SEASONAL VARIATION OF MILK IN CENTRAL VALLEY CALIFORNIA AND
THE ASSOCIATION OF MILK VARIATION WITH THE COMPOSITION
AND TEXTURE OF LOW MOISTURE PART SKIM MOZZARELLA

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Vaideki Jai,

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COMMITTEE MEMBERSHIP

TITLE: Seasonal Variation of Milk in Central Valley California and the Association between Milk Composition and the Chemical Composition and Texture of Low Moisture Part Skim Mozzarella

AUTHOR: Vaideki Jai

DATE SUBMITTED: December 2014

COMMITTEE CHAIR: Rafael Jiménez-Flores, Ph.D.,
Professor of Dairy Products Technology
California Polytechnic State University,
San Luis Obispo, California.

COMMITTEE MEMBER: Ulric J. Lund, Ph.D.,
Professor of Statistics,
California Polytechnic State University,
San Luis Obispo, CA.

COMMITTEE MEMBER: Amy Lammert, Ph.D.,
Assistant Professor of Food Science and Nutrition,
California Polytechnic State University,
San Luis Obispo, CA.

ABSTRACT

Seasonal Variation of Milk in Central Valley California and the Association between Milk Composition and the Chemical Composition and Texture of Low Moisture Part Skim Mozzarella

Vaideki Jai

The chemical composition of milk (specifically casein, fat, and calcium) is known to affect the quality and functional properties of Mozzarella cheese. Therefore, concentrations of total nitrogen, casein nitrogen, non-casein nitrogen, non-protein nitrogen, true nitrogen, casein nitrogen to total nitrogen ratio, casein nitrogen to true nitrogen ratio, fat, total calcium, total solids, somatic cells, and pH were measured in silo milk samples collected weekly over 18-months from a large dairy plant in Central Valley, California from July 2008 to December 2009 to verify changes and correlate to low moisture part skim Mozzarella (LMPS) characteristics. LMPS mozzarella cheese from the same plant was also collected biweekly during the same period and analyzed five days post manufacture for total nitrogen, water soluble nitrogen, total calcium, water soluble calcium, salt, pH, fat in dry matter and total solids and texture properties (i.e., hardness (g), cohesiveness, springiness, chewiness (g), aggregation index (AGI), and percentage cheese loss during shredding). Significant seasonal variations of total nitrogen, non-protein nitrogen, casein nitrogen, casein nitrogen to total nitrogen ratio, casein nitrogen to true nitrogen ratio, and total calcium in milk were explained using a linear model equivalent to a basic single cosinor model with sine and cosine of week (converted into radians) as predictors. Correlation studies were done between milk composition and cheese composition, milk composition and cheese textural characteristics as well as cheese composition and cheese texture, showing that

concentration of total calcium and nitrogen fractions in cheese milk significantly affected the texture and composition of LMPS mozzarella. Also, the cheese total nitrogen, total calcium and water soluble calcium affected the cheese texture. The LMPS Mozzarella that was firmer and more cohesive had less loss during shredding and aggregated to a lesser extent. The milk total nitrogen, non-protein nitrogen, casein nitrogen, casein to total protein ratio, casein to true protein ratio, and total calcium had positive correlation with each other. However, the milk non-casein nitrogen did not significantly correlate with other nitrogen fractions and total calcium of milk. In addition, there was a significant increase of water soluble nitrogen, percent loss in shredding and aggregation index, and a significant decrease of hardness, and chewiness of LMPS Mozzarella ripened at 8.9⁰ C in comparison to the cheese ripened at 3.3⁰ C for 21 days.

Keywords: Seasonal variation, milk composition in California, Low Moisture Part Skim (LMPS) Mozzarella, LMPS Mozzarella chemical composition, LMPS Mozzarella texture characteristics, ripening study of LMPS Mozzarella

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I. INTRODUCTION

Milk is nature's most nutritious food, and its composition determines the ability to make good quality dairy products. With milk prices being based on the composition (milk fat, true protein, and other dairy solids) dairy farmers have changed their farming practices to produce milk with high protein, fat, and solids (Henrichs *et al.*, 2005). Milk components especially casein, fat, calcium, and pH influence the cheese making aspects, composition, and yield (Fox & Cogan, 2004). Therefore, the composition of milk is of great importance for the dairy industry due to the interest in changing the composition of milk to suit the processors and consumers requirements. Seasonal variation of milk composition is due to a combination of factors like heat stress, breed differences, stage of lactation, feeding practices and photoperiod (Laben, 1963). These changes are more pronounced in countries like New Zealand, Ireland, and parts of Australia countries where milk is produced from spring calving herds fed on pasture (Heck *et al.* 2009). In California, the seasonal variation in milk composition is thought to be minimal because of the large herd sizes, even calving pattern all year round, and feeding mostly concentrates versus pasture (Bruhn & Franke, 1977). However, in California, some researchers have reported seasonal variation of milk components. Nickerson (1960) found that 18 of 23 milk components varied with season except proteose-peptone, non-protein nitrogen, two minor phosphorus compounds, and soluble calcium. He also reported that variations in most of the constituents were lowest in May through July and highest in November through January (Laben, 1963). Bruhn & Franke (1991) found that protein, fat, and solid not fat in milk were higher in winter and lower in summer months in California. Bruhn & Franke (1977) studied the variation of gross composition of California milk due to breed differences and environmental conditions. They observed

that the fat and protein concentrations for all breeds were significantly lower from May through August and higher from November through February. To the contrary, Frank *et al.* (1987) found that there was no variation of protein or its fractions in milk with season in the four regions in California (North Bay, L.A. Basin, S. San Joaquin Valley, and North West Coast) from where milk samples were collected.

Low moisture part skim (LMPS) Mozzarella is a variety of pasta-filata cheese used widely in pizzas in the United States. The extensive use of LMPS Mozzarella in pizzas and other related foods is due to their longer shelf life and good shredding properties. These essential quality attributes are due to their low moisture content ($\leq 52\%$) and fat in dry matter ($< 45\%$) when compared with other types of Mozzarella (Kindstedt *et al.*, 1999). In 1985, about 75% of all Mozzarella produced was used as an ingredient in food service mainly in pizza (Kindstedt, 1999). The production of low moisture Mozzarella has increased worldwide due to the increase in demand for pizza and related foods. Large-scale production of cheese requires a precise control and monitor of all aspects of cheese making process (Kindstedt *et al.*, 1999). Along with the good manufacturing practices, raw milk quality plays an important role in the Mozzarella yield and quality (Barbano, 1987a). Even though, in California, seasonal variations in milk composition are said to be less, the impact of the variation of milk composition remains in question pertaining to utilization in cheese.

Therefore, in this study, the composition of silo milk (total nitrogen, casein nitrogen, non-casein nitrogen, non-protein nitrogen, true nitrogen, casein, casein to total nitrogen ratio, casein to true nitrogen ratio, fat, total calcium, total solids, somatic cells, and pH) collected weekly from a plant in Central Valley, California from July 2008 to December

2009 was analyzed for any seasonal variation using linear regression analysis. Also, the LMPS Mozzarella manufactured in the same plant during the same period was analyzed on a biweekly basis to see if the seasonal variation of milk composition had any effect on the cheese composition (total nitrogen, water soluble nitrogen, total calcium, water soluble calcium, salt, pH, fat in dry matter and total solids) and un-heated textural characteristics (hardness, cohesiveness, springiness, chewiness, aggregation index and percentage loss in shredder). Correlation analysis was done between milk components and cheese composition and texture for the sampling period (July 2009 – April 2010). To observe if there was any association within milk components, mozzarella components and texture characteristics correlation analysis was done as follows: 1) Between different milk components for the entire sampling period (July 2008 to December 2009) 2) Between different cheese components for the sampling period (July 2008 to November 2009) 3) Between cheese textural characteristics for the sampling period (July 2008 to November 2009) 4) Between cheese composition and textural properties for the sampling period (July 2008 to November 2009). Finally, a ripening study was done to analyze the impact of storage temperature (3.3°C and 8.9°C) on pH, water-soluble nitrogen, and the above mentioned textural characteristics of LMPS Mozzarella.

II. REVIEW OF LITERATURE

A. Brief Outline

This review consists of different parts in relation to the objective of the study. In the first part, how different components of milk affect the cheese composition and quality are discussed briefly. In the next part, factors that affect the milk quality, especially the effect of seasonal variation on milk are reviewed in depth. Then, the manufacture of Low Moisture Part Skim (LMPS) Mozzarella and characteristics of the cheese structure in relation to its composition are discussed. Next, the functional properties of LMPS Mozzarella before heating such as shreddability, matting behavior, and textural properties are briefly summarized. Finally, the chemical and functional changes that Mozzarella undergoes during proteolysis are examined.

B. Milk Composition

Milk is a complex fluid matrix consisting of nutritious components that makes it a “Complete Food”. The composition of milk with approximate concentrations is shown in Figure 1 (Walstra *et al.*, 2006a).

Figure 1. Approximate composition of milk (Adapted from Walstra et al., 2006a)

C. Quality of Milk affecting Cheese Quality

In Italy, Mozzarella was traditionally made from buffalo milk due to its characteristic aroma and physical attributes. Due to the decreasing water buffalo herd numbers, a transition from buffalo milk to cow milk was made in the 1950s (Rankin *et al*, 2006). In the US, LMPS Mozzarella cheeses are made from cow's milk. Milk components, especially casein, fat, calcium, and pH, influence the cheese making aspects, composition, and yield (Fox & Cogan, 2004). Therefore, the effect of each constituent on cheese is discussed briefly below.

1. Somatic Cell Count (SCC)

To make good quality cheese, the raw milk should have low bacterial and somatic cell counts. During udder infections and damage, somatic cells are released from the blood.

However, milk from healthy cows also contains low numbers of somatic cells in the form of epithelial cells (<100,000 cells/ml). Apart from infections, late stage of lactation, heat stress, and poor feeding practices contribute to high levels of SCC (Rankin *et al.*, 2006). An increased SCC in raw milk affects the constituents of milk and thereby affects rennet coagulation properties, syneresis, and cheese yield (Verdi *et al.*, 1987).

Epithelial cells or other somatic cells in milk from non-mastitis or normal cows constitute 80% of the SCC (SCC < 100,000 cells/ml) (Barbano *et al.*, 1987a). In milk from cows with subclinical mastitis (SCC $\geq$ 500,000 cells/ml) or mastitis (SCC > 1,000,000 cells/ml), neutrophils are present more than 26 fold and lymphocytes are present more than 3.9 fold when compared to normal milk (SCC < 100,000 cells/ml) (see Table 1). Neutrophils are a type of white blood cells that carry very active proteases, lipases, phospholipases, and specific chemicals to fight infection and tissue damage. During cheese making, levels of protease and lipase enzymes makes the curd weak due to break down of casein, fat, etc., and hence causes a lot of shattering during cutting and milling. This causes more fines, impaired whey drainage, and higher moisture content in the cheese (Barbano *et al.*, 1987a).

Table 1. Change in types of somatic cells present in milks with increasing somatic cell counts (Taken from Barbano et al. 1987a)

Milk type (cells per ml)	Somatic Cell type			
		Lymphocytes	Neutrophils	Epithelial
Normal (<100,000)	% of Total	6.1	9.1	84.8
	Number	6061	9091	84,848
Subclinical Mastitis (≥500,000)	% of Total	4.8	47.6	47.6
	Number	23,809	238,095	238,095
	Increase	3.9X	26X	2.8X
Clinical Mastitis (≥1,000,000)	% of Total	2.6	71.6	25.8
	Number	25,848	716,000	258182
	Increase	4.3X	79X	3.0X

In full-blown mastitis, there is usually leakage of blood plasma into the milk because of the damaged cells in the udder. The blood plasma contains the proteolytic enzyme “Plasmin” that breaks down the casein during processing and storage (Barbano *et al.*, 1987a). Plasmin can survive ultra-high temperatures and also plasmin gets activated from the inactive form “Plasminogen” at pasteurizing temperatures which makes it challenging to control during processing (Barbano *et al.*, 1987a).

In California, the legal limit for SCC in commercial milk is less than or equal to 750,000 cells/mL (Rankin *et al.*, 2006). Apart from affecting the casein and fat content, increased somatic cell count also decreases the starter activity, which affects the quality of cheese (Rankin *et al.*, 2006; Barbano *et al.*, 1987a; Verdi *et al.*, 1987). Mixing of high somatic cell count milk with low count milk also causes breakdown of milk casein and fat but storing at lower temperatures would slow down the damage (Barbano *et al.*, 1987a).

2. *Protein*

Total protein consists of three main fractions - casein, whey protein and non-protein, and these constitute 77.9%, 17.2% and 4.9% respectively, (Walstra *et al.*, 1999). In cheese making, during coagulum formation, casein forms the main structural framework by trapping fat and moisture. This network formation and its properties determine to a larger extent the amount of other milk constituents retained in cheese (Walstra *et al.*, 2006b). Bovine casein is present in milk as aggregates and contains the colloidal calcium phosphates (CCP). Casein precipitates out at pH 4.6, and as the pH is lowered, the CCP dissolves into soluble phase (Walstra *et al.*, 2006c). Casein micelle consists of four individual components known as α_{s1} - casein, α_{s2} - casein, β - casein and κ - casein in the approximate ratios of 4:1:4:1 (w/w) (Banks, 2007). A portion of the β -casein is divided into gamma casein and proteose peptone by the action of proteolytic enzymes. Each of the four main casein components varies due to the degree of phosphorylation, glycosylation, disulphide bonding, proteolysis and genetic polymorphism (Walstra *et al.*, 2006c). Banks (2007) reported that the BB genotypes of β - lactoglobulin and κ - casein have a good effect on the renneting properties, cause higher recoveries of fat, less loss of fines in whey, and high yields. The casein fractions and some properties important to cheese making are shown in Table 2.

Table 2. Casein fractions and importance to cheese making (Taken from Goff, H.D., 2009)

Name	Casein (%)	Properties
α_{s1} -casein	38	Binds calcium strongly Sensitive to break down by rennet Resists the milk protease, plasmin
α_{s2} – casein	10	Most calcium sensitive Binds calcium strongly
β - casein	34	Partially soluble in cold milk Breakdown by plasmin not by rennet
κ - casein	15	Stabilizes casein particles against coagulation Bonds with whey proteins during heating

The cheese milk is standardized to constant casein to fat ratio to get good cheese quality and yield. Though casein plays an important role in cheese making, Lacroix *et al.* 1996 mentioned, “There is not a simple, accurate and automated procedure that could be applied for the casein determination in milk in industry.” In dairy plants, caseins are often estimated by assuming constant casein to total protein ratio of 0.78 (Walstra *et al.*, 2006b). Lacroix *et al.* (1996) observed monthly variation of casein to total protein ratio (CP) and casein to true protein ratio (CP_t) in Quebec in commingled milk from seven plants for 14 months. The average monthly difference of CP and CP_t was 2.29% and 1.94% respectively for the 14 months. In California, Nickerson (1960) and Bruhn & Frank (1977) reported variation of protein over the season (high in summer months and low in winter months), and hence there might be variation of casein over the different seasons as well. This variation calls for a close monitor of casein to protein ratios when standardizing milk. However, Frank (1987) reported no variation of protein over the seasons in California.

3. *Fat*

Fat is present in milk as small globules, and its size is known to vary according to the cow breed. During the maturation process, fat via lipolysis imparts texture, flavor, and aroma to cheese. In the coagulum, fat is trapped by the casein network, and its presence in the curd inhibits syneresis thereby influencing the moisture retention of the curd (Banks, 2007). Low fat cheese is hard in texture and lacks flavor. Even one percent fat can produce considerable flavor in cheese and this is widely exploited in the low fat cheese industry (Neil, 2010).

4. *pH*

The pH of milk plays an important role in cheese making, and the milk composition, especially the amount of salts, influence the pH value. The pH of natural milk is about 6.7 but varies slightly (Kelly, 2007a). The use of refrigeration in milk handling and storage has minimized the acid producing bacteria thereby maintaining the pH close to 6.7. The pH increases slightly in late lactation milk, mastitis milk, and during storage due to loss of CO₂. This increase in pH is not suitable for the action of chymosin, which requires an acidic pH optimum function (Kelly, 2007a). Pre-acidification of milk is done at the start of the cheese making process to offset the increase in pH. The milk is pre-acidified by adding acid (acidogen, gluconic acid lactone, etc.) or by limited growth of lactic acid starter followed by pasteurization (Fox & Cogan, 2004).

5. *Salts*

Salts are present in low quantities in milk as metallic components (sodium, potassium, calcium, magnesium, manganese, iron, and copper) and non-metallic elements such as sulphur, chlorine, and phosphorous (Neil, 2010).

Calcium is the most important salt in cheese making, and it is partitioned between the colloidal phase (calcium phosphate within the casein micelles) and the soluble phase of milk. After rennet coagulation, the formation of the rennet coagulum is dependent on the amount of soluble calcium and insoluble calcium present in the milk. Calcium ions neutralize the negative charges on the casein micelles by forming bonds between negatively charged phosphate groups on the casein micelles. The coagulation time and firmness depend on the calcium ion activity (Kelly, 2007b).

The partition of calcium into soluble and insoluble form depends on the pH. As the pH decreases, the colloidal calcium becomes more soluble and is completely solubilized at pH 4.6. The amount of calcium retained in curd will depend on the pH at which the whey is drained. Calcium chloride is often added to cheese milk to fasten the rate of coagulation and increase the firmness of the curd (Fox and Cogan, 2004).

6. *Miscellaneous Components*

Lactose acts as the substrate for the starter cultures to produce lactic acid, and hence, aids in acid production during cheese making. Approximately 10% of the lactose is used by the starter bacteria to produce lactic acid, and the remaining is lost with the whey (Neil, 2010). For cheeses used in pizza, the galactose component of the lactose molecule gives the desired browning. Galactose is obtained by adding appropriate cultures that utilizes

only the glucose molecule of the disaccharide lactose for acid production (McMahon & Oberg, 2011).

Different enzymes gain entry into milk through bacteria present in the teats canals or from organisms present in the environment. These enzymes affect the quality of raw milk and can affect the fats and proteins during ripening to impart delicate flavors and aromas in aged cheese (Neil, 2010). Milk fat contains the fat-soluble vitamins (A, D, E and K), and most of the water-soluble vitamins (B complex and vitamin C) are lost during the draining of whey. These vitamins act as food for the bacteria to grow during cheese ripening (Neil, 2010).

7. Milk Components and Cheese Making

Figure 2. Coagulum formation in cheese making (Adapted from Wedholm, 2008 and Walstra et al., 2006b)

The chemistry behind cheese making is summarized below. κ -casein, which has a negative charge, is present on the surface of the casein micelles that make the casein

micelles repel each other in the milk serum. The casein molecules also trap the milk fat globules (Figure 2, step a). The clotting steps occur in two steps. First, when rennet is added, it cleaves the Phe₁₀₅-Met₁₀₆ bond of κ -casein into para- κ -casein, which stays with the casein micelle and a hydrophilic caseinomacropeptide (CMP) that ends up in the whey fraction (Figure 2, step b). The para- κ -casein micelles, which have a neutral net charge, form small-elongated shaped aggregates (Figure 2, step c). This is followed by the non-enzymatic second stage in which a three dimensional network is formed by the aggregation of para- κ -casein micelles under the influence of calcium ions. A gel/coagulum is formed that traps the fat and moisture. This coagulum gets firmer as more bonds are formed by the hydrophobic and electrostatic interactions between the micelles and also by calcium phosphate linkages between the micelles (Figure 2, step d). The clotting process is influenced by calcium concentration, pH and temperature. Syneresis (expelling of the whey) occurs when the gel is cut, and it is further enhanced during the cooking, stirring, and cheddaring process (Wedholm, 2008; Fox & McSweeney, 1998; Walstra *et al.*, 2006b). Due to the pressure applied, some bonds are broken, and new ones are formed, and hence, expelling the whey out in the process. Other factors that influence syneresis are increased temperature and reduced pH of milk. In the mozzarella process, the milled curd is plasticized, kneaded, and stretched with heat. The curd fibers reorganize and orient themselves in a unidirectional direction giving Mozzarella the characteristic texture (Figure 2, step f) (Kindstedt *et al.*, 1994).

D. Factors affecting Milk Composition

As described earlier, milk components play an important role in cheese making. Milk composition varies within a country and from one region to another (Barbano, 1987a).

This variation is caused by a combination of genetic and environmental factors like age, stage of lactation, feeding, health status, and climatic conditions (Fox & McSweeney, 1998). The various factors that bring about changes in the milk composition are discussed briefly below.

1. Genetic

Selective breeding of dairy cattle is done to increase the milk yield and produce milk with more fat and protein. In the US, 90% of the dairy herd consists of Holsteins. The Holsteins are known for producing large volumes of milk and have dominated milk production since 1945. Jersey represents 7% and Ayrshire, Brown Swiss, Guernsey, and Milking Shorthorn makes up 2% of the milking herd population (Wendroff & Paulus, 2011). Milk composition varies between breeds, and breed selection has decreased this variability to a great extent (Huppertz & Kelly, 2009).

Table 3. Composition (g/100g) of cow's milk from various breed (Taken from Huppertz and Kelly, 2009)

Breed	Fat	Protein	Lactose	Ash	Total solids
Ayreshire	4.0	3.3	4.6	0.7	12.7
Brown Swiss	3.8	3.2	4.8	0.7	12.7
Guernsey	4.6	3.5	4.8	0.8	13.7
Holstein	3.6	3.0	4.6	0.7	11.9
Jersey	5.0	3.7	4.7	0.8	14.2

Nitrogen composition of milk varies between breeds. Holstein milk has the lowest casein and true protein, and jersey cows have the highest values (Depeters & Ferguson, 1992).

Apart from breeding, environment and various physiological factors also influence the yield and composition of milk (Glantz *et al.*, 2009).

2. Interval between Milkings

The fat content of milk varies between the morning and evening milk due to a shorter interval between the morning and evening milking than between the evening and morning milking. Milking of cows at regular intervals will reduce this variability. SNF and protein content does not vary much with the milking interval (Walstra *et al.* 2006a).

3. Completeness of Milking

Fat droplets tend to accumulate in the upper portions of the alveoli due to their low specific gravity. Hence, the first milk drawn from the udder is low in fat while the last drawn milk is high in fat. However, there is no net loss of fat as it is picked up in subsequent milkings. Also, mixing of milk tends to even out this variability (Nickerson, 1999).

4. Age and Stage of Lactation

As the cow ages, with each successive lactation, the fat and solid not fat content decreases by about 0.02% and 0.12%, respectively (Laben, 1963). The fat, lactose, and protein contents of milk vary according to stage of lactation as shown in (Figure 3).

Solids-not-fat (SNF) content is usually highest during the first two to three weeks, and then a slight decrease is observed. The protein content of milk tends to be higher during the initial and later part of the lactation period and decrease at about 60 days of the lactation cycle. The fat content tends to increase during the later part of the lactation. However, the protein to fat ratio is highest at about 60 days of lactation (peak of

lactation) and lowest at the end of lactation period (www.irli.org). The proportion of alpha-caseins decreases during lactation while the beta-casein increases, which affects the cheese ripening and flavor (Goff, H.D. 2009).

Figure 3. Changes in the concentrations of fat, protein and lactose over a lactation of a cow (Taken from www.irli.org)

5. Feeding Regime

Diet of the cows can alter fat and milk protein content of milk. Fat is the most sensitive component to dietary changes and varies by 3.00 percentage units whereas the protein content changes only about 0.60 percentage units. The lactose and mineral content of milk do not vary much with dietary manipulations (Looper, www.uaex.edu). Generally less roughage and high energy feeds will encourage higher fat content with a little increase in protein content to provide a higher protein to fat ratio (Schroeder, 2012).

6. Disease

Disease can raise the normal body temperature of lactating cow, and this can affect the milk yield and composition. Laben (1963) observed that mastitis could reduce the yield of milk, SNF, and protein up to 10 to 12%. Though other diseases tend to affect milk composition, mastitis is the most widely studied disease that affects the milk composition. Even subclinical mastitis is also known to increase the somatic cell count, sodium, chloride, free fatty acids, and levels of blood constituents in milk and decrease fat, solids-not-fat, and lactose. Mastitis does not change the total protein content significantly, but a decrease in the level of casein and an increase in the levels of albumin and immunoglobulin have been reported (Dohoo & Meek, 1982). These changes in the milk from mastitis cows decrease the cheese yield and alter the quality of cheese produced (Looper, www.uaex.edu).

7. Seasonal Variation

Milk generally has higher fat and protein content during the winter and fall months and lowest during the spring and summer months. This variation is attributed mainly to change in climatic conditions and feeding regime. During spring, the green pastures tend to provide low fiber in diet, which decreases the fat and protein content in milk. In summer months, the heat stress reduces the dry matter intake resulting in decrease of fat and protein content (Looper, www.uaex.edu). Apart from diet and hot weather, stage of lactation, calving patterns, humidity, photoperiod, somatic cell count, etc., also contribute to variation in milk composition. Hence, it is difficult to single out one factor as the main cause to seasonal variation is due to a combination of multiple factors (Fox & Cogan, 2004).

7.a. Studies on Seasonal Variation of Milk Composition

Bernabucci *et al.* (2002) studied the variation of milk composition with regard to the environmental temperature for two seasons: spring and summer. The study comprised of 40 mid lactating Holstein cows in central Italy. The feed during the study was a ration based feed with concentrates given by self-feeders. The cows were balanced for genetic index, housed, and milked in the same way for the entire study. During the experiment, the average temperature was $11.6^{\circ} \pm 2.6^{\circ}$ C in spring during daytime and $6.4^{\circ} \pm 3.5^{\circ}$ C during nighttime. In summer, the temperature was $29.9^{\circ} \pm 2.9^{\circ}$ C during daytime and $21.9^{\circ} \pm 4.1^{\circ}$ C during nighttime. They found that milk yield during the summer was 10% lower ($p\text{-value} < 0.01$) than during the spring. Milk protein percentages were 9.9% lower ($p\text{-value} < 0.01$) in the summer than in the spring (3.01% vs. 3.31%, respectively). Casein percentage and casein number were lower ($p\text{-value} < 0.01$) in the summer than in the spring (2.18% vs. 2.58% and 72.4 % vs. 77.9% for casein content and casein number, respectively). α_s -casein and β -casein content were lower ($P < 0.01$) in the summer milk. There was no difference found for κ -casein and somatic cells between seasons. They found that summer cows consumed less dry matter, protein, and energy than spring cows due to heat stress, which contributed to less milk yield and protein in the summer milk. Ozrenk & Inci (2008) studied the seasonal effect on milk composition in Van Province Turkey. They collected milk from 12 points in the region during winter (January – March) and summer (June – August). They found that milk fat, protein, and total solid percentages were significantly higher during the winter than the summer months. They suggested that these changes might be due to the feeding pattern (high grains diet and low fiber diets in winter), difference in photo light period and temperature differences.

