with ultrafiltration (UF) retentate for the manufacture of reduced salt (~ 1%) NaCl Cheddar cheese. Control cheese (1% NaCl, with no UF retentate fortification) was acidic, bitter, pasty and lacked typical Cheddar cheese flavor, while cheeses fortified with whole milk UF retentate exhibited good to excellent quality.

Application of high hydrostatic pressure (**HHP**) processing to cheese influences cheese microflora, acid development, proteolysis and protein interactions (Martinez-Rodriguez et al., 2012). Ozturk et al. (2013a) investigated the effect of HHP on regular (5.3%), reduced (2.5%), low (1.9%), and no NaCl (0.2%) added Cheddar cheeses. One wk after cheese manufacture, cheeses were subjected to HHP at ~405 MPa for 3 min. There were no significant differences in proteolysis, pH values or sensory profiles of cheeses made with the same NaCl levels. The effects of HHP primarily depend on the pressure/time treatment and the age of the cheese when HHP treatment is applied (Juan et al., 2007). Juan et al. (2007) reported that cheeses that were

HHP treated at 1 d had higher pH values than non-treated cheeses, while cheeses HHP treated at 15 d did not exhibit any pH differences compared to non-treated cheese. Previous studies indicated that pressures > 400 MPa resulted in lower levels of lactic acid and proteolysis (via decreased residual activity of chymosin and bacterial proteinases) (Malone et al., 2003; Juan et al., 2007).

It is generally agreed that the activity of residual coagulant is significantly involved in bitterness development in cheese (Guinee and Fox, 2004; Grant, 2011). A novel type of coagulant (i.e. fermentation produced camel chymosin) that had less residual proteolytic activity was successfully used to decrease bitterness in low fat (Govindasamy-Lucey et al., 2010) and low sodium cheeses (Grant, 2011; Moller et al., 2013). Therefore, we hypothesized that decreasing the activity of residual coagulant with HHP could assist in the manufacturing of good quality low sodium cheese.

In this study, we wanted to explore the combination of cheese milk fortification with UF retentate, along with HHP treatment of cheese, on the quality of low sodium Cheddar cheeses manufactured with fermentation produced camel chymosin.

4.3 MATERIALS AND METHODS

Cheese Manufacture

Licensed Wisconsin cheese makers manufactured 5 independent batches of Cheddar cheeses at the University of Wisconsin-Madison dairy processing plant. On each cheesemaking day, 4 cheese vats were utilized to manufacture two salt in moisture (**S/M**) level cheeses. Cheese milk (12.4 ± 1.5% total solids (**TS**), 2.4 ± 0.3% casein (**CN**), 3.7 ± 0.5% fat) in two vats were fortified with ultrafiltration (**UF**) retentate (27.5 ± 1.5% TS, 7.7 ± 0.3% CN, 11.6 ± 0.5% fat) to give blended cheese milk (17.2 ± 0.6% TS, 4.0 ± 0.1% CN, 6.5 ± 0.2% fat). For two unfortified vats, 227 kg cheese milk was used, while the two UF fortified vats were manufactured with 125 kg of UF fortified cheese milk, to be able to produce similar final curd weights. Starter culture and coagulant were added based on CN content and their ratio were kept constant among cheese vats. The average composition of the cheese milks for each vat, and the manufacturing procedures are given in Table 4.1. Retentate fortified cheese milk tended to clot faster, thus lower (26.7ºC) ripening (gel forming) temperatures were used to adjust (lengthen) the cutting time. Cheese making procedures were modified to try to obtain similar moisture contents among different NaCl levels cheeses. Direct vat-set starter culture containing *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* blend (MA11, Dupont, Madison, WI, USA) was added at the rate of 10 g for 227 kg of unfortified milk, or 125 kg of UF fortified milk, and ripened as outlined in Table 4.1. Fermentation-produced camel chymosin (Chymax® M, Chr. Hansen, Milwaukee, WI, USA) was used as coagulant at the rate of 11.3 g for 227 kg of unfortified milk, or 125 kg of UF fortified milk (2.05 ± 0.5 g chymosin per kg of CN). Coagulum was cut on firmness (~20 min) with 6.35 mm knives and whey was drained when the curd pH reached around 6.05. Cheeses were ripened for 6 mo at 7°C and analyzed at 4 d, 2 wk, 1, 3 and 6 mo.

High Pressure Treatment

One day after manufacture, one block (35 × 28 × 8.5 cm) of the low NaCl, UF fortified cheeses were HHP treated at 500 MPa for 3 min at 6.4 ± 1ºC. High pressure unit (Avure Ultra 215 L, Avure Technologies Inc., WA, USA) reached 500 MPa in around 120 s. The decompression cycle took about 30 s.

Composition Analyses

The UF retentate and cheese milk was analyzed for total solids (Green and Park, 1980), fat (Mojonnier method; AOAC, 2000), protein (total percentage N × 6.38, Kjeldahl method; AOAC, 2000), casein (AOAC, 2000), non-protein nitrogen (AOAC, 2000), lactose (AOAC, 2000), total Ca (Park, 2000), and insoluble Ca (**INSOL Ca**) by the acid–base titration method (Lucey et al., 1993; Hassan et al., 2004). The rennet whey was prepared as described in Lucey et al. (1993) and analyzed for soluble Ca content (Park, 2000). The cheese composition was analyzed at 2 wk of ripening for moisture (Marshall, 1992), fat (AOAC, 2000), protein by the Kjeldahl method (AOAC, 2000), pH using a glass pH electrode (Sam Gray gold electrode; Nelson-Jameson, Marshfield, WI, USA; Marshall, 1992), salt by the chloride electrode method (model 926, Corning Glass Works, Medfield, MA, USA; Johnson and Olson, 1985), water activity (a_w) by the capacitance method (AquaLab Water Activity Meter, Decagon, Pullman, WA), lactose and lactic acid using HPLC method (Zeppa et al., 2001) and total Ca and Na by inductively coupled argon plasma emission spectroscopy (Vista-MPX Simultaneous ICP-OES, Varian Inc., Palo Alto, CA, USA) (Park, 2000). The INSOL Ca content in cheeses were measured at 1 d, 4 d, 2 wk, 1 mo, 3 mo, and 6 mo by the acid–base titration method and calculated as described by Hassan et al. (2004). All analyses were done in triplicate.

Microbiological Analysis

Starter lactococci and nonstarter lactobacilli strains were measured by de Man, Rogosa and Sharpe (MRS) agar and Rogosa SL media, respectively, samples were incubated at 32°C for 48 h under anaerobic conditions (Frank and Yousef, 2004; Ozturk et al., 2013a).

Proteolysis and Urea PAGE Gels

Proteolysis was monitored by water soluble nitrogen (**W-SN**) and 12% trichloroacetic acid soluble nitrogen (**TCA-SN**) levels (AOAC, 2000). Analyses were performed in duplicate. The breakdown of α- and β-caseins during ripening was monitored with urea-polyacrylamide gel electrophoresis (**PAGE**). Urea-PAGE gels were prepared as described by Ozturk et al. (2013a). Pictures of the gels were analyzed with densitometric analysis using image analysis software (Gel Expert 3.5; Nucleo Tech, San Carlos, CA, USA).

Rheological and Textural Measurements

Rheological properties of the cheese samples were measured by dynamic small amplitude oscillatory rheology as described in Ozturk et al. (2013a). The storage modulus (**G′**), loss modulus (**G″)**, and loss tangent (**LT**) were measured with temperature sweep test from 5 to 85°C with the heating rate of 1°C/min at frequency of 0.08 Hz and a strain of 0.5%. The maximum loss tangent (**LTmax**) value observed during heating was also recorded.

Texture profile analysis (**TPA**) and uniaxial compression tests were carried out using a TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA) as described by Ozturk et al. (2013a).

Descriptive sensory analysis

Cheese flavor and texture was evaluated with a mixture of sensory Spectrum and quantitative descriptive analysis (Meilgaard et al., 1999) by ≥ 9 trained (20 h of training) panelists at 1, 3 and 6 mo of ripening. Cheeses cubes (2 × 2 × 2 cm) were evaluated at 12°C.

Samples were identified with random 3 digit numbers. A 15-point scale was used to evaluate the following attributes: firmness, chewiness, acid, bitter, salt, milky, oxidized, metallic, and sulfur (Table 4.2). All sensory panels were conducted in duplicate on two different days.

Experimental Design and Statistical Analyses

Cheeses were manufactured on five replicate cheese making batches. In each batch, 4 treatments were used: one regular salt (2% NaCl) non-UF fortified, no HHP applied (**R-Na**); three low salt (0.8% NaCl); non-UF fortified, no HHP applied (**L-Na**); UF fortified (17.2 ± 0.6% TS), no HHP applied (**L-Na-UF**); and UF fortified, HHP treated (**L-Na-UF-HHP**) (500 MPa for 3 min applied at 1 d).

The effect of treatment (R-Na, L-Na, L-Na-UF, L-Na-UF-HHP) and ripening time and their interactions on pH, INSOL Ca, W-SN, and textural, rheological and sensory properties were monitored using a split-plot design. In the whole-plot factor, cheesemaking day was the blocking factor and treatment was analyzed as discontinuous variable. For the split-plot factor, age and interaction of age and treatment were treated as variables. The interactive term of treatment and cheesemaking day was treated as the error term for the treatment effect. Statistical analyses were carried out using JMP (version 11.0; SAS Institute Inc., Cary, NC, USA). Data were analyzed for analysis of variance (**ANOVA**) with Tukey-HSD multiple comparisons test, and significance was determined at $P < 0.05$. Pearson’s correlation coefficients were estimated between various responses.

4.4 RESULTS AND DISCUSSION

Composition and Microbiology

Moisture content of R-Na Cheddar cheese was lower than all L-Na samples (Table 4.3). Previous studies also reported that reduction in NaCl increased the moisture content of cheeses (Grant, 2011; Rulikowska et al., 2013). R-Na cheese exhibited higher S/M and Na contents (Table 4.3), and lower water activity values (results not shown) compared to all L-Na cheeses, as was expected. There were no significant ($P > 0.05$) differences in S/M and Na values between all L-Na cheeses. Use of HHP treatment and UF retentate fortification significantly ($P < 0.05$) increased the pH values of cheeses during ripening (Table 4.4). The pH value of L-Na-UF-HHP cheeses was similar or higher than R-Na cheese at all ages, while the L-Na cheese exhibited significantly ($P < 0.05$) lower pH values during ripening (Table 4.5). R-Na cheese exhibited higher residual lactose (results not shown) and lower lactic acid content than L-Na cheese during ripening (Table 4.4). High pressure processing decreased the amount of lactic acid produced in cheese during ripening. L-Na-UF-HHP cheese exhibited significantly ($P < 0.05$) lower lactic acid content than L-Na cheese during ripening (Table 4.5). Casal and Gomez (1999) reported that HHP treatment decreased the acidifying activity of lactic acid bacteria. We do not have results to know if the lower lactic acid values of L-Na-UF-HHP were due to an impairment in glycolytic pathway or due to the lower numbers of viable starter lactic acid bacteria in L-Na-UF-HHP cheese (Table 4.5).

Pressure treatment decreased starter culture numbers by ~2 and ~4.5 log, at 4 d and 2 wk of ripening, respectively, compared to all other cheeses (Table 4.4, Table 4.5). Pressure treatment could have created some damaged bacteria cells that could have lost further viability during ripening in the harsh cheese environmental conditions (compared to growth media) during cheese ripening. Starter bacteria numbers of L-Na-UF-HHP cheese remained ~2 log lower than

R-Na cheese and low NaCl cheeses (L-Na and L-Na-UF) until 3 and 6 mo of ripening, respectively.

Rulikowska et al. (2013) reported that NaCl level significantly (*P* < 0.05) negatively influenced numbers of starter bacteria at 1 d of ripening. In our study, NaCl level did not significantly (*P* > 0.05) affected the starter culture numbers; R-Na cheese and L-Na and L-Na-UF cheeses exhibited similar starter culture numbers during ripening (Table 4.5). The difference in the response of starter population to NaCl levels between Rulikowska et al. (2013) and our study may be due to the different salt sensitivity of starter cultures used. Swearingen et al. (2004) reported that various types of Cheddar cheese starter cultures exhibited different salt sensitivity or tolerances. The growth rate of non-starter lactic acid bacteria (**NSLAB**) was unaffected by NaCl content, UF retentate fortification or HHP treatment during ripening (results not shown). Lane et al. (1997) reported that S/M levels ≤ 5.9% did not influence the growth of NSLAB numbers in Cheddar cheese. Ozturk et al. (2013a) reported that HHP treatment (405 MPa for 3 min), performed at 1 wk of ripening, resulted in 1 to 4 log reduction in the number of NSLAB in cheese depending on the NaCl level. However, HHP was applied at 1 d of ripening in the current study, which could have hindered the initial growth of NSLAB during ripening.

Acid-Base Buffering Curves (Insoluble Ca Levels)

Salt content and UF retentate fortification significantly (*P* < 0.05) influenced INSOL Ca levels in Cheddar cheese samples (Figure 4.1, Table 4.4). During the first 3 mo of ripening, L-Na cheese exhibited significantly (*P* < 0.05) lower INSOL Ca values compared to R-Na cheese (Figure 4.1, Table 4.4). Cheese pH affects the amount of INSOL Ca in cheese. Calcium in the INSOL (or colloidal state) becomes soluble as cheese pH decreases (Lucey et al., 2003). Higher lactic acid and lower pH values of L-Na cheese could be responsible for their low levels of INSOL Ca (Table 4.4). The pH values of cheeses were significantly positively correlated ($P < 0.05$) with INSOL Ca levels with an r value of 0.78. L-Na-UF and L-Na-UF-HHP cheeses did not significantly ($P > 0.05$) differ in INSOL Ca values compared to R-Na cheese. UF retentate fortified L-Na cheeses had slightly higher protein content (Table 4.3) that could have helped to increase its INSOL Ca (since INSOL Ca is associated with protein) compared to R-Na cheese. Previous studies reported that cheeses manufactured with UF retentate fortified milk resulted in cheeses with higher buffering capacities (Kosikowski, 1983), which is positively correlated with INSOL Ca levels (Hassan et al., 2004). Our results also indicated that cheeses manufactured from UF retentate fortified milk tend to have higher INSOL Ca levels. The L-Na-UF-HHP cheese exhibited significantly ($P < 0.05$) higher pH values compared to L-Na-UF up to 1 mo of ripening. However, there was no parallel increase in INSOL Ca values for L-Na-UF-HHP cheese; as it exhibited similar INSOL Ca values to L-Na-UF cheese during ripening. Previous studies indicated that HHP did not significantly change the level of INSOL Ca in cheese (Ozturk et al., 2013a) even though there have been reports (Schrader et al., 1997) of reversible solubilization of INSOL Ca in milk with HHP treatment (400 MPa for 5 min).

Proteolysis

Water soluble nitrogen is an indicator of primary proteolysis in cheese (Rank et al., 1985). The level of NaCl did not significantly ($P > 0.05$) influence the concentration of W-SN (Figure 4.2a). Starting from 1 mo of ripening, L-Na-UF-HHP cheese exhibited significantly ($P < 0.05$) lower levels of W-SN (Figure 4.2a, Table 4.4), indicating a possible impairment/reduction in residual coagulant activity. Previous studies suggested that application of pressures > 400

MPa decreased residual calf chymosin activity (Malone et al., 2003; Juan et al., 2007). The aspartic proteinase family exhibit high sequence identity (Chitpinityol and Crabbe, 1998), thus camel and calf chymosins were likely to be similarly affected by HHP treatment. Activity of both residual rennet and microbial proteases contribute to the levels of TCA-SN in cheeses (Rank et al., 1985). The L-Na cheese exhibited significantly ($P < 0.05$) higher TCA-SN levels compared to R-Na cheese during 1 mo of ripening (Figure 4.2b). L-Na-UF and L-Na-UF-HHP did not significantly ($P > 0.05$) differ from R-Na cheese in TCA-SN values until 1 mo of ripening. After 1 mo of ripening, L-Na-UF-HHP exhibited significantly ($P < 0.05$) lower levels of TCA-SN compared to all other cheeses, regardless of UF retentate fortification or NaCl content. Fox (1970) reported that caseins (especially α_{s1}-casein) were more prone to proteolysis with a decrease in the level INSOL Ca phosphate. The activity of residual chymosin on α_{s1}-casein and β-casein is highest at pH values ≤ 5.0 (Mulvihill and Fox, 1978; Grappin et al., 1985). The higher levels of TCA-SN values in L-Na cheese could be due to its lower pH values and/or lower INSOL Ca levels. At 1, 3 and 6 mo of ripening, L-Na-UF-HHP cheese exhibited significantly lower TCA-SN levels compared to all samples (Figure 4.2b). Pressure treatment significantly decreased the number of starter bacteria in our study (Table 4.4). Malone et al. (2003) reported that pressures ≥ 500 MPa decreased the activity or inactivated major proteolytic enzymes in cheese matrix (residual chymosin and starter proteases, such as, cell envelope protease, X-Prolyl-dipeptidyl aminopeptidase, and aminopeptidase N). Thus, the significantly ($P < 0.05$) lower levels of TCA-SN observed for L-Na-UF-HHP cheese may suggest a decrease in the activities of both microbial proteases and residual chymosin due to HHP.

Urea-PAGE electrophoretogram of cheese samples during ripening is shown in Figure 4.3. The hydrophobic β-CN (f1-189) peptide is a significant contributor to bitterness in cheese

(Visser et al., 1983). Salt content and HHP treatment significantly ($P < 0.05$) influenced the intensity of this β-CN (f1-189) band in the experimental cheeses (Figure 4.3, Figure 4.4a). R-Na and L-Na-UF-HHP samples exhibited significantly ($P < 0.05$) lower levels of β-CN (f1-189) peptide compared to L-Na and L-Na-UF samples at 3 and 6 mo of ripening (Figure 4.4a). The concentration of NaCl (S/M) significantly influences residual coagulant activity in cheese (Guinee and Fox, 2004). Previous studies reported that the production of this β-CN (f1-189) peptide significantly increased with a decrease in the NaCl level (Kelly et al., 1996; Moller et al., 2013; Ozturk et al., 2013a). Degradation of α_{s1}-CN was also significantly ($P < 0.05$) lower for L-Na-UF-HHP cheese compared to all other cheeses after 3 mo of ripening (Figure 4.4b). Level of NaCl did not significantly ($P > 0.05$) influence the degradation of α_{s1}-CN; R-Na cheese exhibited similar levels of intact α_{s1}-CN compared to L-Na and L-Na-UF cheeses during ripening (Figure 4.3, Figure 4.4b). Fox and Walley (1970) reported that NaCl concentrations around 5% were optimal for the activity of chymosin on α_{s1}-CN; only very high concentrations of NaCl (15 and 20%) decreased the activity of chymosin on α_{s1}-CN.

Rheological Properties

High pressure treatment and NaCl level significantly ($P < 0.05$) affected the rheological properties of cheese samples (Table 4.6). The crossover point (where the LT = 1) is an indicator of melting temperature. R-Na cheese exhibited the highest melting temperature during ripening while L-Na-UF-HHP cheese had the lowest melting temperature during ripening (Figure 4.5a). Previous studies suggested that HHP treatment reduced hydrophobic interactions in cheese and decreased melting temperature (Messens et al., 2000; Ozturk et al., 2013a; Ozturk et al., 2013b). Cheese milk UF retentate fortification did not influence the melting temperature of L-Na cheeses during the first 3 mo of ripening. However, the melting temperature of L-Na cheese significantly increased ($P < 0.05$) from 3 mo to 6 mo of ripening, while melting temperature of L-Na-UF cheese did not change. Thus, the melting temperature of L-Na and L-Na-UF differed ($P < 0.05$) from each other after 3 mo of ripening (Figure 4.5a). Similar trends in melting temperature of L-Na cheese during ripening were previously reported (Grant, 2011; Ozturk et al., 2013a).

