

An Investigation into Improvement of Low Fat Cheddar Cheese by the  
Addition of Hydrocolloids

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## **Dedication**

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## Abstract

Low fat cheeses (less than 3 g fat/50 g of cheese) suffer from texture defects not apparent in full fat cheese. Polysaccharides have been added to reduced fat cheeses with the goal of replacing the properties fat provides. The goal of this research is to evaluate the incorporation of polysaccharides as a filler gel into the cream portion of cheese milk used to make low-fat Cheddar cheese as a way to improve the texture of the cheese by mimicking the gel-filling property of fat. Five different hydrocolloids were evaluated for incorporation into a filler gel that was added to the cream required to make a 0.5% fat cheese milk, a portion of the skim milk and homogenized. Hydrocolloids evaluated were alginate, xanthan gum pectin, carrageen and Novagel RCN 15 (microcrystalline cellulose and guar gum). The hydrocolloids were mixed with water and a whey protein concentrate (Avonlac 180) that contains a high level of milk fat globule membrane on a high shear mixer. The gel was mixed with the total amount of cream, and an amount of skim which created a blend that was 20% of the total cheese milk. This blend was homogenized on a two-stage Niro Panda homogenizer at 160 bar (1<sup>st</sup>: stage 110 bar; 2<sup>nd</sup> stage: 50 bar). Cheese was made in 10 Kg lab scale batches using a modified low-fat Cheddar stirred curd procedure with pre-acidification of the cheese milk to pH 6.2. Cheese was pressed in small Wilson-style hoops with 40 pounds of pressure. The cheese was evaluated by instructing untrained panelists to place coded samples of cheese (which also contained low and full-fat control) on an unanchored 24 x 24 inch sheet of paper, spatially relative to each other based on flavor and texture differences. All samples were analyzed on a TAXT-Plus Texture analyzer by texture profile analysis. Novagel and Pectin containing

samples most approximated the texture of full-fat Cheddar and were selected for pilot scale processing. Cheese was then made in 1200 pounds batches using the procedure described. Samples were analyzed throughout aging for texture, proteolysis, and organic acids. Descriptive sensory analysis and microscopic evaluation by confocal scanning laser microscopy were conducted at the end of shelf-life. Low fat cheese treated with Novagel and Pectin did not show differences in descriptive sensory analysis. There were no differences in firmness score analyzed by TPA between the treatments however; low fat cheese containing whey protein concentrate had more resilient, gummy and chewy texture. None of the treatments showed any differences in age related proteolysis compared to low fat cheese and full fat cheese. All low fat cheeses were found different ( $P < 0.05$ ) in organic acids content compared to full fat cheese. Pectin treated cheeses had the highest level of lactic acid and Novagel treated cheese had the highest formic acid. Microstructural examination through confocal microscopy indicated that pectin and Novagel were most likely retained in the treatment cheeses. This study describes an effort made to improve low fat Cheddar cheese in bench top and pilot scale production by addition of different hydrocolloids.

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# CHAPTER 1

## 1.1 Introduction

In 1990, no U.S. state had an obese population greater than 15%. By 2007, in all but one state, at least 25% of the population was considered obese (Centers for Disease Control and Prevention, 2008). Full fat Cheddar cheese contains 9 grams of fat per 30 gram serving. This means that based on a 2000 calorie diet, one serving equates to 15% of the recommended daily intake (FDA, 2011). In order to qualify for a low-fat health claim, a block eating cheese such as Cheddar must have less than 3 grams of fat per 50 grams of cheese. This is an 82% reduction in fat from the full fat version of Cheddar cheese (USDA, Nutrient Data Laboratory, 2011). The dietary guidelines and a desire for consumption of low fat products have influenced trends in the market place. This has resulted in an increased demand for low fat products. A recent study conducted by Dairy Management Inc. and Taylor Nelson Sofres found that 16 percent of adults ages between 20 and 54 are restricting cheese in their diet (“cheese restrictors”) (DMI, 2009). The same study also claims that 29 percent of “cheese restrictors” would be willing to incorporate cheese into their diet, if low fat cheese was available without comprising flavor and texture.

For these reasons, there is great interest in developing low fat dairy products to satisfy this demand. Many different manufacturing changes have been evaluated for use in low fat cheese production. These ingredients and processing changes will be highlighted in this chapter.

## **1.2 Literature Review**

Low fat cheese has been researched for many years by many authors. Because of the extreme level of fat reduction to qualify for “low fat” in the United States, a perfect solution has not been found. Many techniques have been investigated to improve the functionality of low fat cheese. These techniques have centered around manufacturing changes, culture selection and ingredient inclusion. In order to understand why these changes have been studied to improve low fat cheese, an understanding of the effect of fat on flavor, texture and chemistry is necessary.