Bertocchi *et al.* (2013) investigated annual, seasonal, and monthly variations in milk characteristics (somatic cell count, total bacterial count, fat, and protein percentage) and thermal humidity index (THI) -milk characteristics relationships over a seven-year period (2003-2009) in Holstein dairy farms in Po Valley, Italy. They found high somatic cell count, total bacterial counts, lower fat, and protein percentage in summer months.

However, they reported that the THI – milk characteristics study suggested that heat load was not the main factor contributing to fat and protein decrease. They speculated that the photo-period and lactation stage might be the contributing factor.

Verdi *et al.* (1987) observed that the milk protein and fat varied seasonally in their two-year study from 24 plants in the New York area. They found that the non-protein nitrogen (Figure 4) and casein (Figure 5) were lowest in July and August, and the highest were in October and November.

Figure 4. Monthly average non- protein nitrogen (NPN) as a percent of total nitrogen (TN) (Taken from Verdi et al. 1987)

Figure 5. Monthly average casein expressed as a percent of total nitrogen (Taken from Verdi et al. 1987)

Verdi *et al.* (1987) also observed that the casein was low in high somatic cell (>500,000 cells/ml) than in low somatic milk (< 200,000 cells/ml) (Figure 6).

Figure 6. Monthly average casein expressed for the high and low somatic cell count milk (Taken from Verdi et al. 1987)

Heck *et al.* (2009) collected bulk milk samples from seventeen milk plants in Netherlands from February 2005 to February 2006. In their study, they observed a higher fat in the winter months than summer months in the milk and attributed this change to silage diet in

winter rather than a pasture diet in summer. They noticed only a minimal change in protein content during the season i.e. more protein during winter and less during summer (Figure 7).

protein (■), fat (◆), lactose (△)

Figure 7. Weekly variation in the concentration of protein, fat, lactose (Taken from Heck et al., 2009)

Larsen *et al.* (2010) reported a difference in the concentrations of fat and carotenoids in different parts of Sweden and variation in fat during the different seasons. They attributed both these differences due to feed changes.

Paval & Gavan (2011) reported a lower fat, protein, solids not fat, somatic cell, and freezing point of milk during the spring than autumn in milk samples collected at research station in Romania. They observed that fat, solid not fat, and somatic cells varied more than protein and speculated that this variation might be partially due to feed and temperature difference in different seasons.

Dohoo & Meek (1982) in their review reported that the somatic counts were lowest during winter and the highest during the summer with high levels usually reported in July and August, and the somatic cell counts were generally higher from April through October. They said that the somatic cells remained high even after the temperature index started to decline and called this “summer carry over.” Dohoo & Meek speculated that the increase in somatic cell count during summer may be not entirely due to elevated temperatures because higher somatic cell counts were not observed in a study where cows were housed in environmentally controlled chambers with increased temperatures. They attributed the increase in somatic cell counts to diet changes as they mentioned that in Scandinavia cows on pasture diet had higher cell counts than cows confined to barns during summer time.

Lacroix *et al.* (1996) collected milk samples from seven plants for a 14- month period (September 1991- October 1992) in Quebec to study the seasonal and regional variations in the nitrogen fractions and their effects on the ratios of casein to total protein (CP) and true protein (CP_t). They used a second order periodic oscillatory Fourier model to test for statistically significant effects of CP and CP_t variation for different seasons. They reported small but highly significant differences in nitrogen fractions of co-mingled milk among different regions and seasons. In addition, lower-total nitrogen (protein) concentrations were observed in May and July with a maximum in September – October (Figure 8). A similar pattern was observed for non-casein nitrogen (NCN) (Figure 9). However, the non-protein nitrogen fraction remained relatively constant from January to April, and then, it increased until September and then decreased (Figure 10). The casein to total protein (CP) and casein to true protein (CP_t) varied inversely to that of total

nitrogen and NCN, with a maximum in April followed by a steep decrease to a minimum in August (Figure 11 and Figure 12). From the model, seasonal variation represented 63.5% and 55.5% of the total variation for CP and CPt respectively. Due to seasonal variations, they suggested that the second order “Fourier Periodic Oscillatory Model” used in this study was found to be more suitable for determining the variation of casein over different seasons instead of using fixed casein to protein ratio throughout the year. They summarized the CP ratios variation from other studies and noted that the mean CP ratios varied between 74.8% to 82.4% (Table 4). When they fitted the CP data from other studies in the “Fourier Periodic Oscillatory Model” used in this study, a significant seasonal variation of CP with a R^2 value of 82% was observed in few studies where the sample size was large. In some other studies they did not find any significant seasonal variation of CP especially where the CP variations were small and/or the CP data was largely scattered.

Figure 8. Variations of total protein in milk (average of seven Quebec cheese plants)
(Taken from Lacroix et al. 1996)

Figure 9. Variations of non-casein fraction in milk (average of seven Quebec cheese plants (Taken from Lacroix et al. 1996)

Figure 10. Variations of non-protein fraction in milk (average of seven Quebec cheese plants) (Taken from Lacroix et al. 1996)

Figure 11. Variation of casein to total protein (CP) ratio (Taken from Lacroix et al. 1996)

Figure 12. Variation of casein to true protein (CPT) ratio (Taken from Lacroix et al. 1996)

Table 4. Seasonal variation of casein to total protein ratio (Taken from Lacroix et al., 1996)

Sampling	Country or region	Length of study	Mean CP (%)	Maximum	Minimum	References
6 plants	California	20 months	76.71	Dec	Sept	Nickerson (1960)
1 plant	Friesland	3 years	76.11	Jan	Mar	Ter Horst (1963)
9 plants	Holland 1 year	1 year	77.9	Dec-Jan	Sept-Oct	Posthumus <i>et al.</i> , (1964)
2 herds	England	2 years	76.02	May	Jul-Aug	Burton (1967)
3 herds	Australia	18 months	76.66	Aug	June	Tucker (1969)
15 regions	Sweden	5 years	78.43	Nov-Feb	Sept	Joost <i>et al.</i> (1973)
24 plants	Ontario	1 year	74.76	Nov-Feb	Sept – Oct	Szijarto <i>et al.</i> (1973)
17 plants	England-Wales	23 years	76.13	Dec – Jan	Aug – Sept	Harding & Royal (1974)
1 herd	The Netherlands	11 months	75.0	Mar	Oct	De Koning <i>et al.</i> (1974)
5 plants	The Netherlands	3 years	76.7	Mar	Jul –Aug	Lolkema (1978)
2 plants	Scotland	1 year	82.44	Oct – Nov	Aug – Sept	Davies & Law(1980)
3 plants	Ireland	1 year	74.83	Aug	Dec	Phelan (1981)
2800 cows from 63 herds	Quebec	17 months	79.77	Feb	Jan	Ng-Kwai-Hang <i>et al.</i> (1982)
7 plants	Vermont	11 months	77.47	Apr	Aug	Kindstedt <i>et al.</i> (1983)
115 farms from one cooperative	New York	2 years	77.02	Nov	May	Verdi <i>et al.</i> (1987)
13 plants	Quebec	15 months	76.1	Feb	Dec	Ng-Kwai-Hang <i>et al.</i> (1987)
4 regions	California	1 year	76.4	NO	NO	Franke <i>et al.</i> (1988)
10 regions	USA	1 year	78.15	Dec-Jan	Jul- Sept	Barbano (1990)
7 regions	Quebec	14 months	78.92	Apr	Jul-Sept	Lacroix <i>et al.</i> (1996)

7.b. Studies on Seasonal Variation of Milk Composition in California

In California, the seasonal variation in milk composition is thought to be minimal because of the large herd sizes, constant production of milk maintained by having equal number of cows freshen up each month, and feeding in dry lot throughout the year (alfalfa and grain in proportion to production). Herds are also monitored regularly by analyzing their milk for fat, protein, and lactose for payment by using Infra-Red Analyzer (Bruhn & Franke, 1977). However, few studies have reported some seasonal changes in the milk composition, and it is briefly discussed below.

Nickerson (1960) collected bulk milk samples from six processing plants in different areas in California (Visalia, Newman, Davis, Willows, Petaluma, and Fernbridge). The samples were collected over a nine-month period (June 1955 to October 1956) and analyzed for 23 milk components. He reported significant compositional difference among the bulk milk samples, both seasonally and within areas. He observed that 18 of 23 components, which include total solids, fat, SNF, total nitrogen, casein, and total calcium varied significantly. Only proteose-peptone and non-protein nitrogen, two minor phosphorus compounds, and soluble calcium failed to vary with season. He also noticed that the milk that had the lowest average protein content also had the lowest calcium and magnesium levels and vice versa. Most constituents that varied were lowest in May through July and highest in November through January.

Frank *et al.* (1987) studied the suitability of California raw milks for the production of cheese during 1983 by measuring the distribution of nitrogen of farm milk from four major dairy regions in California. The four regions are North Bay, L.A. Basin, S. San Joaquin Valley, and North West Coast. Though they observed a regional difference in

total protein and protein fractions, they found that there was no significant variation of protein or its fraction with season in all the four regions.

Bruhn & Franke (1991) compiled the milk composition and cheese yield data from four dairy plants in California for two years (1987 and 1988). The samples were analyzed for protein, fat, and solid not fat using infra-red analyzer. Protein, fat and solid not fat were higher in winter and lower in summer months (Table 5). They noted that the trend observed in this study were similar to that observed in Quebec.

Table 5. Composition of milk received by four California cheese plants (Taken from Bruhn & Franke, 1991)

Month	Milk Fat			Protein			Solids-not-fat		
	1987	1988	Overall	1987	1988	Overall	1987	1988	Overall
January	3.99	4.07	4.03	3.33	3.38	3.36	8.89	8.86	8.88
February	3.93	3.96	3.94	3.27	3.31	3.29	8.82	8.80	8.81
March	3.87	3.84	3.85	3.29	3.27	3.28	8.85	8.79	8.82
April	3.74	3.75	3.75	3.27	3.23	3.25	8.85	8.75	8.80
May	3.70	3.75	3.73	3.21	3.22	3.22	8.77	8.72	8.75
June	3.70	3.70	3.70	3.19	3.21	3.20	8.73	8.67	8.70
July	3.72	3.67	3.70	3.21	3.19	3.20	8.74	8.68	8.71
August	3.75	3.67	3.71	3.22	3.18	3.20	8.74	8.60	8.67
September	3.80	3.77	3.79	3.29	3.24	3.27	8.77	8.70	8.73
October	3.85	3.83	3.84	3.32	3.33	3.32	8.79	8.76	8.77
November	3.97	3.88	3.93	3.50	3.42	3.46	8.86	8.84	8.85
December	4.08	4.00	4.04	3.41	3.45	3.43	8.89	8.89	8.89
Annual mean	3.84	3.82	3.83	3.29	3.29	3.29	8.81	8.75	8.78
Minimum	3.70	3.67	3.67	3.19	3.18	3.18	8.73	8.60	8.60
Maximum	4.08	4.07	4.08	3.50	3.45	3.50	8.89	8.89	8.89

Laben (1963) has mentioned in his review that three years of records from a high-producing herd of 300 cows in the California milk composition study showed distinct differences in seasonal trend of SNF content between years. The summer tests for 1961 failed to show a down-ward trend whereas the 1960 and 1962 tests showed definite drops

in SNF tests from June through August. Milk fat test showed an almost identical fluctuation of winter peaks and summer depressions all three years.

Bruhn & Franke (1977) studied the variation of gross composition of milk due to breed differences and environmental conditions from 1974 to 1975 in California. They collected milk from 15 herds, which comprised 4 breeds in the San Joaquin Valley where the average day-time temperature in summer was 37⁰ C and winter was 13⁰ C. The night variation was 20⁰ C in summer and 11⁰ C in winter. They analyzed the milk composition using infra-red analyzer. The shorthorn breeds produced significantly less fat than other herds. Holstein produced milk with less fat than Jersey and Guernsey herds. The Shorthorn and Holstein produced significantly less protein than Guernsey and Jersey herds. In addition, the fat and protein content concentration for all herds were significantly lower from May through August and higher from November through February (Figure 13 and Figure 14).

Figure 13. Monthly variation of fat from four breeds in California (1974 -1975)
(Adapted from Bruhn & Franke, 1977)

Figure 14. Monthly variation of protein from four breeds in California (1974 -1975)
(Adapted from Bruhn & Franke, 1977)

E. Low Moisture Part Skim (LMPS) Mozzarella

Mozzarella, originally made from buffalo milk in Italy, is one of the prominent members in the pasta-filata cheese. Pasta-filata cheeses are made by plasticizing and kneading fresh curd in hot water, which reorganizes into a unidirectional fibrous structure (Kindstedt *et al.*, 2004). When Italian cuisine, especially pizza, became popular in the U.S, mozzarella began gaining importance. In 1985, 75% of all Mozzarella produced in the US was used for pizza (Kindstedt, 1999). In 2010, 2.5 billion kg of Italian cheeses were sold around the world and U.S accounts for about 2/3rd of global mozzarella production. Most of the Mozzarella produced in the U.S. is targeted for the food service industry (Jones G.M, 2011).

Mozzarella cheese is divided into four separate categories defined by standards of identity in Code of Federal Regulation. It is categorized on the basis of moisture content and fat in dry matter (FDM) as indicated in Table 6. Mozzarella and part skim mozzarella have high moisture content and are soft bodied thereby shred poorly, clump together more, and have a limited shelf life. Hence, these varieties of Mozzarella are mostly consumed as table cheese and are not used often in food service as an ingredient for pizza. In contrast, low moisture and low moisture part skim Mozzarella have much lower water content (typically 47-48%), longer shelf life, and firmer body. These properties impart good shredding and matting properties, and therefore, are used primarily as ingredients for pizza and related foods (Kindstedt, 1999). A FDM of 45% is a critical for the meltability and free oil release of Mozzarella (Rankin *et al.*, 2006). During melting, initial heat causes relaxation of the protein – protein bonds, which increases the movement of casein strands and eventual collapse of the casein matrix, allowing fat to

leak out and coalesce. As the temperature rises further, hydrophobic interactions between casein molecules increase causing the casein network to shrink. This shrinkage of the main framework pushes out water and liquid fat. Studies have reported a biphasic relationship between meltability and FDM. In fact, changes from 18% to 45% FDM have a little increase on melt properties, but a change above 45% FDM causes a rapid increase in meltability. At 45% FDM, the density of casein network reaches a point where the solid to liquid ratio influences melt and the ability for casein to hold liquid fat (Rankin *et al.* 2006). Hence, mozzarella with different moisture and FDM content is made to suit customers' functionality and requirement.

Table 6. Compositional standards for mozzarella in United States
(Adapted from Code of Federal Regulation (CFR) 133.155 to 133.158)

Type	Moisture (%)	Fat in Dry Matter (%)
Mozzarella	> 52 but $\leq$ 60	$\geq$ 45
Low -moisture	> 45 but $\leq$ 52	$\geq$ 45
Low moisture part skim	> 45 but $\leq$ 52	$\geq$ 30 but < 45
Part-Skim	> 52 but $\leq$ 60	$\geq$ 30 but <45

F. Manufacturing of Low Moisture Part Skim (LMPS) Mozzarella

LMPS Mozzarella is manufactured from partially skimmed milk that contains about 2% milk fat. In the United States, its fat content can be from 30% to 45% on a dry basis (FDB), and its moisture content can be from 45% to 52%. As LMPS Mozzarella is mainly used in pizza, it is manufactured to meet mainly the heated functional properties (melt, flow, and browning) specified by each pizza company. However, “string cheese” is one exception in which LMPS Mozzarella is hot extruded with a diameter of about

1.5cm, brined, cut into finger-length pieces, and packaged as individually wrapped pieces. It is a popular snack and generally consumed cold (McMahon & Oberg, 2011). Traditionally, manufacture of low moisture Mozzarella is quite similar to that of cheddar, with some notable changes. To compensate for the variation of milk composition, the milk is standardized to ensure that manufactures meet the required levels of fat in dry matter and moisture content. The milk is standardized to a casein-to-fat ratio of about 1.2 by removing cream or by adding solids in the form of liquid or dried skim milk concentrate (McMahon & Oberg, 2011). The standardized milk is pasteurized, inoculated with starter culture, and then pumped into enclosed vats (Figure 15) (Rankin *et al.*, 2006).

Figure 15. Large scale manufacture of LMPS Mozzarella cheese in enclosed vats
(Taken from McMahon & Oberg, 2011)

Starter culture used can be mesophillic (e.g., *Lactococcus lactis* ssp. *lactis*, *cremoris*) or thermophilic starter culture, which comprises *Streptococcus salavarius* ssp. *thermophilus* in combination with *Lactobacillus delbrueckii* ssp. *bulgaricus* or *Lactobacillus helveticus* (Kindstedt *et al.*, 2004). Chemical acidification instead of starter cultures is done for mozzarella used as table cheeses where high moisture content of 55% – 60% is required.

Chemical acidification is not used extensively for making low moisture Mozzarella (McMahon & Oberg, 2011). Usually, thermophilic starters are used in the production of low moisture part skim mozzarella. As this cheese has a mild flavor, the main function of the starter is to produce lactic acid, which will solubilize the casein associated calcium so that the curd will melt and stretch in hot water to give the desired texture and functional properties. Then inoculated cheese milk is coagulated with rennet. Since thermophilic cultures are used for LMPS Mozzarella, the milk is set at about 35⁰ C (McMahon & Oberg, 2011). Before the renneting process, calcium chloride may be added to enhance rennet properties, namely reduce the gelling time, and increase curd firmness. The amount of calcium chloride depends on the quality of milk (Rankin *et al.*, 2006). However, addition of calcium chloride 40% (w/w) was found to cause weeping, water loss, reduction in pH, and a more aggregated para casein matrix. This indicates that calcium addition reduces the casein hydration and hence reduces the levels of moisture and impacts the texture of mozzarella (Guinee & Fox, 2004).

Figure 16. Curd formation after renneting of milk inside an enclosed vat (Adapted from McMahon & Oberg, 2011)

The set curd (Figure 16) is cut to 2 inch pieces, stirred, and heated to about 40⁰ C. At pH 6.2–6.3, it is pumped to a large enclosed conveyor belt system (Figure 17) where the whey is drained, and the curd is stirred until a pH of 5.1 to 5.4 is reached (McMahon & Oberg, 2011).

Figure 17. Curd mass cut into pieces as it exits the draining, matting and cheddaring belt (Taken from McMahon & Oberg, 2011)

If salting of the curd is done at this step, the curd is removed at a slightly higher pH, and salt is added. The milled curds, unsalted, partially salted or salted, are stretched

mechanically in hot brine or hot water and mechanically worked in a cooker/stretching machine to produce the plastic consistency characteristic of pasta-filata cheeses (McMahon & Oberg, 2011). In an industry, stretching and plasticization is usually done in a two-step process. In the first step, the curd enters a reservoir of hot water at the front of the mixer where the curd temperature increases to 50° - 55° C. The curd at this temperature is transformed into a plastic workable consistency. In the second stage, the plasticized curd is kneaded and stretched by single or twin-screw augers as shown in Figure 18 (Kindstedt *et al.*, 2004).

Figure 18. Curd being mechanically stretched in hot water (Taken from McMahon & Oberg, 2011)

Cheese exits the cooker/stretcher at about 55° – 65° C as a smooth plastic mass (Figure 19) with a fibrous structure.

Figure 19. Hot mass exiting the cooker/stretcher (Taken from McMahon & Oberg, 2011)

The hot mass of cheese is either filled under pressure into molds (Figure 20) or extruded as a continuous ribbon, which is cooled in cold water, cold brine, or in a cooling tunnel. Dry salt can be added before stretching or as hot cheese mass exits the cooker/stretcher, or 5-10% hot brine solution is used as a part of the stretching process. Mechanization of the cheese making process allows virtually any shape of block to be formed (Kindstedt *et al.*, 2004). The cheese blocks can also be cooled and then salted in a cool 5-10% brine solution (Figure 21). Then the cheese block is dried and vacuum packaged (McMahon & Oberg, 2011).

Figure 20. Mechanical molding of hot cheese into rectangular blocks (Taken from McMahon & Oberg, 2011)

Figure 21. Blocks of LMPS mozzarella entering the brining tank (Taken from McMahon & Oberg, 2011)

G. Characteristics of LMPS Mozzarella Cheese Structure

Milk, which is a fluid suspension of fat globules and casein micelles, is converted into coagulum by aggregation casein micelles. This clumping of casein micelles occurs due to

addition of rennet and lowering of the pH. Cutting and stirring of the coagulum with a drop in pH causes the whey to ooze out, and the curd shrinks. When the curd is stretched under heat, the curd transforms from an open-celled structure consisting of a network of protein strands, containing interspersed serum and fat globules (Figure 22), into parallel fibrous protein fibers separated by long channels of accumulated fat and free serum (Figure 23). During stretching/cooking, as the curd melts and is mechanically mixed, the protein strands fuse together except at places where fat and moisture are present. After the protein strands cool, the channels separating the protein fibers are filled with close-packed fat globules. When fat is removed from mozzarella cheese, the protein strands fuse together to a homogenous mass due to less serum phase (McMahon & Oberg, 2011).

Figure 22. Scanning electron micrograph of curd after whey is drained (Taken from McMahon & Oberg, 2011).

In Figure 22, the small arrow indicates cavities in which fat globules and serum were removed during sample preparation.

Figure 23. Scanning electron micrograph of hot LMPS mozzarella after stretching (Taken from McMahon & Oberg, 2011).

In Figure 23, the formation of protein network into parallel strands separated by serum channels is shown. The black spaces in Figure 23 represent the fat globules and serum phases that were removed during sample preparation. The physical properties of Mozzarella like texture, meltability, stretchability, and color are affected by initial cheese milk composition, manufacturing protocol, and ripening conditions. The most important factors influencing these properties are the amount of casein, fat moisture, and calcium associated with casein, interaction between and within molecules, and the extent of proteolysis. These are in turn influenced by various other conditions such as pH development, temperature, and ionic strength (Lucey *et al.*, 2003, and McMahon & Oberg, 2011). Fat-serum interrupts the para-casein fibers, and the amount of fat influences the initial structure and gives softness to LMPS Mozzarella especially when heated. As fat content decreases, the casein strands become thicker with little fat-serum pools between them. This makes the cheese rubbery and very firm (Kindstedt *et al.*, 2004).

The casein-associated calcium influences the ability of the curd to plasticize in hot water and reorganize into a unidirectional fibrous structure when stretched. The total calcium of the curd and distribution of the calcium between the soluble and insoluble states plays an important role during stretching. Too much casein associated calcium makes the curd tougher curd, and it fractures easily during stretching whereas too little calcium results in a softer curd and during stretching it becomes fluid-like due to complete loss of structure (Kindstedt *et al.*, 1999).

This decrease in calcium binding to casein is attributed to a decrease in hydrophobic binding sites of submicelles, which results in weakening of the extent of binding strength between submicelles (Kimura *et al.*, 1992). The decrease in calcium mediated protein – protein interactions also allows for increased hydration of the protein matrix, and hence a higher moisture cheese is produced and the serum expressed is also reduced (Joshi *et al.*, 2004).

The pH of the curd also plays an important role in the distribution of the calcium between the soluble and insoluble states. At lower pH, the calcium moves into the soluble phase and is subsequently released from the curd during syneresis. The solubilization of the colloidal calcium of the curd depends on the timing of acidification. Also, the amount and type of acid influence the solubilization of casein bound calcium (McMahon & Oberg, 2011). Usually milk is pre-acidified to pH 6.2-6.4 prior to renneting. Calcium losses are high when the pH drop is rapid before coagulation and cutting as in the case of directly acidified Mozzarella (before syneresis). Therefore, directly acidified mozzarella contains very low levels of calcium. The ratio of casein bound calcium to soluble calcium is a function of pH, and it influences the characteristics of the curd during stretching process

(Kindstedt *et al.*, 2004). To attain a good texture, a curd with high calcium content should be plasticized at lower pH, and low calcium content curd should be plasticized at a higher pH. Curd made from demineralized skim milk produces good texture cheese if it is stretched at a higher pH. Thus, plasticization and stretching can be achieved over an extraordinarily broad range of total curd calcium content (Kindstedt *et al.*, 2004).

Another factor playing an important role during stretching is the temperature. Kindstedt *et al.* (2004) suggested that a higher stretching temperature could contribute to a increased hydrophobically mediated aggregation, contraction of para-casein, and also calcium could shift to the casein-associated state, resulting in a more highly calcium cross linked, stronger, and more elastic fibers.

The conditions of low pH and high temperature contribute to limited aggregation of para-casein and form high tensile para-casein fibers (Taneya *et al.*, 1992).

H. Functional Properties of LMPS Mozzarella

LMPS Mozzarella is mainly used as an ingredient in prepared foods; therefore, functional properties are essential features of this cheese (Kindstedt *et al.*, 2004). The functional properties can be divided into properties before heating (shredding and firmness) and heat-induced properties (meltability, stretchability, oiling off, and browning). In this study, we concentrate on the properties before heating, and thereby they are discussed briefly below.

1. Shreddability and Matting Behavior

LMPS Mozzarella is manufactured in blocks, so shredding is needed before using it in pizzas or other foods to ensure uniform distribution and melting (Rankin *et al.*, 2006). As

explained by Kindstedt *et al.* 2004, the term shreddability refers to many functional properties such as:

1. The ability of block to be passed through the shredder easily
2. Geometry and integrity of the shreds
3. Property of the curd not to shatter and form fines during shredding
4. Ability of the cheese to be free flowing and not clump together after shredding.