The LTmax values are another indicator of cheese meltability, higher LTmax values indicates more liquid-like structure during the heating process (Lucey et al., 2003). The L-Na-UF-HHP cheese exhibited significantly ($P < 0.05$) higher LTmax values at 4 d compared to all other cheeses and hardly changed during ripening (Figure 4.5b). All cheeses exhibited similar LTmax values by 3 mo (Figure 4.5b). Acharya and Mistry (2004) reported that cheese meltability decreased with an increase in the concentration of total solids in the cheese milk; unless corrective steps were taken during cheese manufacture (Govindasamy-Lucey et al., 2005). In cheese making, the addition of coagulant proportional to the CN content of milk could help with the producing similar proteolysis, and thereby similar melting behaviors of L-Na and L-Na-UF cheese samples, up to 3 mo of ripening (Govindasamy-Lucey et al., 2005).

At 6 mo of ripening, L-Na cheese exhibited significantly ($P < 0.05$) lower LTmax values compared to all other treatments. Above 50^{o}C, both G′ and G″ values increased gradually for L-Na cheese from 3 mo to 6 mo of ripening and a sharp increase was observed in G′ after 70^{o}C, indicating formation of some new type of interactions (Figure 4.6). On the other hand, G′ values of all other cheeses did not exhibit a similar behavior as L-Na cheese, i.e. no increase in G′ values at temperatures ≥70 ^{o}C was observed (results not shown). The decrease in LTmax for L-Na cheese was unusual because cheese meltability is expected to increase with age due to proteolysis (Lucey et al., 2003). There are several studies that reported increased G′ values above

70^{o}C (Lee et al., 2005; Grant, 2011; Ozturk et al., 2013a). All these studies either had low pH or low S/M content (or both), this indicate the importance of electrostatic forces on rheological properties. Lee et al. (2005) reported that G′ values of low pH Cheddar cheeses measured at 5, 40, and 80^{o}C (pH values < 5.0) increased during ripening (from 4 to 12 wk), even though the pH or INSOL Ca values did not exhibit any significant change. Grant (2011) reported that after 3 mo of ripening of Cheddar cheese made with 2.1% S/M content, the G′ values exhibited sharp increase at temperatures above 70^{o}C. In this study and above mentioned studies, pH values, INSOL Ca contents, and NaCl contents of cheese samples did not change after 1 mo ripening, this suggests that proteolysis contributed to the increased G′ values at higher temperatures (i.e. decreased LTmax values). Electrostatic repulsion between proteins decreases as the pH approaches isoelectric point. Lucey at al. (2003) suggested that decreased electrostatic repulsion between caseins in low pH (pH < 5) cheeses led to increased protein-protein interactions and thereby less melt. Low ionic strength increases the rate of interaction of renneted micelles (Horne and Lucey, 2014). Thus, the decrease in LTmax values of L-Na cheese appear to be related to enhanced casein interactions at high temperature that are promoted by the high hydrolysis of caseins, low pH and low ionic strength.

Textural Properties

R-Na sample exhibited higher TPA hardness values (P < 0.05) compared to all L-Na cheeses at all ripening periods (Figure 4.7). There were some slight differences in moisture content among cheeses. However, even though the moisture content of R-Na and L-Na-UF cheeses were not significantly different (Table 4.3), the R-Na cheese was significantly (P < 0.05) harder even at 4 d. Previous studies also indicated that reduction in NaCl decreased cheese hardness (Pastorino et al., 2003; Grant, 2011) even when moisture contents of cheeses were modified to be similar (Grant, 2011; Moller et al., 2012). Among L-Na cheeses, L-Na-UF exhibited significantly ($P < 0.05$) higher hardness values compared to both L-Na-UF-HHP and L-Na cheeses, until 1 mo of ripening. Kosikowski (1983) also reported that UF retentate fortification of cheese milk increased hardness in reduced NaCl cheese. The INSOL Ca content could affect cheese hardness (Lucey et al., 2003). UF retentate fortification of cheese milk increased the initial INSOL Ca level for L-Na-UF cheese (Figure 4.1), resulting in higher initial hardness values. Even though L-Na-UF-HHP cheese was also manufactured with UF retentate fortified milk, it exhibited similar hardness values compared to L-Na cheese at any time point. Previous studies suggested that HHP treatment decreased cheese hardness via reducing hydrophobic interactions in the protein network (Messens et al., 2000; Ozturk et al., 2013b). All low NaCl cheeses exhibited similar hardness values after 1 mo. At 2 wk of age, the L-Na cheese had significantly ($P < 0.05$) lower INSOL Ca values compared to L-Na-UF cheese; however, these cheeses exhibited similar INSOL Ca values from 1 mo of ripening. Reduction in the levels of INSOL Ca tends to weaken the cheese network (Lucey et al., 2003). Thus, we might have observed similar hardness values between L-Na-UF and L-Na cheeses after 1 mo of ripening as a result of their similar INSOL Ca levels by this time point.

Uniaxial compression (obtained from 80% compression of cheese) profiles showed that L-Na cheeses exhibited higher strain to fracture during 1 mo of ripening compared to R-Na cheese (results not shown). Ozturk et al. (2013a) also reported an increase in fracture strain with salt reduction in Cheddar cheese. Previous studies suggested that as salt content increased, cheeses became harder, shorter and brittle (Guinee, 2004; Guinee and Fox, 2004). From 3 mo of age, L-Na-UF and L-Na-UF-HHP cheeses exhibited fracture at significantly ($P < 0.05$) higher strains compared to L-Na and R-Na cheeses (results not shown). Lucey et al. (2003) reported that cheese pH is closely related to strain at fracture. As the electrostatic repulsion between caseins decreases, cheese becomes short and brittle. The decrease in fracture strain of L-Na cheese with ripening could be due to its lower pH values compared to UF fortified L-Na cheeses.

Sensory analysis

Panelists scored R-Na cheese significantly ($P < 0.05$) firmer than all L-Na cheeses (Table 4.7). UF retentate fortification or HHP treatment did not significantly ($P > 0.05$) influence the sensory firmness of L-Na cheese samples. Sensory chewiness values of all cheeses were not affected by NaCl level, UF retentate fortification or HHP treatment (Appendix III).

R-Na cheese received significantly ($P < 0.05$) higher scores for saltiness compared to all L-Na cheeses (Table 4.7), as was expected. In general, all samples developed only very low bitterness values (< 2.5 out of 15 point scale) during ripening. Previous studies also reported that cheeses manufactured with camel chymosin exhibited lower levels of bitterness compared with cheeses made with calf chymosin (Govindasamy-Lucey et al., 2010; Grant, 2011). The L-Na-UF-HHP cheese did not significantly ($P > 0.05$) differ from R-Na cheese in acidity and bitterness scores at 3 or 6 mo of ripening, while the L-Na cheese were significantly ($P < 0.05$) more bitter and acid than R-Na cheese (Table 4.7, Table 4.8). The L-Na-UF cheese exhibited acid and bitterness scores intermediate between L-Na and L-Na-UF-HHP cheeses. The lower level of bitterness in L-Na-UF-HHP cheese was in agreement with our observations of reduced levels of β-CN (f1-189) peptide (Figure 4.3, Figure 4.4a). There were no significant differences in sweetness values in any cheese sample (Appendix III). Panelists perceived threshold (< 1.75 out of 15 point scale) metallic note in all samples (Table 4.7). The metallic taste was significantly (*P*

< 0.05) higher for L-Na cheese compared to R-Na cheese during ripening. At 6 mo of ripening, L-Na cheese exhibited significantly ($P < 0.05$) higher metallic taste compared to R-Na, L-Na-UF, and L-Na-UF-HHP cheeses (Table 4.7). L-Na cheeses are often reported to exhibit more off-flavors than R-Na cheeses (Grant, 2011).

4.5 CONLUSIONS

High pressure treatment and UF retentate fortification significantly affected the texture, rheology, microflora, and sensory properties of L-Na cheese. Rheological and textural properties of UF retentate fortified L-Na cheese were altered after HHP treatment and remained fairly unchanged during 6 mo of ripening. These results indicated that 500 MPa HHP treatment helped to minimize ripening related changes in L-Na cheese. Starter culture numbers significantly decreased with HHP treatment while NaCl content of the experimental cheeses did not influence the number of starter bacteria present in cheese during ripening. Pressure application decreased the levels of W-SN, TCA-SN, and the production of a key bitter β-CN (f1-189) peptide. UF retentate fortification of cheese milk prevented L-Na cheeses from exhibiting an excessive pH drop that may be due to the higher INSOL Ca phosphate content (buffering) observed in these cheeses. UF retentate fortification of cheese milk increased initial cheese hardness (i.e. until 1 mo of ripening), which could be helpful in improving the initial texture of L-Na cheeses.

Even though UF retentate fortification and HHP treatment decreased acidity and bitterness in L-Na cheeses, these cheeses failed to develop characteristic Cheddar cheese flavor. Using starter adjuncts, salt replacers or flavor enhancers along with UF retentate fortification and HHP processing may help the cheese industry to produce L-Na Cheddar cheeses with comparable flavor characteristics of R-Na Cheddar cheese. The reduction in proteolysis and microbial numbers with HHP could help to provide increase shelf-life for L-Na cheeses. The combination of HHP and UF retentate fortification appears to be promising to improve the quality of L-Na cheese.

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Table 4.1. Cheese milk composition and cheese manufacturing procedures that were used to manufacture Cheddar cheese

	Regular NaCl	Low NaCl	Low NaCl UF fortified	Low NaCl UF fortified[1]
Total Solids (%)	11.8 ± 0.5	11.8 ± 0.5	17.1 ± 0.6	17.1 ± 0.6
Casein to Fat Ratio	0.66 ± 0.01	0.66 ± 0.01	0.63 ± 0.03	0.63 ± 0.03
Ripening Temperature (°C)	32.2	33.9	26.7	26.7
Ripening Times (min)	60	60	60	60
Cutting the gel	3 slash passes	15 slash passes	15 slash passes	15 slash passes
Stacking procedure during Cheddaring	With stacking	Single layer (no stacking)	Single layer (no stacking)	Single layer (no stacking)
Wait time between salt applications (min)	5	20	20	20
Wait time between salting and hooping (min)	20	80	80	80
Salt (g NaCl/kg cheese)	53.1	9.4	9.4	9.4

[1] Cheeses obtained from this vat was subjected to high hydrostatic pressure at 1 d after manufacture.

Table 4.2. Definition of the attributes used by the trained panelists to evaluate the texture and flavor of the Cheddar cheeses at 12^{o}C using a combination of Spectrum and quantitative descriptive analysis

Attribute	Definition and evaluation procedure	References used, preparation instructions, and anchor points (0-15)
Texture		
Hand firmness	Force required to compress the cheese between finger and thumb. Place the cheese cube between thumb and forefinger. Compress cheese cube; do not fracture.	Green-colored Thera-Putty (#5075, Sammon Preston) = 4.5 Blue-colored Thera-Putty (#5077, Sammon Preston) = 7.0 Flesh-colored Thera=Putty (Graham-Field Inc.) = 9.5 Grey eraser (Primacolor Kneaded Rubber) = 12.0 White eraser (School Select White) = 15.0
Chewiness	The total amount of energy required to masticate the sample to a state pending swallowing. Place cheese cube between molars, chew cheese cube at an even rate, both sides of mouth may be used. Measure total energy required. Chewiness is a product of cohesiveness, hardness and springiness. The longer time required to chew, the chewier the sample.	Philadelphia Full-fat Cream Cheese (Kraft Foods) = 1.0 Beef frankfurters (Hebrew National Brand) = 4.0 Gum Drops (Dots Brand) = 9.0 Beef Jerky (Jack Links Brand) = 13.5
Flavor[1]		
Salt	Basic taste sensation elicited by salt	none to pronounced
Acid	Basic taste sensation elicited by acids	none to pronounced
Bitter	Basic taste sensation elicited by bitter compounds	none to pronounced
Metallic	Aromas and flavors associated with metal	none to pronounced

[1] Attributes were evaluated using quantitative descriptive analysis (Meilgaard et al., 1999).

Table 4.3. Composition of two-week old Cheddar cheese manufactured with two different NaCl levels with or without UF retentate fortification of cheese milk and HHP treatment[1]

	Regular NaCl	Low NaCl	Low NaCl UF fortified	Low NaCl UF fortified-HHP treated[2]
Moisture (%)	34.7 ± 0.7^{a}	38.2 ± 0.8^{d}	36.0 ± 0.7^{ab}	36.2 ± 0.7^{bc}
Fat (%)	34.7 ± 0.6^{a}	33.1 ± 0.5^{bc}	33.9 ± 0.5^{ab}	33.4 ± 0.5^{bc}
Protein (%)	25.1 ± 0.5^{ab}	24.4 ± 0.4^{a}	25.9 ± 0.6^{bc}	26.0 ± 0.6^{bc}
Salt in moisture (%)	5.8 ± 0.4^{a}	2.1 ± 0.1^{b}	2.3 ± 0.2^{b}	2.2 ± 0.1^{b}
MNFS[3] (%)	53.1 ± 0.9^{a}	57.0 ± 0.9^{b}	54.3 ± 1.2^{a}	54.3 ± 0.9^{a}
FDM[4] (%)	53.1 ± 0.8^{a}	53.6 ± 0.8^{a}	52.8 ± 1.1^{a}	52.4 ± 0.9^{a}
NaCl[5] (%)	2.0 ± 0.2^{a}	0.8 ± 0.0^{b}	0.8 ± 0.1^{b}	0.8 ± 0.1^{b}
Na[6] (mg/100g)	759 ±33^{a}	278 ± 21^{b}	282 ± 17^{b}	273 ± 22^{b}
Ca[6] (mg/100g)	694 ± 39^{a}	660 ± 25^{a}	698 ± 26^{a}	695 ± 27^{a}

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

[1] Values are means (n = 5)

[2] HHP: high hydrostatic pressure, 500 MPa for 3 min, performed on cheese at 1 d of age.

[3] Moisture in nonfat substance.

[4] Fat in dry matter.

[5] Determined using salt analyzer.

[6] Determined using ICP method (Park, 2000).

Table 4.4. Probabilities and R^2 values for proteolysis, insoluble calcium, pH, lactic acid, starter culture numbers and accumulation of β-CN (f1-189) peptide for Cheddar cheeses during 6 mo of ripening

Factor[1]	df[2]	% W-SN[3]	% TCA-SN[3]	% INSOL Ca[4]	pH	Lactic acid	Starter (log)	df	β-CN (f1-189)[6]
Whole-plot									
Treatment (T)	3	<0.0001	<0.0001	0.004	<0.0001	<0.0001	<0.0001	3	<0.0001
Day of Cheesemaking (D)	4	<0.0001	0.085	0.05	0.07	0.006	0.052	4	0.035
Error (T × D)	12							12	
Split-plot									
Age (A)	4	<0.0001	<0.0001	0.035	<0.0001	<0.0001	0.09	1	0.02
A× T	12	<0.0001	<0.0001	0.23	0.06	0.34	<0.0001	3	0.5
Error	64	0.59	0.19	9.78	0.003	0.012	0.43	16	1.2
R^2		0.99	0.99	0.87	0.86	0.91	0.88		0.91

[1] Split-plot design with the 4 treatments (R-Na, L-Na, L-Na-UF, L-Na-UF-HHP) were analyzed as a discontinuous variable and cheesemaking day was blocked (a 4 × 5 block design).Subplot included the effect of aging of cheese (A), and age × treatment as variables.

[2] Degrees of freedom differed for β-CN (f1-189) as we only run urea-PAGE for cheeses at 1 d, 3 and 6 mo.

[3] Water and trichloroacetic acid soluble nitrogen as a percentage of total nitrogen.

[4] Percentage of insoluble calcium as a percentage of total calcium.

[5] Non-starter lactic acid bacteria.

[6] Accumulation of β-CN (f1-189) peptide as a percentage of intact β-CN at 1 d measured by Gel Expert.

Table 4.5. Starter bacteria numbers, pH and lactic acid (w/w %) values for regular NaCl (R-Na), low NaCl (L-Na), UF fortified low NaCl (L-Na-UF), and UF fortified HHP treated low NaCl (L-Na-UF HHP) Cheddar cheeses at 4 d, 2 wk, 1, 3 and 6 mo of ripening[1]

	Ripening time (day)	R-Na	L-Na	L-Na-UF	L-Na-UF-HHP[2]
Starter (log cfu/g)	4	8.9 ± 0.2^{a}	9.0 ± 0.2^{a}	9.0 ± 0.2^{a}	7.0 ± 1.0^{b}
	14	9.0 ± 0.2^{a}	9.3 ± 0.1^{a}	9.2 ± 0.1^{a}	4.8 ± 1.9^{b}
	30	8.9 ± 0.2^{a}	9.4 ± 0.5^{a}	9.2 ± 0.3^{a}	5.2 ± 1.3^{b}
	90	8.2 ± 0.8^{b}	9.1 ± 0.5^{a}	9.1 ± 0.3^{a}	6.5 ± 0.6^{c}
	180	7.6 ± 0.7^{ab}	8.7 ± 0.3^{a}	8.6 ± 0.3^{a}	7.2 ± 0.4^{b}
pH	4	5.13 ± 0.05^{a}	4.94 ± 0.08^{c}	5.06 ± 0.04^{b}	5.22 ± 0.02^{a}
	14	5.12 ± 0.06^{b}	4.99 ± 0.05^{c}	5.09 ± 0.03^{b}	5.23 ± 0.06^{a}
	30	5.22 ± 0.11^{a}	5.01 ± 0.07^{b}	5.16 ± 0.04^{ab}	5.21 ± 0.04^{a}
	90	5.26 ± 0.13^{a}	5.01 ± 0.06^{b}	5.16 ± 0.03^{ab}	5.24 ± 0.08^{a}
	180	5.27 ± 0.13^{a}	5.09 ± 0.05^{b}	5.23 ± 0.06^{a}	5.29 ± 0.04^{a}
Lactic acid	4	0.70 ± 0.20^{c}	1.28 ± 0.22^{a}	1.16 ± 0.12^{ab}	1.03 ± 0.05^{b}
	14	0.88 ± 0.18^{c}	1.40 ± 0.22^{a}	1.25 ± 0.23^{b}	1.18 ± 0.25^{b}
	30	1.17 ± 0.12^{c}	1.62 ± 0.05^{a}	1.50 ± 0.04^{ab}	1.40 ± 0.11^{b}
	90	1.21 ± 0.15^{c}	1.69 ± 0.08^{a}	1.58 ± 0.01^{ab}	1.46 ± 0.09^{b}
	180	1.51 ± 0.17^{c}	1.79 ± 0.01^{a}	1.69 ± 0.02^{ab}	1.61 ± 0.07^{ab}

[1] Values are means (n = 5)

[2] HHP: high hydrostatic pressure, 500 MPa for 3 min, performed on cheese at 1 d of age.