### **1.2.1 Definition, classification, and composition of cheese**

According to FAO (1978), “Cheese is the fresh or mature solid or semi-solid product obtained by coagulating milk, skimmed milk, partly skimmed milk, cream, whey cream, or butter milk or any combination of these materials through the action of rennet or other suitable coagulating agents and by partially draining the whey resulting from such coagulation.”

Cheese can be classified many ways based on various characteristics such as composition, method of ripening, fat content, moisture content, and mode of coagulation. Table 1 depicts three ways to classify cheese based on moisture content, fat content, and ripening characteristics.

Table 1. Classification of cheese by moisture content, fat content, and ripening characteristics (Sammis, 1948; Davis, 1965; Fox, 2004).

MFFB <sup>1</sup> (%)	Term I		Term II		Term III
	Description based on firmness	FDB <sup>2</sup> (%)	Description based on fat	Description as per main curing characteristics	
< 51	Extra hard	> 60	High fat	1. Cured or ripened (a) Mainly surface (b) Mainly interior	
49-56	Hard	45-50	Full fat		
54-63	Semi hard	25-45	Medium fat	2. Mold cured/ripened (a) Mainly surface (b) Mainly interior	
61-69	Semi soft	10-25	Low fat		
> 67	Soft	< 10	Skim	3. Uncured/unripened	

<sup>1</sup>**MFFB:** Moisture on fat-free basis

<sup>2</sup>**FDB:** Fat on dry basis

The trend of consuming low calorie/low fat food has created a demand for reduced and low fat cheese products (Sandrou and Arvanitoyannis, 2000). Table 2 illustrates the variation in composition of Cheddar cheese when formulated to different fat levels as described by several authors.

Table 2. Composition of Cheddar cheese (Metzger and Mistry, 1994; Oommen et al., 2000; Nurcan Koca, 2004; Kucukoner and Haque, 2006; Rogers et al., 2009).

Constituent	Type of cheese		
	Full fat	Reduced fat	Low fat
Fat (%)	32 - 37	15 - 18	5 - 6
Moisture (%)	36 - 39	42 - 48	50 - 55
Protein (%)	24 - 26	28 - 32	30 - 35
Ash (%)	3.7	3.5 - 3.6	3.4
pH	5.2 - 5.4	5.0 - 5.2	4.9 - 5.2

Manufacturing of reduced and low fat Cheddar cheese poses a great challenge to the cheese industry since the reduction of fat in cheese affects the texture, flavor, and functional properties such as melting and stretching. These effects on the properties of cheese, and the various strategies which have been tried to address these effects are discussed in the next section.

### **1.2.2 Cheese making procedures**

Cheese manufacturing involves two distinct phases; manufacturing and ripening. The casein, fat, and colloidal salts of milk are concentrated 6-12 fold during the removal of water from milk, and this concentration factor is based on the cheese variety. The

manufacturing phase involves the removal of 90% of the water and a substantial amount of the lactose, whey proteins, and soluble milk salts (Scott, 1981).

Most cheese varieties are comprised of five basic cheese making steps. These steps include acidification (by addition of starter culture or food grade acid), coagulation of proteins (by a proteinase), removal of water (manipulation of the coagulum by cutting, heating and acidification of the curd), salting and shaping (molding, pressing, curd manipulation) (Fox, 2004). Figure 1 illustrates the fundamental manufacturing process of cheese.