The problems with clogging the machines during shredding and causing the cheese to mat after shredding often occur when the cheese is soft and pasty. Firm and dry cheese cause the cheese to fracture easily and produce shattered shreds and fines (Childs *et al.*, 2007).

Chen (2003) reported that mozzarella cheese to be shredded must be firm in texture and not adhesive. He reported that firmness does not vary over aging (a typical decrease is about one unit through three months of aging). He noted that adhesiveness changes to a greater extent with proteolysis. A firmer cheese with better shredding properties at higher moisture content of 50% can be obtained if the curd temperature after the stretching process was increased from 135⁰ F to 150⁰ F. They did not observe this effect at 47% moisture. They also reported that without lowering the moisture content, firmer cheese could be obtained if the final pH of the cheese is higher, and the mineral (calcium) retention is increased.

Researchers found the firmer and less adhesive the cheese, the higher the quality of shreds. Commercial shredders use centrifugal force to direct cheese cubes into stationary blades, thus converting cheese cubes into shreds. A firm textured cheese has less deformation, and blades are able to make cleaner cuts. In addition, a firm textured cheese

cube maintains a uniform speed, and blades can shred the entire length of the cube. On the other hand, a soft textured cheese bends and deforms around the blade, slowing the portion of the cheese cube in contact with the blade. The opposite side of cheese cube is moving faster and has greater momentum. The momentum directs the cube away from the blade before the blade cuts a full block length producing shorter and thinner shreds. An adhesive cheese sticks to the blades slowing the portion of the cheese in contact with the blade and resulting in shorter shreds (Rankin *et al.*, 2006).

In low moisture Mozzarella, cheese composition, mainly high moisture content and fat content (FDM of 45%), causes cheese to clog in the shredder. The increase in moisture content and fat in cheese causes a decrease in modulus of elasticity, which causes difficulty during shredding (Kindstedt *et al.*, 2004).

Age of cheese at the time of shredding also affects the quality of shreds. Newly manufactured cheese has a lot of moisture on the surface than within the body of cheese and hence shreds poorly. Four to five days of ageing causes the moisture to absorb into the cheese due to hydration of the para-casein matrix, and thereby the shredding quality improves. Due to proteolysis, Mozzarella cheese that is aged too long will become too soft and gummy and thereby will clog in the shredder and will not produce high quality shreds (Kindstedt *et al.*, 2004).

Table 7. Factors influencing the shreddability of cheese (Adapted from Childs et al. 2007)

Factor	Effect	Shred-ability
High moisture	Matting shreds	Decreases
High Fat ($\geq$ fat% in DM)	Matting shreds	Decreases
Too young (Mozzarella day 1)	Excessive free moisture at the surface causes matting.	Decreases
Too old (20 days post manufacture)	Ragged edges, fines, matting, produces gummy balls	Decreases
Too firm, too dry	Shatter into fines and small particles	Decreases

Physical properties, such as firmness and adhesiveness, impact the behavior of the cheese during shredding. It is difficult to cleanly shred a hard cheese because it has a relatively low fracture strain. Also, it is challenging to evenly cut an over-acid cheddar cheese because it fractures and breaks at the edges (Guinee *et al.*, 2002).

Aggregation index is an empirical test to measure clumping of properties and is based on the ability of the cheese to pass through a stack of sieves of decreasing mesh size without matting. Sticky cheese that mats a lot is retained by larger sieves whereas the cheese that does not clump together flows to the bottom of the stack relatively easily. The aggregation value (sum of (sieve size x mass retained by each sieve)) increased during storage and with higher fat content, indicating increased matting (Kindstedt *et al.*, 2004). Banville *et al.* (2013) reported that aging did not significantly affect the adhesion of the cheese to the blade while shredding. However, they noticed an average of 18% difference

in adhesion for cheese stored at eight and thirty-six days, indicating a trend that ageing increased adhesion. In addition, more adhesion was observed when the cheese was shredded at room temperature (22⁰ C) than at 4⁰ C and 13⁰ C. A lower renneting pH was found to increase the adhesion on the blade when shredding is done at room temperature, causing the cheese to adhere more to the blade. However, lower renneting pH decreased fines during the shredding process. They also observed that matting was highest in room temperature 22⁰ C and lowest at 4⁰ C.

Childs *et al.* (2007) evaluated adhesive properties of cheese by measuring tack energy, which is the energy required to separate two materials that are not bound permanently. They concluded that an increase in tack energy was associated with an increase in cheese adherence to the cutting blade. Tack energy for Monterey jack and process cheese was greater than the tack energy for mozzarella cheese, which indicates that a three- month Monterey jack had greater adhesion to the blade than the young Mozzarella. They found that the tack energy and visco-elastic properties were the best indicators of shredding defects, and adherence to the blade was positively associated with cheese viscosity, and the production of fines was associated with increases in firmness.

2. Texture of Cheese

Fox *et al.* 2002 stated, “Rheology involves those properties that respond to stress or strain that is applied during processing and consumption of cheese.” These properties include elasticity, viscosity, fracture stress, and firmness among others. Texture is defined as a sensory attribute resulting from the perceptions perceived by senses like sight, touch, chewing, and swallowing (Fox *et al.*, 2000).

Sensory evaluation of cheese texture requires extensive training, and therefore it is time consuming. Also, the wide variation in the test results can affect the conclusion of the study. As a result, instrumental methods were developed to quantify the sensory perceptions of texture (Fox *et al.*, 2000).

Many empirical and imitative instrumental tests have been developed to measure the sensory texture descriptors, but by far the most popular imitative test is the uniaxial double bite compression test at a constant speed, using texture analyzers like Instron Universal testing Machine, TA.XT2 and TA.HD texture analyzers (Callaghan & Guinee, 2004). The texture profile analysis (TPA) graph obtained using the double bite compression test is shown and explained in the material and methods section.

Many studies have shown high correlation of mechanical parameters with sensory parameters like mechanical hardness with sensory hardness. Hence, by using instrumental methods, the small changes in physical characteristics of cheese can be quantified (Callaghan & Guinee, 2004). However, because of factors like complexity of mastication, differences between individuals in the perception of texture, and the effect of time of day, the texture analyzer cannot become a complete substitute to sensory evaluation (Halmos, 2000).

The hardness or firmness of mozzarella studied using the similar uniaxial compression tests have shown that low moisture mozzarella became significantly softer with increasing age (proteolysis), higher levels of fat and moisture content, and decreasing levels of calcium and pH (Kindstedt *et al.*, 2004).

Yun *et al.* (1993) reported that TPA hardness of un-melted low moisture mozzarella appeared to decrease with decreasing cooking temperature and increasing moisture

content of the cheese. The TPA hardness and TPA springiness for all cheeses decreased with age. The TPA cohesiveness increased slightly during the first two weeks and then remained constant.

I. Proteolysis and Ageing of Cheese

A lot of researchers have shown that in low moisture mozzarella hardness and apparent viscosity decrease, and meltability, stretch-ability, and oiling off increase after the first few weeks of manufacture (Kindstedt *et al.*, 2004). These functional changes are also due to the changes in structure of the cheese as a result of proteolysis, changes in serum phase, and an increase in the water binding capacity of the casein (Kindstedt *et al.*, 2004, Kindstedt and Guo, 1997).

A brief ripening period of LMPS mozzarella (usually less than a month) is required for the cheese to get the desired functional properties (shredding and meltability) to be used as a pizza ingredient. Newly manufactured cheese has a lot of moisture at the surface and within the body of cheese, hence it will not shred well. Four to five days of ageing will allow the cheese to absorb the moisture back, and this will improve the shredding quality (McMahon & Oberg, 2011). When cheese is maintained at cold temperatures for prolonged time of about two to three weeks, the cheese shreds better because the caseins become more hydrated and swollen (Figure 24) due to free water being absorbed back into the protein matrix (Kindstedt *et al.*, 2004).

Several investigators have shown that the amount of serum, expressed from cultured low moisture mozzarella by centrifugation or pressing, decreased from levels of 20%-40% to no serum after two to three weeks of ageing (Kindstedt *et al.*, 2004). Though, the serum decreases on ageing, the soluble calcium increases due to a decrease in hydrophobic

interaction between the protein and calcium molecules. This allows more movement of the proteins in relation to each other, and hence the cheese melts easily when heated. As ageing continues, protein-protein interactions decreased because of the breakdown of protein through proteolysis, and thus the cheese melts too quickly and loses its stretch (McMahon & Oberg, 2011).

Salt addition of 0.5% to 2.0% is known to increase the casein solubility (McMahon & Oberg, 2011); therefore a NaCl – mediated redistribution of water at a microstructure level occurs during ageing (Kindstedt *et al.*, 2004).

Figure 24. Scanning electron micrograph of cheese after four weeks of storage
(Taken from McMahon and Oberg, 2011).

The initial breakdown of casein to larger peptides in low moisture mozzarella cheese occurs mainly through the action of the coagulant. The starter culture contributes to secondary proteolysis, which is degradation of the products of primary proteolysis to small peptides and amino acids (Kindstedt *et al.*, 2004). Cheeses made with typical strains of *St. thermophilus* have very little proteolytic activity than cheeses made with *St.*

thermophilus combined with *Lactobacillus helveticus*. This is because *Lactobacillus helveticus* has a more proteolytic activity whereas strains of *St. thermophilus* have very little proteolytic activity. *Lactobacillus delbruekii* also causes proteolysis of α_{s1} -casein. The proteolysis of β -casein occurs very slowly with most of the β -casein remaining intact (McMahon & Oberg, 2011). However, there is evidence of plasmin activity in mozzarella cheese during ripening, and this causes degradation of the β -casein to form the γ -casein (Creamer, 1976).

The proteolysis occurs in the first month of storage depending on cheese composition, residual coagulant, and manufacturing methods. Cheeses with higher moisture content undergo faster proteolysis. Increasing or decreasing the residual coagulant level in the cheese has a corresponding effect on the rate of initial cleavage of caseins. The extent of proteolysis varies widely depending on the temperature and time of hot water stretching and the consequent thermal inactivation of the starter culture. If the curd is stretched at a higher temperature, the residual coagulant and starter activity in the cheese will be limited; hence the cheese will have to be stored for a longer time to get the desirable functional properties (McMahon & Oberg, 2011).

Feeney *et al.* (2001) reported that water-soluble nitrogen (WSN) soluble at pH 4.6 increased in low moisture mozzarella as it matured at different temperatures for 70 days (Figure 25). After 15 days, there was significant difference in pH 4.6 WSN between cheese ripened at 10⁰ C, 15⁰ C, and cheese ripened at 0⁰, 4⁰ C. Urea- PAGE of the pH 4.6 insoluble portion showed that there was α_{s1} -casein degradation during ripening and increasing temperatures affected the degradation significantly. The maximum proteolysis of α_{s1} -casein was observed at 10⁰ C and 15⁰ C.

Low Moisture Mozzarella ripened at 0°C (●), 4°C (○), 10°C (▲) and 15°C (△)

Figure 25. Level of pH4.6 soluble nitrogen expressed as a percentage of total nitrogen in low moisture mozzarella (Adapted from Feeney et al., 2001).

Yun *et al.* 1993 also demonstrated that higher cooking temperature resulted in a slower rate of proteolysis during storage. Soluble nitrogen contents increased with refrigerated storage time for all cheeses. The influence of refrigerated storage time was much greater than cooking temperature on proteolysis. The amount of α_s -casein as a percentage of total protein decreased significantly for all cheeses during refrigerated storage, and the rate of decrease was significantly affected by the differences in cooking temperature and with cheese age. The higher cooking temperature retarded the breakdown of α_s -caseins. There was not much breakdown of β -casein due to increased cooking temperature and during the refrigerated storage.

III. MATERIALS AND METHODS

A. Sample collection

1. Milk Sampling

Composite milk samples were collected every week at a plant in Central Valley California from July 2008 to December 2009. A total of seventy-six milk samples were collected every week over a period of 18 months. A weekly composite milk sample was made from silo milk collected every day. One tablet of 18 mg broad spectrum Microtabs II was added to the 40 ml milk as a preservative, and the milk sample was stored at 4⁰ C. Two weekly composite milk samples were then shipped to Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo, California (DPTC, Cal Poly) over night in a cooler box. The schematic diagram of the milk sampling plan is shown in Figure 26.

Figure 26. Milk sampling plan

2. Low moisture part skim mozzarella (LMPS) sampling

Cheese samples were collected from the same plant as that of the milk samples, but the samples were collected for a shorter period (July 2008 to mid-November 2009). A total of 33 cheese samples were collected biweekly whereas the milk samples were collected weekly. Three ten-pound blocks were sampled from the same vat/ batch of manufacture. The blocks were vacuumed packed and shipped to DPTC, Cal Poly, in a cooler box overnight. One block of cheese sample was analyzed exactly on the fifth day after the date of manufacture. The other two blocks were subjected to ripening for 21 days at different temperatures. The milk samples were a representation of the cheese milk used to make that week's cheese sample. As the milk was only a representation and not the exact

milk from which the weekly cheese was made, the conclusions that can be drawn from this study are limited.

B. Analysis of Milk Samples

The milk samples received were stored at 4⁰ C. At the time of analysis the samples were heated to 38⁰ C (Lacroix *et al.*, 1996) and mixed well by swirling the sample cup for two minutes and subjected to the following analysis:

1. Total Nitrogen

The total nitrogen (TN) content of milk samples was determined in duplicates by Kjeldhal method, and the results were expressed as a percentage protein. The percent total nitrogen TN (%) was calculated using the below formula

$$\frac{1.4007 \times (\text{Volume of HCl in sample} - \text{Volume of HCl in blank}) \times \text{Normality of NaOH}}{\text{Weight of Sample}}$$

Total Nitrogen was converted to percent protein by multiplying by 6.38. This method is from AOAC Official method 991.20, AOAC 2000

2. Non- protein Nitrogen (NPN)

NPN is urea and other non-protein compounds such as creatine, creatinine, uric acid, etc. Protein was precipitated by 15% trichloroacetic acid (TCA), and precipitated milk protein was removed by filtration. The filtrate contained the NPN and was used to determine non- protein nitrogen in duplicates by Kjeldhal method. Results were expressed as the percentage of non-protein nitrogen (% NPN) and calculated using the below formula

$$\frac{1.4007 \times (Vs - Vb) \times M}{(Wf \times Wm)/(W - (Wm \times 0.065))}$$

Where,

V_s = ml of HCl titrant used for test portion

V_b = ml of HCl titrant used for blank

M = Molarity of HCl solution

W_f = Weight (g) of 20 ml filtrate

W_m = Weight (g) of milk

W_t = Weight (g) of milk plus 40 ml of 15 % TCA solution

This method is from AOAC Official method 991.21, AOAC 2000

3. *Non-casein Nitrogen (NCN)*

NCN was extracted from milk according to AOAC Official method 998.05, AOAC 1998.

Casein was precipitated from milk at pH 4.6 using an acetic acid and sodium acetate solution. Precipitated milk casein was removed by filtration. The nitrogen content in the filtrate that contained the non-casein nitrogen components was determined by Kjeldhal in duplicates. Results were expressed as the percentage of non-casein nitrogen (% NCN), which was calculated using the below formula

$$\frac{1.4007 \times (V_s - V_b) \times M \times 2 \times factor}{W_m}$$

Where,

V_s = ml of HCl titrant used for test portion

V_b = ml of HCl titrant used for blank

M = Molarity of HCl solution

W_m = Weight (g) of milk

The factor is determined by the following formula

$$Factor = 1 - \frac{(0.11 \times fat\%)}{100} + \frac{(0.07 \times casein\%)}{100}$$

As the fat and casein content of milk was about 2.6% in calculation the factor was approximated to be 1.0. This method is from AOAC method 991.21, AOAC 2000

4. True Nitrogen

The true nitrogen (TrN %) was calculated from subtracting non -protein nitrogen (NPN) from total nitrogen (TN - NPN) and true protein (%) was obtained by multiplying TrN (%) with 6.38 (Tremblay *et al.*, 2003).

5. Casein Nitrogen

The casein nitrogen (CN %) was calculated as the difference between total nitrogen and non-casein nitrogen (TN – NCN) and the casein protein (%) was obtained by multiplying CN (%) with 6.38 (Tremblay *et al.*, 2003).

6. Total Solids

Total solids were determined in duplicates by weighing milk (initial weight), drying milk, and weighing dried milk residue (final weight). 5 grams of the test samples were dried for approximately 4 hours at $100^{\circ}\text{C} \pm 1^{\circ}\text{C}$ in a forced air oven till a constant weight is reached between 5 minutes of drying. Total solids content of milk was the weight of dried milk residue and was expressed as a percentage of original milk weight. The percent moisture content was calculated using the below formula:

$$\frac{(Initial\ weight - Final\ weight) \times 100}{Initial\ weight}$$

And the percent total solid was calculated as $(100 - \% \text{ Moisture content})$. This method is from AOAC Official method 990.20, AOAC 2000

7. *Fat Content*

Fat was extracted using Mojonnier method where a mixture of ethers was used to extract fat from known weight of milk. Then the ether extract was decanted into dry weighing dish, and ether was evaporated. Extracted fat was dried to constant weight. Result was expressed as a percentage of fat by weight (AOAC Official method 989.05, AOAC 2000). The samples were analyzed in duplicates. The percent fat was calculated using the below formula:

$$\frac{((\text{Weight of dish} + \text{fat}) - \text{Weight of dish}) \times 100}{\text{Weight of milk used for test}}$$

8. *Somatic Cells*

Somatic cells were measured using a Delaval Somatic Cell Counter DCC. Milk sample was filled in a cassette and inserted in the Delaval Somatic Cell Counter DCC. The cassette has a DNA specific fluorescent reagent that stains the nuclei. Then a digital camera counts the stained nuclei while taking picture of the somatic cells' nuclei one by one. The readings were then displayed as the number of cells per micro liter of milk (Delaval cell counter DCC manual). The samples were run in triplicates.

9. *pH Measurement*

The pH of milk was measured in duplicates by using an Orion pH meter (model 410). Each time the pH meter was calibrated with buffers pH 4 and pH 7, and the slope was always 98% to 100%.

10. Total Calcium

Total calcium (%) was measured in duplicates according to the method by Metzger *et al.* (2000a), followed by Varian 55B Atomic Absorption Spectrophotometer. A sample size of 0.75 gram of milk was added to 29.25 gram of 40% trichloroacetic acid. After 30 minutes, the mixture was filtered through Whatman Number 541. Ten grams of filtrate was added to 9.6 grams of distilled water and 0.4 gram of 5% lanthanum oxide. This final extract was then aspirated into Varian 55B Atomic Absorption Spectrophotometer fitted with a calcium lamp for calcium determination.

C. Analysis of Cheese Parameters

One block of 10 lb cheese sample was analyzed on the fifth day after manufacture. If the cheese samples arrived early, the cheese was stored at 3.3⁰ C before analysis. All the cheese samples were brought to a core temperature of 3.3⁰ C before sampling for analysis. The analysis carried out on the cheese on a biweekly basis is described below.

1. Total Nitrogen

Total nitrogen (%) was measured in duplicates according to AOAC official method 920.123, AOAC (2000). Two grams of cheese were taken and analyzed using Kjeldhal method, which follows the same procedure as the total protein determination of milk (see B. 1).

2. Water Soluble Nitrogen (WSN)

Water soluble nitrogen was extracted by homogenizing cheese with distilled water in the ratio of 1:2. The homogenized mixture was incubated at 40⁰ C for one hour. Then the mixture was filtered using Whatman filter paper No. 1 (Kuchroo & Fox, 1982). Five

grams of the obtained filtrate were analyzed in duplicates for nitrogen content using Kjeldhal. The procedure followed in analysis of the filtrate was similar to that of total protein determination of milk (see B.1).

3. *pH Measurement*

The pH of cheese was measured in duplicates on 1:1 slurry of the cheese made with distilled water. The pH was measured using Orion pH meter model 410.

4. *Moisture Content*

The moisture content of cheese was measured in duplicates using vacuum oven method (AOAC Official Method 948.12, AOAC 2000). Two to three grams of cheese were weighed in a flat –bottomed metal dish, and the dish was weighed (initial weight was noted). The dish was dried to constant weight in a vacuum oven at 100⁰ C. The dishes were removed and placed in a desiccator, cooled, and weighed (final weight). The percent moisture content was calculated using the below formula.

$$\frac{(Initial\ weight - Final\ weight) \times 100}{Initial\ weight}$$

5. *Fat*

The fat content of cheese was analyzed in duplicates using the Babcock method by Wehr & Frank (2004). Ten ml of hot water were pipetted into a Babcock bottle containing 9.00 grams of cheese. The sample was thoroughly mixed and about 15 ml of sulfuric acid was added in three ml aliquots in intervals. The bottle was mechanically shaken till the lump of cheese was broken down. Then, the bottle was filled with hot water and centrifuged to separate out the fat. The bottle was then incubated in a water bath at 60⁰ C, and the

readings that marked read on the bottle corresponded to the percentage of fat in the cheese.

6. *Salt Content of Cheese*

The salt content of cheese was measured in duplicates using Nelson Jameson Chloride Analyzer M 925. Approximately five grams of the sample were measured and added to a blender jar. Taking into account the moisture of the cheese, distill water was added to yield a total of 100 grams.

$$\text{Amount of water used} = 100 - (\text{Sample weight} \times \% \text{ Moisture in sample}).$$

The mixture was blended at a medium speed for 30 seconds. Then, the mixture was filtered using Whatman #1 filter paper. The chloride analyzer was calibrated according to the owner's manual. Then, 250 µl of the filtrate was added and titrated using the chloride analyzer, which provided the results in mg/l. The obtained reading was multiplied by 0.4 to get the percentage of salt in the cheese sample. Calibration of the machine and sample analysis was done according to Arnold (2008a) and the Nelson Jameson Chloride Analyzer M 925 manual

7. *Fat in Dry Matter (FDM)*

FDM (%) was calculated using the formula

$$\frac{\% \text{ Fat of cheese}}{\% \text{ Total solids in cheese}} \times 100$$

8. *Total Calcium in Cheese*

Total calcium (%) was measured in duplicates according to the method described by Metzger *et al.* (2000a). A sample size of 1.00 gram of cheese was blended with 30 gram

of 40% trichloroacetic acid using a blender for 30 seconds. After 30 minutes, the mixture was filtered through Whatman Number 541. One gram of the obtained filtrate was added with nine grams of 40% trichloroacetic acid, 9.6 grams of distilled water, and 0.4 gram of 5% lanthanum oxide. This final extract was then aspirated into Varian 55B Atomic Absorption Spectrophotometer fitted with a calcium lamp for calcium determination.

9. Water Soluble Calcium in Cheese

The water soluble calcium in cheese filtrate (%) was measured in duplicates according to the method described in Metzger *et al.* (2000a) and Metzger *et al.* (2000b). Five grams of cheese were blended with 50 grams of distilled water at 60⁰ C for 30 seconds using a blender. The cheese filtrate was then filtered through Whatman No. 1 filter paper (Metzger *et al.* 2000b). A final extract was prepared using the obtained filtrate as described by Metzger *et al.* (2000a) (except one gram of cheese filtrate was used to prepare the final extract instead of 0.75 gram of milk (see B.10)). The extract was then aspirated into Varian 55B Atomic Absorption Spectrophotometer fitted with a calcium lamp for calcium determination.

10. Texture Attributes of LMPS Mozzarella

10. a. Textural Profile Analysis (TPA)

Ten cubes of 2 cm x 2 cm x 2 cm were cut from different locations in a block of LMPS Mozzarella. Then, the cubes were covered and conditioned in the refrigerator for two hours followed by 30 minutes in room temperature. Five cubes were used to measure hardness, cohesiveness, springiness, and chewiness by using a TAX-T2 Texture Analyzer, Texture Technologies Corp., Scarsdale, NY. The cheese sample preparation and parameters were set in the TAX-T2 Texture Analyzer are according to procedure

given by Arnold, 2008b. The analyzer is hooked up with a computer on which the following parameters are set up.

Table 8. Specific settings selected for TPA with the TA-XT2 texture analyzer.

Parameter Selected	Settings
Test Mode	T.P.A
Pre Test Speed	1.2 mm/s
Post Test Speed	1.2 mm/s
Test Speed	2 mm/s
Distance	10 mm
Compression	50%
Time	5 s
Force	5 g

The computer generated Force vs. time profile is shown in Figure 27.

Figure 27. The texture profile analysis curve for cheese using TAX-T2 texture analyzer (Adapted from TTC Texture Technologies, 2009).

Definition of Texture Parameters and Physical Definitions

Hardness

The force required to compress a cheese between the molar teeth or between the tongue and palate to a given deformation. The hardness was calculated as the peak force of the first compression of the product (peak of the first curve in Figure 27) (TTC Texture Technologies, 2009).

Cohesiveness

According TTC texture technologies website (2009) cohesiveness is “the extent to which a cheese can be deformed before it ruptures.” Cohesiveness is basically how good the cheese withstands a second deformation relative to the first deformation. It was measured as the area of work during the second compression divided by the area of work during the first compression (Refer to Area 2/Area 1 in Figure 27) (TTC Texture Technologies, 2009).

Springiness

Springiness is defined as the extent to which the cheese physically springs back after it has been deformed. The cheese springs back to an extent after the first compression, so the wait time between the first and second compression can be relatively important. In some cases, a long wait time will allow a product to spring back more than it might under the conditions being researched.

Springiness was measured as the distance of the height of the cheese on the second compression (Length 2), divided by the original compression distance (Length 1) (Refer Length 1 and 2 in Figure 27) (TTC Texture Technologies, 2009).

Chewiness

The length of time or the number of chews required to masticate a cheese ready for swallowing. Chewiness is a product of hardness, cohesiveness and springiness (TTC Texture Technologies, 2009).