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

Table 4.6. Probabilities and R^2 values for rheological properties and hardness determined by texture profile analyzer (TPA) for Cheddar cheeses during 6 mo of ripening

Factor[1]	df[2]	LTmax[3]	Temperature at LTmax[3]	df	LT = 1[4]	df	TPA hardness[5]
Whole-plot							
Treatment (T)	3	0.0002	<0.0001	3	<0.0001	3	<0.0001
Day of Cheesemaking (D)	4	0.011	0.25	4	0.11	4	0.044
Error (T × D)	12			12		12	
Split-plot							
Age (A)	4	<0.0001	<0.0001	4	<0.0001	4	0.07
A× T	12	<0.0001	<0.0001	12	<0.0001	12	0.05
Error	64	0.032	10.06	61	1.23	60	6.94
R^2		0.88	0.77		0.96		0.89

[1] Split-plot design with the 4 treatments (R-Na, L-Na, L-Na-UF, L-Na-UF-HHP) were analyzed as a discontinuous variable and cheesemaking day was blocked (a 4 × 5 block design).Subplot included the effect of aging of cheese (A), and age × treatment as a variables.

[2] Degrees of freedom differed among measured parameters as some cheeses did not reach loss tangent value of 1 and one time point of one trial was dropped due to a technical error at the measurement of TPA hardness values.

[3] Maximum loss tangent values.

[4] Temperature at which loss tangent value = 1.

[5] TPA hardness was measured by texture analyzer.

Table 4.7. Sensory texture and flavor intensities (on 0-15 point scale) for regular NaCl, low NaCl control, UF fortified low NaCl, and UF fortified-HHP treated low NaCl Cheddar cheeses at 1, 3 and 6 mo of ripening[1]

Ripening Time	Attribute	R-Na	L-Na	L-Na-UF	L-Na-UF-HHP[2]
1 mo	Firmness	13.2 ± 0.3^{a}	10.9 ± 0.3^{b}	11.4 ± 0.3^{b}	11.3 ± 0.2^{b}
	Salt	5.5 ± 0.8^{a}	2.9 ± 0.3^{b}	2.6 ± 0.3^{b}	2.5 ± 0.2^{b}
	Acid	4.0 ± 0.6^{a}	5.3 ± 0.3^{b}	4.5 ± 0.7^{ab}	4.1 ± 0.2^{a}
	Bitter	0.4 ± 0.2^{a}	0.6 ± 0.1^{a}	0.6 ± 0.1^{a}	0.5 ± 0.1^{a}
	Metallic	0.6 ± 0.2^{a}	1.0 ± 0.2^{b}	0.7 ± 0.2^{ab}	0.7 ± 0.2^{ab}
3 mo	Firmness	13.2 ± 0.3^{a}	11.9 ± 0.2^{b}	11.5 ± 0.3^{b}	11.6 ± 0.4^{b}
	Salt	5.8 ± 0.7^{a}	3.3 ± 0.3^{b}	3.0 ± 0.5^{b}	2.8 ± 0.4^{b}
	Acid	4.7 ± 0.4^{a}	6.0 ± 0.3^{c}	5.4 ± 0.2^{bc}	5.0 ± 0.4^{ab}
	Bitter	0.7 ± 0.2^{a}	1.6 ± 0.3^{c}	1.3 ± 0.2^{bc}	1.0 ± 0.4^{ab}
	Metallic	1.0 ± 0.3^{a}	1.3 ± 0.1^{b}	1.3 ± 0.1^{b}	1.3 ± 0.2^{ab}
6 mo	Firmness	13.0 ± 0.2^{a}	11.9 ± 0.2^{b}	11.5 ± 0.2^{b}	11.6 ± 0.2^{b}
	Salt	5.7 ± 0.3^{a}	3.5 ± 0.2^{b}	3.5 ± 0.1^{b}	3.3 ± 0.2^{b}
	Acid	4.8 ± 0.7^{a}	5.7 ± 0.3^{c}	5.2 ± 0.2^{bc}	4.8 ± 0.3^{ab}
	Bitter	1.2 ± 0.4^{a}	2.4 ± 0.6^{b}	2.2 ± 0.8^{ab}	1.6 ± 0.4^{ab}
	Metallic	1.3 ± 0.1^{a}	1.8 ± 0.2^{b}	1.5 ± 0.2^{a}	1.5 ± 0.2^{a}

[1] Values are means (n = 5)

[2] HHP: high hydrostatic pressure, 500 MPa for 3 min, performed on cheese at 1 d of age.

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

Table 4.8. Probabilities and R^2 values for firmness (sensory), salt, acid bitter, and metallic for Cheddar cheeses during 6 mo of ripening

Factor[1]	df[2]	Firmness	Salt	Acid	Bitter	Metallic
Whole-plot						
Treatment (T)	3	<0.0001	<0.0001	0.0005	<0.0001	<0.0001
Day of Cheesemaking (D)	4	0.01	0.002	0.82	0.011	0.22
Error (T × D)	12					
Split-plot						
Age (A)	2	0.003	<0.0001	<0.0001	<0.0001	<0.0001
A× T	6	0.001	0.18	0.93	0.11	0.32
Error	32	0.06	0.11	0.12	0.16	0.03
R^2		0.96	0.96	0.86	0.84	0.89

[1] Split-plot design with the 4 treatments (R-Na, L-Na, L-Na-UF, L-Na-UF-HHP) were analyzed as a discontinuous variable and cheesemaking day was blocked (a 4 × 5 block design).Subplot included the effect of aging of cheese (A), and age × treatment as a variables.

[2] Degrees of freedom.

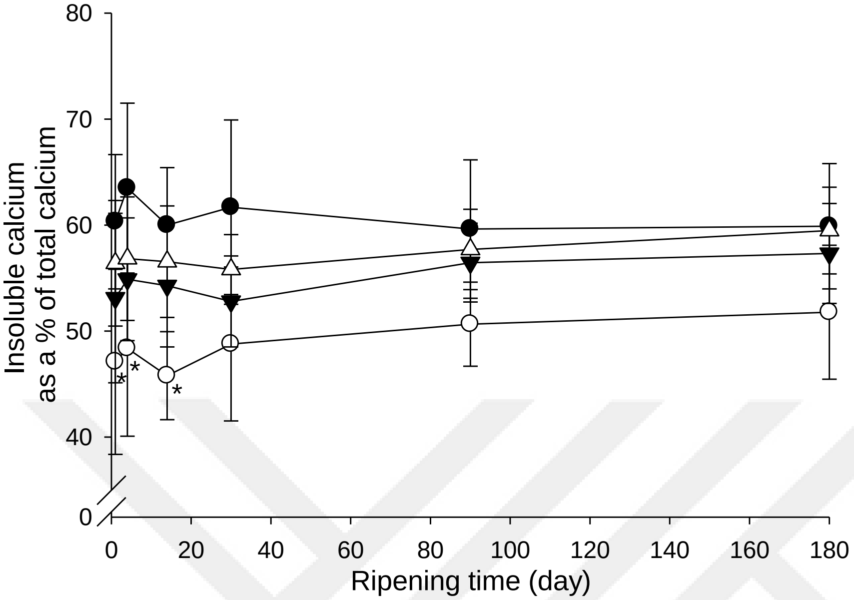

Figure 4.1. Insoluble calcium, expressed as a percentage of total calcium, for regular NaCl (●), low NaCl (○), UF fortified low NaCl (▼), and UF fortified-HHP treated low NaCl (Δ) Cheddar cheeses during 6 mo of ripening at 7°C. Vertical bars represent standard deviations.

*significantly ($P < 0.05$) different from regular NaCl cheese at the indicated ripening time point.

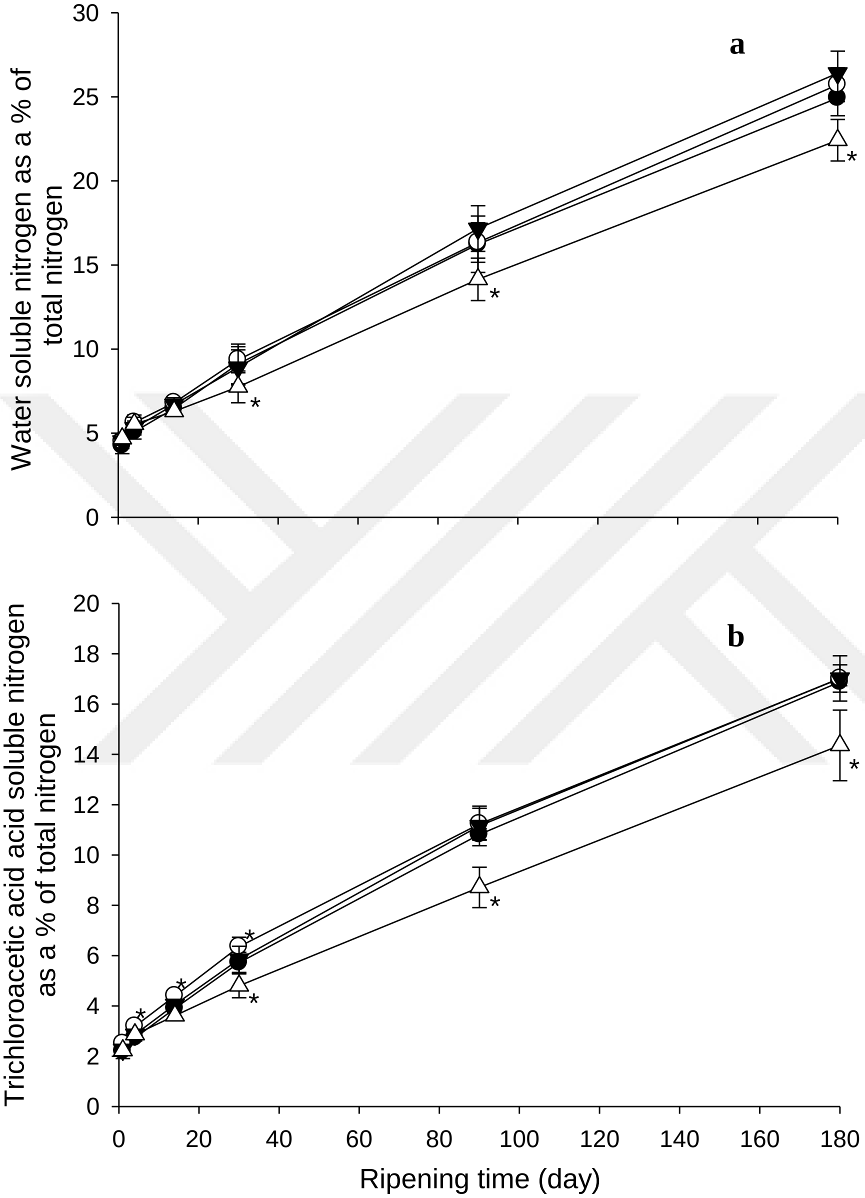

Figure 4.2. Water soluble nitrogen (a) and trichloroacetic acid soluble nitrogen (b) as a percentage of total nitrogen values for regular NaCl (●), low NaCl (○), UF fortified low NaCl (▼), and UF fortified-HHP treated low NaCl (Δ) Cheddar cheeses during 6 mo of ripening at 7^{o}C. Vertical bars represent standard deviations.

*significantly (*P* < 0.05) different from regular NaCl cheese at the indicated ripening time point.

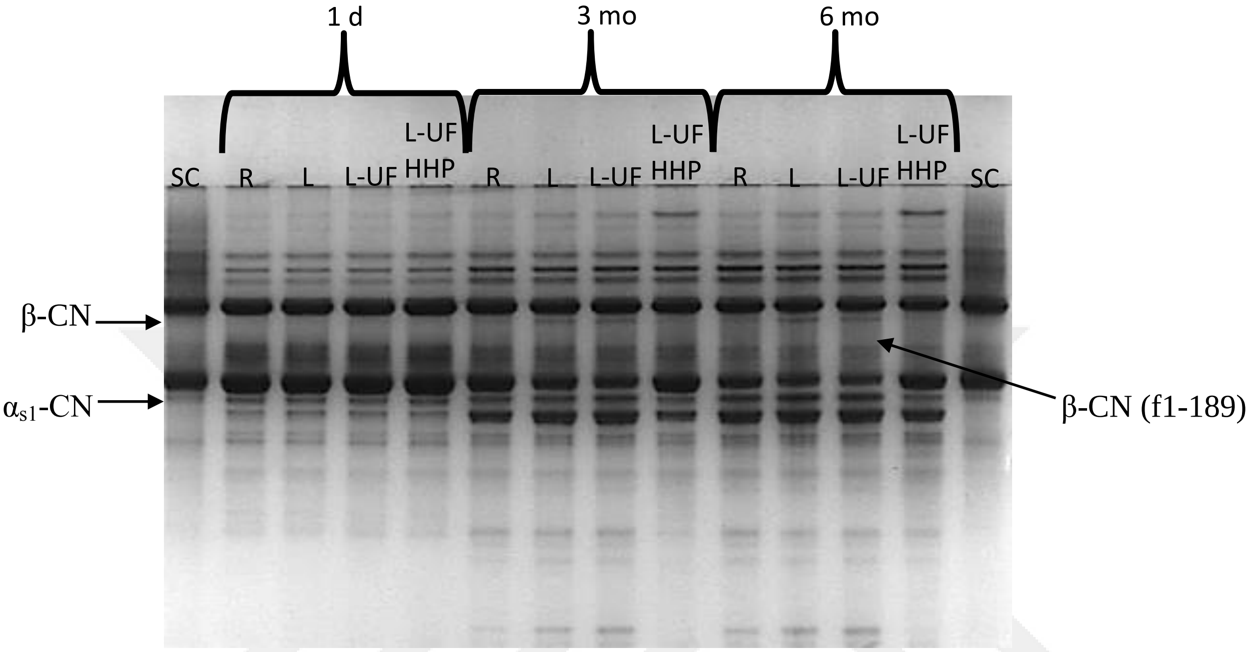

Figure 4.3. Urea-PAGE electrophoretograms showing the breakdown of caseins in regular NaCl (R), low NaCl (L), UF fortified low NaCl (L UF), and UF fortified-HHP treated low NaCl (L UF HHP) Cheddar cheeses at 1 d, 3, and 6 mo of ripening at 7°C. Lanes with SC refer to sodium caseinate (standards).

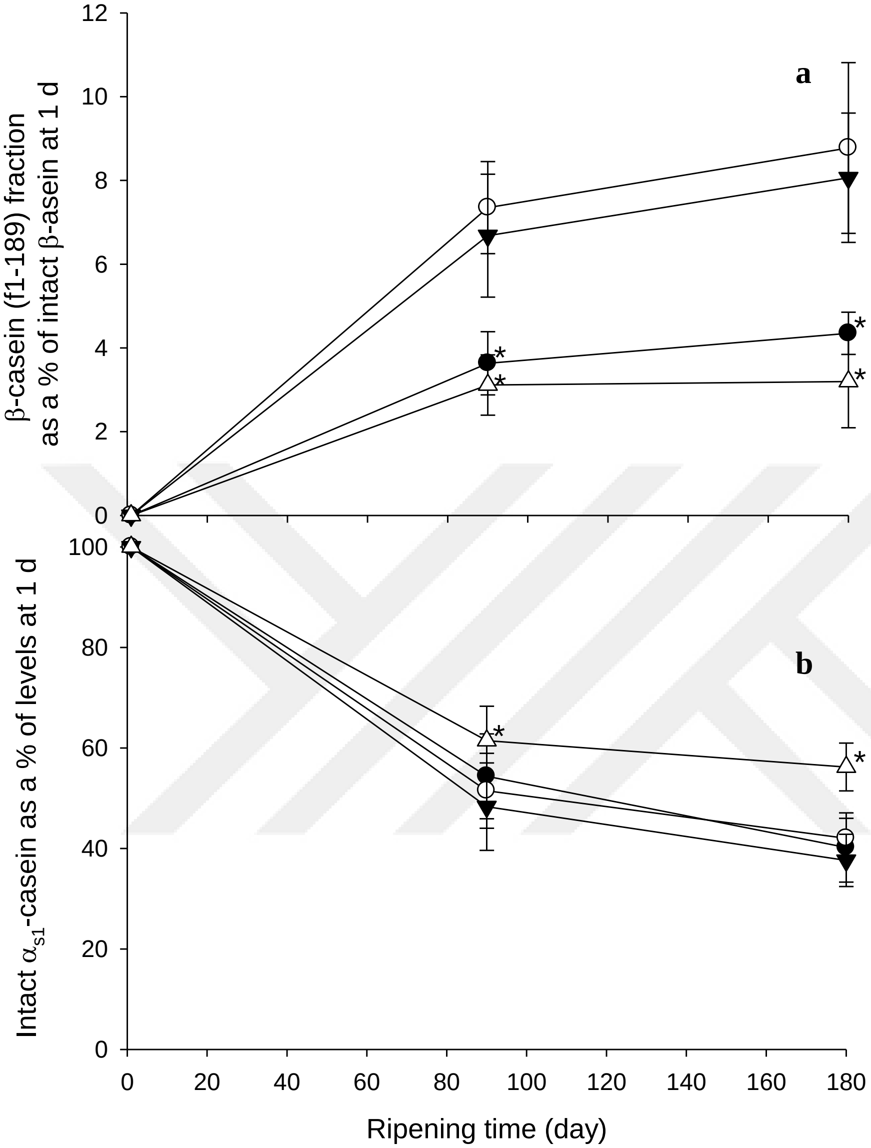

Figure 4.4. Accumulation of β-casein (f1-189) fraction as a percentage of the intact β-casein level at 1 d (a), and level of intact α_{s1}-casein as a percentage of the level at 1 d (b) calculated from area (pixel intensity) by the Gel Expert program from the urea-polyacrylamide gels for regular NaCl (●), low NaCl (○), UF fortified low NaCl (▼), and UF fortified-HHP treated low NaCl (Δ) Cheddar cheeses at 1 d, 3, and 6 mo of ripening at 7°C. Vertical bars represent standard deviations.

*significantly (*P* < 0.05) different from regular NaCl cheese at the indicated ripening time point.

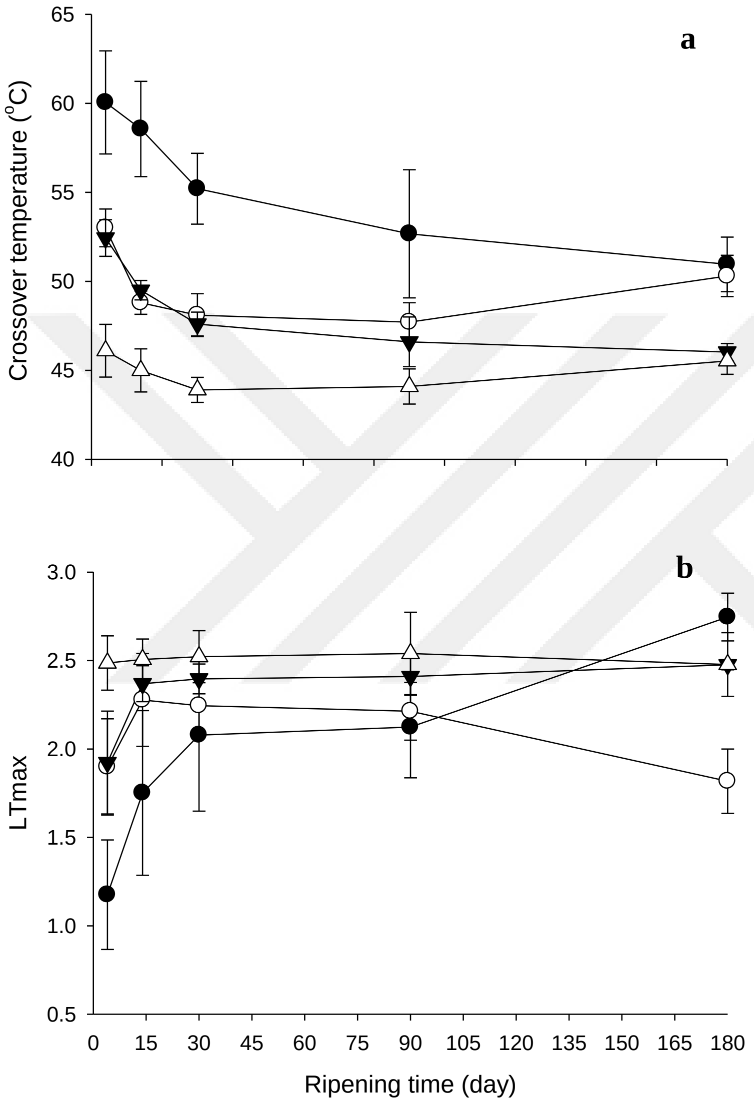

Figure 4.5. Temperature of the crossover point (where LT = 1) (a), and the maximum loss tangent values (b) for regular NaCl (●), low NaCl (○), UF fortified low NaCl (▼), and UF fortified-HHP treated low NaCl (Δ) Cheddar cheeses at 1 d, 3, and 6 mo of ripening at 7°C. Vertical bars represent standard deviations.