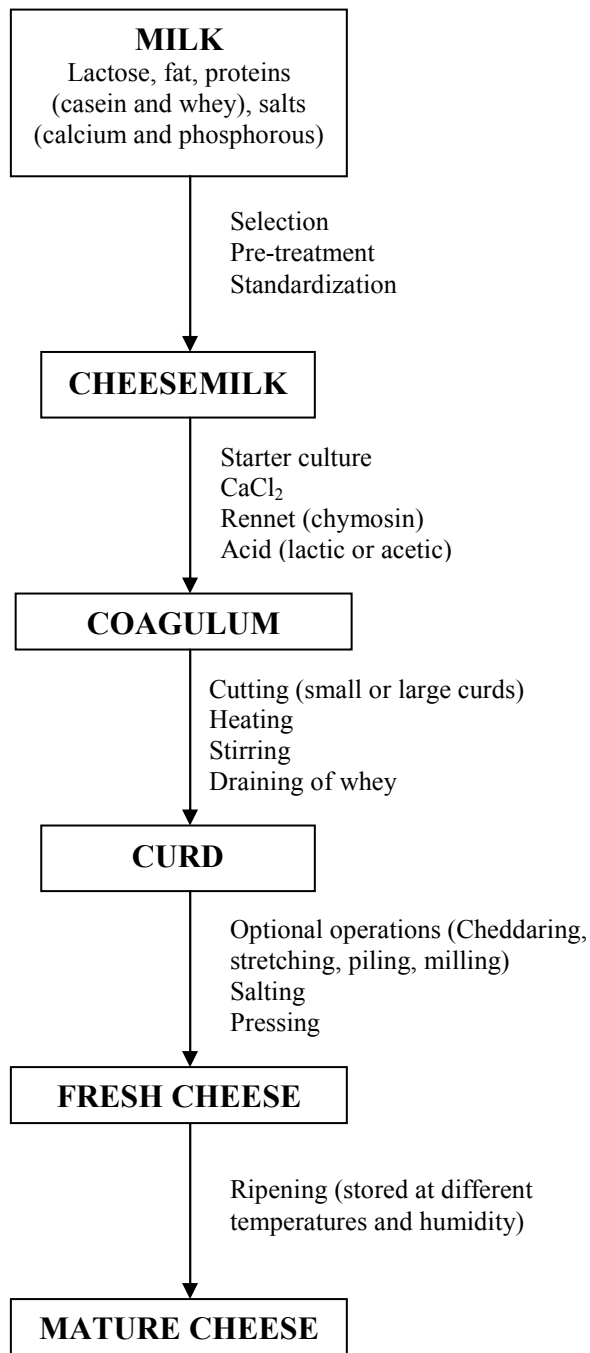


Figure 1. Cheese manufacturing process (adapted from Upadhyay, 2003; Fox and Cogan, 2004)

### 1.2.3 Formation of milk coagulum

When raw or pasteurized milk is left unrefrigerated for a period of time, milk becomes sour which indicates that it has fermented. The bacterial action in the milk causes this change. When a curd forms, it is known as coagulation or curdling. Caseins, the predominant protein type found in milk, can be precipitated by lactic acid produced by bacteria that ferment lactose. When the pH approaches the isoelectric point (approximately pH 4.6) the charge on the casein micelle will approach its minimum, and the casein micelles will aggregate and a coagulum will be formed if done quiescently. This is the process by which acid coagulated cheeses like cottage and cream cheese are produced.

The casein proteins are associated in a structure called the casein micelle. It is composed of the different casein proteins ( $\alpha_{s1}$ ,  $\alpha_{s2}$ ,  $\beta$ ,  $\kappa$ ), salt components (calcium, magnesium, phosphate, and citrate), and peptide fragments (Gordon and Kalan, 1965; Brule and Lenoir, 1987). This association protects the caseins from precipitating due to the calcium in milk.

The enzymatic coagulation of milk is the basis for rennet coagulated cheeses, like Cheddar. In contrast to acid coagulation, the pH of rennet coagulated cheese does not need to be significantly lowered. Rennet coagulation is a 2-stage process, which involves an enzymatic phase and non enzymatic phase (Figure 2).

## Enzymatic Phase (Primary)

Casein + Chymosin = Para  $\kappa$ -casein + Glycomacropeptide

$\approx 90\%$   $\kappa$ -casein hydrolyzed  
Ionic Calcium  
>20 °C



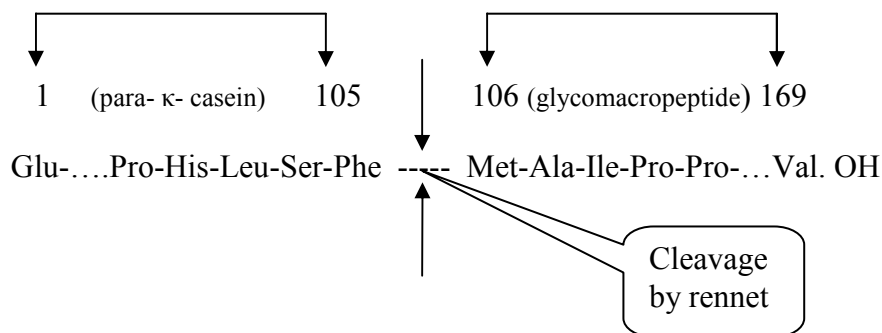
## Non- Enzymatic Phase (Secondary)

Aggregation of micelles due to loss of steric hindrance (the carbohydrate portion no longer provides charge, preventing aggregation)

Figure 2. Mechanism of rennet coagulation (adapted from Fox et al., 2000b)

There are several factors described for the stability of the micelle in milk. Primary factors include the net charge and degree of hydration of the casein micelle. Secondary factors are related to micellar composition (such as the amount of  $\kappa$ - casein), and concentrations of calcium and phosphate (Brule and Lenoir, 1987).