10. b. Aggregation Index (AGI)

Aggregation index (AGI) is an empirical test to measure the matting behavior of cheese based on its ability to penetrate down through a stack of vibrating sieves of decreasing mesh size (Kindstedt *et al.*, 2004). The procedure followed to measure AGI is adapted and modified version given in Kindstedt *et al.*, 2004. A cheese sample of 500 grams was shredded from a block of cheese using Univex Shredder (Salem, NH). From the same block, another 500 grams of cheese was cut into 2 cm x 2 cm x 2 cm cubes. The temperature of cheese during the process was maintained at $3.3^{\circ}\text{C} \pm 1^{\circ}\text{C}$. Then, the cubes and shreds were placed in the top most sieve in a stack of sieves that had been arranged in a decreasing sieve size from top to bottom. The sieve sizes used were 6.35 mm, 5.6 mm, 4.74 mm, 3.35 mm, 2.36 mm, 1.00 mm and pan. The stack of sieves along with cheese was shaken in a mechanical shaker for 20 seconds. Sticky cheese that matted excessively was retained by larger sieves whereas cheese that remained free flowing penetrated to the bottom of the stack. Larger AGI indicated that the cheese matted excessively and vice versa (Kindstedt *et al.*, 2004).

The aggregation index value of mozzarella cheese was calculated as a weighted average of sieve size x mass of cheese retained by each sieve divided by the total amount of cheese i.e.,

$$AGI = \sum \frac{\text{Sieve size} \times \text{mass retained by each sieve after shaking}}{\text{total amount of cheese placed in the top most sieve before shaking}}$$

10. c. Percentage Loss during Shredding

A block of 2 pounds of cheese was shredded in a Univex Shredder (Salem, NH). The temperature of the cheese during shredding was maintained at $3.3^{\circ}\text{C} \pm 1^{\circ}\text{C}$. The percentage of cheese lost in shredder is calculated using the below formula.

$$\frac{\text{Initial weight of cheese} - \text{Weight of cheese after shredding}}{\text{Initial weight of cheese}} \times 100$$

D. Statistical Analysis for Modeling the Seasonal Variation

Seasonal variation of components in milk were analyzed using a multiple linear regression model equivalent to a basic single cosinor model ($Y = m + A \cos(t - \Phi) + \varepsilon$) with sine and cosine of week as predictors

$$y = \beta_0 + \beta_1 \cos(\text{time}) + \beta_2 \sin(\text{time}) + \varepsilon$$

Where y was the milk component and time was week between 1 and 52. Time was converted into an angular variable by multiplying it by $2\pi/52$, β_0 is the intercept, β_1 and β_2 are the slopes of the cosine and sine functions respectively, and ε is the residual error term.

Peck & Devore, 2008, stated the basic assumptions of the multiple linear regression model as follows:

1. “The distribution of ε at any particular x value has a mean or average of zero.
2. The standard deviation of ε is the same of any particular value of x.
3. The distribution of ε at any particular x value is normal.

4. The random deviations $\varepsilon_1, \varepsilon_2, \varepsilon_3, \dots, \varepsilon_n$ associated with different observations are independent of one another”.

Lacroix *et al.* (1996) used a second order periodic oscillatory Fourier model to analyze the seasonal variations in casein to total protein and casein to true protein ratios. The Fourier model is similar to the multiple linear regression model used in this study and is as follows:

$$y = ao + \sum_{i=1}^{n=1} ai \cos(i\omega t) + bi \sin(i\omega t)$$

Where y was the response variable, t was the time (predictor), ω was the frequency and $\omega=360/12=30^0$, ai and bi were the regression coefficients, and ao was the intercept or the mean value for y.

Statistical analysis was done using Minitab version 17.0. The assumptions were checked using residual plots. To meet the model assumptions, the residuals were checked to have no systematic patterns.

1. Interpretation of R-squared value in Multiple Linear Regression Model

Coefficient of determination (R-squared value) is the statistical measurement that indicates how well the data fits the observed data. Frost (2013) defined R-squared value as the “percentage of the response variable variation that is explained by regression model.”

$$R\text{-squared} = (Explained\ variation)/(Total\ variation)$$

Frost (2013) explained that R-square valued lies between zero and 100%, where 0% and 100% means that the model explains no variability and all variability, respectively, of the

response data around its average value. The higher the R-squared indicates that the model fits the data better.

2. Interpretation of the p-value in Multiple Linear Regression Analysis

The null hypothesis (H_0) in linear regression analysis is that the R-squared is equal to zero indicating that the model does not explain any variability in the response. The alternative hypothesis (H_a) is that the model explains at least some variability in the response. A p-value < 0.05 indicates that the H_0 can be rejected. This would show that the model explains some variability in the response (y value). The p-values for individual predictors can also be examined to test whether each predictor variable is significantly associated with the response, after controlling for all other predictors in the model. A p-value < 0.05 for such a test on an individual predictor indicates that it is a meaningful addition to the model because changes in the x value were associated with changes in the response variable (y). However, a p-value > 0.05 indicates that the null hypothesis cannot be rejected and hence changes in the predictor were not related with changes in the response variable (Frost, 2013).

E. Correlation Studies

Correlation studies were carried out between milk components, cheese composition, cheese texture, and the milk composition and the corresponding week cheese composition and textural aspects using Minitab version 17.0. Correlations, except between milk composition and cheese composition and texture, were done for the 18-month period. The correlation analysis between milk composition and cheese composition, and texture was done only for a ten-month period because from May 2009 changes in the production

protocol of cheese were done to increase the firmness of cheese, thereby reducing the loss during shredding. The *p-values* and Pearson correlation coefficient were noted.

1. Interpretation of the Correlation or Pearson Correlation Coefficient

The correlation coefficient measures the strength of a linear relationship between two variables. The correlation coefficient is always between -1 and +1. The closer the correlation is to ± 1 , the closer the two variables are to having a perfect linear relationship (Peck & Devore, 2008). The correlation coefficient interpreted by Ratner, (<http://www.dmstat1.com/res/TheCorrelationCoefficientDefined.html>) is as follows:

- 1.0 to -0.7 - strong negative association
- 0.7 to -0.3 - moderate negative association
- 0.3 to +0.3 - little or no association
- + 0.3 to + 0.7 - moderate positive association
- +0.7 to +1.0 - strong positive association

F. Ripening Studies

Two blocks of 10 lb vacuum packed cheese samples were subjected to ripening studies.

One block was ripened at 3.3⁰ C and the other at 8.9⁰ C for 21 days from the date of manufacture. After 21 days, both the cheese blocks ripened at different temperatures were brought to a core temperature of 3.3⁰ C before carrying out analysis.

The ripened samples were analyzed for pH, WSN, and textural studies, which include Texture Profile Analysis (TPA), % loss shredder, and aggregation index.

1. Statistical Analysis in Ripening Study

The parameters of the cheese, ripened at different temperatures were compared using paired t-test analyzed using Minitab version 17.0. This test determines whether the parameters' means differ from each other in a significant way under the assumptions that the paired differences are independent and identically normally distributed. The data was analyzed using Minitab 17.0 and assumptions were checked using a normality plot. If the p-value from the normality plot is greater than 0.05, it means that the data may follow a normal distribution.

2. Urea PAGE

Proteolysis of the samples ripened at 3.3⁰ C and 8.9⁰ C was assessed by comparing with the fresh samples.

2. a. Sample Preparation

For ripening study, fresh cheese sample C08090110 (Jan 09) and 21 day cheese sample (same lot C08090110) ripened at 3.3⁰ C and 8.9⁰ C were taken and subjected to analysis. Sodium caseinate was used as reference standards for both the studies. The samples were prepared according to the procedure consolidated by Arnold, 2008c. A ten mg sample was mixed with 1.0 ml of 1X sample buffer (0.75 g tris base, 49 g urea, 0.4 ml conc. HCl, 0.7 ml beta mercaptoethanol, 0.15 g bromophenol blue, and distilled water). For standard, five mg of sodium caseinate was mixed with 1.0 ml of 1X sample buffer. The samples were incubated in a water bath for 30 minutes.

2. b. Gel Preparation

10% Poly acrylamide resolving gels were prepared by making a solution of 5.0 ml of 40% poly acrylamide, 14.0 ml of separating gel buffer (32.15 g of tris (hydroxymethyl) methylamine, 150 g urea, 2.86 ml of concentrated HCl which is made up to 500 ml with water (pH 8.9)), 10 µl TEMED, 75.2 µl of ammonium persulfate, 0.100 g of N, N' methylene bisacrylamide and 1.0 ml of distilled water. The Bio-Rad Mini Protean gel spacer plates were assembled according to the manufacturer's instruction on the Mini-PROTEAN 3 Multi-Casting Chamber. The prepared solution was then carefully pipetted in between the plates until they were 3/4th full. Then, distilled water was pipetted on top and the gel was allowed to polymerize for 30 minutes. Once the resolving gel was polymerized, the water layer was poured off, and the plates were filled with 4% acrylamide stacking gel solution (9.0 ml of stacking buffer (4.15 g of tris (hydroxymethyl) methylamine, 150 g urea, 2.2 ml of concentrated HCl which is made up to 500 ml with water), 10 µl TEMED, 60 µl of ammonium persulfate, pH 7.6), 0.1 ml of SDS ml, 1 ml of 40% acrylamide, 50 micro L of 10% APS, 5 micro L of TEMED). A Mini Protean Comb was inserted to generate the wells and was allowed to polymerize for 15 minutes. The gel was prepared according to the procedure consolidated by Matt R. Arnold, 2008c.

2. c. Running Gel

The above prepared gel plates were removed from Mini-PROTEAN 3 Multi-Casting Chamber and were mounted in XCell Surelock™ Mini-Cell chamber (EI0001). The chamber was then filled with electrode buffer (3.0 g tris base, 14.6 g glycine, and 1000 ml of distilled water, pH 8.4). The cell unit was connected to a Bio-Rad power unit (PAC300, BioRad) and was pre run for 15 minutes at 150 V. Then, the gel was loaded

with 4 μ l of the samples and standards. The gel was run at 100 V for 90 minutes or until the tracking buffer line reached the end of the plate. The gel was removed from the cell unit and spacer plates. The gel was immersed in a staining solution (2.5 g Coomassie Brilliant Blue R -250) and incubated in this solution overnight on a rotary incubator (Max 2000 E-class, Barnstead, IA) at room temperature. After 24 hours the gels were removed from staining solution and de-stained with several changes of distilled water. The gel was prepared according to the procedure consolidated by Arnold, 2008c.

IV. RESULTS AND DISCUSSION

The results are summarized in parts and are briefly discussed below. In the first part of the study, the seasonal variation and correlation between milk components in weekly bulk milk samples (76 samples) collected from a plant in Central Valley, California were studied for an 18- month period. Secondly, the thirty-three low moisture part skim mozzarella samples collected on a biweekly basis from the same plant during the same period was analyzed for any variation in their composition and un-melted textural properties (shreddability, aggregation index, % loss in shredder, and TPA characteristics). Correlation analyses were done among the cheese composition, cheese textural characteristics', and between cheese composition and texture. In the third part, the association between milk composition and cheese composition and texture were studied using correlation analysis. Finally, the association between ripening temperature and the cheese pH, water soluble nitrogen, and un-melted textural properties of LMPS Mozzarella was studied.

This was an observational study, and hence no cause and effect conclusions can be drawn as there are a lot of variables that were not controlled.

A. Analysis of Milk

1. Milk Composition

The average data for milk composition for seventy-six bulk milk samples obtained from silos on a weekly basis from a plant in Central Valley, California from July 2008 to December 2009 are shown in Table 9.

Table 9. Descriptive statistics for milk composition:

Milk components	Sample size	Mean	SD	Min.	Max.
Total protein (%)	76	3.29	0.115	3.04	3.48
Total nitrogen (TN) (%)	76	0.52	0.173	0.48	0.55
True protein (%)	76	3.13	0.093	2.89	3.31
True nitrogen (%)	76	0.49	0.015	0.45	0.52
Non-Protein Nitrogen (NPN) (%)	76	0.025	0.0042	0.016	0.035
Non-Casein Nitrogen (NCN) (%)	76	0.11	0.008	0.089	0.128
Casein nitrogen (%)	76	0.41	0.021	0.36	0.44
Casein nitrogen (CN) (%)	76	2.63	0.134	2.32	2.85
Casein/ total protein (CP)	76	0.799	0.018	0.76	0.829
Casein/true protein (CPt)	76	0.84	0.02	0.79	0.881
Fat (%)	74	2.56	0.65	1.15	3.87
Total solids (%)	75	11.54	0.680	9.65	12.87
Total calcium (%)	76	0.11	0.0058	0.09	0.12
pH	76	6.66	0.039	6.53	6.71
Somatic cells / μ L	76	262.61	79.22	122.00	508.67

The mean $\pm$ standard deviations of total protein and true protein for the bulk milk samples were $3.29\% \pm 0.12\%$ and $3.13\% \pm 0.09\%$, respectively (Table 9). The total protein of milk was close to the mean total protein values reported by Nickerson (1960), Bruhn and Franke (1987), and Franke *et al.* (1987) in California milk. Other researchers reported a range of total protein content of 3.32% to 3.02 % for milk produced by Holstein cows (Bruhn & Franke, 1977). The range 3.04% to 3.48% for total protein of milk in this survey falls close to that of milk produced from Holstein cows.

The mean values of non-protein content and non-casein nitrogen, 0.025 % and 0.1056 %, respectively (Table 9), were similar to the values reported by Lacroix *et al.* (1996) in Quebec milk. However, Nickerson (1960) reported a NPN with a mean $\pm$ standard deviation of $0.0314\% \pm 0.0016\%$ in California milk, which was higher than what was observed in this study.

The casein fraction represented $79.91\% \pm 1.7\%$ of the total protein (CP) and $83.97\% \pm 2.14\%$ of true protein (CPt) (Table 9), which was in range with the values (CP 78.92 %) and CPt (83.12%) reported by Lacroix *et al.* (1996) in Quebec milk samples. However, the mean CP value of 79.91% is higher than the mean value of 76.71 % and 76.4% reported by Nickerson (1960) and Franke *et al.* (1987), respectively, in milk from California. The larger range of standard deviation observed for CPt compared with CP indicates that casein varied more to true protein than that of total protein. This larger variation of casein may due to variation of NPN along with TN. As standardization of milk is done with a target of casein to fat ratio, hence a variation of casein or fat in milk will affect the hardness of cheese unless the moisture content of the curd is increased or decreased to accommodate the changes in hardness (Gunasekaran and Ak, 2000a).

In this study, the average $\pm$ standard deviation of fat content in bulk milk samples was found to be $2.56\% \pm 0.66\%$ (Table 9). The mean value of fat content was low compared to mean values observed 3.82% and 3.67% by Nickerson (1960) and Bruhn & Franke (1991), respectively, in California milk. The minimum was found to be as low as 1.15% and high as 3.87% (Table 9). The milk samples were heated to 38°C before analysis to allow fat to get distributed evenly. However, the variation of milk fat was very high with a $\pm 0.66\%$ standard deviation during the entire sampling period.

The average value for total solids was 11.543% with a standard deviation of $\pm 0.680\%$ (Table 9), which was close to $11.18\% \pm 1.432\%$ reported by Ozrenk & Inci (2008).

Some researchers reported the average total solids content of milk as 12.5% (Walstra *et al.*, 2006). The total solids like any other milk components were found to vary according

to breed and other factors (Nickerson, 1960 and Ozrenk &Inci, 2008). The standard deviation of total solids was $\pm 0.68 \%$ (Table 9), which was close to that of fat $\pm 0.66 \%$. The mean $\pm$ standard deviation of total calcium in silo milk samples was found to be $0.11\% \pm 0.006 \%$ (Table 9), which was quite in range with the values reported by Metzger *et al.* (2000a) (0.114%) and Fox and Cogan (0.12%). Calcium plays an important role in rennet coagulation and firmness of milk coagulum during the cheese making process. Also in mozzarella, the inherent casein associated calcium and phosphate influences the ability of curd to plasticize and form unidirectional fibrous structure (Kindstedt *et al.*, 1999).

The average somatic cell count (SCC) along with the standard deviation in the bulk milk samples for the 18 month period was 262.61 ± 79.22 cells/ μ l, which was within the range for healthy cows ($SCC < 750,000$ cells/ml). Barbano *et al.* (1987) reported that the milk from sub clinical mastitis has $SCC \geq 500,000$ cells/ml and this could affect the quality of milk, in turn affecting the yield and quality of cheese. The maximum value during the study obtained was 508,000 cells/ml. This somatic cell count greater than 500,000 cells/ml was found only for two samples (May 2009 and October 2008 - Appendix 1). Other than the two milk samples, most of the milk samples had somatic cell count below 500,000 cells/ml, which indicates that all the milk samples were free from mastitis. The average $\pm$ standard deviation of pH for the milk samples was found to be 6.66 ± 0.039 , which is within the normal range of 6.7 (Kelly, 2000a).

2. Correlation of Milk Composition

The data of milk components collected from weekly milk samples for 18 months was subjected to correlation analysis in Minitab version 17.0 to see if the variation of the components had an association with each other. The results are summarized in Table 10.

Table 10. Correlation between milk components

	TN	Tr N	NPN	NCN	CN	CP	CPt	Fat	TS	Total Ca
Tr N	0.975 0.000									
NPN	0.601 0.000	0.408 0.000								
NCN	0.081 0.486	0.025 0.830	0.243 0.034							
CN	0.945 0.000	0.920 0.000	0.573 0.000	-0.181 0.118						
CP	0.652 0.000	0.635 0.000	0.393 0.000	-0.547 0.000	0.864 0.000					
CPt	0.703 0.000	0.626 0.000	0.638 0.000	-0.399 0.000	0.882 0.000	0.958 0.000				
Fat	-0.175 0.134	-0.162 0.164	-0.134 0.253	-0.132 0.258	-0.126 0.283	-0.022 0.848	-0.052 0.655			
TS	-0.021 0.858	-0.023 0.847	-0.005 0.967	-0.140 0.232	0.047 0.689	0.139 0.236	0.121 0.303	0.932 0.000		
Total Ca	0.502 0.000	0.462 0.000	0.404 0.007	0.119 0.325	0.487 0.000	0.351 0.003	0.407 0.000	-0.114 0.350	-0.035 0.776	
pH	-0.060 0.609	-0.078 0.500	0.037 0.751	0.202 0.081	-0.099 0.397	-0.141 0.225	-0.106 0.360	0.041 0.728	-0.002 0.985	-0.105 0.389
SCC/ μL	0.010 0.934	-0.007 0.950	0.065 0.574	-0.156 0.179	0.064 0.583	0.136 0.241	0.138 0.233	0.047 0.687	0.110 0.347	0.017 0.892

Cell content: Pearson correlation coefficient on top and *p*-value on the bottom. The yellow markings indicate significant correlation at the 5% significance level.

The total nitrogen (TN), non-protein nitrogen (NPN), casein nitrogen (CN), casein to total protein ratio (CP), casein to true protein ratio (CPt), and total calcium had positive correlation ($r > 0.3$ and $p\text{-value} < 0.05$) with each other (Table 10). However, the non-casein nitrogen (NCN) did not significantly correlate with other nitrogen fractions and total calcium (Table 10). The total solids had a strong positive correlation ($r > 0.7$, p -

value < 0.05) only with the fat and not with the other components (Table 10, Figure 28). Hence the variation of total solids followed similar pattern. Also, any error in analysis of fat can be ruled out because the variation was also seen in total solids.

Figure 28. Variation of total solids with respect to total nitrogen, fat, total calcium and casein nitrogen.

3. Variation of Milk Composition

All the milk components (total protein, true protein, casein, casein/total protein, casein/true protein, fat, total solids, total calcium, pH, and somatic cells) were plotted versus time (July 2008 to December 2009) to see if there was any variation over the 18 months for the seventy-six samples. The curves in the graphs (Figure 29, Figure 30, Figure 31, and Figure 32) just trace the trend and are not the fitted curve of the linear regression model used in analysis.

Figure 29. Variation of Nitrogen fractions (total nitrogen (TN), non-protein nitrogen (NPN), and non-casein nitrogen (NCN)) in milk

Figure 30. Variation of casein, casein/total protein ratio, and casein/true protein ratio

Figure 31. Variation of fat, total solids, and total calcium in milk

Figure 32. Variation of pH and somatic cell count

From Figure 29, Figure 30, and Figure 31, a cyclic pattern was observed for total nitrogen, non-protein nitrogen, casein, casein to total protein ratio (CP), casein to true

protein ratio (CPT), and total calcium. Higher total nitrogen was observed during winter months (November – February) (Figure 29) and lowest ones during summer (May – August). Bernabucci *et al.* (2002), Ozrenk & Inci (2008), Bertocchi *et al.* (2013), Verdi *et al.* (1987), and Lacroix *et al.* (1996) found similar variation of total nitrogen in different parts of world. In California, Nickerson (1960), Bruhn & Franke (1991), and Bruhn & Franke (1977) observed that the fat and protein content were significantly lower from May through August and higher from November through February. However, Frank *et al.* (1987) reported that they did not observe any seasonal variation of total protein in California milk in 1983.

The non-protein nitrogen (NPN) was found to be high during winter (October 2008 - February 2009) (Figure 29) and lowest values were observed during summer (May – August). Similar seasonal variation of NPN was observed by Nickerson (1960) and Verdi *et al.* (1987). On the contrary, Lacroix *et al.* (1991) observed a different trend; the NPN remained constant from January to April then increased until September and decreased thereafter. In the later part of this study, from October 2009 to December 2009, there was no increase of NPN and it remained relatively constant (Figure 29). Laben (1963) mentioned in his review that in a three-year study in California solid not fat varied for two years (1960, 1962), but in 1961 no seasonal variation was observed. A similar variation (higher for one winter and no increase for the next year) was observed for NPN. Hence, an extended study would have given more information on this variation.

The non-casein nitrogen variation did not show any particular pattern in the scatter plot (Figure 29). In contrast to this observation, Lacroix *et al.* (1991) observed a seasonal

variation (high for winter months and low for summer months) of non-casein nitrogen in their study.

The casein (calculated TN - NCN) showed a cyclic pattern with highs during the winter months and low values during the summer months (Figure 30). In spite of NCN being almost constant, casein nitrogen varied due to the variation contributed by the total nitrogen. A similar seasonal variation of casein was reported by Nickerson (1960) in California. On the other hand, Franke *et al.* (1987) did not find any significant seasonal variation of casein in California milk in 1983.

Casein to total protein ratio (CP) and casein to true protein ratio (CPt) also followed a similar variation as that of the total nitrogen and casein. Lacroix *et al.* (1991) in their study found that CP and CPt values were high during the winters and spring. In their study, they compiled the CP values and variation by various researchers, and most of them showed that the high values were in winter and spring, and low ones were in summer. However, Franke *et al.* (1987) reported that in California milk, there was no significant variation of CP during their survey in 1983.

Total calcium also had a cyclic pattern with highs in January to April (winter – spring months) and low in May – July (summer) and again peaking up around October (Figure 31). Highest level calcium of 0.12 % was observed in January 09 month, and a low level of 0.09% was observed in June 2009. Nickerson (1960) also observed similar seasonal variation of total calcium in milk during 1955 to 1958 in California. In this study, the total calcium variation followed a similar trend to that of total protein (Figure 29, Figure 30). This observation was also in line with that of Nickerson (1960) who noticed that

milk that had the lowest average protein content also had the lowest calcium content and milk with the highest average protein had the highest calcium content.

Even though fat and total solids varied considerably between samples with a standard deviation $\pm 0.65\%$ and $\pm 0.68\%$ respectively (Table 9), there was no particular high and low values for any season. Their variation was random as shown in Figure 31. In contrast, a lot of researchers reported low fat in milk during summer and highs in winter (Ozrenk & Inci 2008; Verdi *et al.* 1987; Pavan & Gavan 2011; Heck *et al.* 2009; Larsen *et al.* 2010). Also, Heck *et al.* (2009) and Larsen *et al.* (2010) noticed that milk fat varied more with season compared to other components. They attributed this variation to change in diet during different seasons and noticed that fat varies to a greater extent with change in diet than other milk components. In California, Nickerson (1960), Bruhn & Franke (1991), and Bruhn & Franke (1977) observed that the fat content was significantly lower from May through August and higher from November through February. However, Frank *et al.* (1987) reported that he did not observe any seasonal variation of total protein in California milk in 1983. Ozrenk & Inci (2008) and Nickerson (1960) found that the total solids also varied seasonally, with high levels in winter and low values in summer. The somatic cell count and pH variation did not show any seasonal patterns (Figure 32). However, Bertocchi *et al.* (2013) and Dohoo & Meek (1982) have reported that the somatic counts vary seasonally (lowest counts during winter and the highest counts during the summer). They attributed the increase in somatic cells due to change in diet and heat stress.

4. Modeling of Milk Components for Seasonal Variation

The results of the multiple linear regression (single cosinor) model, used to estimate the seasonal variation in milk components, is given below. More complete output for this statistical analysis and the residual plots are in Appendix 2.

Table 11. Regression analysis results of milk components

Predictors	t-values and p-values		Fit for the whole model	
	Sine (week)	Cosine (week)	F –values and p-values	R ² values (%)
Total nitrogen	-3.73 0.009	10.69 0.000	63.40 0.000	63.5
True nitrogen	-5.00 0.000	9.48 0.000	56.6 0.000	60.80
Casein nitrogen	-5.55 0.000	12.04 0.000	86.71 0.000	70.4
Casein/Total Protein (CP)	-5.29 0.000	7.60 0.000	42.12 0.000	53.58
Casein/True Protein (CPT)	-3.38 0.001	8.51 0.000	41.38 0.000	53.13
Total Calcium	2.01 0.088	9.59 0.000	48.86 0.000	59.32
Non- casein nitrogen	2.31 0.023	-0.24 0.813	2.70 0.074	6.88
Non- protein nitrogen	2.62 0.011	0.860 0.000	27.29 0.000	42.78
Fat	-1.95 0.055	-0.04 0.971	1.91 0.156	5.04
Total solids	-2.73 0.008	1.26 0.211	4.50 0.014	11.11
pH	0.150 0.883	-0.59 0.554	0.19 0.831	0.51
Somatic Cells/μl	-0.57 0.572	0.08 0.938	0.16 0.850	0.45

After modeling these components with the linear regression model with cosine (week) or sine (week) as predictors, p-values < 0.05 for both or one of the predictors was obtained for total nitrogen, true protein, non-protein nitrogen, CP, CPT, and total calcium. The R²

>50% (Table 11) for total nitrogen, true nitrogen, casein nitrogen, CP, CPt, and total calcium shows that more than 50% of the data fitted well in the model. The R^2 value for non- protein nitrogen was 42.78% (Table 11), which shows that 42.78% of data fitted well in the model. These findings indicate that significant seasonal variations in total nitrogen, true nitrogen, casein, non-protein nitrogen, total calcium of casein/total protein, casein/true protein, and non-protein nitrogen in silo milk during the sampling period (July 2008 – December 2009) can be explained using the linear regression model with time as an angular variable. The model showed less than 12% variation for pH, fat, total solids, and somatic cells; thereby, no significant seasonal variation could be explained for these components as the p-values > 0.05%. The hottest months in Central Valley, California, during 2008 and 2009 were from June to September with temperatures reaching above 100⁰ F (refer Appendix 3). In addition, these years (2008 and 2009) had very little precipitation (refer Appendix 4). Hence, the variation of total nitrogen, casein nitrogen, non-protein nitrogen and total calcium can be attributed to longer photoperiods and high temperatures (above 100⁰ F), which causes less food intake due to heat stress during the summer months compared to the winter months. Therefore, the accuracy of fixed casein/total protein ratio often used in dairy plants to estimate casein content of milk throughout different seasons is questionable. Thus, it may be concluded that estimating casein from true protein or casein from total protein while accounting for monthly variations of total nitrogen and non-protein nitrogen should be more accurate.