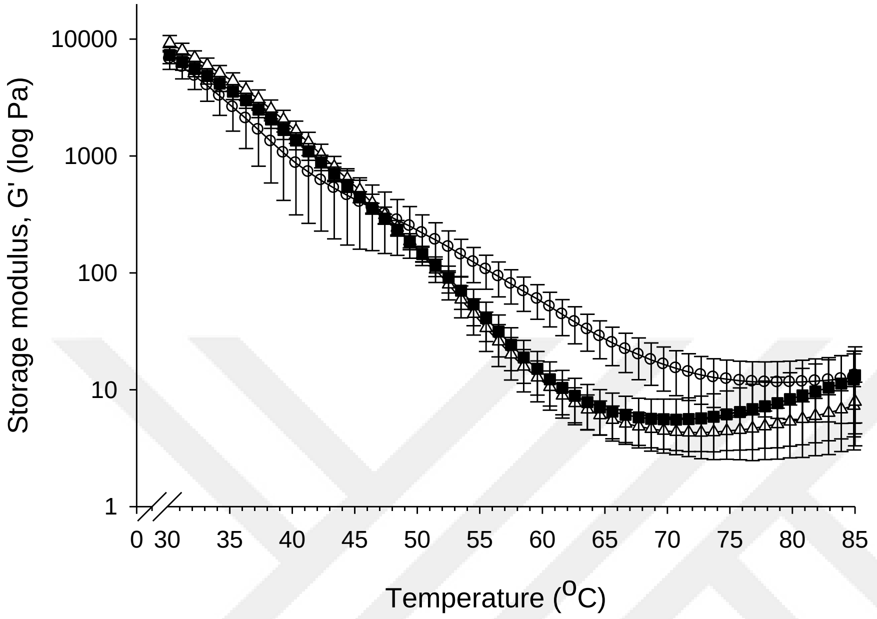

Figure 4.6. Storage modulus (G′) as a function of temperature for L-Na cheese at 4 d (○), and 3 (Δ), and 6 mo (■). Cheese was heated from 5 to 85 °C at 1°C/min. Vertical bars represent standard deviations.

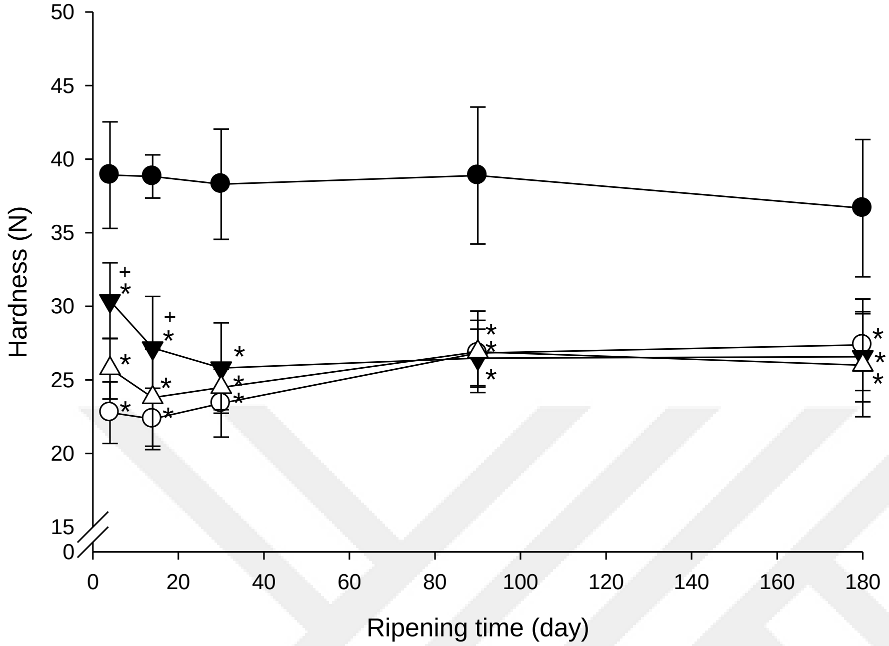

Figure 4.7. Hardness (N) from texture profile analysis testing (20% compression of cheese cylinders compared to original height) for regular NaCl (●), low NaCl (○), UF fortified low NaCl (▼), and UF fortified-HHP treated low NaCl (Δ) Cheddar cheeses during 6 mo of ripening at 7°C. Vertical bars represent standard deviations.

*significantly ($P < 0.05$) different from regular NaCl cheese at the indicated ripening time point.

$^{+}$significantly ($P < 0.05$) different from low NaCl cheese at the indicated ripening time point.

Chapter 5.

Investigating the Properties of High Pressure Treated, Reduced Sodium, Low-Moisture, Part-Skim Mozzarella Cheese during Refrigerated Storage

5.1 ABSTRACT

We proposed that the performance and sensory properties of reduced Na, LMPS-Mozzarella cheese could be extended by decreasing microbial and enzymatic activity with the application of high hydrostatic pressure (HHP). Fermentation-produced camel chymosin was also used as a coagulant to help reduce cheese proteolysis. Average composition of reduced Na (1.0 ± 0.1% NaCl), LMPS-Mozzarella cheeses were 48.6 ± 0.6% moisture, 22.5 ± 0.4% fat, and 24.5 ± 0.6% protein. Cheeses were divided into three groups randomly after manufacture and stored at ~4^{o}C. One group was non-pressurized and kept as control. Two wk after manufacture, the two groups of cheese samples were HHP treated at 500 or 600 MPa for 3 min. Analysis was performed at 2, 4, 6, 8, 12, 16, and 20 wk after cheese manufacture. Texture profile analysis (TPA) and dynamic low-amplitude oscillatory rheology was used to monitor cheese functionality during ripening. Quantitative descriptive analysis was conducted with 9 trained panelists to evaluate texture and flavor attributes of unmelted and melted cheeses on pizzas using a 15 point scale. Pressure treatments at 500 and 600 MPa resulted in ~1 and ~2 log reduction in starter culture numbers at 2 wk of ripening, respectively, compared to control cheese. High pressure treatment of LMPS-Mozzarella cheese resulted in an initial (at 2 wk of ripening) increase in pH values; however, by 4 wk of ripening we did not observe any statistical difference in pH values between control and HHP-treated samples. At 2 and 4 wk of storage, HHP treated cheeses exhibited significantly lower TPA and sensory (unmelted) hardness; however, after 16 wk the 600 MPa HHP treated cheese had significantly higher TPA and sensory (unmelted) hardness compared to control. Sensory panels also indicated that after 16 wk of age, the 600 MPa HHP-treated sample was significantly firmer than the control. Cheeses HHP treated at 600 MPa and 500 MPa exhibited significantly lower water soluble nitrogen values compared to control cheese after 8 and 12 wk, respectively. The level of intact α_{s1} casein was significantly higher in HHP treated cheeses after 12 wk compared to control. Pizza panels indicated that 600 MPa HHP-treated cheese was significantly chewier and exhibited lower blister quantity and higher strand thickness compared to control. Pressures of 600 MPa produced reduced Na, LMPS-Mozzarella cheese with acceptable performance on pizza for a greatly extended refrigerated storage period.

KeyWords: High pressure, reduced sodium, camel chymosin

5.2 INTRODUCTION

Low-moisture, part-skim (LMPS) Mozzarella cheese is mostly consumed as an ingredient for pizza. Physical, or performance, properties of LMPS Mozzarella cheese are acceptable for only a relatively short time period (e.g.3-4 wk) when stored under refrigeration conditions (4^oC) (Kindstedt, 1993a). Protein breakdown, at some extent, is desired in LMPS Mozzarella cheese (Kindstedt, 1993b). During longer term storage (such as for exporting), cheese becomes soft and pasty due to physicochemical changes in para-casein matrix and ongoing protein breakdown, which results in poor shredding properties for unmelted cheese (Kindstedt, 1993a). Also, melted cheese loses its consistency and becomes excessively fluid, which is undesirable for pizza. Kindstedt et al. (1991) reported that coagulants from various sources survived cooking and stretching (curd temperature 57^oC) and they were still active in cheese. Heat treatment during cooking and stretching decreases residual chymosin activity, leading to reduced primary proteolysis and more intact α_{s1}-casein in cheese (Feeney et al., 2002; Lucey et al., 2003). Previous studies suggested that cheese firmness and stretchability were closely related with the level of intact α_{s1}-casein (Creamer, 1976; DeJong, 1976). Moynihan et al. (2014) investigated the effect of fermentation-produced bovine and camel chymosin on LMPS Mozzarella cheese. They reported that cheeses manufactured with camel chymosin exhibited lower primary proteolysis compared to the cheeses manufactured with calf chymosin. Cheeses made with camel chymosin maintained their unmelted and melted performance during 84 d of storage.

Several other methods have been investigated to improve performance shelf-life of Mozzarella cheese. Bertola et al. (1996) reported that the viscoelastic and sensory properties of Mozzarella cheese could be maintained by frozen storage, although not all researchers agree (Dahlstrom, 1978; Oberg et al., 1992). Cervantes et al. (1983) reported that freezing/thawing cycle was critical parameter on frozen stored LMPS Mozzarella. However, frozen storage causes extra costs for cheese manufacturers, such as, investment for freezing, maintenance costs, and most importantly energy costs.

There is an increased consumer demand on less ingredient (preservative) foods, thus high hydrostatic pressure (HHP) processing has become more widely used in food industry to maintain product quality for longer time periods. The number of HHP units has been exponentially increasing in the past 20 years; correspondingly, the cost of HHP has become more feasible for industrial use (Bermudez-Aguirre and Barbosa-Canovas, 2011). Currently HHP treatment costs around 0.35-0.80 U.S Dollars per kg of product (depending on the size and the packaging of the product) (Z. Greg, American Pasteurization Company, Milwaukee, WI, USA, personal communication). Textural, rheological, and sensory characteristics of cheeses can be modified with HHP depending on the magnitude of pressure/holding time (Malone et al., 2003; Ozturk et al., 2013a). Malone et al. (2003) reported that activity of major glycolytic and proteolytic enzymes (including residual chymosin) reduced or inhibited with pressures ≥ 500 MPa, suggesting that HHP could be useful tool to arrest cheese ripening. Recently, Evert-Arriagada et al. (2014) reported that 500 MPa HHP treatment of starter-free fresh cheese increased the shelf-life from 7-8 d to 19-21 d.

There is a current trend in food industry to reduce sodium in processed foods as high sodium intake is a concern for adverse health effects (especially for elder people), even though further research is required to demonstrate long term health impacts (Johnson et al., 2009; Taylor et al., 2011). Salt (NaCl) influences conformation of caseins, final moisture content of cheese, hydration of protein network, and microbial and enzymatic activity in cheese (Cervantes et al., 1983). Grant (2011) reported that decreasing NaCl content of Cheddar cheese caused some quality defects, such as, soft and pasty texture, lower meltability and off-flavor development. These NaCl reduction associated defects would be a major quality problem for reduced Na, LMPS Mozzarella cheese as shreddability, meltability and clean background flavor are important for the end use (pizza).

LMPS Mozzarella cheese requires aging around 1 to 3 wk at refrigeration temperature (4^{o}C) to reach the optimal melt and stretch (Kindstedt, 1993b). After the desired characteristics are reached, minimizing the biochemical changes in cheese matrix may extend the shelf-life of the LMPS Mozzarella cheese. Therefore, we hypothesized that reduced sodium, LMPS Mozzarella cheese could be stored at refrigeration temperature (4^{o}C) without loss in quality for more than normal 4-6 wk when manufactured with a less proteolytic coagulant (camel chymosin) and HHP treated at pressures ≥ 500 MPa for 3 min.

5.3 MATERIALS AND METHODS

Cheese Manufacture

Two licensed Wisconsin cheese maker manufactured five independent batches of reduced Na, LMPS Mozzarella cheeses at the University of Wisconsin-Madison Dairy Plant over a period of 5 wk. At each manufacture time, three independent cheese vats (272 kg of milk capacity) were utilized to have better control on factors (especially stretching, cooling and brining) that might affect cheese final composition. All three cheese vats were manufactured with the same procedure and cheeses were randomized (except one 2.3 kg block from each vat to check for coliforms) after manufacture. Partially skimmed (2.55 ± 0.05) milk was pasteurized at 73°C for 19 s, cooled to 33.3°C. Cheese milk was inoculated with a direct-vat-set thermophilic culture (a blend of *Streptococcus thermophilus* and *Lactobacillus helveticus*) (Tempo 303; Cargill Texturizing Solutions, Waukesha, WI, USA) at the rate of 23 g per 272 kg of milk. After ripening of cheese milk for 45 min, fermentation-produced camel chymosin (Chymax® M, Chr. Hansen, Milwaukee, WI, USA) was added to the milk at the rate of 10 g per 272 kg milk. The coagula was set around 45 min and cut on firmness (subjectively evaluated by the cheesemaker) (~pH 6.5) with 1.9 cm knives. Curd was given a 10 min healing time after cutting, then the temperature of the vats were increased to 41°C over 30 min. The whey was drained once the curd pH reached 5.9. At pH 5.2, cheeses were milled and pre-salted at the rate of 210 g of salt per vat (272 kg milk at the start). Curds were stretched in a cooker (Supreme Filata Mixer, Stainless Steel Fabricating Inc., Columbus, WI,USA) for ~8 min. After hot curd (~58°C) exited the cooker, it was placed in rectangular molds (9 × 9 × 27 cm). Cheese blocks were cooled in a cold

(~9°C) water bath for 1 h before brine salting (in 25% salt brine at 4°C for 45 min). After brine salting, cheeses were vacuum packed, randomized, separated into 3 groups, and stored at 4°C during 20 wk. Analysis were performed at 2, 4, 6, 8, 12, 16, and 20 wk.

High Hydrostatic Pressure Treatment

Cheeses were HHP treated 2 wk after manufacture. For each HHP condition (500 MPa and 600 MPa for 3 min) 10 blocks (~2.3kg, with the dimensions of 9 × 9 × 27 cm) of reduced Na, LMPS cheese were used. A commercial high pressure unit (Avure Ultra 215 L, Avure Technologies Inc., WA, USA) was used for this study, and the capacity of the HHP unit was 215 L (~150 kg depending on the product size and packaging) of product per cycle. High pressure unit used water (~9^{o}C) as the pressure transfer medium. The pressure come up time was around 2 and 3 min for 500 and 600 MPa, respectively. Ten blocks of reduced Na, LMPS Mozzarella cheeses were non-pressurized and kept as a control for each trial.

Compositional Analyses

Cheese milk was analyzed for protein (total percentage N × 6.38, Kjeldahl method; AOAC, 2000), casein (AOAC, 2000), fat (Mojonnier method; AOAC, 2000), lactose (AOAC, 2000), total solids (Green and Park, 1980), total Ca by inductively coupled argon plasma emission spectroscopy (Vista-MPX Simultaneous ICP-OES, Varian Inc., Palo Alto, CA) (Park, 2000), and insoluble calcium (**INSOL Ca**) by the acid–base titration method (Lucey et al., 1993; Hassan et al., 2004).

To minimize the sampling differences in a cheese block, cheeses were sampled by cutting a block (~2.3 kg) in two pieces from the center. One half was used for sensory analysis, the other half was used to evaluate textural properties and cheese composition (at 2 wk). Cheeses were analyzed for protein by the Kjeldahl method (AOAC, 2000), fat (Mojonnier method; AOAC, 2000), moisture (Marshall, 1992), salt by the chloride electrode method (model 926; Corning Glass Works, Medfield, MA; Johnson and Olson, 1985), and lactose, galactose and lactic acid by using HPLC method (Zeppa et al., 2001). Cheese pH was measured at 2, 4, 6, 8, 12, 16, and 20 wk by using a pH electrode (Sam Gray gold electrode; Nelson-Jameson, Marshfield, WI; Marshall, 1992). The INSOL Ca content in cheeses was monitored until 12 wk of storage by acid-base titration (Lucey et al., 1993; Hassan et al., 2004).

Microbiological Analysis

Starter and non-starter lactic acid bacteria numbers were measured by de Man, Rogosa and Sharpe (MRS) agar and Rogosa SL media, respectively, incubated at 32°C for 48 h under anaerobic conditions (Frank and Yousef, 2004; Ozturk et al., 2013b).

Proteolysis and Urea PAGE Gels

Water soluble nitrogen (**W-SN**) levels were measured to monitor cheese proteolysis (AOAC, 2000). Urea-polyacrylamide gel electrophoresis (**PAGE**) was used to monitor α and β-casein breakdown during storage. Preparation of urea-PAGE gels was described previously (Ozturk et al., 2013b). Gel images were analyzed and protein bands were quantified by using image analysis software (Gel Expert 3.5; Nucleo Tech, San Carlos, CA, USA).

Rheological and Textural Measurements

Dynamic small amplitude oscillatory rheology was used to measure rheological properties of the cheese samples. Temperature sweep test (from 5 to 85°C at a heating rate of 1°C/min) was used with a frequency of 0.08 Hz and a strain of 0.5% to measure the storage modulus (**G′**), loss modulus (**G″)**, and loss tangent (**LT**) values.

Texture profile analysis (**TPA**) test were carried out using a TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA) as described by Ozturk et al. (2013b).

Fluorescence Microscopy

Cheese samples were prepared for confocal laser scanning microscopy as described by Ozturk et al., 2013a). Cheese samples were cut from the center of cheese block in the same direction of the cheese as it exited from cooker (so as to be able to observe paracasein fibers).

Descriptive sensory analysis

Sensory texture and flavor properties of unmelted cheese cubes and melted cheese shreds (on baked pizza) were monitored as described by Moynihan et al. (2014). A mixture of sensory Spectrum and quantitative descriptive analysis (Meilgaard et al., 1999) were used to monitor sensory properties of the reduced Na, LMPS Mozzarella cheeses by 9 trained (20 h of training) panelists. Cheese cubes were tempered at 12^{o}C and evaluated for texture (firmness and cohesiveness of mass) and flavor (acid, salt, bitter, buttery).

Pizza crust (30.5cm in diameter; Arrezio Thin & Crisp Par-Baked, Sysco Food Services, Baraboo, WI, USA) were homogeneously covered with 30g tomato sauce (Contadina Roma-style tomato sauce, Metcalfe’s Market, Madison, WI, USA). Approximately 300 g of mechanically shredded cheeses (Cuisinart Prep 11 Plus, Madison, WI, USA) were placed on the pizza crust and baked in a forced-air commercial oven (Impinger ovens, Lincoln Foodservice Products Ins., Ford Wayne, IN) at 260°C for 5 min. The surface characteristics (free oil release, blister color, blister quantity and skinning), stretch characteristics (strand length and strand thickness of stretched cheese), texture (hardness, chewiness and cohesiveness of mass) and flavor attributes (acid, salt bitter, and buttery) of melted cheeses were evaluated as described by Moynihan et al. (2014).