The casein micelle is formed by the casein proteins associating via hydrophobic interactions, and calcium-phosphate bridges between phospho-serine amino acids. The micelle has a high concentration of  $\kappa$ - casein on the outer surface, while  $\alpha_{s1}$ -  $\alpha_{s2}$  and  $\beta$  – caseins are predominantly in the interior (Walstra, 1984). The peptide backbone of  $\kappa$ - casein, when cleaved by the action of chymosin, consists of two segments; para-  $\kappa$ - casein, and the glyco-macropeptide. The glyco-macropeptide (106-169 residues) has a high negative charge, is hydrophilic in nature, and is lost to the whey in cheese. The N terminal end (1-105 residues) carries a positive charge and is hydrophobic (Brule and Lenoir, 1987). It becomes part of the cheese coagulum. The primary phase of coagulation involves the rapid hydrolysis of  $\kappa$  –casein by rennet on a specific site that causes the destabilization of casein micelle (Figure 3).



As a consequence of cleaving the peptide bond between amino acids 105 -106, the net charge on the micelles is reduced as a result of the loss of the hydrophilic C terminal end which results in destabilization of the micelle from solution (Fox et al., 2000b).

The secondary phase of coagulation starts when almost 85% of  $\kappa$ -casein has been hydrolyzed. The secondary phase of rennet coagulation is unclear, but it is possible that the calcium induces cross-linkages and aggregation of casein micelles (Guinee and O'Brien, 2010).

The casein micelles bind together in the presence of calcium via serine phosphate residues or by electrostatic interactions (attractive forces). Hydrophobic interactions also play a role in aggregation as casein micelles ( $\alpha_{S1\&2}$ - and  $\beta$ -caseins) are rich in hydrophobic regions (Harboe et al., 2010). The aggregation of destabilized micelles eventually forms a three dimensional network, referred to as a coagulum. This gel formation increases in viscosity as the hydrolysis of  $\kappa$ -casein and aggregation increases (Scott, 1981). The coagulum is considered to be a gel in which fat globules and water are entrapped in the three dimensional casein network, so the fat act as a filler. Fat exists as globules surrounded by a structured membrane, described as the milk fat globule membrane (MFGM). During cheese making these MFGM gets damaged due to several processing conditions, and free fat will float on the surface of the milk. The free fat is not entrapped in the coagulum and hence lost in the whey (Desai and Nolting, 1995).

#### **1.2.4 Role of fat in cheese making**

Cheese consists mainly of water, fat, protein, and minerals. The cheese matrix may be viewed as proteins forming the body building structure of the matrix, with water entrapped inside the matrix and fat acting as inert filler (Le et al., 2011).

The size of fat globules and composition influences the incorporation of fat, and affects the amount of fat retained in cheese (Scott, 1998). The composition and quantity of milk fat varies widely depending on species, breed, stage of lactation, season, and environment. These factors lead to the variations in the quantity and quality of fat, thus influencing cheese making (Goff and Hill, 1993). The composition of fat affects the melting point and hence there may be release of molten fat because of processing conditions during cheese making. Larger fat globules may ooze out when curd is pressed at temperatures above 27 °C. Larger fat globules are more likely to get damaged and distorted as a result may exist as free fat (no MFGM) entrapped in the protein matrix as compared to smaller fat globules. Fat affects several aspects of cheese; composition, yield, microstructure, biochemical, textural, functional and rheological properties. Fat also contributes to flavor development through lipolysis as free fatty acids serve as precursors for other flavor compounds (Collins et al., 2003).

Fat acts as a plasticizer and affects cheese texture. The plasticizing effect of fat improves the fluidity of the cheese matrix. The fat globules present in the cheese matrix reduce the density and continuity of casein matrix (Rajah, 2002). During cheese making fat globules aggregates as the casein matrix dehydrates with certain processing conditions like cooking of the curd. As the fat content reduces in low fat cheese, the absence of fat globules results in the casein matrix; being more elastic and continuous (Guinee and McSweeney, 2006). The addition of ingredients, such as hydrocolloids, when manufacturing low fat cheese may mimic the properties of fat in the casein matrix, and prevent the continuous, dense network thereby creating a softer bodied cheese.