Calibration of infra-red instruments has been traditionally done using total nitrogen by Kjeldahl as the reference (Barbano, 1994). A systematic bias error of 0.03% - 0.06% total protein can be created between infra-red milk analyzers as a result of differences in the

mean non-protein nitrogen (NPN) as a percentage of total nitrogen between calibrations. In addition, variation in NPN as a percentage of total protein can cause systematic differences as large as 0.1% protein at specific protein concentrations (Lacroix *et al.* 1996). Now days, mid-infra-red transmission spectroscopy is used in measurement of milk composition by Dairy Herd Improvement Association (DHIA) and payment testing laboratories. With the recent developments of technology involving near infra- red analyzer and mid Fourier Transform Infrared analyzers, which use full spectral calibration instead of the traditional calibration, minor components like urea can be measured, and the interference of NPN in calibration can be overcome (Barbano and Wojciechowski, 2012). The components of milk, especially casein, fat, pH, and calcium have a major impact on several aspects of cheese manufacturing, cheese composition and yield (Fox & Cogan, 2004). As seasonal variations for casein and total calcium were observed, a close monitor for these components may be beneficial to improve the quality of cheese.

B. LMPS Mozzarella Analysis

The cheese samples collected from the Central Valley, California, for the sixteen and half - month period (July 2008 - mid November 2009) were analyzed for its composition and texture five days after manufacture. A wait period of five days after manufacture was chosen because newly manufactured cheese has excessive moisture at the surface and within the body of cheese and hence will not shred well. Four to five days of ageing will allow the cheese to absorb the moisture back and thereby the shredding quality improves (Kindstedt *et al.*, 2004; McMahon & Oberg, 2011). The cheeses were stored at 4⁰ C before analysis.

1. Analysis of LMPS Mozzarella Composition

The average composition data for the thirty-three cheese samples collected biweekly from July 2008 to November 2009 from a plant in Central Valley California is given in Table 12.

Table 12. Descriptive statistics for LMPS mozzarella after five days of manufacture

Cheese components	Sample size	Mean	SD	Min.	Max.
Total Nitrogen (%)	33	3.65	0.12	3.45	3.88
Water Soluble Nitrogen (%)	33	0.33	0.04	0.27	0.45
Total Solids (%)	33	51.98	0.60	50.37	52.99
Moisture (%)	33	48.02	0.60	47.01	49.63
Fat (%)	33	21.33	0.71	19.58	22.27
Fat in Dry Matter (%)	33	41.05	1.51	36.943	43.08
Total calcium (%)	33	0.59	0.03	0.50	0.63
Water Soluble Calcium (%)	33	0.27	0.02	0.24	0.33
Salt (%)	33	2.14	0.10	2.36	1.88
pH	33	5.47	0.04	5.41	5.53

The cheese composition (total nitrogen, water soluble nitrogen, total solids, moisture, fat in dry matter (FDM), total calcium, water soluble calcium, salt, and pH) was plotted versus month. The curves in the graphs (Figure 33, Figure 34 and Figure 36) just trace the trend and are not the fitted curve of the linear regression model used in analysis.

Figure 33. Variation of cheese total nitrogen (TN%), water soluble nitrogen (WSN%)

The average total nitrogen content of the cheese sample was $3.6452 \pm 0.12\%$ (Table 12), which has a mean of 22.9% protein. The mean protein content reported by other researchers was 26% (Feeney *et al.*, 2001; Joshi *et al.*, 2004; Banville *et al.*, 2013). However, Guinee *et al.* (2002) reported a lower mean protein content of 23.52% in directly acidified mozzarella, which was close to the average protein content of 22.9% from this study. The total nitrogen content had a maximum of 3.88% (Table 12), which was seen around winter months (December 2008 – January 2009) (Figure 33). The total nitrogen content was low (3.43%) in summer months (July – September 2008, May – July 2009) (Figure 33). As a dip in the nitrogen content was observed during May and June 2009, changes during manufacturing (non-fat dry milk was added) were made to increase protein content in August 2009. The water soluble nitrogen (WSN %) had a mean of 0.33% (Table 12), which was 8.8% of the total nitrogen. The WSN was low during 2008 summer but remained almost constant during the remaining study (Figure 33).

Figure 34. Variation of cheese fat in dry matter (FDM %), moisture (%) and salt (%)

The mean $\pm$ standard deviation for moisture and fat in dry matter (FDM) of LMPS mozzarella was 48.024 ± 0.599 % and 41.05 ± 1.508 %, respectively (Table 12). The percent moisture content and FDM were within the limits for standard of identity of LMPS Mozzarella specified by CFR 133.157. Very high and low levels of salt can decrease protein solubility and affect the melting properties. A 2% salt content is the optimal level as it increases the casein solubility to a sufficient level, thereby giving the required meltability in mozzarella (McMahon & Oberg, 2011). The mean $\pm$ standard deviation of salt content was found to be 2.14 ± 0.10 % (Table 12). From Figure 34, the FDM, moisture content and salt were almost constant during the entire sampling period.

Figure 35. Variation of cheese total calcium (%), water soluble calcium (WSC %), pH

The total calcium had a mean $\pm$ standard deviation of $0.5911 \pm 0.0301\%$ (Table 12). The total calcium levels in cultured low moisture mozzarella were 0.673% (Feeney *et al.*, 2001). However, the total calcium levels in this study were close to that of 0.578% , which was obtained when the milk was pre-acidified to pH 5.8 with citric acid (Metzger *et al.*, 2000a). Even though the protein and calcium levels were close that of the directly acidified low moisture mozzarella, the LMPS Mozzarella in this study was made by adding cultures. The total calcium content was relatively constant during the entire sampling period (Figure 35).

The mean $\pm$ standard deviation for water soluble calcium (WSC) was $0.2713 \pm 0.0181\%$ (Table 12). The WSC % obtained by Metzger *et al.* (2000b) was between 40% -50% of the total calcium. The WSC (%) was measured using the method followed by Metzger *et al.* (2000b), and the average WSC% was found to be 47.36% of the total calcium. The

water soluble calcium levels were low during the fall and winter months and were found to increase during the spring and summer (Figure 35).

The pH and levels of calcium in the curd during the stretching process impacts the ability of the curd to plasticize and stretch. In order to plasticize and obtain the required stretch, a high calcium content curd that is 3.0% of protein is stretched at a higher pH (5.6 – 5.7), and low calcium content of 2.2% of protein is stretched at a lower pH of 5.1 – 5.3

(Kindstedt *et al.*, 2004). The stretching pH usually determines the final pH of the cheese.

The amount of calcium associated with casein available to crosslink the para-casein matrix influences the ability of the curd to plasticize in hot water and reorganize into a unidirectional fibrous structure (Kindstedt *et al.*, 2004; Guinee *et al.*, 2002). The total calcium content in this study was 2.5% of protein content (calculated using the formula - (mean total calcium/mean protein content) * 100), and the average pH was 5.4 ± 0.038 , which was not very low or high. From Figure 35, a slight increase of pH during the fall and winter months compared to the summer months was observed. From Figure 35, the pH was found to vary inversely with WSC (%). In addition, a significant moderate negative correlation ($r = -0.447$ with a p-value of 0.030 (Table 13)) between pH and WSC % indicated that as the pH increased, WSC (%) decreased, and vice versa. The distribution of calcium between the soluble and insoluble states is a function of pH because at lower pH, the calcium moves into the soluble phase (Kindstedt *et al.*, 2004; Guinee *et al.*, 2002; Joshi *et al.*, 2004). The pH of the cheese was found to influence the amount of water soluble calcium and also as a drop in pH and increase in water soluble calcium in May 2009 was observed. As the total calcium of the curd and distribution of the calcium between the soluble and insoluble influences the firmness of cheese and

hence the texture of cheese (Kindstedt *et al.*, 2004), the pH of the finished cheese was increased above 5.45 from August 2009 to prevent the water soluble calcium from decreasing.

Table 13. Correlation between LMPS mozzarella compositions

Cheese parameters	WSN	Total solids	Fat	FDM	Total Ca	WSC	Salt	pH
Total nitrogen	0.299 <i>0.091</i>	0.071 <i>0.696</i>	-0.364 <i>0.041</i>	-0.335 <i>0.041</i>	-0.088 <i>0.639</i>	-0.169 <i>0.364</i>	0.145 <i>0.419</i>	-0.062 <i>0.733</i>
WSN		-0.528 <i>0.002</i>	0.090 <i>0.623</i>	0.294 <i>0.097</i>	-0.063 <i>0.735</i>	0.025 <i>0.894</i>	0.239 <i>0.180</i>	-0.093 <i>0.608</i>
Total solids			-0.163 <i>0.373</i>	-0.294 <i>0.097</i>	-0.205 <i>0.269</i>	-0.03 <i>0.870</i>	-0.210 <i>0.242</i>	0.153 <i>0.395</i>
Fat				0.950 <i>0.000</i>	-0.059 <i>0.756</i>	0.331 <i>0.069</i>	-0.158 <i>0.380</i>	0.178 <i>0.322</i>
FDM					-0.288 <i>0.116</i>	0.304 <i>0.097</i>	-0.075 <i>0.678</i>	0.110 <i>0.544</i>
Total Ca						-0.639 <i>0.000</i>	-0.076 <i>0.683</i>	-0.319 <i>0.080</i>
WSC							-0.028 <i>0.882</i>	-0.447 <i>0.030</i>
Salt								-0.203 <i>0.257</i>

Cell content: Pearson correlation coefficient on top and p- value on the bottom. The yellow markings indicate significant correlation.

2. Textural Analysis of LMPS Mozzarella

The LMPS Mozzarella samples were analyzed for un-melted textural properties such as textural profile analysis (hardness, cohesiveness, springiness, and chewiness), loss in shredder, and aggregation index for the 18-month period. The average textural data obtained during this study is given in Table 14.

Table 14. Descriptive statistics for textural properties of mozzarella after five days of manufacture

Textural Properties	Sample size	Mean	SD	Min.	Max.
Hardness (g)	33	4135	624	3088	5502
Cohesiveness	33	0.51	0.045	0.42	0.66
Springiness	33	0.67	0.049	0.58	0.75
Chewiness (g)	33	1445.5	344.4	852.4	2229.5
Loss in shredder (%)	33	9.71	1.395	7.303	12.76
Aggregation Index	33	6.04	0.22	5.49	6.38

The textural properties were plotted versus month (Figure 36, Figure 37), and correlation between cheese textural parameters and cheese composition (Table 15) and between cheeses' textural properties were done (Table 16). The curves in the graphs (Figure 36, Figure 37) just trace the trend and are not the fitted curve of the linear regression model used in analysis.

Table 15. Correlation between cheese composition and cheese texture

Cheese parameters	Total nitrogen	WSN	Moisture	FDM	Total Ca	WSC	Salt	pH
Hardness	0.876 0.000	0.211 0.238	-0.189 0.292	-0.363 0.038	0.369 0.040	-0.250 0.174	0.083 0.646	0.274 0.122
Cohesiveness	0.322 0.067	-0.093 0.608	-0.188 0.295	-0.312 -0.079	0.355 0.049	-0.403 0.025	-0.081 0.654	0.172 0.340
Springiness	0.405 0.019	-0.179 0.318	-0.353 0.044	-0.436 0.011	0.426 0.017	-0.515 0.003	-0.011 0.950	0.110 0.541
Chewiness	0.782 0.000	0.060 0.740	-0.313 0.076	-0.496 0.003	0.470 0.008	-0.475 0.007	0.020 0.914	-0.280 0.115
AGI	-0.353 0.044	0.018 0.920	0.356 0.042	0.302 0.088	-0.126 0.501	0.194 0.295	0.193 0.281	-0.081 0.653
Loss in shredder	-0.489 0.003	0.151 0.418	0.204 0.272	0.335 0.057	-0.402 0.025	0.471 0.007	-0.100 0.593	-0.238 0.197

Cell content: Pearson correlation coefficient on top and p- value on bottom. The yellow markings indicate significant correlation.

Figure 36. Variation cheese TPA (hardness (H (g)), cohesiveness (C), springiness (Sp), and chewiness (Ch (g)))

The TPA hardness, springiness, and chewiness were found to have a similar variation. They had low values in summer months and high ones during winter (Figure 36). The mean $\pm$ standard deviation of hardness, springiness, and chewiness is given in Table 14. Cohesiveness was found to remain relatively constant (Figure 36). Hardness and chewiness had a significant strong positive correlation with total nitrogen content of cheese ($r > 0.7$ and $p\text{-value} < 0.05$) (Table 15). This indicates that with an increase in total protein content of cheese the firmness and chewiness of cheese were found to increase. During winter, the total nitrogen content in cheese was 0.45% higher than summer (Figure 36, Table 12) and also the hardness and chewiness of cheese was higher by 2414 g and 1337.1 g, respectively (Figure 36, Table 14). Fat in dry matter (FDM) also had a significant weak negative correlation with hardness, springiness, and chewiness ($-0.7 < r < -0.3$ and $p\text{-value} < 0.05$) (Table 15), indicating that as fat content decreased the

TPA hardness, springiness and chewiness increased. Though there was a variation of fat in dry matter by 6.14% (Table 12) during the sampling period, no seasonal variation was observed (Figure 34). Also, moisture had a weak negative correlation with springiness and chewiness ($-0.7 < r > -0.3$ and $p\text{-value} < 0.05$) (Table 15). As moisture content in cheese decreased, the springiness and chewiness were found to increase. Though there was a variation of moisture content by 2.59% (Table 12) during the sampling period, no seasonal variation was observed (Figure 34). Total calcium had a significant positive weak correlation ($0.7 < r > 0.3$ and $p\text{-value} < 0.05$) (Table 15) with hardness, cohesiveness, springiness and chewiness, as total calcium increased the hardness, springiness and chewiness increased. Though there was a variation of total calcium by 0.14% (Table 12) during the sampling period, no seasonal variation was observed (Figure 35). Cohesiveness, springiness and chewiness were found to decrease with water soluble calcium. i.e., WSC had a significant weak negative correlation ($-0.7 > r > -0.3$ and $p\text{-value} < 0.05$) (Table 15) with cohesiveness, springiness and chewiness. The hardness of cheese was not found to have any significant correlation with the WSC (%). During the sampling period, the water soluble calcium varied by 0.08% (Table 12); higher values were observed during summer and low ones in winter (Figure 35). Therefore, most of the TPA characteristics of cheese were dependent on the cheese total nitrogen, total calcium, FDM, water soluble calcium, and moisture content.

Figure 37. Variation of aggregation index (AGI) and % loss in shredder

Aggregation index (AGI) is an empirical test to measure matting behavior based on the ability of the cheese to pass through a stack of sieves of decreasing mesh size. Sticky cheese that mats excessively is retained by larger sieves whereas the cheese particles that are free flowing may penetrate to the bottom of the stack. The larger the AGI, more the cheese aggregates (Kindstedt *et al.*, 2004). From Figure 37, AGI and loss in shredder increased during summer months and decreased during winter months. This shows that in the summer months, cheese aggregated more than the winter months. From Table 15, there was a negative weak correlation ($-0.7 < r > -0.3$ and $p\text{-value} < 0.05$) between percent loss in shredder and total nitrogen, total calcium, and a weak positive correlation ($0.3 > r > 0.7$ and $p\text{-value} < 0.05$) between percentage loss in shredder and FDM, water soluble calcium. This indicates that as total nitrogen and total calcium increased the percentage loss in shredder decreased, and when FDM and water soluble calcium increased the percentage loss in shredder increased. Hence a variation of 0.45% of total nitrogen during

the winter compared to summer months brought about a loss of 5.45% (Table 14) during shredding of cheese between the different seasons. However total calcium had a variation of during of 0.13% during the sampling period, but the variation was not seasonal.

Kindstedt *et al.* (2004) reported that in low moisture mozzarella, high moisture content and fat content (FDM of 45%) cause cheese to clog in the shredder. In this study, we did find that higher FDM caused more loss during shredding. There was no significant correlation between moisture content and shredding loss. However, many researchers have reported that one of the reasons high moisture mozzarella cannot be shredded was due to the moisture content, which is greater than 52%. In this study, there was no significant correlation between moisture and shredding loss because the maximum moisture content of 49.63% (Table 12) did not exceed the threshold moisture content of 52%; therefore, it's the association between shredding and moisture content could not be observed. Hence, the loss in shredder was affected by the cheese composition, mainly the total nitrogen content, FDM, total calcium, and water soluble calcium. Like % loss in shredder, total nitrogen and water soluble calcium varied seasonally. Total nitrogen was 0.45% higher in winter (Figure33, Table 12), and WSC was 0.14% (Figure 35, Table 12) higher in summer. Though during the sampling period FDM and moisture had a variation of 6.14% and 2.59% respectively (Table 12), their variation was found to be random and not seasonal (Figure 34).

From Table 15, there was a significant weak negative correlation ($-0.7 < r > -0.3$ and p - value < 0.05) between AGI and total protein content, and a significant positive correlation between moisture and AGI. As the moisture increased, there was more aggregation, and also as the total protein content increased the cheese aggregated less.

Therefore, the cheese aggregation was mostly affected by the total nitrogen and moisture. The AGI was higher in winter by 0.8 than summer (Table 14, Figure 37).

Table 16. Correlation between cheese textural parameters

	Cohesiveness	Springiness	Chewiness	AGI	Loss in shredder
Hardness	0.141 0.435	0.369 0.035	0.777 0.000	-0.365 0.037	-0.516 0.003
Cohesiveness		0.718 0.000	0.703 0.000	-0.501 0.003	-0.590 0.000
Springiness			0.801 0.000	-0.652 0.000	-0.824 0.000
Chewiness				-0.618 0.000	-0.774 0.000
AGI					0.574 0.001

Cell content: Pearson correlation coefficient on top and p – value on the bottom. The yellow markings indicate significant correlation.

According to Chen (2003), mozzarella cheese to be shredded must be firm in texture and not adhesive. From Table 16, there was a weak significant negative correlation ($-0.7 < r > -0.3$ and $p - \text{value} < 0.05$) between TPA parameters (hardness, cohesiveness, springiness, and chewiness) and percentage loss in shredder. The same trend was observed for the AGI and TPA parameters, showing that firmer, more cohesive, springy, and chewy cheese aggregated less, and thus, the loss during shredding was less.

3. Effect of Milk Composition on Cheese Composition and Cheese Texture

Correlation analyses were done between milk composition and cheese composition and texture using Minitab version 17.0 from July 2008 to April 2009. The correlation analysis was done only for a ten- month period because from May 2009 changes in the production protocol of cheese were done to increase the firmness of cheese, thereby reducing the loss

during shredding. The corresponding week milk and cheese samples were correlated for the ten- month period, and the results are summarized in Table 17 and Table 18.

Table 17. Correlation between cheese composition and milk composition

Milk Components	Cheese Composition							
	TN	WSN	Moisture	FDM	Total Ca	WSC	Salt	pH
TN	0.725 0.003	0.263 0.262	-0.549 0.012	-0.552 0.012	0.050 0.845	-0.171 0.498	-0.048 0.841	-0.090 0.707
True N	0.635 0.003	0.187 0.431	-0.531 0.016	-0.522 0.018	-0.012 0.961	-0.242 0.333	-0.056 0.816	-0.100 0.675
NPN	0.728 0.000	0.400 0.080	-0.505 0.023	-0.499 0.025	0.306 0.095	0.073 0.773	-0.017 0.944	0.083 0.727
NCN	0.056 0.814	0.014 0.953	-0.454 0.045	-0.296 0.205	0.590 0.010	0.327 0.185	0.103 0.665	0.397 0.083
Casein	0.744 0.000	-0.308 0.187	-0.410 0.072	-0.464 0.039	-0.061 0.809	-0.262 0.293	-0.086 0.718	-0.186 0.433
CPt	0.648 0.002	0.319 0.171	-0.125 0.598	-0.255 0.277	-0.217 0.388	-0.355 0.148	-0.136 0.568	-0.307 0.188
CP	0.759 0.000	0.393 0.086	-0.217 0.358	-0.337 0.146	-0.109 0.667	-0.248 0.321	-0.113 0.635	-0.258 0.272
Fat	-0.149 0.543	-0.115 0.640	0.166 0.498	0.411 0.080	-0.524 0.031	-0.229 0.377	-0.037 0.881	0.125 0.610
TS	0.064 0.394	-0.005 0.984	0.088 0.727	0.261 0.281	-0.518 0.033	-0.290 0.258	0.009 0.862	-0.064 0.794
Total Ca	0.624 0.003	0.577 0.008	-0.176 0.450	-0.219 0.355	0.430 0.045	0.252 0.314	0.246 0.297	-0.056 0.81
Somatic cells/ μ L	0.339 0.144	-0.098 0.680	-0.344 0.137	-0.231 0.328	-0.281 0.258	-0.191 0.447	0.007 0.977	0.252 0.283
pH	-0.297 0.213	-0.411 0.072	-0.401 0.080	-0.047 0.846	0.189 0.453	0.112 0.657	0.056 0.813	0.331 0.154

*Cell content: Pearson correlation coefficient (r) on top and p – value on the bottom.
The yellow -markings indicate significant correlation.*

The raw milk quality, especially the casein, pH, total calcium, fat, and somatic cells, has an important impact on the quality and yield of cheese (Barbano, 1987; Kindstedt *et al.*, 2004). Cheese milk was standardized to a ratio of casein to fat of 1.2 to get a good yield and meet the standard of identity of LMPS Mozzarella (Rankin *et al.*, 2006). In cheese making, casein plays an important role in cheese composition, texture, and yield. It forms

the structural network in which fat and moisture are trapped. The formation and properties of the coagulum derived from casein network determines the efficiency of milk constituents' retention in cheese (Walstra *et al.*, 2006). Therefore, a significant strong positive correlation ($r > 0.7$ and $p\text{-value} < 0.05$) (Table 17) was observed between total nitrogen, true nitrogen, casein, non-protein nitrogen content and casein to total protein (CP) of milk and total nitrogen content of cheese. Also, there was a moderate positive correlation between ($0.7 > r > 0.3$ and $p\text{-value} < 0.05$) (Table 17) between total calcium true nitrogen, and casein to true protein (CPT) ratio and total nitrogen content of cheese. From Table 17, there was a moderate positive correlation between ($0.7 > r > 0.3$ and $p\text{-value} < 0.05$) between total calcium in milk and cheese. From Table 18, milk total nitrogen, true nitrogen NPN, casein, CP, CPT and had a significant strong to moderate positive correlation with the TPA characteristics of cheese ($r > 0.6$ and $p\text{-value} < 0.05$). Also, a significant strong moderate negative correlation ($-0.8 > r > -0.4$ and $p\text{-value} < 0.05$) between milk total nitrogen, true nitrogen NPN, casein, CP, CPT NPN, casein, CP, and CPT with loss in shredder and AGI were observed. The total calcium content in milk correlated positively ($r > 0.4$, $p\text{-value} > 0.2$) (Table 18) with hardness in cheese. As the total nitrogen content, true nitrogen, casein and non- protein content of milk varied by 0.06 %, 0.066% 0.53% and 0.019% respectively (Table 9), there was variation of 0.45% of total nitrogen (Table 12), 5.45% loss in shredder, 0.90 of AGI in cheese between seasons (Table 14), with higher values being in summer and lower ones in summer. In milk, the variation of NPN over different seasons was found to have an association on the total nitrogen content, casein, and true nitrogen, thereby influencing the CP and CPT ratios.

As the fat in silo milk was reduced to 2% fat, there was no correlation between fat in silo milk and FDM in cheese. There was a significant moderate positive correlation ($0.7 < r > 0.3$ and $p\text{-value} < 0.05$) between total calcium in milk and total calcium, water soluble nitrogen, and total nitrogen in cheese.

Table 18. Correlation between milk composition and cheese texture

Milk Components	Cheese Texture					
	Hardness	Cohesiveness	Springiness	Chewiness	AGI	Loss in Shredder
TN	0.805 0.000	0.664 0.001	0.734 0.000	0.840 0.000	-0.579 0.007	-0.525 0.025
NPN	0.853 0.025	0.601 0.005	0.698 0.001	0.866 0.000	-0.613 0.004	-0.476 0.046
NCN	0.252 0.283	-0.073 0.761	-0.054 0.823	-0.089 0.621	0.077 0.748	0.116 0.647
True N	0.721 0.000	0.644 0.002	0.686 0.001	0.770 0.000	-0.561 0.010	-0.500 0.034
Casein	0.781 0.000	0.741 0.007	0.808 0.000	0.857 0.000	-0.693 0.000	-0.611 0.000
CPt	0.611 0.004	0.734 0.003	0.782 0.000	0.736 0.000	-0.757 0.000	-0.628 0.005
CP	0.735 0.000	0.742 0.000	0.825 0.000	0.830 0.000	-0.745 0.000	-0.634 0.005
Fat	-0.269 0.265	-0.030 0.903	0.027 0.912	-0.194 0.426	-0.035 0.886	-0.103 0.695
Total Solids	-0.059 0.811	0.249 0.305	0.293 0.223	0.072 0.771	-0.285 0.238	-0.364 0.049
Total Ca	0.605 0.005	0.188 0.427	0.276 0.239	0.494 0.027	-0.151 0.525	-0.122 0.629
Somatic cells/ μ L	0.196 0.408	0.075 0.753	0.101 0.671	0.100 0.578	-0.129 0.587	-0.282 0.258
pH	-0.214 0.364	-0.099 0.677	-0.083 0.728	-0.165 0.490	-0.016 0.948	-0.059 0.816

Cell content: Pearson correlation coefficient (r) on top and p – value on the bottom. The yellow markings indicate significant correlation

Milk total solids were found to have a weak negative correlation with loss in shredder (Table 18). Milk pH, fat, non-casein nitrogen, and somatic cells appeared to have no association with the textural aspects of cheese.