Experimental Design and Statistical Analyses

Five replicate batches of reduced Na, LMPS Mozzarella cheeses were manufactured. In each batch, 3 vats of cheeses were made. Cheeses from 3 vats were randomized and separated into 3 groups after manufacture. Two of these 3 groups of cheese were HHP treated either at 500 MPa or 600 MPa for 3 min and 1 group was kept as control. The effect of the treatments (control, 500 MPa, 600 MPa) and ripening time and their interactions on pH, starter culture numbers, NSLAB nubers, INSOL Ca, proteolysis, rheological, textural and functional properties were monitored using a split-plot design. In the whole-plot factor, cheesemaking day was the blocking factor and treatment was analyzed as discontinuous variable. For the split-plot factor, age and interaction of age and treatment were treated as variables. The interactive term of treatment and cheesemaking day was treated as the error term for the treatment effect. Statistical analyses were carried out using JMP (version 11.0; SAS Institute Inc., Cary, NC, USA). Data were analyzed for analysis of variance (**ANOVA**) with Tukey-HSD multiple comparisons test, and significance was determined at $P < 0.05$. Pearson's correlation coefficients were estimated between various responses.

5.4 RESULTS AND DISCUSSION

Composition and Acid-Base Buffering Curves

The compositions of the cheeses were 48.6 ± 0.6% moisture, 22.5 ± 0.4% fat, and 24.5 ± 0.6% protein and 1.0 ± 0.1% salt. There were no significant ($P > 0.05$) differences in residual lactose and galactose contents and the amount of lactic acid produced in cheeses (results not shown). Cheeses were HHP treated at 2 wk of age, thus the residual lactose has already been depleted in cheese samples before the HHP treatment. The pH values of cheeses were significantly ($P < 0.05$) influenced by HHP treatment (at 2 wk) (Table 5.1); pH values were 5.23 ± 0.03, 5.33 ± 0.03, and 5.34 ± 0.01 for control, 500 MPa and 600 MPa HHP treated cheeses, respectively, at 2 wk. However, HHP treated cheeses and control cheese exhibited similar pH values after 4 wk. Increase in cheese pH with HHP treatment was previously reported (Johnston and Darcy, 2000; Rynne et al., 2008; Ozturk et al., 2013a). Rynne et al. (2008) proposed two possible mechanisms to explain high pH values in HHP treated cheeses; the starter bacteria or their glycolytic enzymes could be inactivated with HHP treatment before complete fermentation of lactose, and/or dissociation of ionisable groups of proteins with HHP. Residual lactose was depleted in all cheese samples before the application of HHP, thus some other mechanism was responsible for the higher pH values of HHP treated cheeses at 2 wk. Decreased INSOL Ca levels ($P < 0.05$) with HHP treatment may explain the immediate increase in pH values (Figure 5.1, Table 5.1). Several researchers studied the state of Ca in HHP treated milk and cheeses. Desobry-Banon et al. (1994) reported that HHP up to 200 MPa resulted in a slight decrease in the levels of INSOL Ca in milk, but higher pressures (up to 700 MPa for 22 min) did not influence the level of INSOL Ca. Hupertz and de Kruif (2007) had a different approach to investigate the impact of HHP on INSOL Ca; they modified micelles by cross-linking caseins with transglutaminase. Pressure treatment solubilized INSOL Ca linearly with increasing pressure from 100 to 400 MPa. Pressure driven solubilization of INSOL Ca was concentration dependent i.e. less solubilization was observed with higher casein concentrations. This may partially explain why Messens et al. (1998) and Ozturk et al. (2013b) did not observe significant changes in the level of INSOL Ca with the application of ~400 MPa. Cheese is a much concentrated system than what Hupertz and de Kruif (2007) studied (25 g cross-linked casein in 1 L solution), thus HHP treatments higher than 400 MPa might be required to solubilize INSOL Ca in cheese. The INSOL Ca levels of all HHP treated cheese samples were numerically ($P > 0.05$) lower than non-pressurized cheeses at 2 wk in Ozturk et al. (2013b), indicating HHP at 405 MPa for 3 min for cheese might not be large enough to cause significant changes in INSOL Ca levels. In this study, cheeses were HHP treated at 500 and 600 MPa, resulting in a significant ($P < 0.05$) change in the levels of INSOL Ca at 2 wk. The level of INSOL Ca did not significantly ($P > 0.05$) change for 500 and 600 MPa HHP treated cheeses during storage (Figure 5.1). However, the level of INSOL Ca decreased for control cheese, reaching to similar INSOL Ca levels as 500 and 600 MPa HHP by 6 wk of storage.

Microbiology

Pressure treatment at 600 MPa for 3 min is the conditions used in industry to commercially pasteurize (5 log reduction) foods. However, 600 MPa for 3 min HHP treatment of reduced Na, LMPS Mozzarella cheese resulted in ~ 2 log reduction in starter culture numbers in our study (Table 5.2). Reduction in starter culture numbers with HHP depend on bacterial strain, cheese type, and cheese composition besides the magnitude of the HHP treatment and holding time (Rynne et al., 2008; Moschopoulou et al., 2010; Ozturk et al., 2013b).

Even though the culture we used in cheese manufacture contained *Lactobacilli*, non-starter lactic acid bacteria (**NSLAB**) numbers were < 10 cfu/g in all cheeses at 2 wk (Table 5.2). Growth of NSLAB numbers was slower in HHP treated cheeses. At 6 and 8 wk of storage, control cheese had significantly ($P < 0.05$) higher numbers of NSLAB compared to 600 MPa HHP treated cheese (Table 5.2); however, there was no difference in NSLAB numbers among all samples after 12 wk of storage. Ozturk et al. (2013a) reported HHP between 50 and 225 MPa slowed down the development of NSLAB in reduced fat Cheddar cheese, but NSLAB numbers steadily decreased during ripening by the application of HHP ≥ 350 MPa. Even though the HHP conditions used in this study were much higher than Ozturk et al. (2013a), we did not observe a reduction in NSLAB numbers with age, which may be due to the lower initial NSLAB numbers we had (< 10 cfu/g) in our cheeses compared to Ozturk et al. (2013a) (~10^6cfu/g) and/or different procedures used in the manufacture of Cheddar and Mozzarella (e.g. curd heating and stretching for Mozzarella). Presumably some pressure resistant NSLAB was growing in our cheese samples during storage; however, we did not investigate the species of the bacteria.

Proteolysis

Water soluble nitrogen in cheese contains large, medium and small peptides and amino acids that are produced by the activity of residual coagulant, milk proteinases and milk microflora. However, W-SN is mostly used as an indicator of residual coagulant activity in cheese (Rank et al., 1985). There are conflicting results on how HHP influences cheese proteolysis. Several factors seems to be important on this phenomenon, such as, the type and composition of cheese, the age of cheese when HHP applied and the magnitude of HHP and holding time. High pressure treatment caused a slight ($P < 0.05$) increase in W-SN levels at 2 wk (Table 5.1, Figure 5.2). At 2 wk, we did not observe any major differences in breakdown of caseins in Urea-PAGE electrophoretogram analysis (Figure 5.3); thus, small peptides and amino acids might have contributed the higher W-SN levels of HHP treated cheeses at 2wk of storage. Saldo et al. (2002) reported that HHP treatment of 400 MPa for 5 min did not influence the peptide and casein profiles of ewes' milk cheese in urea-PAGE electrophoretograms compared to control; however, level of free amino acids (measured by reverse-phase high performance liquid chromatography) were higher for HHP treated cheese compared to non-pressurized control. Even though the amount of W-SN was higher in HHP treated cheeses at 2wk, 600 and 500 MPa HHP treated cheeses exhibited significantly ($P < 0.05$) lower W-SN values compared to control starting from 8 wk and 12 wk, respectively (Figure 5.2, Table 5.1). Also the amount of W-SN was significantly ($P < 0.05$) lower in 600 MPa HHP treated cheese compared to 500 MPa HHP treated cheese after 12 wk (Figure 5.2). Densitometric analysis of urea-PAGE electrophoretograms revealed that degradation of α_{s1}-casein was significantly ($P < 0.05$) lower for 500 and 600 MPa HHP treated cheese compared to control after 12 wk (Figure 5.4). Intact α_{s1}-casein was significantly ($P < 0.05$) higher in 600 MPa HHP treated cheese than 500 MPa HHP treated cheese after 12 wk. Malone et al. (2003) reported that the activity of major proteolytic enzymes in cheese (residual chymosin (bovine) and bacterial proteases, such as, cell envelope protease, X-Prolyl-dipeptidyl aminopeptidase, and aminopeptidase N) were decreased or inactivated at HHP ≥ 500 MPa.

Rheological Properties

Rheological properties of reduced Na, LMPS Mozzarella cheeses were significantly (*P* < 0.05) affected by HHP treatment (Table 5.3). The crossover temperature (LT = 1), which is an indicator of melting point, decreased (*P* < 0.05) with the application of 500 and 600 MPa HHP and remained lower than control until 20 wk of storage (Figure 5.5a). The decrease in melting temperature could be partially as a result of decreased INSOL Ca levels observed in HHP treated cheeses (Figure 5.1). However, melting temperatures remained lower for HHP treated cheeses during storage even though we did not observe significant differences (*P* > 0.05) in INSOL Ca levels between HHP treated cheeses and control after 4 wk. Proteolysis is another factor that effect melting temperature of cheeses (Lucey et al., 2003). Even though HHP treated cheeses exhibited higher levels of W-SN at 2 wk, the level of W-SN were similar or lower for HHP treated cheeses compared to control after 4 wk of storage. Messens et al. (2000) reported that HHP treatment of 400 MPa decreased hydrophobic interactions in Gouda cheese. Thus, lower melting temperature of HHP treated cheeses could be as a result of both their low INSOL Ca levels and decreased hydrophobic interactions in these cheeses. At 20 wk of storage, all cheeses exhibited similar melting temperature. Melting temperature of control cheese decreased linearly during storage (R^2 = 0.84) as expected. However, melting temperature of 500 and 600 MPa treated samples increased during storage with the R^2 value of 0.94 and 0.83, respectively. These results may suggest that hydrophobic interactions have partially recovered during storage in HHP treated cheeses. Messens et al. (2000) reported that the rheological differences between HHP treated Gouda cheese and non-pressurized control became smaller during ripening due to the structural rearrangement of caseins and recovery/addition of new bonds.

The maximum loss tangent (**LTmax**) value is used as an indicator of cheese meltability, cheese with a higher LTmax value tend to melt more during heating (Lucey et al., 2003).

Pressure application resulted in an immediate increase ($P < 0.05$) in LTmax values (at 2 wk) (Figure 5.5b, Table 5.3). This could be as a result of solubilization of INSOL Ca in HHP treated cheeses. Surprisingly, LTmax values significantly ($P < 0.05$) decreased in all cheeses during storage. This was unusual because cheese was expected to be more meltable as proteolysis increased. The cheese exhibited the lowest proteolysis rates (600 MPa HHP treated sample) exhibited significantly ($P < 0.05$) higher LTmax values at 12 and 16 wk of storage ($P = 0.054$ at 20 wk) compared to control and 500 MPa HHP treated cheese. Decrease in LTmax values in low and reduced NaCl cheeses was previously reported (Grant, 2011; Ozturk et al., 2013b). Horne and Lucey (2014) reported that low ionic strength increased the intereaction rate of the renneted micelles. Therefore, high casein hydrolysis at low ionic strength environment appears to enhance casein interactions, leading to lower LTmax (higher G′) and lower meltability.

Textural Properties

High pressure treated cheeses exhibited significantly ($P < 0.05$) lower TPA hardness values compared to control until 12 wk of storage (Figure 5.6, Table 5.3). Reduction in cheese hardness with HHP has been previously reported (Messens et al., 2000; Ozturk et al., 2013a). Softening of HHP treated cheeses could be as a result of solubilization of INSOL Ca; however, HHP treated cheeses exhibited significantly ($P < 0.05$) lower hardness values even after INSOL Ca levels were not different from control cheese (i.e. at 8 and 12 wk). Messens et al. (2000) and Ozturk et al. (2013a) explained the reduction in cheese hardness with HHP with reduced hydrophobic interactions, which may explain lower ($P < 0.05$) hardness values of HHP treated cheeses at 8 and 12 wk of storage in this study. At 20 wk of storage, 600 MPa HHP treated cheese exhibited significantly ($P < 0.05$) higher TPA hardness values compared to control, while

500 MPa HHP treated cheese had hardness values in between control and 600 MPa HHP treated cheese (Figure 5.6). DeJong (1976) reported that cheese hardness was closely related to level of intact α_{s1}-casein as it was higher in 600 MPa HHP treated cheese compared to control and 500 MPa HHP treated cheese at 20 wk (Figure 5.3, Figure 5.4).

Cheeses exhibited similar TPA adhesiveness values during storage; however, both 500 and 600 MPa HHP treated cheeses exhibited significantly ($P < 0.05$) lower TPA cohesiveness compared to control at 2 and 4 wk of storage (results not shown). Lucey et al. (2003) reported that reduction in INSOL Ca levels (with acid or chelating agents), reduces attractive interactions in protein network, leading to less cohesive cheeses. We did not observe significant ($P > 0.05$) differences in TPA cohesiveness between HHP treated cheeses and control after 4 wk of storage.

Fluorescence Microscopy

Confocal laser scanning microscopy (**CLSM)** images demonstrated number and the size of the void spaces and fat droplets decreased with HHP treatment of cheeses at 2 wk (Figure 5.7). O’Reilly et al. (2002) reported that protein matrix was more continuous, and fat was more dispersed in 400 MPa HHP treated low-moisture Mozzarella cheese in CLSM images. Johnston and Darcy (2000) investigated 1 d old fresh Mozzarella cheese microstructure with scanning electron microscopy. They reported that serum filled void spaces disappeared after HHP treatment, while serum-fat channels were clearly visible in control cheese. Johnston and Darcy (2000) and O’Reilly et al. (2002) reported that HHP treatment of Mozzarella cheese accelerated storage related microstructural changes in cheese matrix. At 4 wk of storage, we did not observe any major differences in the microstructure of control and HHP treated cheeses.

Sensory analysis: Unmelted cheese

High pressure treatment significantly influenced firmness, cohesiveness (degree to which the cheese holds together as a mass) of reduced Na, LMPS Mozzarella cheeses (Table 5.4, Table 5.5). Pressure treatment decreased firmness and increased cohesiveness of unmelted cheese until 8 wk of storage (Table 5.5). After 16 wk of storage, 600 MPa HHP treated cheese was scored significantly (P < 0.05) firmer than control and 500 MPa HHP treated cheeses by sensory panelists (Table 5.5). Cohesiveness of HHP treated cheese samples did not significantly (P < 0.05) change during 20 wk of storage while control cheese became more cohesive during storage (Table 5.5). Sensory firmness of the cheese cubes were in agreement with the TPA hardness results. However, TPA and sensory panel gave opposite cohesiveness result at 2, and 4 wk, i.e. while HHP treated cheeses exhibited lower TPA cohesiveness compared to control, sensory cohesiveness of HHP treated cheeses were higher compared to control. Statistical analysis of the cheeses revealed that HHP treatment did not affect (P > 0.05) sensory adhesiveness, and TPA adhesiveness values (results not shown except for bitterness and buttery). Foegeding and Drake (2007) reported that the hardness or firmness was the easiest parameter to measure with an instrument or sensory panel, but parameters that assessed by chewing, such as, cohesiveness were more complicated. Several factors could contribute to low correlation between TPA cohesiveness and sensory cohesiveness, such as, speed of chewing, shape of molar, and saliva (Bryant et al., 1995; Foegeding and Drake, 2007). In general, HHP treatment did not alter the sensory flavor profiles of the cheeses (results not shown) except for bitterness and buttery. All cheeses received similar scores for sweetness, saltiness, and acidity. Pressure treatment significantly (P < 0.05) influenced bitterness scores; however, all cheeses received very low bitterness scores (< 1 out of 15 point scale) during 20 wk of storage (Appendix IV). Recent studies reported that cheeses manufactured with camel chymosin exhibited lower bitterness scores compared to cheeses manufactured with calf chymosin (Govindasamy-Lucey et al., 2010; Grant, 2011; Moller et al., 2013). Application of HHP slightly decreased ($P < 0.05$) buttery flavor of unmelted cheese (Table 5.4, Table 5.5). Overall, control cheese exhibited the highest and 600 MPa HHP treated cheese exhibited the lowest buttery flavor, while 500 MPa HHP treated cheese exhibited buttery flavor in between control and 600 MPa HHP treated cheeses.

Sensory analysis: Melted cheese

Shredded reduced Na, LMPS Mozzarella cheese was baked on pizza crust to evaluate the melted cheese characteristics (surface, stretch, texture, and flavor). Blister color, formation of free oil and skinning were not influenced by HHP treatment during storage (Appendix IV). Blister quantity increased in cheeses with storage (Table 5.4, Table 5.5). Pressure treatment significantly ($P < 0.05$) decreased blister quantity (Table 5.4, Table 5.5), HHP treated cheeses exhibited less blister quantity after 12 wk of storage. Oberg et al. (1991) reported that Mozzarella cheeses manufactured with proteinase deficient starter cultures exhibited less browning. Reduction in the proteolytic activity in HHP treated cheeses could explain lower blister quantity after 12 wk of storage.

Stretch quality of cheese was analyzed by measuring strand length and thickness. Stretch was described as the ability of continuous casein network to maintain its integrity under an applied elongational stress (Lucey et al., 2003). All reduced Na, LMPS Mozzarella cheeses exhibited similar ($P > 0.05$) strand thickness and length until 12 wk of storage. After 12 wk of storage, 600 MPa HHP treated cheese sample exhibited significantly ($P < 0.05$) higher strand length and thickness compared to control and 500 MPa HHP treated cheeses (Table 5.4, Table

5.5). Lucey et al. (2003) reported that stretch is decreased in cheese as proteolysis increased as a result of the discontinuity of protein network due to excessive casein hydrolysis. Oberg et al. (1991) reported that proteinase deficient cultures retained more stretch during ripening. Thus, higher intact α_{s1}-casein levels, and lower W-SN values could be responsible for greater stretchability of 600 MPa HHP treated cheese over control and 500 MPa HHP treated cheeses after 12 wk of storage.

Pressure treatment did not significantly ($P > 0.05$) affect the acidity and bitterness of the melted cheeses (Appendix IV). Unlike unmelted cheese, buttery flavor of melted cheese significantly ($P < 0.05$) increased with 600 MPa HHP treatment while control and 500 MPa treated cheeses exhibited significantly ($P < 0.05$) lower buttery flavor compared to 600 MPa HHP treated cheese ($R^2 = 0.51$) (Appendix IV). Hydrophobic interactions increases as cheese heated (Lucey et al., 2003) and strong hydrophobic interactions may decrease the perception of hydrophobic flavor compounds (Carunchia Whetstine et al., 2006). Messens et al., (2000) reported that HHP treatment decreased hydrophobic interactions, which may explain higher buttery scores of 600 MPa HHP treated cheese when heated. Textural characteristics of melted cheese, such as, hardness, chewiness (Table 5.5), and cohesiveness of mass (Appendix IV) were significantly ($P < 0.05$) affected by HHP treatment.