The flavor of cheese can be directly affected by the fatty acid composition of the triglycerides, which serve as the source of free fatty acids which can be sources of flavor. During aging of some cheeses, fatty acids are hydrolyzed from the glycerol backbone, and these free fatty acids are converted to form flavor compounds such as lactones, esters, methyl ketones, and carbonyls (Fox et al., 2000a). Foda et al. (1974) investigated the role of different kinds of milk fat, singly or in combination with altered fatty acid composition, treated with mineral oil or butter milk solids in the development of flavor in Cheddar cheese. They found that cheese made with natural milk fat, and covered with MFGM that had the native enzymes played a role in flavor development. Similar findings were reported by Fox et al. (2000a).

In reduced and low fat cheeses the distinctive cheese flavor is lacking. Also, the texture of reduced and low fat cheeses is not comparable to full fat cheeses (Drake and Swanson, 1995). Gunasekaran and Ak (2003) conveyed the role of fat as such an important characteristics that even if the moisture is higher in low fat Cheddar cheese, the texture will be hard due to more compact protein matrix with less open spaces.

### **1.2.5 Changes in milk pretreatment when reducing fat**

The critical parameters contributing to the body and texture of any cheese are the casein matrix pH, charge repulsion, proteolytic activity, saturation level of fat, fat content, and moisture content (Johnson and Law, 2010). The increase in moisture in low fat cheese is one of the several practices advocated in making low fat cheese more comparable to full fat. Several authors have tried to increase the moisture content of low fat cheeses to mimic the properties of full fat cheeses in an effort to improve the texture

(Banks et al., 1989; Johnson and Chen, 1995; Oommen et al., 2000; Lobato Calleros et al., 2001; Konuklar et al., 2004; Dabour et al., 2006) .

One of the pretreatments researched is homogenization (Metzger and Mistry, 1995; Rudan et al., 1998; Nair et al., 2000; Oommen et al., 2000). The process of homogenization results in the reduction in size of fat globules in smaller fat globules leading to increase in globular surface area (Walstra, 2005). In general practice, homogenization of milk is preferred to avoid clumping of larger fat globules which afterwards float to the surface of milk.

Usually the practice of homogenization of milk for fluid milk production is done by warming the milk (40° C – 60° C) to allow for increased homogenization efficiency, but cold homogenization (refrigerated temperature) is preferred in the case of improving low fat cheese texture to avoid the denaturation of proteins and the interaction of whey protein with  $\kappa$ -casein, resulting in the retention of whey proteins in cheese.

Homogenization at higher temperatures promotes disulfide linkages between  $\beta$ -lactoglobulin and  $\kappa$  – casein which adversely affects renneting (Malin et al., 1995). The sites available for chymosin to cleave on specific sites of  $\kappa$  –casein will not be as easily accessible when it interacts with  $\beta$ -lactoglobulin (Huppertz and Kelly, 2006).

Homogenization is not usually practiced in the manufacture of most cheese varieties except cream, blue, and some soft varieties (Upadhyay, 2003). Several approaches have been made for improving the functional properties of cheese through homogenization (Tunick et al., 1993; Metzger and Mistry, 1994; Nair et al., 2000). Homogenization of cream used in making Cheddar cheese enhanced the melting properties of cheese during ripening (Oommen et al., 2000). In another study,

homogenization of cream in Cheddar cheese led to decreased free oil, increased emulsification of fat, and improved the texture of reduced fat cheese (Metzger and Mistry, 1995). They reported an increase in surface area of the fat globules, and an increased amount of denatured casein and whey proteins associated with the surface of the fat, which increases the amount of water they bind (Malin et al., 1995).

Homogenization of cream and milk for reduced fat mozzarella cheese was studied and the cheeses had less free oil, had a whiter appearance and directionally lower TPA hardness values than the control cheeses with no homogenization (Rudan et al., 1998). Researchers have also investigated the effect of high pressure homogenization (HPH) of milk at 100 MPa for making Caciotta cheese (a mild soft Italian cheese) (Lanciotti et al., 2006). The HPH treatment of milk resulted in extensive proteolytic activity evidenced by low molecular weight peptides. HPH treated caciotta cheese received significantly lower bitterness scores than the untreated pasteurized and raw milk cheeses.

Preacidification of milk prior to renneting is another way researchers have attempted to achieve a softer body in low fat cheeses (Metzger et al., 2000; Farkye, 2004; Sheehan and Guinee, 2004). Reducing the pH of milk at refrigerated temperature dissociates calcium from the casein micelle. Reducing pH of milk prior to renneting by organic acids decreases the calcium content of cheese thus increasing the moisture in low fat mozzarella cheese (Metzger et al., 2001).