Therefore, during the manufacture of cheese, the calcium, total protein, and casein content in cheese milk should be monitored closely as they are found to have a significant association with the texture and composition of LMPS mozzarella. But as the milk is only a representation and not the exact milk from which the weekly cheese was made, the conclusions that can be drawn from the study were limited.

C. The Effect of Temperature on Ripening

The ripening temperature influences the rate of proteolysis, the cheese composition, cheese texture, and micro flora. Most of the studies have concentrated on cheddar and Dutch varieties (Creamer, 1976; Kindstedt *et al.*, 2004). In low moisture mozzarella, Feeney *et al.* observed that after 15 days of ripening, there was significant difference in pH 4.6 WSN between cheeses ripened at 10⁰ C and 15⁰ C and cheese ripened at 0⁰ C and 4⁰ C. Also extensive degradation of α_{s1} -casein was observed at 10⁰ C and 15⁰ C. The study indicated that changing the ripening temperature provides a convenient means of controlling proteolysis without altering the type of proteolysis. A lot of researchers have reported that they saw a significant difference in proteolysis when cheeses were ripened above 10⁰ C, or if the cheese was ripened for more than 50 days at temperatures below 10⁰ C (Jana & Mandal, 2011).

A brief ripening period of LMPS mozzarella (usually less than a month) is required for the cheese to get the desired functional properties to be used as a pizza ingredient. When cheese is maintained at cold temperatures for about two to three weeks, the caseins become more hydrated and swollen due to free water being absorbed back into the protein matrix, and the cheese protein matrix expands into the fat-serum channel giving the cheese the desired meltability and oiling off properties (Kindstedt *et al.*, 2004). Newly

manufactured cheese has excessive moisture at the surface and within the body of cheese, hence it will not shred well. Four to five days of ageing will allow the cheese to absorb the moisture back, and this will improve the shredding quality (McMahon & Oberg, 2011). Thus, the cheese is shredded typically two to three weeks after manufacture. Therefore, in this study the main focus was to see if there was an association between temperature (below 10⁰ C) and the LMPS mozzarella unheated textural properties, pH, and water soluble nitrogen when ripened for a short period of time (21 days). Thereby, two blocks of 10 lb vacuum packed cheese samples shipped from the plant in Central Valley, California, were subjected to ripening, one block at 3.3⁰ C and the other at 8.9⁰ C for 21 days from the date of manufacture. After 21 days, both the cheese blocks ripened at different temperatures were brought to a core temperature of 3.3⁰ C before carrying out the analysis. The results are summarized in Table 19.

Table 19. Statistical analysis of fresh and ripened cheese

Cheese parameters	Age	N	Mean	SD	Min.	Max.	* t - value p-value
H (g)	5 days	33	4135	624	3088	5502	
	21 days / 3.3⁰C	33	3760.1	545.5	2593.8	4820	
	21 days /8.9⁰C	33	3557	598	2523	4620	5.73 0.000
C	5 days	33	0.51	0.04	0.42	0.66	
	21 days / 3.3⁰C	33	0.48	0.029	0.42	0.55	1.60
	21 days /8.9⁰C	33	0.48	0.029	0.421	0.53	0.120
Sp	5 days	33	0.67	0.0488	0.58	0.75	
	21 days / 3.3⁰C	33	0.63	0.04	0.56	0.70	0.72
	21 days /8.9⁰C	33	0.63	0.04	0.54	0.69	0.475
Ch (g)	5 days	33	1445.5	344.4	852.4	2229.5	
	21 days / 3.3⁰C	33	1151.9	257.4	735.3	1677	7.34
	21 days /8.9⁰C	33	1068.3	246.2	663.3	1563.6	0.000
Loss in shredder (%)	5 days	33	9.71	1.40	7.30	12.76	
	21 days / 3.3⁰C	32	12.66	2.13	9.52	16.74	-2.86
	21 days /8.9⁰C	31	14.19	2.24	10.53	18.97	0.008
AGI	5 days	33	6.04	0.22	5.49	6.38	
	21 days / 3.3⁰C	32	6.14	0.17	5.81	6.39	-2.77
	21 days /8.9⁰C	32	6.18	0.16	5.81	6.45	0.009
WSN (%)	5 days	33	0.32	0.0401	0.27	0.45	
	21 days / 3.3⁰C	30	0.38	0.0352	0.31	0.45	-7.60
	21 days /8.9⁰C	29	0.41	0.038	0.34	0.498	0.008
pH	5 days	33	5.47	0.0381	5.41	5.53	
	21 days / 3.3⁰C	33	5.53	0.053	5.44	5.66	0.380
	21 days /8.9⁰C	30	5.52	0.044	5.43	5.60	0.708

*- the t-value and p-value were from the paired t test done only for the cheese ripened at 3.3⁰ C and 8.9⁰ C for 21 days. The t-value is above and p-value is below in that column. In the table N- Sample size, H- Hardness, C –Cohesiveness, Sp-Springiness, Ch-Chewiness, AGI-Aggregation Index, WSN-Water soluble nitrogen

The mean hardness, cohesiveness, springiness, chewiness decreased, and the average percentage loss in shredder, aggregation index, WSN, and pH increased after 21 days of

manufacture in comparison to the five day old cheese. Yun *et al.* (1993c) reported a decrease in textural characteristics namely hardness, cohesiveness, and springiness when cheese was ripened for 50 days at 4⁰ C. As LMPS mozzarella ripened, Jana & Mandal (2011) reported that there was a significant decrease in the concentration of intact casein and firmness. Kindstedt *et al.* (1994) observed that for low moisture mozzarella the water soluble nitrogen increased significantly during seven weeks of ripening, but the rate of increase depended on the different coagulants. The increase in water soluble nitrogen can cause an increase in pH (Jana & Mandal, 2011). Hence, the decrease in intact casein during proteolysis would be the contributing factor for a decrease in hardness, cohesiveness, springiness, chewiness, and an increase in WSN and pH during the 21-day ripening period. In commercial shredders, where centrifugal force is used for shredding, a firm textured cheese has less deformation, and blades are able to make cleaner cut. In addition, a firm textured cheese cube maintains a uniform speed, and blades can cut shreds the length of the cube. On the other hand, a soft textured cheese bends and deforms around the blade, slowing the portion of the cheese cube in contact with the blade (Rankin *et al.*, 2006). Hence, the decrease in firmness would have attributed to the increase in percentage loss of shredder. There was an increase in aggregation index, which indicates that the shredded cheese clumped and aggregated more in the 21-day ripened cheese than the 5-day old cheese. However, the increase in aggregation index (AGI) was very minimal (approximately 2%), and this could be overcome by application of anti-caking agents (Jana & Mandal, 2011).

From Table 19 the average hardness, cohesiveness, springiness and chewiness were greater in cheese ripened at 3.3⁰ C than the cheese ripened at 8.9⁰ C. The mean loss in shredder, AGI, and water soluble nitrogen was less in cheese ripened at 3.3⁰ C. The mean aggregation index (AGI) and pH were similar in cheese ripened at different temperatures. Paired t- test analyses were done using Minitab Version 17.0 to analyze whether there was any significant difference (at $\alpha=0.05$) between the hardness, cohesiveness, springiness, chewiness, aggregation index, percentage loss in shredder, pH, and WSN. The data was independent and the *p-values* were all greater than 0.05 in the normality plots indicating that the data was normally distributed (Appendix 8). There was a significant difference in the water soluble nitrogen (WSN), hardness, chewiness, aggregation index, and percent loss in shredder with a *p-value* < 0.05 when ripened at different temperatures (3.3⁰ C and 8.9⁰ C) (Table 19). At 95% confidence limit there was no significant difference in the pH, cohesiveness, and springiness of cheese.

Figure 38. Urea- PAGE of sodium caseinate and LMPS mozzarella ripened at different temperatures. Lane 1 & 2 – Sodium caseinate standard. Lane 3, 4, and 5 – Fresh cheese. Lane 6 and 7 – Cheese Ripened at 3.3⁰ C. Lane 8 and 9 – Cheese ripened at 8.9⁰C

Urea-polyacrylamide gel electrophoretogram of the fresh and cheese ripened at different temperatures are shown in. Overall, the trends observed with PAGE are consistent with those of water soluble nitrogen (p -value <0.05 (Table 19)), which showed that proteolysis was significantly affected by ripening temperature. Increasing the ripening temperature resulted in an increase in the rate of degradation of α_{s1} -casein where most of the degradation was observed at 8.9⁰C. In agreement with previous studies on Mozzarella (Yun *et al.*, 1993a; Yun *et al.*, 1993b; Fox P.F., 1989), there was very little hydrolysis of β -casein over the 21- day period. The relatively low degree of age-related degradation of β -casein, compared to as α_{s1} -casein, has also been observed for other cheese varieties

such as Cheddar, Gouda, and Blue-cheese (Creamer, 1976). Hence, in this study we can conclude that there was an increase in the rate of proteolysis at 8.9⁰ C than at 3.3⁰ C. This rate of proteolysis affected the water soluble nitrogen and the textural properties mainly hardness, chewiness, aggregation index, and percentage loss during shredding of LMPS Mozzarella

V. CONCLUSIONS

In this study, seasonal observations for total nitrogen, casein nitrogen, non-protein nitrogen, true nitrogen, casein to total nitrogen ratio, casein to true nitrogen ratio, and total calcium were measured and analyzed in the silo milk collected from a plant in Central Valley, California, from July 2008 to December 2009. The seasonal changes showed that all the above constituents had highest values during winter months and low ones in summer months. In addition, 2008 and 2009 had very less precipitation (Appendix 4), and the hottest months were in June to September with temperatures crossing over 100⁰ F (Appendix 3). Hence, the variation of total nitrogen, casein nitrogen, non-protein nitrogen and total calcium can be attributed to the high temperatures (above 100⁰ F), less food intake due to heat stress, and longer photoperiods during the summer months compared to the winter months.

Therefore, the accuracy of fixed casein to total protein ratio often used in dairy plants to estimate casein content of milk throughout different seasons is questionable. Thus, it may be concluded that estimating casein from true protein or casein from total protein while accounting for monthly variations of total nitrogen and non-protein nitrogen should be more accurate. As there was a seasonal variation for casein and total calcium, a close monitor of these components may be beneficial to improve the quality and consistency of cheese throughout the year.

The total nitrogen and pH of low moisture part skim (LMPS) mozzarella was found to have higher values in winter months and decrease during summer. The water soluble calcium of the LMPS mozzarella was found to be less in winter months than summer months. As the pH of cheese was found to decrease, the water soluble calcium levels increased and vice versa. The hardness, chewiness and springiness of cheese were found

to have high values in winter and low ones in summer. The loss during shredding and aggregation of LMPS Mozzarella were more in summer than winter months.

The total nitrogen, total calcium, and water soluble calcium of the cheese were found to have significant correlation with the hardness, chewiness, aggregation index and loss in the shredder. Also, the total calcium, total protein, casein, and non-protein nitrogen content in cheese milk were found to have a significant association on the texture and composition of LMPS mozzarella. As the total nitrogen content, true nitrogen, casein, non-protein content, and total calcium of milk varied by 0.06 %, 0.066% 0.53%, 0.019%, and 0.03% respectively (Table 9), there was a variation of 0.45% of total nitrogen, 0.13% total calcium 0.088% water soluble calcium, (Table 12), 2414 g of hardness, 5.45% loss in shredder, and 0.90 of AGI (Table 14) in LMPS Mozzarella between seasons. The pH of the cheese did not significantly correlate with the textural characteristics of the cheese. However, as pH of cheese had a moderate negative with water soluble calcium and loss in shredder correlated positively with the water soluble calcium. pH of cheese was increased to above 5.45 during summer months to prevent the water soluble calcium levels from increasing, and hence the loss in shredder was minimized. The total nitrogen content of cheese during the summer months was also increased by changing the manufacturing protocol. Hence, the firmness of cheese was increased, and the aggregation index and percentage losses in shredder were minimized during the summer months.

Therefore, higher total nitrogen content, mainly the casein content and total calcium of milk, brought about higher total nitrogen content of LMPS Mozzarella and firmer cheese, which in turn contributed to less loss in shredder and less aggregation in LMPS

Mozzarella. Since the milk samples were only a representation and not the exact milk from which the cheese was made, the conclusions that can be drawn from this study are limited.

Also, there was a significant difference ($\alpha = 0.05$) in the water soluble nitrogen (WSN), hardness, chewiness, aggregation index, and percent loss in shredder with a *p-value* < 0.05 when LMPS Mozzarella was ripened at different temperatures (3.3°C and 8.9°C) for 21 days (Table 19). At 95% confidence limit, there was no significant difference in the pH, cohesiveness, and springiness of cheese when the cheese was ripened at different temperatures. Increasing the ripening temperature resulted in an increase in the rate of degradation of α_{s1} -casein where most of the degradation was observed at 8.9°C . There was relatively low degree of age-related degradation of β -casein, compared to α_{s1} -casein. Hence, in this study we can conclude that there was an increase in the rate of proteolysis at 8.9°C than at 3.3°C . This rate of proteolysis affected water soluble nitrogen content of cheese and the textural properties of LMPS Mozzarella mainly the hardness, chewiness, aggregation index, and percentage loss during shredding.

VI. RECOMMENDATIONS FOR FUTURE WORK

- In this study, the seasonal variation of milk components was analyzed by collecting milk samples from only one plant in the Central valley, California. To eliminate limitations', milk samples should be taken from different plants and different areas in California to give us more information on the seasonal variation of milk composition.
- Also, the milk used for making cheese should be analyzed to study the association of milk composition on cheese composition and texture. In this study the milk from silo was only a representation and not the exact milk from which the cheese was made, hence the conclusions that can be drawn from the study were limited.
- As this was an observational study, no cause and effect conclusions can be drawn. Hence a well-designed experimental study would give more substantial conclusions

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APPENDICES

Appendix 1. Milk Raw Data

Month	Sample No.	Number of week in a year	Total Nitrogen %	True Nitrogen %	Non-Protein Nitrogen %	Casein Nitrogen %	Non-Casein Nitrogen %
Jul-08	MO5	27	0.5017	0.4794	0.0223	0.3796	0.1221
	MO6	28	0.4921	0.4707	0.0214	0.3884	0.1037
	MO7	29	0.4982	0.4767	0.0214	0.3962	0.1020
	MO8	30	0.4920	0.4706	0.0213	0.3881	0.1038
Aug-08	MO9	31	0.4981	0.4777	0.0204	0.3913	0.1068
	MO10	32	0.4983	0.4780	0.0203	0.3902	0.1081
	MO11	33	0.5032	0.4821	0.0211	0.4068	0.0964
	MO12	34	0.5209	0.4970	0.0239	0.4180	0.1029
Sep-08	MO13	35	0.5071	0.4828	0.0243	0.4152	0.0920
	MO14	36	0.5137	0.4922	0.0215	0.4154	0.0983
	MO15	37	0.5050	0.4814	0.0236	0.4093	0.0957
	MO16	38	0.5056	0.4824	0.0232	0.4068	0.0988
	MO17	39	0.5154	0.4926	0.0228	0.4045	0.1108
	MO18	40	0.5022	0.4802	0.0220	0.4052	0.0970
	MO19	41	0.5083	0.4886	0.0197	0.4142	0.0940
	MO20	42	0.5312	0.5053	0.0259	0.4402	0.0910
Nov-08	MO21	43	0.5264	0.5004	0.0260	0.4364	0.0900
	MO22	44	0.5275	0.4969	0.0305	0.4366	0.0909
	MO23	45	0.5335	0.5019	0.0316	0.4302	0.1267
	MO24	46	0.5354	0.5041	0.0313	0.4372	0.1248
	MO25	47	0.5375	0.5061	0.0314	0.4302	0.1280
	MO26	48	0.5393	0.5071	0.0323	0.4447	0.1140
Dec-08	MO27	49	0.5435	0.5115	0.0320	0.4441	0.1144
	MO28	50	0.5402	0.5069	0.0333	0.4465	0.1037
	MO29	51	0.5423	0.5105	0.0318	0.4438	0.1132
	MO30	52	0.5424	0.5078	0.0346	0.4443	0.1201
Jan-09	MO31	1	0.5441	0.5136	0.0305	0.4418	0.1023
	MO32	2	0.5405	0.5117	0.0287	0.4392	0.1079
	MO33	3	0.5454	0.5142	0.0312	0.4441	0.1153
	MO34	4	0.5247	0.4950	0.0297	0.4241	0.1002
Feb-09	MO35	5	0.5182	0.4870	0.0312	0.4185	0.0997
	MO36	6	0.5258	0.4964	0.0294	0.4295	0.0963
	MO37	7	0.4853	0.4603	0.0250	0.3719	0.1134

Month	Sample No.	Number of week in a year	Total Nitrogen %	True Nitrogen %	Non-Protein Nitrogen %	Casein Nitrogen %	Non - Casein Nitrogen %
	MO38	8	0.4767	0.4526	0.0241	0.3637	0.1130
Mar-09	MO39	9	0.5061	0.4772	0.0289	0.3970	0.1091
	MO40	10	0.5100	0.4859	0.0241	0.4009	0.1091
	MO41	11	0.5003	0.4759	0.0243	0.4068	0.0934
	MO42	12	0.4882	0.4654	0.0228	0.3938	0.0944
Apr-09	MO43	13	0.5147	0.4907	0.0240	0.4105	0.1041
	MO44	14	0.5089	0.4847	0.0242	0.3927	0.1162
	MO45	15	0.5092	0.4844	0.0248	0.3948	0.1144
	MO46	16	0.5087	0.4867	0.0220	0.3937	0.1150
May-09	MO47	17	0.5034	0.4795	0.0239	0.3870	0.1163
	MO48	18	0.5074	0.4826	0.0248	0.3932	0.1142
	MO49	19	0.4995	0.4743	0.0252	0.3868	0.1127
	MO50	20	0.5030	0.4768	0.0262	0.3940	0.1090
Jun-09	MO51	21	0.5021	0.4786	0.0235	0.3906	0.1115
	MO52	22	0.4876	0.4620	0.0256	0.3790	0.1086
	MO53	23	0.4850	0.4598	0.0252	0.3825	0.1025
	MO54	24	0.5087	0.4832	0.0255	0.3940	0.1147
	MO55	25	0.5158	0.4892	0.0267	0.4038	0.1120
	MO56	26	0.5068	0.4793	0.0275	0.3996	0.1072
Jul-09	MO57	27	0.5015	0.4768	0.0247	0.3999	0.1016
	MO58	28	0.4959	0.4719	0.0240	0.3915	0.1045
	MO59	29	0.4947	0.4730	0.0216	0.3893	0.1053
	MO60	30	0.5041	0.4839	0.0201	0.4027	0.1014
Aug-09	MO61	31	0.5142	0.4875	0.0267	0.4083	0.1060
	MO62	32	0.5009	0.4802	0.0208	0.3929	0.1080
	MO63	33	0.5025	0.4810	0.0215	0.4067	0.0958
	MO64	34	0.5025	0.4861	0.0164	0.4050	0.0975
Sep-09	MO65	35	0.5063	0.4884	0.0178	0.4060	0.1003
	MO66	36	0.5066	0.4849	0.0217	0.4089	0.0977
	MO67	37	0.5196	0.4991	0.0205	0.4064	0.1132
	MO68	38	0.5298	0.5086	0.0212	0.4197	0.1101
Oct-09	MO69	39	0.5227	0.5007	0.0220	0.4125	0.1102
	MO70	40	0.5243	0.5023	0.0220	0.4167	0.1076
	MO71	41	na	na	na	na	na
	MO72	42	na	na	na	na	na
	MO73	43	0.5211	0.4969	0.0242	0.4110	0.1101
	MO74	44	0.5235	0.5014	0.0221	0.4225	0.1010
Nov -09	MO75	45	0.5376	0.5193	0.0183	0.4355	0.1021

Month	Sample No.	Number of week in a year	Total Nitrogen %	True Nitrogen %	Non-Protein Nitrogen %	Casein Nitrogen %	Non-Casein Nitrogen %
	MO76	46	0.5345	0.5144	0.0201	0.4303	0.1042
	MO77	47	0.5365	0.5044	0.0321	0.4352	0.1013
	MO78	48	0.5371	0.5039	0.0332	0.4370	0.1001
Dec-09	MO79	49	0.5323	0.5036	0.0287	0.4342	0.0981
	MO80	50	0.5321	0.5087	0.0234	0.4401	0.0920
	MO81	51	0.5381	0.5083	0.0298	0.4458	0.0923
	MO82	52	0.5398	0.5127	0.0271	0.4397	0.1001

Month	Sample No.	Number of week in a year	Casein/ Total Nitrogen	Casein/True Nitrogen	Fat %
Jul-08	MO5	27	0.7566	0.7918	3.18
	MO6	28	0.7893	0.8251	3.215
	MO7	29	0.7953	0.8311	3.225
	MO8	30	0.7890	0.8247	2.545
Aug-08	MO9	31	0.7856	0.8191	2.41
	MO10	32	0.7831	0.8163	3.085
	MO11	33	0.8084	0.8438	2.855
	MO12	34	0.8025	0.8410	3.275
Sep-08	MO13	35	0.8186	0.8599	3.305
	MO14	36	0.8087	0.8440	3.16
	MO15	37	0.8104	0.8501	3.215
	MO16	38	0.8046	0.8432	3.38
	MO17	39	0.7849	0.8213	3.215
	MO18	40	0.8068	0.8438	3.425
	MO19	41	0.8150	0.8478	2.645
	MO20	42	0.8287	0.8711	3.425
Nov-08	MO21	43	0.8291	0.8722	1.97
	MO22	44	0.8277	0.8785	3.245
	MO23	45	0.8064	0.8571	2.91
	MO24	46	0.8166	0.8673	2.345
	MO25	47	0.8005	0.8502	1.56
	MO26	48	0.8245	0.8770	2.6
Dec-08	MO27	49	0.8171	0.8682	2.38
	MO28	50	0.8266	0.8809	3.3
	MO29	51	0.8184	0.8694	1.45
	MO30	52	0.8193	0.8751	2.73
Jan-09	MO31	1	0.8120	0.8603	1.61
	MO32	2	0.8125	0.8582	1.985
	MO33	3	0.8143	0.8637	1.995
	MO34	4	0.8084	0.8569	3.03
Feb-09	MO35	5	0.8076	0.8594	3.485
	MO36	6	0.8169	0.8653	3.39
	MO37	7	0.7663	0.8079	3.46
	MO38	8	0.7629	0.8034	3.375
Mar-09	MO39	9	0.7845	0.8319	1.83
	MO40	10	0.7861	0.8251	na

Month	Sample No.	Number of week in a year	Casein/Total Nitrogen	Casein/True Nitrogen	Fat %
Mar-09	MO41	11	0.8132	0.8548	1.98
	MO42	12	0.8066	0.8461	2.875
Apr-09	MO43	13	0.7977	0.8366	1.415
	MO44	14	0.7716	0.8102	2.425
	MO45	15	0.7753	0.8150	2.3
	MO46	16	0.7738	0.8089	2.885
May-09	MO47	17	0.7689	0.8071	1.87
	MO48	18	0.7750	0.8148	2.42
	MO49	19	0.7743	0.8154	2.29
	MO50	20	0.7833	0.8263	2.95
Jun-09	MO51	21	0.7779	0.8162	1.435
	MO52	22	0.7773	0.8204	2.43
	MO53	23	0.7886	0.8318	2.18
	MO54	24	0.7745	0.8153	2.405
	MO55	25	0.7828	0.8255	2.02
	MO56	26	0.7885	0.8337	2.675
Jul-09	MO57	27	0.7974	0.8386	1.87
	MO58	28	0.7894	0.8296	1.99
	MO59	29	0.7871	0.8231	1.66
	MO60	30	0.7989	0.8322	2.815
Aug-09	MO61	31	0.7940	0.8374	2.23
	MO62	32	0.7844	0.8183	2.49
	MO63	33	0.8093	0.8455	1.64
	MO64	34	0.8060	0.8332	3.295
Sep-09	MO65	35	0.8019	0.8312	1.85
	MO66	36	0.8071	0.8432	2.535
	MO67	37	0.7821	0.8143	2.165
	MO68	38	0.7922	0.8252	2.43
Oct-09	MO69	39	0.7892	0.8239	2.17
	MO70	40	0.7948	0.8296	3.01
	MO71	41	na	na	na
	MO72	42	na	na	na
	MO73	43	0.7887	0.8271	4.07
	MO74	44	0.8071	0.8427	3.87
Nov-09	MO75	45	0.8101	0.8387	2.165
	MO76	46	0.8050	0.8365	2.43

Month	Sample No.	Number of week in a year	Casein/Total Nitrogen	Casein/True Nitrogen	Fat %
	MO77	47	0.8112	0.8628	2.17
	MO78	48	0.8136	0.8672	3.01
Dec-09	MO79	49	0.8157	0.8622	1.41
	MO80	50	0.8271	0.8651	2.315
	MO81	51	0.8285	0.8770	1.15
	MO82	52	0.8146	0.8576	2.525