Melt cohesiveness, the degree to which a melted cheese sample adheres to itself after chewing, significantly ($P < 0.05$) increased after HHP treatment (at 2 wk); however, there was no significant ($P > 0.05$) difference was observed in melt cohesiveness among cheeses after 2 wk (results not shown). Cohesiveness of melted cheese generally increases with age for LMPS Mozzarella cheese (Chen et al., 2009; Moynihan et al., 2014). O’Reilly et al. (2002) reported that HHP treatment (400 MPa for 25 min) increased water holding capacity, enhanced age-related swelling of protein matrix and accelerated the ripening of low-moisture Mozzarella cheese. We also observed similar structural changes in protein matrix in CLSM images as O'Rielly et al. (2002), but we did not measure water holding capacity in our study. Thus, higher melt cohesiveness values of the HHP treated melted cheeses could be related to the changes in protein matrix. Hardness and chewiness of melted cheese was significantly ($P < 0.05$) influenced by HHP treatment (Table 5.4, Table 5.5). Similar melted cheese hardness and chewiness were observed in all melted cheeses until 12 wk of storage. Pressure treatment increased ($P < 0.05$) melt hardness and chewiness after 12 wk of storage (Table 5.4, Table 5.5). Hardness of the melted cheese, which is the force required to bite through the melted cheese with molar, was significantly ($P < 0.05$) higher for 600 MPa HHP treated cheese compared to control at 16 and 20 wk. Similar trends were observed for melted cheese hardness values compared to TPA analysis and sensory firmness of unmelted cheese except for 2 wk, where HHP treatment decreased the TPA hardness and sensory firmness of unmelted cheeses but melted cheese hardness was not affected by HHP (Figure 5.6, Table 5.4). Likewise, chewiness of melted cheese was significantly ($P < 0.05$) higher for 600 MPa HHP treated cheese at 16 and 20 wk. Lower levels of proteolysis observed in 600 MPa HHP treated cheese could have led to higher melted cheese hardness and chewiness values after 12 wk of storage.

5.5 CONCLUSIONS

High pressure treatments at 500 and 600 MPa for 3 min significantly affected microbiology, texture, rheology, and pizza performance of reduced Na, LMPS Mozzarella cheese. Commercial HHP pasteurization conditions (600 MPa for 3 min) only resulted in ~2 log reduction in starter bacteria numbers, which indicate that HHP processing is still need to be deeply studied in foods with various composition and bacterial microflora before it can be interchangeably used with heat treatment to meet legal criterions. The INSOL Ca was solubilized with HHP treatment in cheese, leading to cheeses with lower hardness and melting temperatures, and higher meltability at 2 wk. However, HHP related changes were more pronounced in cheeses after 12 wk of storage. Pressure application of 500 and 600 MPa resulted in cheeses with similar pizza performance to control cheese until 12 wk of storage; however, after 12 wk, 600 MPa HHP treated cheese exhibited better pizza performance (e.g. lower blister quantity, higher hardness, chewiness and strand thickness) compared to control and 500 MPa HHP treated cheese. Pressure treatment of 600 MPa was significantly ($P < 0.05$) more efficient to hinder cheese proteolysis compared to 500 MPa. This study demonstrated that using camel chymosin as a coagulant and HHP treatment of 600 MPa for 3 min post-manufacture successfully increased the shelf-life performance of reduced Na, LMPS Mozzarella cheese. The shelf-life of LMPS Mozzarella cheeses can be extended from 4-6 wk to 20 wk by using camel chymosin as a coagulant and application of HHP at 600 MPa for 3 min.

5.6 ACKNOWLEDGMENTS

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Table 5.1. Probabilities and R^2 values for pH, starter bacteria numbers, non-starter lactic acid bacteria (NSLAB) numbers, insoluble calcium, and proteolysis for reduced sodium, low-moisture, part-skim Mozzarella cheese during 20 wk of storage.

Factor[1]	df[2]	pH	Starter (log)	NSLAB (log)[3]	df	INSOL Ca[4] (%)	Proteolysis[5] (%)
Whole-plot							
Treatment (T)	2	<0.0001	0.001	0.006	2	0.0005	0.0007
Day of Cheesemaking (D)	4	0.074	0.086	0.004	4	0.012	0.0014
Error (T × D)	8						
Split-plot							
Age (A)	6	0.002	0.03	<0.0001	4	<0.0001	<0.0001
A× T	12	0.59	0.76	0.66	8	0.0005	<0.0001
Error	72	0.002	1.13	2.86	48	7.28	0.36
R^2		0.47	0.59	0.61		0.81	0.99

[1] Split-plot design with the 3 treatments (control, 500 MPa, 600 MPa) were analyzed as a discontinuous variable and cheesemaking day was blocked (a 3 × 5 blocked design). Subplot included the effect of aging of cheese (A), and age × treatment as a variables.

[2] Degrees of freedom differed for insoluble calcium and proteolysis as the time points for analysis during storage were different.

[3] Non-starter lactic acid bacteria.

[4] Percentage of insoluble calcium as a percentage of total calcium.

[5] Percentage of water soluble nitrogen as a percentage of total nitrogen

Table 5.2. Starter and non-starter lactic acid bacteria numbers for control, 500 and 600 MPa HHP treated reduced sodium, low-moisture, part-skim Mozzarella cheeses at 2, 4, 6, 8, 12, 16, 20 wk of storage[1]

Attribute	Storage time	Control	500 MPa	600 MPa
Starter (log)	2 wk	7.05^{a}	6.13^{ab}	5.23^{b}
	4 wk	6.41^{a}	6.05^{a}	5.31^{a}
	6 wk	6.35^{a}	5.82^{ab}	4.82^{b}
	8 wk	6.44^{a}	5.62^{ab}	4.77^{b}
	12 wk	6.58^{a}	5.25^{a}	4.50^{a}
	16 wk	6.68^{a}	5.12^{a}	5.07^{a}
	20 wk	6.51^{a}	4.39^{ab}	3.40^{b}
NSLAB (log)	2 wk	0.70^{a}	0.70^{a}	0.70^{a}
	4 wk	4.78^{a}	3.33^{a}	2.87^{a}
	6 wk	5.57^{a}	3.89^{ab}	2.87^{b}
	8 wk	5.51^{a}	2.84^{b}	2.27^{b}
	12 wk	3.99^{a}	3.76^{a}	4.09^{a}
	16 wk	5.31^{a}	5.10^{a}	5.00^{a}
	20 wk	4.78^{a}	3.51^{a}	2.67^{a}

[1] Values are means (n = 5); values within the same row not sharing a common superscript letter differ ($P < 0.05$).

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

Table 5.3. Probabilities and R^2 values for rheological properties and hardness determined by texture profile analyzer (TPA) for reduced sodium, low-moisture, part-skim Mozzarella cheeses during 20 wk of storage

Factor[1]	df[2]	TPA hardness[3]	LTmax[4]	Temperature at LTmax[4]	LT = 1[5]
Whole-plot					
Treatment (T)	2	0.0007	0.017	0.0002	<0.0001
Day of Cheesemaking (D)	4	0.023	0.098	0.032	0.015
Error (T × D)	8				
Split-plot					
Age (A)	6	0.49	<0.0001	<0.0001	0.13
A× T	12	<0.0001	<0.0001	<0.0001	<0.0001
Error	72	1.04	0.026	2.23	1.49
R^2		0.57	0.9	0.89	0.85

[1] Split-plot design with the 3 treatments (control, 500 MPa, 600 MPa) were analyzed as a discontinuous variable and cheesemaking day was blocked (a 3 × 5 blocked design). Subplot included the effect of aging of cheese (A), and age × treatment as variables.

[2] Degrees of freedom.

[3] TPA hardness was measured by texture analyzer.

[4] Maximum loss tangent values.

[5] Temperature at which loss tangent value = 1

Table 5.4. Probabilities and R^2 values for sensorial properties of unmelted reduced sodium, low-moisture, part-skim Mozzarella cheese and when cheeses were melted on pizzas in a forced-air commercial oven during 20 wk of storage

		Unmelted cheese			Melted cheese				
Factor[1]	df[2]	Firmness	Cohesiveness of mass	Buttery	Blister quantity	Strand thickness	Strand length	Hardness	Chewiness
Whole-plot									
Treatment (T)	2	0.0025	0.004	0.005	0.009	0.0008	0.003	0.009	0.01
Day of Cheesemaking (D)	4	0.0004	0.0003	<0.0001	0.37	0.2	0.4	0.004	0.12
Error (T × D)	8								
Split-plot									
Age (A)	5	0.001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
A× T	10	0.1	0.0005	0.67	<0.0001	0.008	<0.0001	0.57	0.31
Error	60	0.54	0.26	0.1	1.21	0.96	36.8	0.11	0.17
R^2		0.63	0.72	0.72	0.68	0.83	0.79	0.68	0.77

[1] Split-plot design with the 3 treatments (control, 500 MPa, 600 MPa) were analyzed as a discontinuous variable and cheesemaking day was blocked (a 3 × 5 block design). Subplot included the effect of aging of cheese (A), and age × treatment as variables.

[2] Degrees of freedom.

Table 5.5. Sensory analysis results for control, 500 and 600 MPa HHP treated, unmelted, reduced sodium, low-moisture, part-skim Mozzarella cheese and when the cheeses were melted on pizza in a forced-air commercial oven during 20 wk of storage period.

		Treatment		
Attribute	Ripening time (wk)	Control	500 MPa	600 MPa
Unmelted cheese				
Firmness	2	$8.6^{a,A}$	$7.6^{b,AB}$	$7.9^{ab,A}$
	4	$8.6^{a,AB}$	$7.3^{b,AB}$	$8.1^{ab,A}$
	8	$8.1^{a,AB}$	$8.0^{a,A}$	$8.4^{a,A}$
	12	$7.2^{a,AB}$	$6.6^{a,B}$	$7.6^{a,A}$
	16	$6.9^{b,B}$	$7.2^{b,AB}$	$7.9^{a,A}$
	20	$7.4^{b,AB}$	$7.3^{b,AB}$	$8.1^{a,A}$
Cohesiveness	2	$8.5^{b,A}$	$9.9^{a,A}$	$9.8^{a,A}$
	4	$9.3^{b,AB}$	$10.5^{a,A}$	$10.2^{a,A}$
	8	$10.3^{a,BC}$	$10.5^{a,A}$	$10.0^{a,A}$
	12	$10.2^{a,BC}$	$10.3^{a,A}$	$10.3^{a,A}$
	16	$10.7^{a,C}$	$10.5^{a,A}$	$10.0^{b,A}$
	20	$10.6^{a,C}$	$10.7^{a,A}$	$10.1^{a,A}$
Buttery	2	$3.9^{a,AB}$	$4.1^{a,A}$	$3.9^{a,AB}$
	4	$4.0^{ab,AB}$	$4.1^{a,A}$	$3.7^{b,AB}$
	8	$4.2^{a,A}$	$4.2^{a,A}$	$4.2^{a,A}$
	12	$4.4^{a,A}$	$4.1^{ab,A}$	$3.9^{b,AB}$
	16	$3.3^{a,C}$	$3.3^{a,B}$	$3.4^{a,B}$
	20	$3.6^{a,BC}$	$3.4^{a,B}$	$3.3^{a,B}$
Melted cheese				
Blister quantity	2	$5.1^{a,A}$	$6.0^{a,A}$	$6.4^{a,A}$
	4	$5.6^{a,AB}$	$6.0^{a,A}$	$6.5^{a,A}$
	8	$6.7^{a,B}$	$6.5^{a,A}$	$6.4^{a,A}$
	12	$9.4^{a,C}$	$7.3^{b,A}$	$6.0^{b,A}$
	16	$9.6^{a,C}$	$7.6^{a,A}$	$5.7^{b,A}$
	20	$8.9^{a,C}$	$7.7^{a,A}$	$6.7^{a,A}$
Strand thickness	2	$6.7^{a,A}$	$7.0^{a,A}$	$7.1^{a,A}$
	4	$6.7^{a,A}$	$7.2^{a,A}$	$6.8^{a,A}$
	8	$4.7^{a,B}$	$5.3^{a,AB}$	$6.3^{a,A}$
	12	$3.7^{b,B}$	$4.4^{b,B}$	$6.3^{a,A}$
	16	$3.6^{b,B}$	$4.0^{ab,BC}$	$5.2^{a,A}$
	20	$1.3^{b,C}$	$2.2^{b,C}$	$5.0^{a,A}$

Table 5.5 (Continued). Sensory analysis results for control, 500 and 600 MPa HHP treated, unmelted, reduced sodium, low-moisture, part-skim Mozzarella cheese and when the cheeses were melted on pizza in a forced-air commercial oven during 20 wk of storage period.

Attribute	Ripening time (wk)	Treatment		
		Control	500 MPa	600 MPa
Melted cheese				
Strand length[1]	2	45.1^{a,A}	40.9^{a,A}	37.0^{a,A}
	4	39.3^{a,AB}	39.3^{a,A}	40.1^{a,A}
	8	36.7^{a,B}	37.0^{a,AB}	37.1^{a,A}
	12	26.4^{b,CD}	26.0^{b,BC}	40.9^{a,A}
	16	23.8^{b,C}	27.3^{b,ABC}	39.8^{a,A}
	20	13.8^{b,D}	17.1^{b,C}	38.3^{a,A}
Hardness	2	3.2^{a,A}	2.9^{a,AB}	3.2^{a,A}
	4	3.1^{a,A}	3.2^{a,A}	3.3^{a,A}
	8	2.8^{a,AB}	3.1^{a,A}	3.2^{a,A}
	12	3.1^{a,A}	3.2^{a,A}	3.4^{a,A}
	16	2.6^{b,AB}	2.7^{ab,AB}	3.1^{a,A}
	20	2.0^{b,B}	2.4^{a,B}	2.7^{a,A}
Chewiness	2	7.2^{a,A}	7.1^{a,A}	7.1^{a,A}
	4	7.1^{a,A}	6.9^{a,AB}	7.3^{a,A}
	8	6.3^{a,B}	6.5^{a,ABC}	6.6^{a,AB}
	12	6.1^{a,BC}	6.2^{a,ABC}	6.6^{a,AB}
	16	5.6^{b,CD}	5.9^{b,BC}	6.2^{a,B}
	20	5.2^{b,D}	5.6^{b,C}	6.3^{a,B}

[a, b] Means within the same row not sharing a common lowercase superscript differ ($P < 0.05$; comparing the effect of treatment at a single storage time).

[A-C] Means within the same column (for a particular attribute) not sharing a common uppercase superscript differ ($P < 0.05$; comparing the effect of storage time at a single treatment).

[1] Strand lengths (how far the cheese elongates before it breaks) were reported in centimeters.

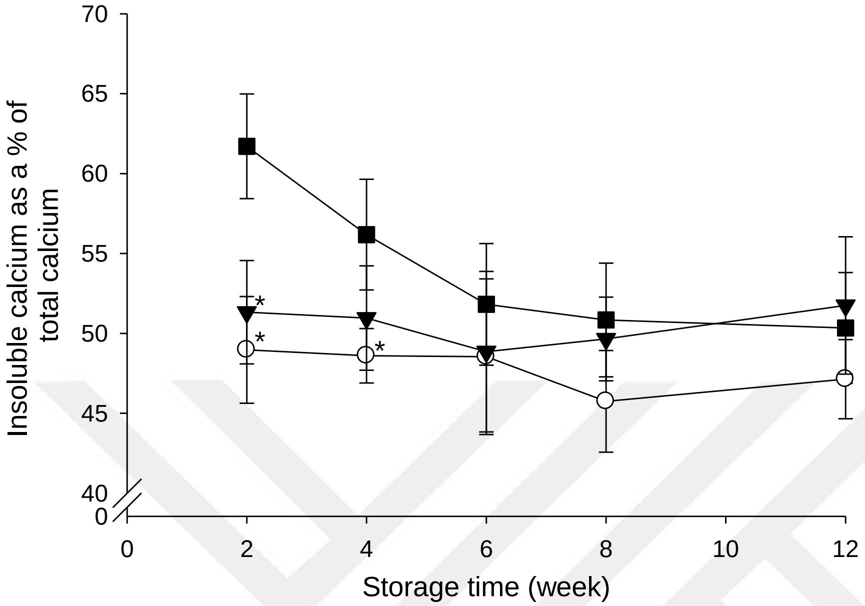

Figure 5.1. Insoluble calcium, expressed as a percentage of total calcium, for reduced sodium, low-moisture, part-skim Mozzarella cheeses for control (■), or cheeses HHP treated at 500 MPa (○), and 600 MPa (▼) during 12 wk of storage at 4^{o}C. Vertical bars represent standard deviations.

*significantly ($P < 0.05$) different from control cheese at the indicated ripening time point.

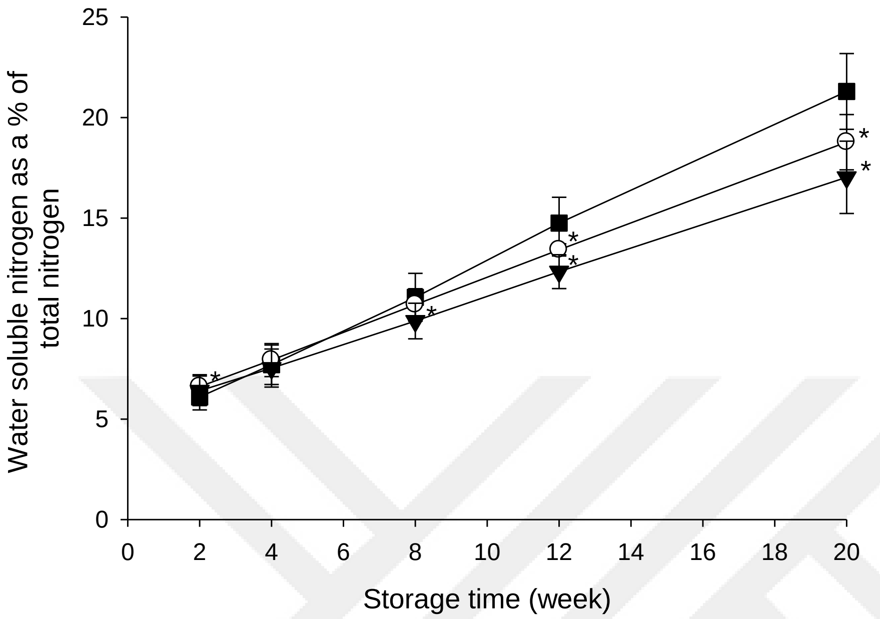

Figure 5.2. Water soluble nitrogen as a percentage of total nitrogen values for reduced sodium, low-moisture, part-skim Mozzarella cheeses for control (■), or cheeses HHP treated at 500 MPa (○), and 600 MPa (▼) during 20 wk of storage at 4°C. Vertical bars represent standard deviations.

*significantly ($P < 0.05$) different from control cheese at the indicated ripening time point.