Reducing the pH also affects the dissolution of colloidal calcium phosphate which affects the gel strength and final amount of calcium in the finished cheese. As a result, the ratio of soluble calcium to colloidal calcium increases leading to hydration of paracasein. This influences the textural properties of cheese during proteolysis (Fox et al., 2000).

### **1.2.6 Selection of starter cultures and adjunct cultures**

The type and inoculation rate of starter cultures is one of the areas researchers have investigated for increasing the moisture content and slowing acid development (Drake and Swanson, 1995). The most commonly used starter cultures are *Lactococcus* spp., and adjunct cultures are *Lactobacillus* spp. (Drake and Swanson, 1995).

Several researchers have studied the effect of exopolysaccharides (EPS) producing strains in an effort to improve the functionality of cheese with reduced fat (Dabour et al., 2005; Agrawal and Hassan, 2007; Hassan et al., 2007). Costa et al., (2010) reported that a ropy capsular EPS producing strain of *L. lactis* spp. *cremoris* had significantly increased moisture resulting in higher yields than non EPS producing strains. They also reported improved texture and melting properties that were similar to full fat Cheddar cheese. In another study, exopolysaccharide producing strains of Lactococci (both ropy and capsular) were used in the manufacture of reduced fat Cheddar cheese (Dabour et al., 2006). Both types of strains showed positive texture modifying properties, with the ropy strain showing an open and weak structure in the ripened cheese.

### **1.2.7 Changes to cheese making procedures when reducing fat to increase moisture, and reduce calcium retention**

In order to improve the properties of cheese when reducing fat, the first step is modification to cheese making procedures to replace the fat lost. As already mentioned, increasing the moisture is desirable. Modifications to the cook temperature (lowering), curd size (increasing) and pH target (increasing) are ways to increase the moisture. Johnson and Chen (1995) studied the effect of washing the curd (no wash, 22° C and 35°

C wash water temperature) on moisture, fat and pH of the Cheddar cheese. They reported an increase in moisture with curd washed at 22° C and a higher pH was obtained with no curd wash treatment.

Kowalchuk and Olson (1977) studied the effects of pH and temperature on the secondary phase of milk clotting by rennet. Reducing the pH of milk from 6.8 to 6.28 prior to renneting and increasing temperature of milk during renneting from 31° C to 40° C increased firmness of the curd (Kowalchuk and Olson, 1977) (so the opposite approach can be used to decrease firmness). Banks et al. (1989) reported that reducing the cook temperature from 39° C to 35° C and stirring for 30 min resulted in an increase in moisture content. Guinee et al. (1998) demonstrated that a higher pasteurization temperature (88° C for 16 seconds) and higher pH (5.7) at milling resulted in high moisture and softer reduced fat cheese but the higher pasteurization temperature adversely affected the rennet coagulation.

The effect of rennet concentration for making low fat Iranian white cheese was evaluated by Madadlou et al., (2005). Doubling the amount of rennet addition (9.0 international milk clotting units (IMCU) of Chy-Max /kg of milk) from control (4.5 IMCU) had positive effects on the sensory and rheological properties of the cheese. The study further reported that tripling the amount of rennet had adverse effects on the firmness and overall quality of cheese. Increased amount of rennet up to three fold significantly increased the calcium content and the TCA soluble nitrogen of the cheese. TCA soluble nitrogen correlates directly with proteolysis in the cheese. Tripling the amount of rennet did not affect flavor and appearance of the cheese in this study.

pH and calcium affect the basic structure of cheese, and changes made to increase the moisture (increasing the pH target) can have the unintended consequence of increasing the amount of calcium in the cheese. Lawrence et al., (1987) explained that as the pH at renneting, and the final pH target increases, retention of calcium in the casein matrix increases. This results in a firm, elastic texture as seen in Swiss and Gouda cheeses.

Lucey et al., (2003) discussed that washing curd or diluting whey during the cooking step helps in reducing soluble calcium in the cheese. They also summarized that the addition of salt in the wash water decreased the ionic strength which resulted in a cheese with a softer texture, and better melt and flow properties. Lee et al., (2005) examined the role of insoluble calcium during acid development in cheese ripening. They found that cheese with higher pH (> 5.0) and low insoluble calcium, resulted in a less dense matrix, and provides cheese with improved melting properties.

### **1.2.8 Ingredients to replace fat**

Ingredients used as fat replacers in food products are usually protein or carbohydrates based, and mimic the sensory and physical functions of fat (Vorhagen, 1998). A key purpose of these ingredients is to bind the water that is added to provide softness when fat is removed.