Month	Sample No.	Number of week in a year	Total Solids%	Total calcium %	pH	Somatic Cells/μ L
Jul-08	MO5	27	12.3800	0.1013	6.6	271.7
	MO6	28	12.2500	0.1025	6.69	238.3
	MO7	29	12.3450	0.1054	6.69	242.7
	MO8	30	11.8450	0.1059	6.71	190.7
Aug-08	MO9	31	11.3100	0.1040	6.66	224.0
	MO10	32	11.9250	0.1052	6.66	241.3
	MO11	33	11.7750	0.1045	6.68	424.7
	MO12	34	12.1100	0.1055	6.68	281.7
Sep-08	MO13	35	12.1550	0.1068	6.64	264.7
	MO14	36	12.1000	0.1066	6.64	241.0
	MO15	37	12.0900	0.1110	6.71	262.7
	MO16	38	12.3800	0.1104	6.71	122.0
	MO17	39	12.2950	0.1101	6.64	155.7
	MO18	40	12.3950	0.1078	6.71	317.7
	MO19	41	11.8450	0.1110	6.53	508.7
	MO20	42	12.5600	0.1090	6.6	258.7
Nov-08	MO21	43	11.1800	0.1070	6.67	243.0
	MO22	44	12.4300	0.0970	6.69	244.7
	MO23	45	12.1350	0.0982	6.7	210.3
	MO24	46	11.5550	0.1164	6.68	219.7
	MO25	47	10.8300	0.1146	6.66	223.3
	MO26	48	11.8800	0.1148	6.67	370.3
Dec-08	MO27	49	11.6450	0.1133	6.68	324.3
	MO28	50	12.5400	0.1116	6.65	266.3
	MO29	51	10.7350	0.1116	6.63	255.0
	MO30	52	11.9200	0.1200	6.69	260.3
Jan-09	MO31	1	10.9300	0.1160	6.71	162.0
	MO32	2	11.2650	0.1134	6.66	155.0
	MO33	3	11.1550	0.1153	6.68	257.7
	MO34	4	12.1750	0.1163	6.65	435.0
Feb-09	MO35	5	12.4900	0.1121	6.65	342.7
	MO36	6	12.3950	0.1092	6.63	260.0
	MO37	7	12.3750	0.1110	6.69	238.3
	MO38	8	12.2750	0.1126	6.71	229.7
Mar-09	MO39	9	10.7900	0.1139	6.66	207.0
	MO40	10	na	0.1139	6.68	224.0

Month	Sample No.	Number of week in a year	Total Solids%	Total calcium %	pH	Somatic Cells/μ L
Mar 09	MO41	11	10.9750	0.1179	6.6	241.3
	MO42	12	11.7750	0.1163	6.57	264.7
Apr-09	MO43	13	10.3550	0.1106	6.64	241.0
	MO44	14	11.0750	0.1108	6.69	262.7
	MO45	15	9.6450	0.1107	6.7	122.0
	MO46	16	11.8050	0.1032	6.69	155.7
May-09	MO47	17	10.8150	0.1105	6.66	317.7
	MO48	18	11.3400	0.1125	6.67	508.7
	MO49	19	11.2150	0.1115	6.68	258.7
	MO50	20	11.8400	0.1069	6.66	243.0
Jun-09	MO51	21	10.3800	0.0924	6.65	244.7
	MO52	22	11.3200	0.0937	6.66	210.3
	MO53	23	10.9750	0.1056	6.65	219.7
	MO54	24	11.2650	0.1046	6.63	223.3
	MO55	25	10.9400	0.1056	6.69	370.3
	MO56	26	11.4450	0.1032	6.71	324.3
Jul-09	MO57	27	10.7600	0.1021	6.66	266.3
	MO58	28	10.8800	0.1045	6.68	255.0
	MO59	29	10.5500	0.1048	6.65	260.3
	MO60	30	11.6400	0.1050	6.65	162.0
Aug-09	MO61	31	11.1000	0.1057	6.63	155.0
	MO62	32	11.3650	0.1081	6.69	257.7
	MO63	33	10.6050	0.1057	6.71	435.0
	MO64	34	12.1200	0.1067	6.66	342.7
Sep-09	MO65	35	10.8050	0.0959	6.68	260.0
	MO66	36	11.4000	0.0965	6.6	238.3
	MO67	37	11.1000	0.1127	6.57	229.7
	MO68	38	11.3750	0.1152	6.64	223.3
Oct-09	MO69	39	11.2100	0.1114	6.69	370.3
	MO70	40	12.0500	0.1091	6.7	324.3
	MO71	41	na	na	na	na
	MO72	42	na	na	na	na
	MO73	43	12.7000	0.1150	6.69	255.0
	MO74	44	12.8700	0.1142	6.66	260.3
Nov-09	MO75	45	11.1000	0.1169	6.67	162.0
	MO76	46	11.3750	0.1072	6.64	155.0

Month	Sample No.	Number of week in a year	Total Solids%	Total calcium %	pH	Somatic Cells/μL
	MO77	47	11.2100	0.1056	6.71	257.7
	MO78	48	12.0500	0.1112	6.53	435.0
Dec-09	MO79	49	10.5500	0.1132	6.6	342.7
	MO80	50	11.4150	0.1168	6.67	260.0
	MO81	51	10.2950	0.1124	6.68	238.3
	MO82	52	11.6100	0.1123	6.65	229.7

Appendix 2. Milk Statistics (analyzed in Minitab 17.0)

1. Descriptive Statistics of Milk Composition

Variable	Total Count	Mean	StDev	Minimum	Maximum
Total Protein%	76	3.2861	0.1105	3.0414	3.4799
Total Nitrogen%	76	0.51507	0.01733	0.47671	0.54544
True Protein %	76	3.1259	0.0968	2.8878	3.3131
True Nitrogen%	76	0.48995	0.01517	0.45263	0.51929
Non-Protein Nitrogen%	76	0.025117	0.004225	0.016426	0.034592
Casein %	76	2.6271	0.1339	2.3202	2.8488
Casein Nitrogen %	76	0.41177	0.02099	0.36367	0.44652
Non-Casein Nitrogen%	76	0.10556	0.00876	0.08999	0.12804
Casein/Total Protein	76	0.79908	0.01773	0.75660	0.82906
Casein/True Protein	76	0.84003	0.02141	0.79177	0.88089
Fat %	76	2.5604	0.6553	1.1500	4.0700
Total Solids%	76	11.543	0.680	9.645	12.870
Total calcium %	76	0.10881	0.00582	0.09239	0.12002
pH	76	6.6612	0.0394	6.5300	6.7100
Somatic Cells/ μ l	76	262.61	79.22	122.00	508.67

2. Regression Analysis of Milk for Total Nitrogen

Total Nitrogen(%) of milk versus Sine(week), Cosine(week)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	2	0.014289	0.007144	63.40	0.000
Sine(week)	1	0.001571	0.001571	13.94	0.000
Cosine(week)	1	0.012872	0.012872	114.23	0.000
Error	73	0.008226	0.000113		
Lack-of-Fit	49	0.006999	0.000143	2.79	0.004
Pure Error	24	0.001227	0.000051		
Total	75	0.022515			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0106153	63.46%	62.46%	60.01%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.51367	0.00127	405.54	0.000	
Sine(week)	-0.00676	0.00181	-3.73	0.000	1.00
Cosine(week)	0.01821	0.00170	10.69	0.000	1.00

Regression Equation

Total Nitrogen% = 0.51367 -0.00676 Sine(week) +0.01821
Cosine(week)

Fits and Diagnostics for Unusual Observations

Obs	Total Nitrogen%	Fit	Resid	Std Resid	
33	0.48533	0.52068	-0.03535	-3.42	R
34	0.47671	0.51845	-0.04174	-4.04	R
38	0.48820	0.50915	-0.02095	-2.03	R
51	0.51585	0.49477	0.02107	2.03	R

R Large residual

Normal Probability plot of Residuals for Total Nitrogen%

Residuals vs Fits for Total Nitrogen%

True Nitrogen% versus Sine(week), Cosine(week)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	2	0.010488	0.005244	56.60	0.000
Sine(week)	1	0.002316	0.002316	25.00	0.000
Cosine(week)	1	0.008323	0.008323	89.84	0.000
Error	73	0.006763	0.000093		
Lack-of-Fit	49	0.005461	0.000111	2.05	0.029
Pure Error	24	0.001302	0.000054		
Total	75	0.017251			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0096252	60.80%	59.72%	57.08%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.48829	0.00115	425.16	0.000	
Sine(week)	-0.00821	0.00164	-5.00	0.000	1.00
Cosine(week)	0.01465	0.00155	9.48	0.000	1.00

Regression Equation

True Nitrogen% = 0.48829 - 0.00821 Sine(week) + 0.01465
Cosine(week)

Fits and Diagnostics for Unusual Observations

Obs	True Nitrogen%	Fit	Resid	Std Resid	
33	0.46031	0.49186	-0.03154	-3.36	R
34	0.45263	0.48985	-0.03722	-3.97	R

R Large residual

Normal probability plot of Residuals for True Nitrogen%

Residuals vs Fits for True Nitrogen%

Casein Nitrogen % versus Sine(week), Cosine(week)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	2	0.023255	0.011628	86.71	0.000
Sine(week)	1	0.004134	0.004134	30.83	0.000
Cosine(week)	1	0.019429	0.019429	144.88	0.000
Error	73	0.009789	0.000134		
Lack-of-Fit	49	0.008384	0.000171	2.92	0.003
Pure Error	24	0.001406	0.000059		
Total	75	0.033045			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0115803	70.38%	69.56%	67.60%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.40954	0.00138	296.39	0.000	
Sine(week)	-0.01097	0.00198	-5.55	0.000	1.00
Cosine(week)	0.02238	0.00186	12.04	0.000	1.00

Regression Equation

Casein Nitrogen % = 0.40954 -0.01097 Sine(week) +0.02238
Cosine(week)

Fits and Diagnostics for Unusual Observations

Casein						
Obs	Nitrogen %	Fit	Resid	Std Resid		
33	0.37190	0.41617	-0.04427	-3.92	R	
34	0.36367	0.41323	-0.04956	-4.39	R	

R Large residual

Normal probability plot of Residuals for Casein Nitrogen %

Residuals vs Fits for Casein Nitrogen %

Casein to Total Nitrogen Ratio versus Sine(week), Cosine(week)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	2	0.012633	0.006316	42.12	0.000
Sine(week)	1	0.004191	0.004191	27.95	0.000
Cosine(week)	1	0.008650	0.008650	57.69	0.000
Error	73	0.010946	0.000150		
Lack-of-Fit	49	0.008013	0.000164	1.34	0.222
Pure Error	24	0.002933	0.000122		
Total	75	0.023579			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0122455	53.58%	52.30%	49.52%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.79687	0.00146	545.37	0.000	
Sine(week)	-0.01104	0.00209	-5.29	0.000	1.00
Cosine(week)	0.01493	0.00197	7.60	0.000	1.00

Regression Equation

Casein Nitrogen /Total Nitrogen = 0.79687 -0.01104Sine(week)
+0.01493 Cosine(week)

Fits and Diagnostics for Unusual Observations

Casein/Total					
Obs	Nitrogen	Fit	Resid	Std Resid	
1	0.75660	0.78338	-0.02678	-2.23	R
33	0.76628	0.79850	-0.03223	-2.70	R
34	0.76287	0.79626	-0.03339	-2.80	R
67	0.78873	0.81359	-0.02486	-2.06	R

R Large residual

Normal probability plot of Residuals for Casein to Total Protein ratio

Residuals vs Fits for Casein to Total Protein ratio

Casein to True Nitrogen ratio versus Sine(week), Cosine(week)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	2	0.018274	0.009137	41.38	0.000
Sine(week)	1	0.002519	0.002519	11.41	0.001
Cosine(week)	1	0.015972	0.015972	72.34	0.000
Error	73	0.016118	0.000221		
Lack-of-Fit	49	0.010674	0.000218	0.96	0.562
Pure Error	24	0.005444	0.000227		
Total	75	0.034392			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0148593	53.13%	51.85%	49.13%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.83828	0.00177	472.79	0.000	
Sine(week)	-0.00856	0.00254	-3.38	0.001	1.00
Cosine(week)	0.02029	0.00239	8.51	0.000	1.00

Regression Equation

Casein/True Nitrogen = 0.83828 - 0.00856 Sine(week)
+ 0.02029 Cosine(week)

Fits and Diagnostics for Unusual Observations

	Casein/True				
Obs	Nitrogen	Fit	Resid	Std Resid	
33	0.80792	0.84532	-0.03740	-2.58	R
34	0.80345	0.84276	-0.03931	-2.72	R

R Large residual

Normal Probability plot of Residuals for Casein/True Nitrogen

Residuals vs Fits for Casein/True Nitrogen

Total calcium % versus Sine(week), Cosine(week)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	2	0.000821	0.000411	48.86	0.000
Sine(week)	1	0.000028	0.000028	3.32	0.088
Cosine(week)	1	0.000774	0.000774	92.04	0.000
Error	73	0.001501	0.000021		
Lack-of-Fit	49	0.000877	0.000018	0.69	0.868
Pure Error	24	0.000625	0.000026		
Total	75	0.002542			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0028994	59.32%	58.11%	55.51%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.109990	0.000359	306.77	0.000	
Sine(week)	0.000941	0.000517	2.01	0.048	1.00
Cosine(week)	0.004630	0.000483	9.59	0.000	1.00

Regression Equation

Total calcium % = 0.109990 + 0.000941 Sine(week)
+ 0.004630 Cosine(week)

Fits and Diagnostics for Unusual Observations

Obs	Total calcium %	Fit	Resid	Std Resid	
37	0.117902	0.112012	0.005890	2.09	R
42	0.103170	0.109228	-0.006058	-2.15	R
64	0.115250	0.108497	0.006752	2.37	R
71	0.105581	0.113266	-0.007685	-2.70	R

Normal Probability plot of Residuals for Total calcium %

Residuals vs Fits for Total calcium %

Non-Casein Nitrogen% versus Sine(week), Cosine(week)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	2	0.000396	0.000198	2.70	0.074
Sine(week)	1	0.000393	0.000393	5.35	0.023
Cosine(week)	1	0.000004	0.000004	0.06	0.813
Error	73	0.005362	0.000073		
Lack-of-Fit	49	0.002978	0.000061	0.61	0.927
Pure Error	24	0.002383	0.000099		
Total	75	0.005758			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0085701	6.88%	4.33%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.10621	0.00102	103.87	0.000	
Sine(week)	0.00338	0.00146	2.31	0.023	1.00
Cosine(week)	-0.00033	0.00138	-0.24	0.813	1.00

Regression Equation

Non-Casein Nitrogen% = 0.10621 +0.00338 Sine(week) -0.00033 Cosine(week)

Normal Probability plot of Residuals for Non-Casein Nitrogen%

Residuals vs Fits for Non-Casein Nitrogen%

Non-Protein Nitrogen% versus Sine(week), Cosine(week)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	2	0.000573	0.000286	27.29	0.000
Sine(week)	1	0.000072	0.000072	6.87	0.011
Cosine(week)	1	0.000494	0.000494	47.06	0.000
Error	73	0.000766	0.000010		
Lack-of-Fit	49	0.000410	0.000008	0.56	0.955
Pure Error	24	0.000356	0.000015		
Total	75	0.001339			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0032392	42.78%	41.21%	38.12%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.025377	0.000387	65.66	0.000	
Sine(week)	0.001448	0.000553	2.62	0.011	1.00
Cosine(week)	0.003567	0.000520	6.86	0.000	1.00

Regression Equation

Non-Protein Nitrogen% = 0.025377 + 0.001448 Sine(week) + 0.003567 Cosine(week)

Normal Probability plot of Residuals for Non-Protein Nitrogen%

Residuals vs Fits for Non-Protein Nitrogen%

Fat % versus Sine(week), Cosine(week)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	2	1.6008	0.80039	1.91	0.156
Sine(week)	1	1.5998	1.59975	3.82	0.055
Cosine(week)	1	0.0006	0.00056	0.00	0.971
Error	72	30.1730	0.41907		

Lack-of-Fit	48	19.5105	0.40647	0.91	0.614
Pure Error	24	10.6625	0.44427		
Total	74	31.7738			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.647356	5.04%	2.40%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	2.5148	0.0783	32.11	0.000	
Sine(week)	-0.220	0.113	-1.95	0.055	1.00
Cosine(week)	-0.004	0.104	-0.04	0.971	1.00

Regression Equation

Fat % = 2.5148 - 0.220 Sine(week) - 0.004 Cosine(week)

Normal Probability plot of Residuals for Fat %

Residuals vs Fits for Fat %

Total Solids% versus Sine(week), Cosine(week)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	2	3.8053	1.9026	4.50	0.014
Sine(week)	1	3.1523	3.1523	7.46	0.008
Cosine(week)	1	0.6726	0.6726	1.59	0.211
Error	72	30.4408	0.4228		
Lack-of-Fit	48	19.6277	0.4089	0.91	0.623
Pure Error	24	10.8131	0.4505		
Total	74	34.2461			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.650222	11.11%	8.64%	3.24%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	11.4793	0.0787	145.94	0.000	
Sine(week)	-0.309	0.113	-2.73	0.008	1.00
Cosine(week)	0.132	0.105	1.26	0.211	1.00

Regression Equation

Total Solids% = 11.4793 - 0.309 Sine(week) + 0.132 Cosine(week)

Normal Probability plot of Residuals for Total Solids%

Residuals vs Fits for Total Solids%

pH versus Sine(week), Cosine(week)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	2	0.000590	0.000295	0.19	0.831
Sine(week)	1	0.000034	0.000034	0.02	0.883
Cosine(week)	1	0.000560	0.000560	0.35	0.554
Error	73	0.115803	0.001586		
Lack-of-Fit	49	0.076253	0.001556	0.94	0.580
Pure Error	24	0.039550	0.001648		

Total 75 0.116393

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.0398290	0.51%	0.00%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.66140	0.00475	1401.68	0.000	
Sine(week)	0.00100	0.00679	0.15	0.883	1.00
Cosine(week)	-0.00380	0.00639	-0.59	0.554	1.00

Regression Equation

pH = 6.66140 +0.00100 Sine(week) - 0.00380 Cosine(week)

Normal Probability plot of Residuals for pH

Residuals vs Fits for pH

Somatic Cells/ μ l versus Sine(week), Cosine(week)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	2	2098	1049.00	0.16	0.850
Sine(week)	1	2068	2067.96	0.32	0.572
Cosine(week)	1	39	39.43	0.01	0.938
Error	73	468553	6418.53		
Lack-of-Fit	49	427717	8728.92	5.13	0.000
Pure Error	24	40835	1701.47		
Total	75	470651			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
80.1157	0.45%	0.00%	0.00%

Coefficients

Term	Coef	SE	Coef	T-Value	P-Value	VIF
Constant	261.11	9.56	27.31	0.000		
Sine(week)	-7.8	13.7	-0.57	0.572	1.00	
Cosine(week)	1.0	12.9	0.08	0.938	1.00	

Regression Equation

Somatic Cells/mic L = 261.11 -7.8 Sine(week) + 1.0 Cosine(week)

Normal Probability plot of Residuals for Somatic Cells/ μl

Residuals vs Fits for Somatic Cells/ μl

Appendix 3. Temperature Profile in Visalia and Fresno (Central Valley California) from 2008 -2009 (Obtained from www.weathersource.com)

Appendix 4. The precipitation in Visalia and Fresno (Central Valley California) from 2008 -2009 (Obtained from www.weathersource.com)

Appendix 5. LMPS Mozzarella Composition after 5 Days of Manufacture (raw data)

Month	Sample Code	Total Nitrogen %	Water Soluble Nitrogen %	Total solids %	Moisture %	Fat %	Fat in Dry Matter %
Jul-08	C080712	3.44	0.27	51.76	48.24	21.25	41.05
Jul-08	C080726	3.49	0.27	52.02	47.98	20.50	39.41
Aug-08	C080809	3.49	0.28	51.77	48.23	21.25	41.05
Aug-08	C080823	3.55	0.27	52.32	47.68	20.75	39.66
Sep-08	C080906	3.61	0.32	52.03	47.97	21.65	41.61
Sep-08	C080920	3.58	0.33	51.73	48.27	22.00	42.53
Oct-08	C0801004	3.59	0.28	52.22	47.78	22.25	42.61
Oct-08	C0801018	3.62	0.33	52.23	47.77	21.85	41.83
Nov-08	C0801101	3.68	0.33	52.03	47.97	21.55	41.42
Nov-08	C0801115	3.73	0.33	52.74	47.26	21.50	40.76
Dec-08	C0801213	3.73	0.33	52.59	47.41	21.48	40.84
Dec-08	C0801227	3.79	0.33	52.99	47.01	19.58	36.94
Jan-09	C090110	3.72	0.33	51.76	48.24	20.50	39.61
Jan-09	C090124	3.78	0.32	52.93	47.07	20.50	38.73
Feb-09	C090207	3.71	0.31	51.62	48.38	20.25	39.23
Feb-09	C090221	3.61	0.34	51.76	48.24	22.25	42.99
Mar-09	C090307	3.63	0.34	52.03	47.97	22.25	42.76
Mar-09	C090321	3.63	0.36	50.37	49.63	21.50	42.68
Apr-09	C090404	3.54	0.30	52.91	47.09	21.50	40.64
Apr-09	C090418	3.53	0.30	52.05	47.95	21.25	40.83
May-09	C090502	3.65	0.33	51.84	48.16	21.50	41.48
May-09	C090516	3.50	0.38	51.73	48.27	21.80	42.14
May-09	C090530	3.45	0.38	51.25	48.75	21.89	42.71
Jun-09	C090613	3.50	0.29	52.64	47.36	22.00	41.79
Jun-09	C090627	3.51	0.27	52.40	47.60	21.65	41.32
Jul-09	C090711	4.30	0.30	52.59	47.41	21.25	40.41
Jul-09	C090725	4.32	0.32	52.99	47.01	21.50	40.58
Aug-09	C090808	3.67	0.45	50.76	49.24	21.58	42.51
Aug-09	C090823	3.88	0.35	51.93	48.07	20.75	39.96
Sep-09	C090905	3.72	0.39	51.70	48.30	22.28	43.08
Sep-09	C090919	3.73	0.34	51.73	48.27	20.50	39.63
Oct-09	C091005	3.82	0.36	51.76	48.24	20.50	39.61
Oct-09	C0901031	3.78	0.31	52.30	47.70	20.25	38.72
Nov-09	C090114	3.84	0.29	52.49	47.51	22.25	42.39
Nov-09	C0901128	3.78	0.33	50.86	49.14	21.50	42.28

Month	Sample Code	Cheese Filtrate Calcium %	Total calcium %	Salt %	pH
Jul-08	C080712	na	na	2.19	5.47
Jul-08	C080726	na	na	2.05	5.43
Aug-08	C080809	0.283	0.57	2.235	5.43
Aug-08	C080823	0.277	0.58	2.07	5.45
Sep-08	C080906	0.286	0.59	2.145	5.45
Sep-08	C080920	0.255	0.61	2.235	5.41
Oct-08	C0801004	0.286	0.58	2.095	5.54
Oct-08	C0801018	0.270	0.59	2.06	5.49
Nov-08	C0801101	0.266	0.59	2.005	5.43
Nov-08	C0801115	0.249	0.62	2.125	5.50
Dec-08	C0801213	0.241	0.60	2.03	5.51
Dec-08	C0801227	0.256	0.63	2.135	5.47
Jan-09	C090110	0.268	0.60	2.305	5.51
Jan-09	C090124	0.251	0.59	2.33	5.43
Feb-09	C090207	0.248	0.58	2.19	5.51
Feb-09	C090221	0.261	0.60	2.25	5.49
Mar-09	C090307	0.272	0.59	2.085	5.47
Mar-09	C090321	0.246	0.61	2.055	5.51
Apr-09	C090404	0.278	0.63	1.98	5.43
Apr-09	C090418	0.248	0.63	2.135	5.46
May-09	C090502	0.302	0.62	2.095	5.42
May-09	C090516	0.285	0.55	2.185	5.43
May-09	C090530	0.282	0.54	2.355	5.54
Jun-09	C090613	0.289	0.53	1.88	5.48
Jun-09	C090627	0.271	0.61	2.12	5.44
Jul-09	C090711	na	0.59	2.235	5.45
Jul-09	C090725	na	0.60	2.235	5.49
Aug-09	C090808	0.260	0.61	2.15	5.45
Aug-09	C090823	0.267	0.63	2.16	5.48
Sep-09	C090905	0.328	0.50	2.18	5.55
Sep-09	C090919	0.279	0.58	2.215	5.51
Oct-09	C091005	0.270	0.58	2.095	5.51
Oct-09	C0901031	0.278	0.59	2.225	5.48
Nov-09	C090114	0.275	0.59	2.22	5.47
Nov-09	C0901128	0.272	0.61	2.12	5.43

Appendix 6. LMPS Mozzarella Textural Analysis after 5 Days of Manufacture (raw data)

Month	Sample Code	Hardness (g)	Cohesiveness	Springiness	Chewiness (g)
Jul-08	C080712	3088.37	0.49	0.66	1001.54
Jul-08	C080726	3467.30	0.52	0.66	1192.26
Aug-08	C080809	3409.97	0.52	0.69	1213.72
Aug-08	C080823	3741.80	0.54	0.68	1369.04
Sep-08	C080906	3854.83	0.56	0.70	1515.13
Sep-08	C080920	3741.03	0.52	0.71	1366.58
Oct-08	C0801004	3737.33	0.53	0.70	1405.31
Oct-08	C0801018	4299.93	0.54	0.73	1697.88
Nov-08	C0801101	4264.63	0.54	0.74	1707.63
Nov-08	C0801115	4656.63	0.56	0.75	1942.61
Dec-08	C0801213	4725.17	0.56	0.73	1913.76
Dec-08	C0801227	5353.40	0.56	0.74	2229.54
Jan-09	C090110	4514.60	0.57	0.72	1845.81
Jan-09	C090124	4474.73	0.53	0.71	1663.97
Feb-09	C090207	4212.87	0.53	0.70	1541.58
Feb-09	C090221	3868.03	0.51	0.69	1367.06
Mar-09	C090307	3887.07	0.50	0.69	1342.52
Mar-09	C090321	3973.27	0.49	0.66	1298.46
Apr-09	C090404	3889.67	0.46	0.65	1164.65
Apr-09	C090418	3878.63	0.48	0.64	1208.81
May-09	C090502	3900.53	0.48	0.63	1182.01
May-09	C090516	3414.10	0.49	0.64	1066.17
May-09	C090530	3100.07	0.48	0.58	852.41
Jun-09	C090613	3485.43	0.50	0.61	1055.01
Jun-09	C090627	3491.20	0.49	0.59	1014.55
Jul-09	C090711	6354.03	0.56	0.76	2698.35
Jul-09	C090725	4804.40	0.50	0.73	1752.31
Aug-09	C090808	4671.87	0.48	0.61	1373.51
Aug-09	C090823	4394.93	0.66	0.74	2148.13
Sep-09	C090905	4305.70	0.43	0.58	1076.63
Sep-09	C090919	4387.10	0.42	0.65	1193.98
Oct-09	C091005	4449.87	0.55	0.68	1654.94
Oct-09	C0901031	5452.63	0.47	0.71	1801.58
Nov-09	C090114	5502.37	0.48	0.64	1690.39
Nov-09	C0901128	4845.97	0.52	0.64	1605.07