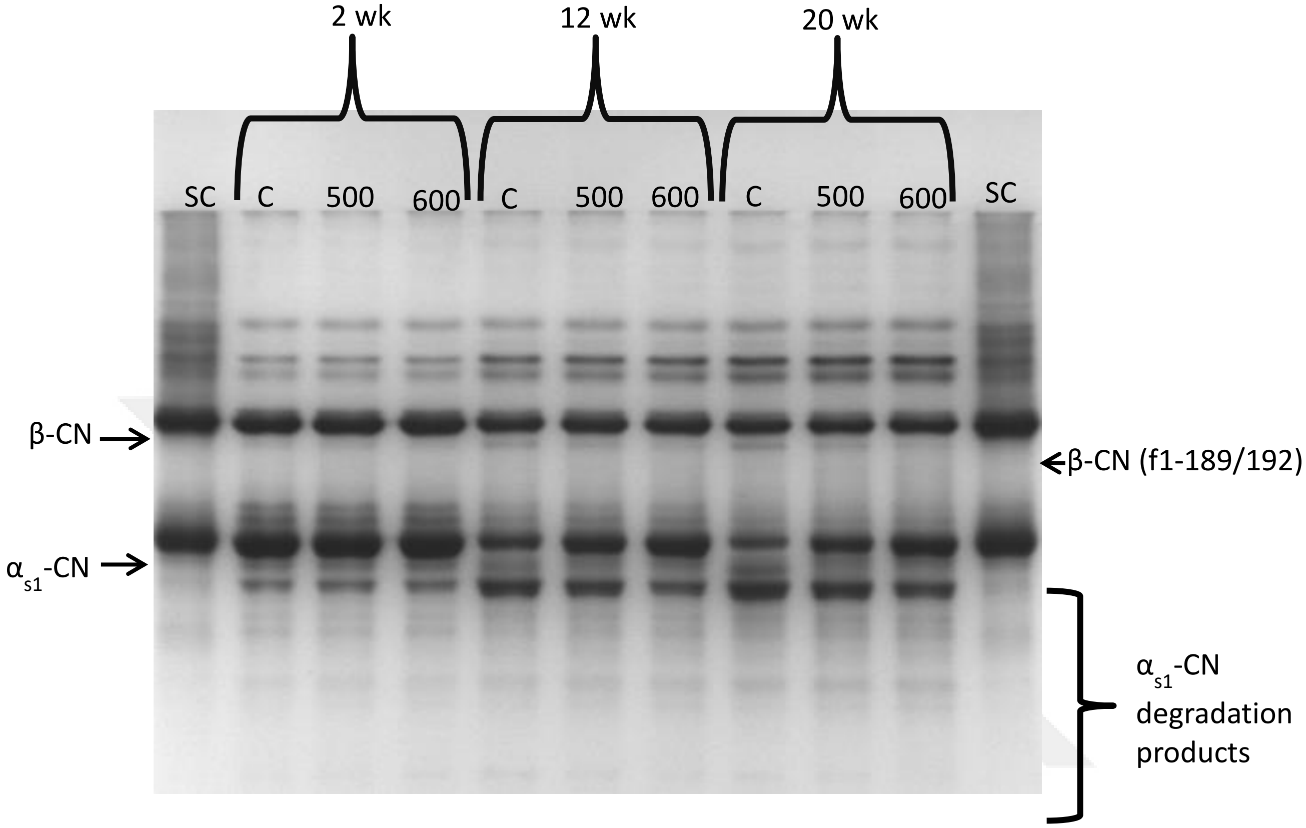

Figure 5.3. Urea-PAGE electrophoretograms showing the breakdown of caseins for reduced sodium, low-moisture, part-skim Mozzarella cheeses for control (C), or cheeses HHP treated at 500 MPa (500), and 600 MPa (600) at 2 wk, 12, and 20 wk of storage at 4°C. Lanes with SC refer to sodium caseinate (standards).

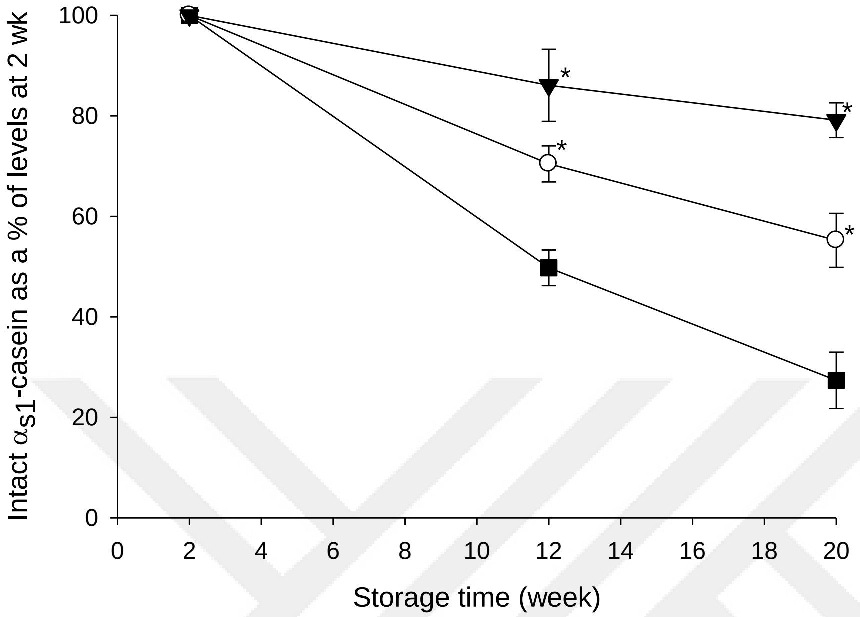

Figure 5.4. Level of intact α_{s1}-casein as a percentage of the level at 2 wk calculated from area (pixel intensity) by the Gel Expert program from the urea-polyacrylamide gels for reduced sodium, low-moisture, part-skim Mozzarella cheeses for control (■), or cheeses HHP treated at 500 MPa (○), and 600 MPa (▼) during 20 wk of storage at 4°C. Vertical bars represent standard deviations.

*significantly ($P < 0.05$) different from control cheese at the indicated ripening time point.

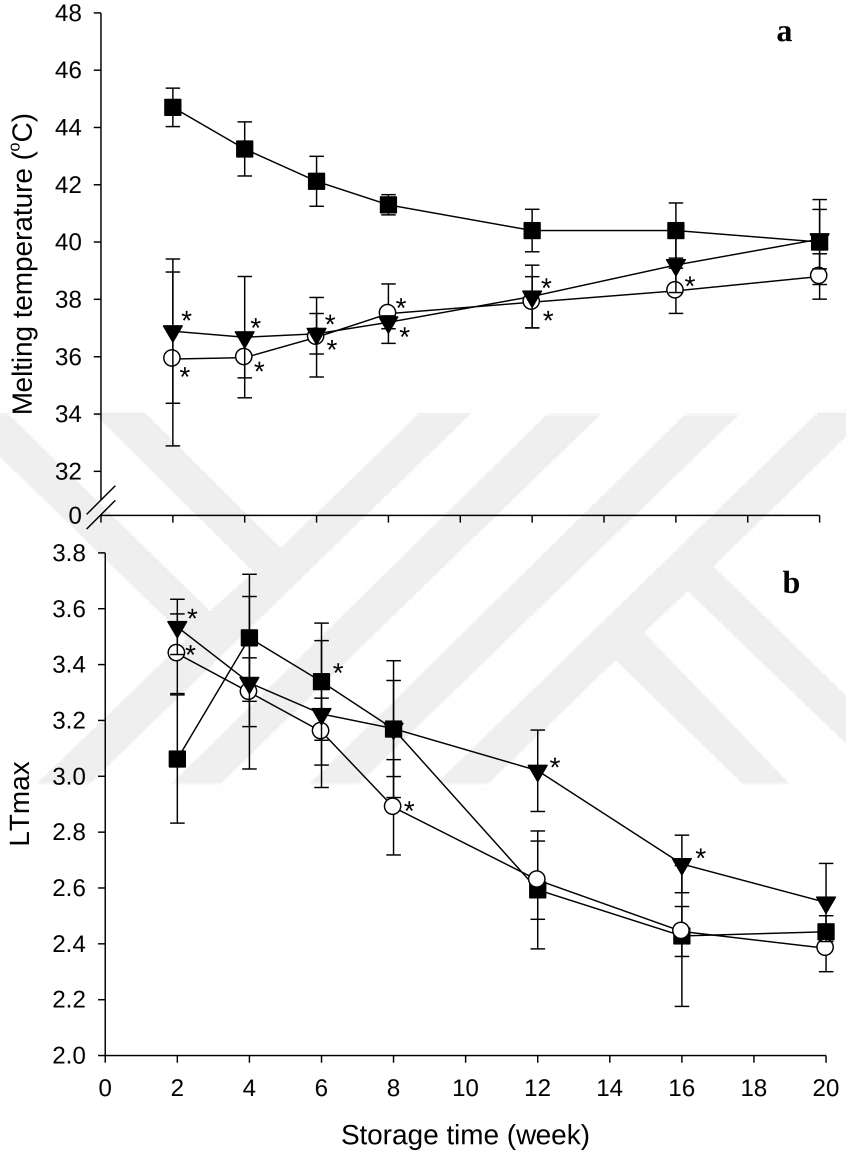

Figure 5.5. Temperature of the crossover point (where LT = 1) (a), and the maximum loss tangent values (b) for reduced sodium, low-moisture, part-skim Mozzarella cheeses for control (■), or cheeses HHP treated at 500 MPa (○), and 600 MPa (▼) during 20 wk of storage at 4°C. Vertical bars represent standard deviations.

*significantly (*P* < 0.05) different from control cheese at the indicated ripening time point.

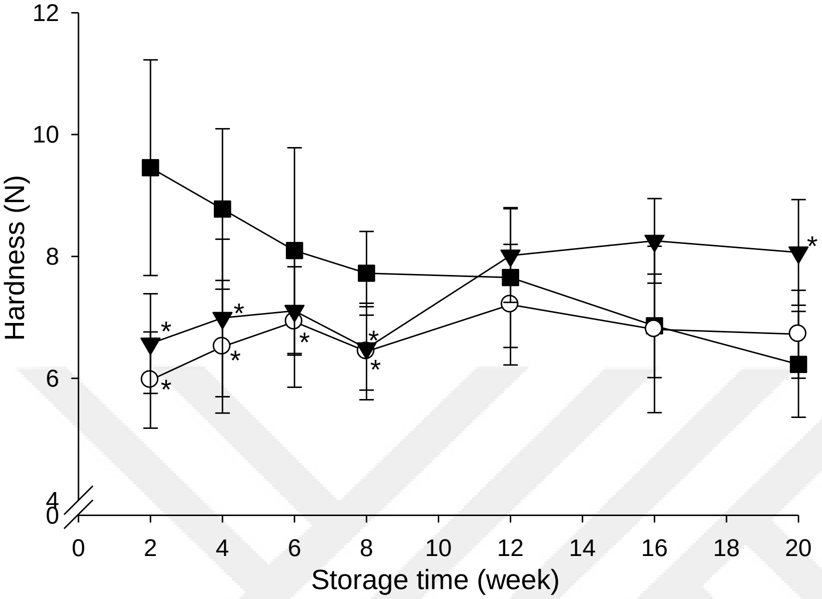

Figure 5.6. Hardness (N) from texture profile analysis testing (20% compression of cheese cylinders compared to original height) for reduced sodium, low-moisture, part-skim Mozzarella cheeses for control (■), or cheeses HHP treated at 500 MPa (○), and 600 MPa (▼) during 20 wk of storage at 4°C. Vertical bars represent standard deviations.

*significantly (*P* < 0.05) different from control cheese at the indicated ripening time point.

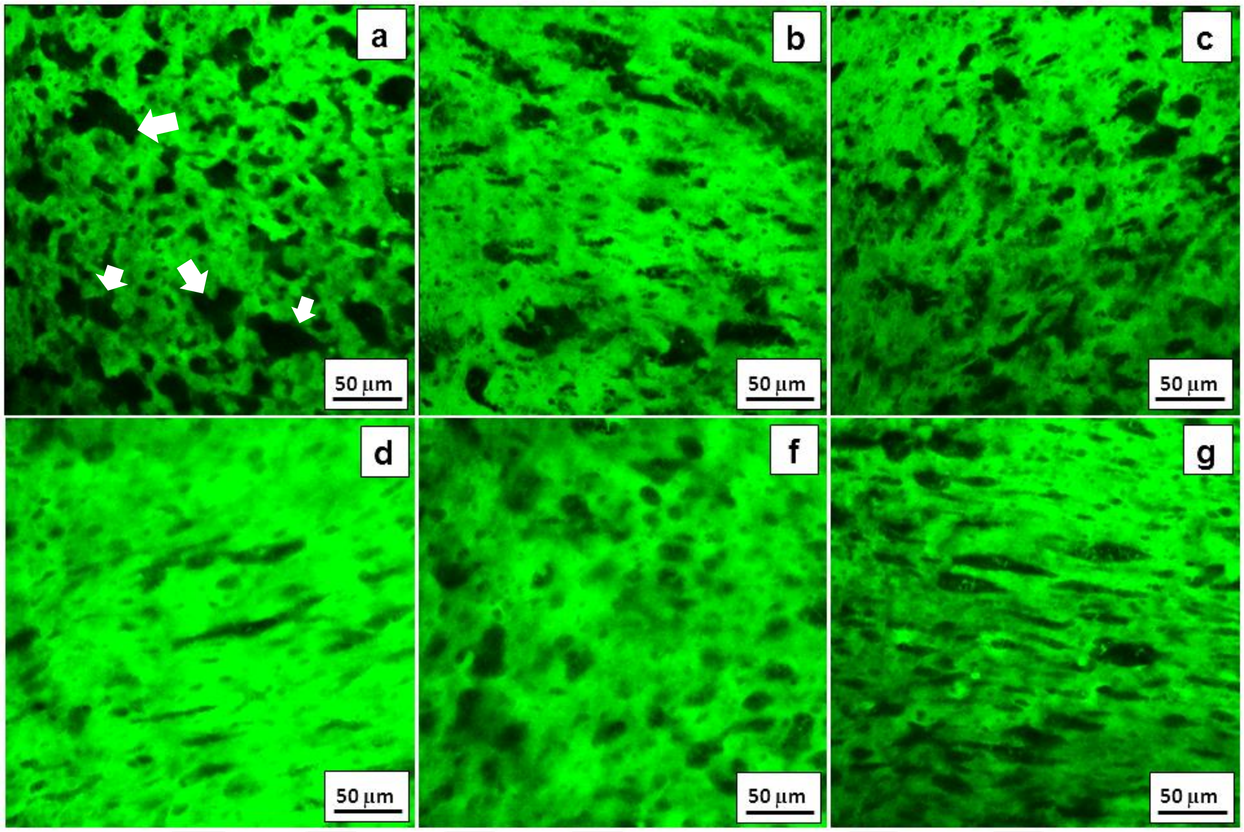

Figure 5.7. Fluorescence micrographs showing fat droplets or void spaces as dark areas (pointed out with arrows) against a light (green) background (protein) for reduced sodium, low-moisture, part-skim Mozzarella cheeses for control (a) (d), or cheeses HHP treated at 500 MPa (b) (e), and 600 MPa (c) (f) at 2 and 4 wk of storage, respectively.

Chapter 6.

Conclusions and recommendations for future research

7.1 CONCLUSIONS

The first study (Chapter 2) in this thesis demonstrated that HHP treatments ≥ 225 MPa significantly affected the texture and rheology of reduced fat Cheddar cheese. Pressure exhibited greater impact on the textural and microbiological attributes than holding time. Le Chatelier's Principle states that only concentration, temperature and pressure can affect the reaction equilibrium, which may partially explain why pressure had greater impact on the cheese texture and rheology compared to holding time. Masi (1988) reported that half of an applied stress relaxes within 30 s in most cheeses (stresses within the linear viscoelastic region). Since the shortest holding time we used in our study was 2.5 min (excluding compression/decompression cycle) and strain rate was most probably beyond the linear viscoelastic region, considerable bond relaxation possibly occurred during this holding periods.

The levels of INSOL Ca phosphate in cheese was not influenced by the conditions studied (48 to 402 MPa) in the first study (Chapter 2). The negative volume change in the system with HHP processing dissociates salts that have negative ionization volumes (Mozhaev et al., 1996). Unfortunately, there is no reported data for dissociation volume of calcium phosphate salts to our knowledge. Van Eldik et al. (1989) reported that HHP ≥ 150 MPa was sufficient to dissociate $CaSO_4$ in water at various temperatures (10 – 25^oC). If we assume that there is some similarity between calcium phosphate and $CaSO_4$, we can make the assumption that INSOL Ca phosphate could be solubilized under HHP ≥ 150 MPa. Likewise, Huppertz and de Kruif (2007) reported that in cross-linked casein micelles the level of INSOL Ca phosphate linearly decreased as HHP treatment increased from 100 to 400 MPa. However, we only observed significant ($P <$ 0.05) reduction in the level of INSOL Ca phosphate at HHP ≥ 500 MPa (Chapter 5). Two factors could be responsible for higher pressure requirements in our system for the solubilization of INSOL Ca phosphate. First, ionization of salts requires much higher compression in non-polar solvents compared to ionization in pure water (Mozhaev et al. 1996). The non-polar compounds, such as, fat and minerals in the soluble phase in our system might have decreased the ionization of INSOL Ca phosphate. Secondly, the solubilization rate of INSOL Ca phosphate with increasing pressures significantly ($P < 0.05$) decreased as the casein concentration of the system increased (Huppertz and de Kruif, 2007). As the cheese is much more concentrated system than the casein micelle solutions (25 g cross-linked casein in 1 L solution) studied by Huppertz and de Kruif (2007), this might explain why a significant ($P < 0.05$) level of INSOL Ca phosphate solubilization of cheese only occurred at HHP ≥ 500 MPa (Chapter 5). Another possible explanation was that some INSOL Ca phosphate that was solubilized during the HHP might have returned to the INSOL Ca phosphate state after pressure release.

Changes in the cheese texture and rheology could be due to disruptions in hydrophobic interactions or physical disruption to the cheese matrix. To understand the effect of HHP on milk fat in cheese, we can make a comparison between HHP treatment of cheese and homogenization of milk, as these two processes have some similarities, such as increase in the kinetic energy of the system, increased mobility and high shear. CLSM images indicated that large size individual fat droplets disappeared after HHP treatment and fat was distributed throughout the protein matrix. The majority of annatto (cheese color) is associated with milk fat globular membrane (MFGM) (Zhu and Damodaran, 2012). The soluble fraction of the cheese (i.e. cheese juice) exhibited great variation in color as a result of the HHP process. In the light of these results, we hypothesized that the shear created during HHP processing resulted in a homogenization-like effect on fat globules within cheeses. Dispersed fat droplets or fragments of MFGM became visible in the moisture phase (juice) due to the association with annatto.

The textural and rheological changes in HHP treated cheeses could be as a result of the shear created during HHP processing. Pressure treatment creates a force applied on each three dimensions which causes a volumetric strain. By the reduction in the volume, a bulk modulus (K) can be determined:

$$K = \frac{PV}{\Delta V}$$

In this equation P is the applied pressure, V is initial volume of the material and ΔV is change in material volume during HHP processing (O’Callaghan and Guinee, 2004). The bulk modulus (K) can be expressed with shear modulus (G), Young’s modulus (E), and Poisson’s ratio (μ):

$$E = 3K\ (1 - 2\mu)$$

$$E = 2G\ (1 + \mu)$$

Thus:

$$K = \frac{2G\ (1 + \mu)}{3\ (1 - 2\mu)}$$

The Poisson’s ratio (μ) is 0.40-0.45 for most cheeses (Gunasekaran and Ak, 2002). For example, G value we obtained from our reduced fat Cheddar cheese control at 2 wk (Chapter 2) is ~0.06 MPa, if we assume that $\mu = 0.45$, K value becomes ~580 kPa, i.e., theoretically cheeses were 100% compressible under all HHP conditions studied. However, the equations that were used above to calculate K value are only valid for the linear elastic region, thus, we do not have information about how much strain was created during HHP treatment.

Results from uniaxial compression test can also give us an idea about the shear created during HHP processing. In Chapter 2, the reduced fat control cheese at 2 wk exhibited 80% strain under 110 N force (this cheese also gave a major fracture around 60% strain where the applied force was ~100 N). As we know the surface area (0.0005 m^2) of the cheese samples we analyzed, we can calculate the pressure that caused 80% strain, which is 2.2 MPa. We should be careful about drawing conclusion from the calculated values above because the data used were obtained from uniaxial compression. Compression from each dimensions would most probably yield much different values. Also, the crosshead speed is a significant factor that influences the force measured by uniaxial testing. Unfortunately, we were unable to control the increase in pressure in the HHP unit, thus, we do not have the data to make assumptions about the compression speed (rate) during HHP.