Hydrocolloids are molecules that can bind water in a food product and allow for the modification of texture by altering the viscosity and gel characteristics of the food. The choice of hydrocolloid used depends on the attributes desired in the product. These can include modifying the sensory attributes, process ability, and other quality parameters (Williams and Phillips, 2000).

Hydrocolloids have been used extensively in the dairy industry in formulated products such as yogurt, sour cream, cheese spreads, ice cream and reduced fat products.

#### **1.2.8.1 Hydrocolloid functional properties, and structure**

Some polysaccharides molecules are linear and some of them are branched. The degree of branching affects the physical properties such as water solubility, viscosity and gelling behavior (Whistler and Smart, 1953). Generally, polysaccharides which are branched are easily soluble in water and have thickening abilities whereas linear molecules perform as structural materials as they are closely packed and form intermolecular cross-linking which makes them rigid and almost insoluble (Misaki, 1994). Some of the usages in food industry are summarized in Table 3.

Table 3. Functions of hydrocolloids in different food products (Glicksman, 1982)

<b>Functions</b>	<b>Example in food products</b>
Adhesion	Glazes, icings, frostings
Binding agent	Pet foods
Bodying agent	Beverages
Crystallization inhibitor	Ice cream, sugar syrups, frozen foods
Clarifying agent (fining)	Beer, wine
Cloud agent	Fruit drinks, beverages
Coating agent	Confectionery, fabricated onion rings
Dietary fiber	Cereals, bread, yogurt, beverages
Emulsifier	Salad dressings
Encapsulating agent	Powdered flavors
Film former	Sausage casings, protective coatings
Foam stabilizer	Whipped toppings, beer
Gelling agent	Puddings, desserts, confectionery
Molding	Gum drops, jelly candies
Protective colloid	Flavor emulsions
Stabilizer	Salad dressings, ice cream
Syneresis inhibitor	Cheese, frozen foods
Thickening agent	Jams, pie fillings, sauces
Whipping agent	Toppings, marshmallows

### 1.2.8.2 Types of hydrocolloids

Table 4. Commonly used hydrocolloids, their sources and applications in food industry  
(Williams and Phillips, 2003)

Hydrocolloid	Source	Functions
Agar	Red Seaweeds ( <i>Gelidium</i> spp.)	Gelation
Alginate	Brown Seaweeds ( <i>Macrocystis</i> , <i>Ascophyllum</i> , <i>Laminaria</i> and <i>Ecklonia</i> spp.)	Thickening, Emulsifying
Carageenan	Red Seaweeds ( <i>Gracilaria</i> , <i>Gigartina</i> and <i>Eucheuma</i> spp.)	Gelation, Viscosity
Carboxymethylcellulose	Cotton Cellulose	Gelation, Surfactant
Chitin, Chitosan	Invertebrates, lower forms of plants; shells of Crustaceans	Adhesion
Galactomannans	Seeds of guar, locust bean	Thickening, Gelling, Stabilizing, emulsification
Gum Arabica	Plant (stem exudates of <i>Acacia</i> <i>Senegal</i> )	Water absorption, decrystallization
Gum Tragacanth	Plant (Stem exudates of <i>Astragalus</i> spp.)	Emulsification, Thickening
Pectin	Citrus fruits, apple and other fruits	Gelation, Thickening
Starch	Cereal grains, tubers	Thickening, Coatings
Xanthan Gum	Biosynthetic ( <i>Xanthomonas</i> <i>campestris</i> )	Stabilizer, Thickening, Pseudoplasticizer)

### 1.2.8.3 Hydrocolloid applications in cheese and dairy products

The textural properties of cheese are formed by the matrix of casein proteins with fat entrapped within the matrix (Haque et al., 2007). Removing large amounts of fat results in a dense product where the protein dominates the texture properties (Fenelon and Guinee, 1997). The texture of cheese has been reported to be improved by an interaction of casein and hydrocolloids (Mistry, 2001).

Alginate occurs in the environment as brown algae (*Phaeophyceae*), as capsular polysaccharides in bacteria (*Azotobacter vinelandii*) found in soil and in several species of *Pseudomonas* (Gimmestad et al., 2009). Sodium salts of alginates are widely accepted because of their range of functional properties in the dairy products such as ice cream (Lal et al., 2006). Carageenan has a synergistic relationship with the casein molecules in milk. Kappa carageenan can interact with positively charged amino acids present in the casein molecules, thus incorporating the casein micelles directly into its gel structure (Piculell et al., 1994; Augustin et al., 1999). Totosaus and Guemes Vera (2008) researched the effect of kappa and gamma carageenan as fat replacers in low fat Oaxaca cheese (a white semi hard cheese from Mexico). They found that cheese with kappa carageenan had improved melting properties and higher yield but no effect on overall quality.