Month	Sample Code	Aggregation Index	% Loss in shredder
Jul-08	C080712	6.23	na
Jul-08	C080726	6.02	na
Aug-08	C080809	6.06	9.78
Aug-08	C080823	6.07	8.66
Sep-08	C080906	5.80	9.15
Sep-08	C080920	5.83	9.79
Oct-08	C0801004	5.76	7.30
Oct-08	C0801018	5.80	8.02
Nov-08	C0801101	5.76	8.95
Nov-08	C0801115	5.49	7.84
Dec-08	C0801213	5.76	8.68
Dec-08	C0801227	5.72	8.33
Jan-09	C090110	6.04	8.18
Jan-09	C090124	5.94	8.70
Feb-09	C090207	6.18	8.70
Feb-09	C090221	6.29	9.09
Mar-09	C090307	6.25	10.00
Mar-09	C090321	6.23	10.45
Apr-09	C090404	6.35	10.87
Apr-09	C090418	6.38	10.45
May-09	C090502	5.98	11.29
May-09	C090516	6.28	11.16
May-09	C090530	6.31	12.50
Jun-09	C090613	5.96	12.76
Jun-09	C090627	6.28	12.12
Jul-09	C090711	6.11	8.05
Jul-09	C090725	5.96	8.13
Aug-09	C090808	6.01	9.33
Aug-09	C090823	6.04	9.20
Sep-09	C090905	6.22	11.76
Sep-09	C090919	6.04	10.71
Oct-09	C091005	5.91	9.48
Oct-09	C0901031	5.92	9.42
Nov-09	C090114	6.23	8.90
Nov-09	C0901128	6.15	9.54

Appendix 7. LMPS Mozzarella Textural Analysis after 5 Days of Manufacture

Variable	Total Count	Mean	StDev	Minimum	Maximum
Total Nitrogen %	33	3.6452	0.1207	3.4362	3.8822
WSN %	33	0.32569	0.04006	0.26776	0.45318
Total solids	33	51.976	0.599	50.370	52.988
Moisture %	33	48.024	0.599	47.012	49.630
Fat %	33	21.335	0.707	19.575	22.275
Fat in Dry Matter %	33	41.054	1.508	36.943	43.084
Water Soluble Calcium	33	0.27130	0.01814	0.24103	0.32846
Total calcium %	33	0.59113	0.03014	0.49764	0.63113
salt %	33	2.1425	0.1011	1.8800	2.3550
pH	33	5.4738	0.0381	5.4067	5.5533
Hardness	33	4135	624	3088	5502
Cohesiveness	33	0.51363	0.04481	0.41887	0.65763
Springiness	33	0.67380	0.04877	0.57697	0.74777
Chewiness	33	1445.5	344.4	852.4	2229.5
Aggregation Index	33	6.0389	0.2192	5.4860	6.3814
% Loss in shredder	33	9.714	1.395	7.303	12.759

Appendix 8. LMPS Mozzarella Ripened at 3.3⁰ C for 21 Days (raw data)

Month	Sample Code.	Hardness (g)	Cohesiveness	Springiness	Chewiness (g)
Jul-08	C080712	2671.27	0.46	0.61	741.97
Jul-08	C080726	3271.43	0.49	0.61	972.23
Aug-08	C080809	3254.07	0.46	0.61	919.85
Aug-08	C080823	3686.37	0.49	0.64	1157.54
Sep-08	C080906	3756.47	0.51	0.67	1293.32
Sep-08	C080920	3604.07	0.47	0.67	1148.78
Oct-08	C0801004	3566.23	0.49	0.67	1171.34
Oct-08	C0801018	3996.97	0.52	0.68	1408.84
Nov-08	C0801101	3762.07	0.51	0.68	1295.19
Nov-08	C0801115	4329.30	0.52	0.70	1595.41
Dec-08	C0801213	4687.43	0.52	0.68	1676.98
Dec-08	C0801227	4820.00	0.53	0.65	1645.23
Jan-09	C090110	4472.33	0.52	0.66	1553.94
Jan-09	C090124	3925.07	0.51	0.65	1304.44
Feb-09	C090207	4038.00	0.50	0.63	1266.62
Feb-09	C090221	3652.17	0.49	0.65	1167.38
Mar-09	C090307	3510.13	0.48	0.64	1066.60
Mar-09	C090321	3706.70	0.47	0.62	1083.97
Apr-09	C090404	3657.10	0.46	0.61	1023.16
Apr-09	C090418	3638.47	0.45	0.61	1008.85
May-09	C090502	3584.07	0.46	0.58	958.77
May-09	C090516	3182.30	0.47	0.61	900.63
May-09	C090530	2770.57	0.46	0.57	735.26
Jun-09	C090613	3270.23	0.49	0.56	900.84
Jun-09	C090627	3016.77	0.46	0.56	773.76
Jul-09	C090711	5355.60	0.47	0.69	1736.13
Jul-09	C090725	4600.87	0.46	0.68	1439.11
Aug-09	C090808	4257.33	0.46	0.61	1205.20
Aug-09	C090823	3790.33	0.42	0.58	926.94
Sep-09	C090905	3627.80	0.47	0.61	1029.67
Sep-09	C090919	3193.73	0.45	0.59	839.16
Oct-09	C091005	3902.07	0.48	0.64	1186.32
Oct-09	C0901031	4490.97	0.55	0.61	1487.22
Nov-09	C090114	4489.00	0.45	0.63	1258.65
Nov-09	C0901128	4589.00	0.46	0.63	1309.71

Month	Sample Code	WSN %	Aggregation index	% Loss in shredder	pH
Jul-08	C080712	0.31	6.39	na	5.58
Jul-08	C080726	0.35	6.17	14.89	5.65
Aug-08	C080809	0.38	6.17	10.37	5.66
Aug-08	C080823	0.33	6.11	11.85	5.47
Sep-08	C080906	0.41	5.81	11.26	5.51
Sep-08	C080920	0.36	6.15	16.73	5.44
Oct-08	C0801004	0.45	5.92	9.91	5.56
Oct-08	C0801018	0.37	5.91	10.68	5.49
Nov-08	C0801101	0.45	5.84	10.08	5.55
Nov-08	C0801115	0.40	5.95	10.05	5.55
Dec-08	C0801213	0.42	5.91	10.00	5.51
Dec-08	C0801227	0.42	5.84	10.48	5.51
Jan-09	C090110	0.40	6.11	9.52	5.44
Jan-09	C090124	0.34	6.20	10.00	5.51
Feb-09	C090207	0.34	6.22	11.36	5.55
Feb-09	C090221	0.36	6.33	12.40	5.48
Mar-09	C090307	0.37	6.39	13.50	5.52
Mar-09	C090321	0.39	6.35	13.04	5.44
Apr-09	C090404	0.37	6.33	12.30	5.54
Apr-09	C090418	0.41	6.41	12.50	5.49
May-09	C090502	na	6.22	13.18	5.54
May-09	C090516	na	6.32	13.16	5.54
May-09	C090530	0.51	6.12	16.00	5.55
Jun-09	C090613	0.39	6.19	14.44	5.63
Jun-09	C090627	0.39	6.01	14.29	5.53
Jul-09	C090711	0.33	6.12	12.12	5.48
Jul-09	C090725	0.42	6.19	12.90	5.49
Aug-09	C090808	na	6.18	15.46	5.55
Aug-09	C090823	0.43	6.13	13.04	5.49
Sep-09	C090905	0.43	6.28	16.67	5.54
Sep-09	C090919	0.38	6.25	15.49	5.55
Oct-09	C091005	0.39	6.09	13.85	5.57
Oct-09	C0901031	0.35	6.29	12.31	5.49
Nov-09	C090114	0.34	6.22	12.50	5.54
Nov-09	C0901128	0.35	6.21	14.29	5.52

Appendix 9. LMPS Mozzarella Ripened at 8.9⁰ C for 21 Days (raw data)

Month	Sample code.	Hardness (g)	Cohesiveness	Springiness	Chewiness (g)
Jul-08	C080712	2593.83	0.47	0.60	730.10
Jul-08	C080726	3183.17	0.49	0.61	937.90
Aug-08	C080809	3160.07	0.46	0.61	897.06
Aug-08	C080823	3593.87	0.47	0.64	1094.41
Sep-08	C080906	3534.53	0.50	0.66	1176.51
Sep-08	C080920	3570.10	0.46	0.66	1084.51
Oct-08	C0801004	3470.80	0.49	0.67	1145.13
Oct-08	C0801018	3753.67	0.52	0.67	1320.81
Nov-08	C0801101	3570.57	0.49	0.67	1188.87
Nov-08	C0801115	4210.83	0.52	0.69	1520.96
Dec-08	C0801213	4477.47	0.52	0.67	1563.59
Dec-08	C0801227	4449.43	0.51	0.66	1496.23
Jan-09	C090110	4330.53	0.53	0.65	1483.13
Jan-09	C090124	3777.23	0.50	0.65	1223.81
Feb-09	C090207	3734.13	0.48	0.64	1149.22
Feb-09	C090221	3563.63	0.51	0.65	1166.79
Mar-09	C090307	3421.73	0.46	0.63	994.77
Mar-09	C090321	3448.57	0.46	0.62	994.24
Apr-09	C090404	3447.20	0.42	0.61	886.07
Apr-09	C090418	3549.43	0.46	0.62	1011.90
May-09	C090502	3275.10	0.45	0.58	854.92
May-09	C090516	2860.30	0.46	0.60	801.32
May-09	C090530	2522.60	0.46	0.57	663.30
Jun-09	C090613	2887.67	0.47	0.56	761.33
Jun-09	C090627	2849.77	0.46	0.54	711.81
Jul-09	C090711	5135.37	0.47	0.65	1559.93
Jul-09	C090725	4352.30	0.46	0.65	1296.96
Aug-09	C090808	4083.50	0.44	0.60	1078.40
Aug-09	C090823	3600.67	0.44	0.59	944.43
Sep-09	C090905	2585.87	0.49	0.60	751.24
Sep-09	C090919	2888.70	0.46	0.61	821.89
Oct-09	C091005	3290.07	0.50	0.62	1023.53
Oct-09	C0901031	4496.63	0.42	0.66	1253.72
Nov-09	C090114	4620.19	0.44	0.62	1261.15
Nov-09	C0901128	4586.85	0.45	0.61	1260.58

Month	Sample Code.	WSN %	Aggregation index	% Loss in shredder	pH
Jul-08	C080712	0.37	6.32	15.24	na
Jul-08	C080726	0.41	6.20	11.32	5.52
Aug-08	C080809	0.38	6.21	14.33	na
Aug-08	C080823	0.36	6.12	13.65	5.52
Sep-08	C080906	0.41	5.88	17.05	5.49
Sep-08	C080920	0.38	6.19	11.59	na
Oct-08	C0801004	0.50	6.07	12.99	5.60
Oct-08	C0801018	0.44	6.00	12.62	5.49
Nov-08	C0801101	0.47	5.81	12.62	5.52
Nov-08	C0801115	0.43	6.04	na	5.58
Dec-08	C0801213	0.45	5.92	11.30	5.52
Dec-08	C0801227	0.44	5.88	12.50	5.49
Jan-09	C090110	0.42	6.14	12.27	5.43
Jan-09	C090124	0.37	6.06	13.18	5.52
Feb-09	C090207	0.39	6.23	14.29	5.57
Feb-09	C090221	0.37	6.25	15.50	5.50
Mar-09	C090307	0.42	6.43	12.50	5.50
Mar-09	C090321	0.42	6.42	14.47	5.43
Apr-09	C090404	0.38	6.38	17.86	5.53
Apr-09	C090418	0.43	6.46	15.42	5.52
May-09	C090502	na	6.26	15.95	5.43
May-09	C090516	na	6.34	17.91	5.55
May-09	C090530	0.52	6.23	17.24	5.60
Jun-09	C090613	0.41	6.23	15.15	5.57
Jun-09	C090627	0.42	6.12	12.60	5.51
Jul-09	C090711	0.40	6.23	13.79	5.49
Jul-09	C090725	0.51	6.23	17.91	5.48
Aug-09	C090808	na	6.18	14.53	5.56
Aug-09	C090823	0.44	6.27	18.97	5.50
Sep-09	C090905	0.47	6.30	13.51	5.56
Sep-09	C090919	0.39	6.25	17.65	5.57
Oct-09	C091005	0.39	6.19	12.68	5.57
Oct-09	C0901031	0.37	6.24	10.53	5.51
Nov-09	C090114	0.34	6.17	12.50	5.52
Nov-09	C0901128	na	6.27	na	5.53

Appendix 10. Statistical Analysis of 21 days Ripened LMPS Mozzarella

1. Descriptive Statistics of Cheese Parameters when Ripened at 3.3^o C

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1
Hardness 38 F	33	0	3760.1	95.0	545.5	2593.8	3390.8
Cohesiveness 38 F	33	0	0.48254	0.00514	0.02953	0.41835	0.45943
Springiness 38 F	33	0	0.62767	0.00636	0.03655	0.56313	0.60702
Chewiness 38 F	33	0	1151.9	44.8	257.4	735.3	942.9
% Loss in shredder 38 F	32	1	12.675	0.375	2.123	9.524	10.527
Aggregation index 38 F	32	1	6.1444	0.0294	0.1663	5.8124	6.0324
WSN % 38 F	30	3	0.38052	0.00644	0.03526	0.31320	0.35134
pH 38 F	33	0	5.5294	0.00924	0.0531	5.4367	5.4883

Variable	Median	Q3	Maximum
Hardness 38 F	3686.4	4147.7	4820.0
Cohesiveness 38 F	0.47625	0.51014	0.54631
Springiness 38 F	0.62533	0.65735	0.70273
Chewiness 38 F	1157.5	1299.8	1677.0
% Loss in shredder 38 F	12.500	14.286	16.735
Aggregation index 38 F	6.1768	6.2705	6.3893
WSN % 38 F	0.37722	0.40792	0.45073
pH 38 F	5.5367	5.5533	5.6600

2. Descriptive Statistics of Cheese Parameters when Ripened at 8.9^o C

Variable	N	N*	Mean	SE Mean	StDev	Minimum	Q1
Hardness 48 F	33	0	3557	104	598	2523	3172
Cohesiveness 48 F	33	0	0.47558	0.00505	0.02898	0.42096	0.45897
Chewiness 48 F	33	0	1068.3	42.9	246.2	663.3	870.5
Springiness 48 F	33	0	0.62596	0.00622	0.03572	0.54463	0.60365
% Loss in shredder 48 F	31	2	14.191	0.402	2.236	10.526	12.500
Aggregation index 48 F	33	0	6.1838	0.0277	0.1591	5.8126	6.0948
WSN % 48 F	29	4	0.40944	0.00706	0.03802	0.33658	0.37660
pH 48 F	30	3	5.5238	0.00808	0.0443	5.4300	5.5025

Variable	Median	Q3	Maximum
Hardness 48 F	3549	3930	4620
Cohesiveness 48 F	0.46725	0.49824	0.52528
Chewiness 48 F	1078.4	1238.8	1563.6
Springiness 48 F	0.62337	0.65620	0.69350
% Loss in shredder 48 F	13.650	15.500	18.966
Aggregation index 48 F	6.2113	6.2695	6.4564
WSN % 48 F	0.41392	0.43298	0.49821
pH 48 F	5.5200	5.5617	5.6033

3. Paired t-tests between Cheese Parameters when Ripened at 3.3⁰ C (38F) and 8.9⁰ C (48F)

Water Soluble Nitrogen(WSN %) when Ripened at 3.3⁰ C (38F) and 8.9⁰ C (48F)

Null Hypothesis Ho: Mean difference = 0; Alternate Hypothesis Mean difference not = to zero

Paired T for WSN % 38 F - WSN % 48 F

	N	Mean	StDev	SE Mean
WSN % 38 F	29	0.38164	0.03534	0.00656
WSN % 48 F	29	0.40944	0.03802	0.00706
Difference	29	-0.02780	0.01971	0.00366

95% CI for mean difference: (-0.03530, -0.02030)

T-Test of mean difference = 0 (vs not = 0): T-Value = -7.60 P-Value = 0.000

At 95% confidence limit ($\alpha = 0.05$); with p-value<0.05 we can reject Ho, hence there is a significant difference in WSN of LMPs Mozzarella when ripened at 3.3⁰ C (38F) and 8.9⁰ C (48F)

Normality check

Null Hypothesis (Ho): The difference of WSN % 38 F and WSN % 48 F follows Normal Distribution

Alternate Hypothesis (H1): The difference of WSN % 38 F and WSN % 48 F does not follow Normal Distribution

From the Normality Plot below we can see that the p-value >0.05 ($\alpha = 0.05$), hence we cannot reject Ho, hence the difference of the data follows Normal Distribution

Normality Plot

Hardness when Ripened at 3.3⁰ C (38F) and 8.9⁰ C (48F)

Null Hypothesis Ho: Mean difference = 0; Alternate Hypothesis Mean difference not = to zero

	N	Mean	StDev	SE Mean
Hardness 38 F	33	3760	545	95
Hardness 48 F	33	3557	598	104
Difference	33	202.8	203.2	35.4

95% CI for mean difference: (130.8, 274.9)

T-Test of mean difference = 0 (vs not = 0): T-Value = 5.73 P-Value = 0.000

At 95% confidence limit ($\alpha = 0.05$); with $p\text{-value} < 0.05$ we can reject Ho, hence there is a significant difference in hardness of LMPs Mozzarella when ripened at 3.3⁰ C (38F) and 8.9⁰ C (48F)

Normality Check

Null Hypothesis (Ho): The difference of Hardness 38 F and Hardness 48 F follows Normal Distribution

Alternate Hypothesis (H1): The difference of WSN % 38 F and WSN % 48 F does not follow Normal Distribution

From the Normality Plot below we can see that the $p\text{-value} > 0.05$ ($\alpha = 0.05$), hence we cannot reject Ho, hence the difference of the data follows Normal Distribution

Normality Plot

Cohesiveness when Ripened at 3.3⁰ C (38F) and 8.9⁰ C (48F)

Null Hypothesis Ho: Mean difference = 0; Alternate Hypothesis Mean difference not = to zero

	N	Mean	StDev	SE Mean
Cohesiveness 38 F	33	0.48254	0.02953	0.00514
Cohesiveness 48 F	33	0.47558	0.02898	0.00505
Difference	33	0.00696	0.02505	0.00436

95% CI for mean difference: (-0.00192, 0.01584)

T-Test of mean difference = 0 (vs not = 0): T-Value = 1.60 P-Value = 0.120

At 95% confidence limit ($\alpha = 0.05$); with p-value > 0.05 we cannot reject Ho, hence there is no significant difference in cohesiveness of LMPS Mozzarella when ripened at 3.3⁰ C (38F) and 8.9⁰ C (48F)

Normality Check

Null Hypothesis (Ho): The difference of Cohesiveness 38 F and Cohesiveness 48 F follows Normal Distribution

Alternate Hypothesis (H1): The difference of WSN % 38 F and WSN % 48 F does not follow Normal Distribution

From the Normality Plot below we can see that the p-value > 0.05 ($\alpha = 0.05$), hence we cannot reject Ho, hence the difference of the data follows Normal Distribution

Normality Plot

Springiness when Ripened at 3.3⁰ C (38F) and 8.9⁰ C (48F)

Null Hypothesis Ho: Mean difference = 0; Alternate Hypothesis Mean difference not = to zero

		N	Mean	StDev	SE Mean
Springiness	38 F	33	0.62767	0.03655	0.00636
Springiness	48 F	33	0.62596	0.03572	0.00622
Difference		33	0.00171	0.01360	0.00237

95% CI for mean difference: (-0.00311, 0.00654)

T-Test of mean difference = 0 (vs not = 0): T-Value = 0.72 P-Value = 0.475

At 95% confidence limit ($\alpha = 0.05$); with p-value > 0.05 we cannot reject Ho, hence there is no significant difference in springiness of LMPS Mozzarella when ripened at 3.3⁰ C (38F) and 8.9⁰ C (48F)

Normality Check

Null Hypothesis (Ho): The difference of Springiness 38 F and Springiness 48 F follows Normal Distribution

Alternate Hypothesis (H1): The difference of WSN % 38 F and WSN % 48 F does not follow Normal Distribution

From the Normality Plot below we can see that the p-value > 0.05 ($\alpha = 0.05$), hence we cannot reject Ho, hence the difference of the data follows Normal Distribution

Normality Plot

Chewiness when Ripened at 3.3⁰ C (38F) and 8.9⁰ C (48F)

Null Hypothesis Ho: Mean difference = 0; Alternate Hypothesis Mean difference not = to zero

	N	Mean	StDev	SE Mean
Chewiness 38 F	33	1151.9	257.4	44.8
Chewiness 48 F	33	1068.3	246.2	42.9
Difference	33	83.6	65.5	11.4

95% CI for mean difference: (60.4, 106.9)

T-Test of mean difference = 0 (vs not = 0): T-Value = 7.34 P-Value = 0.000

At 95% confidence limit ($\alpha = 0.05$); with p-value < 0.05 we can reject Ho, hence there is a significant difference in chewiness of LMPs Mozzarella when ripened at 3.3⁰ C (38F) and 8.9⁰ C (48F)

Normality Check

Null Hypothesis (Ho): The difference of Chewiness 38 F and Chewiness 48 F follows Normal Distribution

Alternate Hypothesis (H1): The difference of WSN % 38 F and WSN % 48 F does not follow Normal Distribution

From the Normality Plot below we can see that the p-value > 0.05 ($\alpha = 0.05$), hence we cannot reject Ho, hence the difference of the data follows Normal Distribution

Normality Plot

Aggregation index when Ripened at 3.3⁰ C (38F) and 8.9⁰ C (48F)

Null Hypothesis Ho: Mean difference = 0; Alternate Hypothesis Mean difference not = to zero

	N	Mean	StDev	SE Mean
Aggregation index 38 F	32	6.1444	0.1663	0.0294
Aggregation index 48 F	32	6.1753	0.1538	0.0272
Difference	32	-0.0309	0.0632	0.0112

95% CI for mean difference: (-0.0537, -0.0081)

T-Test of mean difference = 0 (vs not = 0): T-Value = -2.77 P-Value = 0.009

At 95% confidence limit ($\alpha = 0.05$); with p-value < 0.05 we can reject Ho, hence there is a significant difference in aggregation of LMPS Mozzarella when ripened at 3.3⁰ C (38F) and 8.9⁰ C (48F)

Normality Check

Null Hypothesis (Ho): The difference of Aggregation index 38 F and Aggregation index 48 F follows Normal Distribution

Alternate Hypothesis (H1): The difference of WSN % 38 F and WSN % 48 F does not follow Normal Distribution

From the Normality Plot below we can see that the p-value > 0.05 ($\alpha = 0.05$), hence we cannot reject Ho, hence the difference of the data follows Normal Distribution

Normality Plot

Percentage Loss in Shredder when Ripened at 3.3⁰ C (38F) and 8.9⁰ C (48F)

Null Hypothesis Ho: Mean difference = 0; Alternate Hypothesis Mean difference not = to zero

Paired T for % Loss in shredder 38 F - % Loss in shredder 48 F

	N	Mean	StDev	SE Mean
% Loss in shredder 38 F	30	12.709	2.118	0.387
% Loss in shredder 48 F	30	14.156	2.266	0.414
Difference	30	-1.447	2.771	0.506

95% CI for mean difference: (-2.481, -0.412)

T-Test of mean difference = 0 (vs not = 0): T-Value = -2.86 P-Value = 0.008

At 95% confidence limit ($\alpha = 0.05$); with p-value < 0.05 we can reject Ho, hence there is a significant difference in loss during shredding of LMPs Mozzarella when ripened at 3.3⁰ C (38F) and 8.9⁰ C (48F)

Normality Check

Null Hypothesis (Ho): The difference of %loss in shredder 38 F and %loss in shredder 48F follows Normal Distribution

Alternate Hypothesis (H1): The difference of WSN % 38 F and WSN % 48 F does not follow Normal Distribution

From the Normality Plot below we can see that the p-value > 0.05 ($\alpha = 0.05$), hence we cannot reject Ho, hence the difference of the data follows Normal Distribution

Normality Plot

pH when Ripened at 3.3⁰ C (38F) and 8.9⁰ C (48F)

Null Hypothesis Ho: Mean difference = 0; Alternate Hypothesis Mean difference not = to zero

	N	Mean	StDev	SE Mean
pH 38 F	30	5.52656	0.04626	0.00845
pH 48 F	30	5.52378	0.04426	0.00808
Difference	30	0.00278	0.04027	0.00735

95% CI for mean difference: (-0.01226, 0.01781)

T-Test of mean difference = 0 (vs not = 0): T-Value = 0.38 P-Value = 0.708

At 95% confidence limit ($\alpha = 0.05$); with p-value > 0.05 we can reject Ho, hence there is no significant difference in pH of LMPs Mozzarella when ripened at 3.3⁰ C (38F) and 8.9⁰ C (48F)

Normality Check

Null Hypothesis (Ho): The difference of %loss in shredder 38 F and %loss in shredder 48F follows Normal Distribution

Alternate Hypothesis (H1): The difference of WSN % 38 F and WSN % 48 F does not follow Normal Distribution

From the Normality Plot below we can see that the p-value > 0.05 ($\alpha = 0.05$), hence we cannot reject Ho, hence the difference of the data follows Normal Distribution

Normality Plot