Textural, rheological and microbial properties of reduced and low salt Cheddar cheese were affected by HHP. Application of HHP resulted in lower melt temperatures and softer cheeses at initial stage of ripening. However, the effects of HHP induced textural and rheological changes were dependent on the age of the cheeses. After one month of ripening, there were no differences in textural or rheological between the HHP treated or non-treated cheeses.

The second study (Chapter 3) demonstrated that the application time of HHP treatment to cheese was critical especially for reduced NaCl Cheddar cheeses. In this cheese, the pH value was not influenced by HHP treatment as the residual lactose was already depleted at the time of HHP treatment, and reduced, low and no NaCl added cheeses already reached the a stable INSOL Ca phosphate equilibrium. Another important outcome of this study was that unbalanced starter bacteria proteolytic activity was not the primary reason for the observed bitterness in reduced NaCl cheeses, as HHP treatment caused significant reduction in starter numbers, but cheese bitterness and the production of the key bitter peptide (β-casein (f1-189/192)) were not influenced by HHP treatment, this suggested that 405 MPa may not be sufficient to decrease residual chymosin activity (which was the main contributor to the production of this bitter peptide). Changes in the cheese texture and rheology at 2 wk of ripening could be due to reduced hydrophobic interactions, as we did not observe any significant change in the level of INSOL Ca phosphate or in the rate of proteolysis. Re-formation of protein interactions disrupted by HHP during ripening might have helped to minimize the differences between non-pressurized and HHP treated cheeses.

After observing no improvement in the characteristics of reduced NaCl Cheddar cheeses with the application of 405 MPa HHP for 3 min at 1 wk of ripening (Chapter 3), cheeses were subjected to higher pressure treatment (500 MPa for 3 min) at an earlier stages of ripening (1 d) in Chapter 4. This study demonstrated that 500 MPa HHP treatment can reduce the residual coagulant activity, and the production of the bitter peptide from β-casein (f1-189/192), and thus reduce bitterness in low NaCl Cheddar cheeses. Increased cheese milk buffering capacity, with UF retentate fortification of cheese milk, and decreased starter numbers due to HHP treatment at 1 d after cheese manufacture solved the acid flavor defect in low NaCl Cheddar cheeses. The rheological properties were significantly affected with 500 MPa HHP treatment; however, during 6 mo of ripening, rheological properties of HHP treated cheese remained fairly unchanged, suggesting that HHP treatment modified the protein-protein interactions in cheese and reduced residual proteolytic activity, thus cheese texture properties did not change during ripening.

In the fourth study (Chapter 5), age-related changes in the texture, flavor and functional properties of reduced Na, LMPS Mozzarella cheese was minimized by the application of high HHP treatments. Textural and rheological properties of 500 and 600 MPa HHP treated cheeses changed as a result of solubilization of INSOL Ca phosphate, suggesting that pressures < 500 MPa were not sufficient to make significant changes in the level of INSOL Ca phosphate in high protein environment of cheese. Cheese hardness was lower in HHP treated cheeses compared to non-pressurized control even after the level of INSOL Ca was not different between among cheeses, suggesting that hydrophobic interactions were also affected by HHP treatment (as for suggested for the lower HHP treatments).

Overall, the hydrophobic interactions were reduced in cheeses with pressures ≥ 100 MPa, bacterial numbers in cheese were significantly decreased with pressures ≥ 225, rate of proteolysis and level of INSOL Ca phosphate in cheese was affected by pressures ≥ 500 MPa. This research provides new insights on how modulating microbial and enzymatic activity and protein-protein interaction can be useful to manufacture cheeses with different compositions and functional properties including extended shelf-life.

7.2 RECOMMENDATIONS FOR FUTURE RESEARCH

Present study demonstrated that the level of bacterial inactivation increased with reduction in the S/M content of cheese. Martinez-Rodriguez et al. (2012) suggested that the increased sensitivity of starter bacteria to HHP treatment in high water activity conditions was due to the osmotic effect, i.e., in lower water activity environment bacteria cells shrink and the cell become more resistant to HHP treatment. So, in future studies the HHP treatment of bacteria cells with osmoprotectants (e.g. glycine betaine) at various NaCl levels would be helpful to better understand the mechanism of bacterial lysis during HHP processing of cheese.

This thesis reports there was a decrease in residual coagulant activity based on the proteolysis rates and urea-PAGE results. Even though this result is consistent with those reported in the literature with similar pressure treatments, the coagulant was used in this study was different (camel chymosin). Thus, directly measurement of the residual activity of camel chymosin at similar HHP conditions to this thesis could confirm our results.

A simpler, or model system, could be used to investigate how the level and chemical structure of INSOL Ca phosphate is affected by pressure treatment. This would be helpful to understand how the INSOL Ca phosphate, thus caseins, are modified by pressure application and release.

We observed a dispersion of fat globules in reduced fat cheese due to the application of HHP. This should be confirmed in full fat cheeses. The possible contribution of the dispersed fat material on acceleration of cheese ripening should be explained. For example, some researchers believe that NSLAB can grow/metabolize NFGM material.

7.3 REFERENCES

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APPENDICES

Appendix I

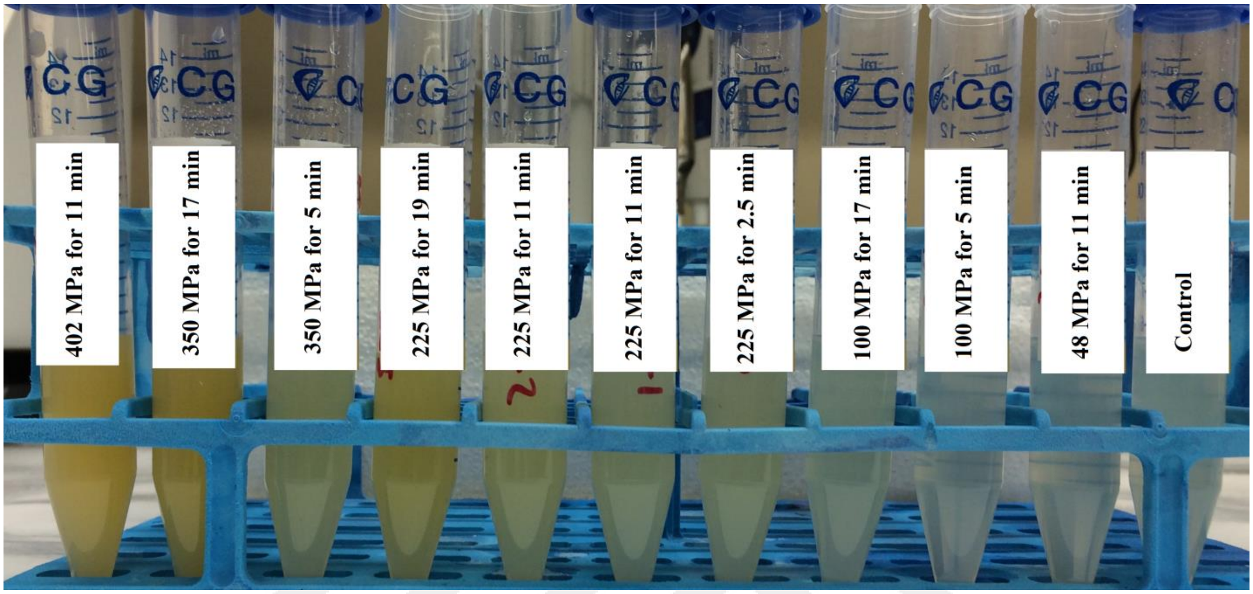

Figure 1. The moisture phase of the cheese obtained by the compression of 2 wk old reduced fat Cheddar cheese and sand mixture for control, or cheeses treated at 11 min 48 MPa, 5 min 100 MPa, 17 min 100 MPa, 2.5 min 225 MPa, 11 min 225 MPa, 11 min 225 MPa-2, 19 min 225 MPa, 5 min 350 MPa, 17 min 350 MPa, 11 min 402 MPa (from right to left, respectively).

Appendix II

Table 1. Sensory flavor intensities (on a 0-15 point scale) for high hydrostatic pressure treated and control Cheddar cheeses made with various S/M levels (n = 4).

		5.3% S/M		2.5% S/M		1.9% S/M		0.2% S/M	
		Control	HHP	Control	HHP	Control	HHP	Control	HHP
Sweet	1 mo	0.3^{ab}	0.3^{a}	0.1^{bc}	0.2^{bc}	0.1^{c}	0.2^{abc}	0.1^{c}	0.1^{c}
	3mo	0.3^{a}	0.3^{ab}	0.1^{bc}	0.1^{abc}	0.1^{bc}	0.1^{c}	0.0^{c}	0.0^{c}
Butter	1 mo	4.8^{a}	5.0^{a}	3.8^{b}	3.4^{bc}	3.3^{c}	3.1^{c}	1.9^{d}	2.0^{d}
	3mo	4.1^{a}	3.9^{ab}	3.2^{abc}	3.0^{bcd}	2.6^{cde}	2.4^{cde}	2.0^{de}	1.7^{e}
Metallic	1 mo	1.0^{de}	0.9^{e}	1.3^{cde}	1.8^{ab}	1.5^{bc}	1.5^{bcd}	2.0^{a}	1.8^{ab}
	3mo	0.5^{c}	0.4^{c}	1.4^{b}	1.5^{b}	2.0^{ab}	1.9^{ab}	2.5^{a}	2.6^{a}
Oxidized	1 mo	0.4^{c}	0.4^{c}	1.0^{b}	1.3^{ab}	1.3^{ab}	1.4^{a}	1.5^{a}	1.6^{a}
	3mo	0.5^{bc}	0.3^{c}	0.9^{abc}	1.0^{abc}	1.1^{abc}	1.1^{ab}	1.4^{a}	1.6^{a}
Sulfur	1 mo	0.5^{ab}	0.4^{abc}	0.4^{abc}	0.5^{ab}	0.3^{bc}	0.6^{a}	0.2^{c}	0.2^{c}
	3mo	0.9^{a}	0.9^{a}	0.9^{a}	0.8^{a}	0.7^{a}	0.9^{a}	0.7^{a}	0.7^{a}

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

Appendix III

Table 1. Sensory texture and flavor intensities (on 0-15 point scale) for regular NaCl, low NaCl control, UF fortified low NaCl, and UF fortified-HHP treated low NaCl Cheddar cheeses at 1, 3 and 6 mo of ripening[1]

Ripening time	Attribute	R-Na	L-Na	L-Na-UF	L-Na-UF-HHP[2]
1 mo	Chewiness	6.0^{a}	5.8^{a}	5.8^{a}	5.9^{a}
	Sweet	0.4^{a}	0.2^{b}	0.2^{b}	0.2^{b}
	Milkfat	4.1^{a}	3.8^{ab}	3.6^{b}	3.6^{b}
	Sulfur	0.6^{c}	1.1^{a}	0.8^{b}	0.9^{ab}
	Cardboard	0.7^{b}	1.0^{ab}	1.0^{ab}	1.3^{a}
3 mo	Chewiness	6.2^{a}	5.9^{b}	6.0^{ab}	6.0^{ab}
	Sweet	0.4^{a}	0.3^{ab}	0.3^{b}	0.3^{ab}
	Milkfat	3.5^{a}	3.2^{a}	3.3^{a}	3.1^{a}
	Sulfur	1.4^{a}	1.4^{a}	1.5^{a}	1.4^{a}
	Cardboard	1.1^{b}	1.1^{b}	1.3^{ab}	1.7^{a}
6 mo	Chewiness	6.1^{a}	6.0^{a}	6.0^{a}	5.9^{a}
	Sweet	0.6^{a}	0.3^{b}	0.3^{b}	0.4^{b}
	Milkfat	3.7^{a}	3.2^{b}	3.3^{b}	3.2^{b}
	Sulfur	2.3^{ab}	2.5^{a}	2.5^{a}	2.0^{b}
	Cardboard	1.0^{b}	1.3^{a}	1.1^{ab}	1.3^{a}

[1] Values are means (n = 5)

[2] HHP: high hydrostatic pressure, 500 MPa for 3 min, performed on cheese at 1 d of age.

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

Appendix IV

Table 1. Sensory analysis results for control, 500 and 600 MPa HHP treated, unmelted, reduced sodium, low-moisture, part-skim Mozzarella cheese and when the cheeses were melted on pizza in a forced-air commercial oven during 20 wk of storage period.

		Treatment		
Attribute	Ripening time (wk)	Control	500 MPa	600 MPa
Unmelted cheese				
Adhesiveness	2	3.5^{b}	4.0^{a}	4.0^{ab}
	4	3.8^{b}	4.5^{a}	4.1^{ab}
	8	4.4^{a}	4.5^{a}	4.5^{a}
	12	4.5^{a}	4.5^{a}	4.6^{a}
	16	4.0^{a}	4.2^{a}	4.2^{a}
	20	4.4^{a}	4.4^{a}	4.4^{a}
Sweet	2	0.3^{a}	0.3^{a}	0.3^{a}
	4	0.1^{a}	0.2^{a}	0.2^{a}
	8	0.1^{a}	0.1^{a}	0.1^{a}
	12	0.1^{a}	0.1^{a}	0.1^{a}
	16	0.1^{a}	0.1^{a}	0.1^{a}
	20	0.1^{a}	0.1^{a}	0.1^{a}
Salt	2	3.4^{a}	3.6^{a}	3.6^{a}
	4	3.5^{a}	3.4^{a}	3.4^{a}
	8	3.9^{a}	3.9^{a}	4.1^{a}
	12	4.1^{a}	4.1^{a}	4.0^{a}
	16	3.5^{a}	3.4^{a}	3.4^{a}
	20	4.0^{a}	3.9^{a}	3.8^{a}
Acid	2	3.9^{a}	4.0^{a}	4.1^{a}
	4	3.7^{a}	3.6^{a}	3.6^{a}
	8	3.3^{a}	3.3^{a}	3.3^{a}
	12	3.5^{a}	3.5^{a}	3.7^{a}
	16	3.3^{a}	3.3^{a}	3.5^{a}
	20	3.5^{a}	3.5^{a}	3.5^{a}

Table 1. (Continued). Sensory analysis results for control, 500 and 600 MPa HHP treated, unmelted, reduced sodium, low-moisture, part-skim Mozzarella cheese and when the cheeses were melted on pizza in a forced-air commercial oven during 20 wk of storage period.

		Treatment		
Attribute	Ripening time (wk)	Control	500 MPa	600 MPa
Unmelted cheese				
Bitter	2	0.6^{a}	0.5^{a}	0.7^{a}
	4	0.3^{a}	0.5^{a}	0.5^{a}
	8	0.3^{a}	0.5^{a}	0.3^{a}
	12	0.2^{b}	0.3^{ab}	0.5^{a}
	16	0.6^{b}	0.7^{ab}	0.8^{a}
	20	0.4^{a}	0.5^{a}	0.4^{a}
Butyric/Rancid	2	0.4^{a}	0.4^{a}	0.5^{a}
	4	0.4^{a}	0.5^{a}	0.5^{a}
	8	0.3^{b}	0.4^{ab}	0.4^{a}
	12	0.3^{a}	0.3^{a}	0.4^{a}
	16	0.7^{b}	1.2^{ab}	1.4^{a}
	20	0.8^{a}	1.2^{a}	1.1^{a}
Metallic	2	1.3^{a}	1.2^{a}	1.5^{a}
	4	1.1^{a}	1.2^{a}	1.1^{a}
	8	1.2^{a}	1.3^{a}	1.4^{a}
	12	1.3^{b}	1.3^{b}	1.6^{a}
	16	1.2^{a}	1.3^{a}	1.6^{a}
	20	1.3^{a}	1.4^{a}	1.8^{a}

Table 1. (Continued). Sensory analysis results for control, 500 and 600 MPa HHP treated, unmelted, reduced sodium, low-moisture, part-skim Mozzarella cheese and when the cheeses were melted on pizza in a forced-air commercial oven during 20 wk of storage period.

		Treatment		
Attribute	Ripening time (wk)	Control	500 MPa	600 MPa
Melted cheese				
Blister color	2	10.1^{a}	10.7^{a}	10.7^{a}
	4	10.7^{a}	11.1^{a}	11.1^{a}
	8	11.0^{a}	10.8^{a}	10.9^{a}
	12	11.7^{a}	11.7^{a}	10.3^{b}
	16	11.4^{a}	10.1^{b}	10.2^{ab}
	20	11.3^{a}	11.0^{a}	10.9^{a}
Free oil	2	7.5^{a}	7.7^{a}	7.2^{a}
	4	7.5^{a}	7.3^{a}	7.7^{a}
	8	7.4^{a}	7.3^{a}	7.9^{a}
	12	5.9^{a}	6.6^{a}	7.7^{a}
	16	6.4^{a}	6.3^{a}	7.6^{a}
	20	5.6^{a}	6.4^{a}	6.7^{a}
Skinning	2	1.1^{b}	1.5^{ab}	1.9^{a}
	4	1.5^{a}	2.0^{a}	1.9^{a}
	8	1.3^{a}	1.6^{a}	1.3^{a}
	12	2.3^{a}	2.0^{a}	1.3^{a}
	16	2.4^{a}	2.1^{a}	1.5^{a}
	20	2.6^{a}	2.1^{a}	1.4^{b}
Cohesiveness	2	8.8^{b}	10.4^{a}	10.2^{a}
	4	10.8^{a}	11.0^{a}	11.0^{a}
	8	11.4^{a}	11.2^{a}	11.0^{a}
	12	11.0^{a}	10.8^{a}	10.9^{a}
	16	11.8^{a}	11.8^{a}	11.4^{a}
	20	11.9^{a}	11.7^{a}	11.9^{a}

Table 1. (Continued). Sensory analysis results for control, 500 and 600 MPa HHP treated, unmelted, reduced sodium, low-moisture, part-skim Mozzarella cheese and when the cheeses were melted on pizza in a forced-air commercial oven during 20 wk of storage period.

		Treatment		
Attribute	Ripening time (wk)	Control	500 MPa	600 MPa
Melted cheese				
Salt	2	4.2^{a}	4.2^{a}	4.4^{a}
	4	4.0^{a}	4.2^{a}	4.2^{a}
	8	4.3^{a}	4.5^{a}	4.4^{a}
	12	4.5^{a}	4.6^{a}	4.9^{a}
	16	4.7^{a}	4.4^{a}	4.5^{a}
	20	4.4^{a}	4.4^{a}	4.9^{a}
Acid	2	3.8^{a}	3.6^{a}	3.7^{a}
	4	3.5^{a}	3.8^{a}	3.8^{a}
	8	3.5^{a}	3.4^{a}	3.3^{a}
	12	3.3^{a}	3.3^{a}	3.3^{a}
	16	3.6^{a}	3.6^{a}	3.5^{a}
	20	3.4^{a}	3.2^{a}	3.5^{a}
Butter	2	0.4^{a}	0.3^{a}	0.3^{a}
	4	0.3^{a}	0.4^{a}	0.3^{a}
	8	0.3^{a}	0.2^{a}	0.2^{a}
	12	0.3^{a}	0.2^{a}	0.3^{a}
	16	0.3^{a}	0.3^{a}	0.2^{a}
	20	0.3^{a}	0.4^{a}	0.3^{a}

$^{a, b}$Means within the same row not sharing a common lowercase superscript differ ($P < 0.05$; comparing the effect of treatment at a single storage time).