Novagel is a mixture of microcrystalline cellulose (MCC) and guar gum derived from fruits and vegetables by FMC Biopolymer (Philadelphia, PA) and can be used as a fat replacer in low-fat processed foods (FMC Corporation, 2010). , McMahon et al., (1996), used Novagel RCN 15 in low fat mozzarella cheese. They reported that addition of Novagel RCN 15 resulted in a less dense protein matrix by replacing the fat globules with cellulose particulates. There was also a significant increase in the moisture content;

however the melting properties were not improved. Romeih et al., (2002) used Simplese® D 100, a microparticulated whey protein concentrate and Novagel™ NC 200 in the making of low fat white brined cheese. Cheese containing Novagel™ NC 200 was less firm than any full fat control cheese. Another study by Hennelly et al., (2006) evaluated inulin, a soluble fiber, as a fat replacer in imitation cheese containing 8% fat. They reported it did not improve the melting properties of the cheese (Hennelly et al., 2006).

Pectin is a naturally occurring structural carbohydrate found in land plants and extracted commercially from citrus fruits and apples (Nussinovitch, 1997). Pectin is classified based on its degree of esterification; high-methoxyl and low-methoxyl pectins (BeMiller, 1986).

High methoxyl pectins are used in foods where calcium plays a role in physico-chemical properties because it allows for interaction between the pectin and food components (Hoefler, 1991). Macku et al., (2008) investigated the effect of different concentrations of pectin in processed cheese. They found that processed cheese made with 0.6 % to 0.8 % pectin was firmer in texture measured by a rheometer. Liu et al., (2008) reported that low fat cheese analogues made with pectin were similar to full fat analogues in terms of sensory and textural properties. Low fat analogues without pectin gel were poorer in texture mouthfeel. There were no significant differences found in aroma or color when compared with full fat cheese analogues.

Xanthan gum is produced by *Xanthomonas campestris* through a fermentation process. This polysaccharide consists of glucose, mannose and glucuronic acid residues.

Since its introduction to the marketplace in the 1960s, it has been used in many food products including dairy applications (Nussinovitch, 1997).

A combination of carageenan, xanthan gum and  $\beta$ -lactoglobulin with exopolysaccharide producing organisms were evaluated in low fat mozzarella cheese (Zisu, 2005). Fat replacers increased the cheese yield and improved the functional characteristics such as melting, stretching and baking performance.

Konuklar et al., (2004) reported that when Nutrim, a  $\beta$ -glucan based product, was added to low fat Cheddar cheese it improved the melting properties and lowered the firmness score. However, it had a negative effect on sensory parameters, such as increased bitterness and metallic flavors.

Many of the studies using hydrocolloids to date have added the ingredients to the full portion of the cheese milk, not considered other hydrocolloids that have had positive effects on texture in other dairy products, or combined them with cheese making modifications such as the homogenization of the fat portion of cheese milk, pre acidification of milk prior to cheese making, lower cook temperature of the curd, and a higher pH target at salting. We hypothesized that by providing a fat like structure by the addition of a filler gel, homogenized into the cream portion of cheese milk, we would create space in the protein matrix, making it less dense. The goal of this research is to evaluate the effect of different hydrocolloids incorporated into the cream portion of the cheese milk, with the addition of a whey protein concentrate with an elevated MFGM concentration, on low fat Cheddar cheese flavor, chemistry and texture.

## CHAPTER 2

### 2.1 Introduction

Full fat cheese contains approximately 9 grams of fat for every 30 gram serving. To qualify for a package claim of “low fat” in the United States, cheeses meant for eating as block cheese like Cheddar have to contain less than 3 g per 50 g of cheese. In the case of Cheddar cheese, this equates to an almost 82 % reduction in fat. This extreme reduction affects both the flavor and texture of the cheese.

The fat entrapped in the network formed by the casein proteins is responsible for much of the texture attributes of cheese (Banks, 2004). Low fat cheese does not inherit the properties of full fat cheese because of the lack of fat and results in a firm textured cheese predominated by the effects of the protein (Drake and Swanson, 1995).

Hydrocolloids have been evaluated in low fat cheese manufacture as a way to improve the textural characteristics, but they have not been homogenized into the cream portion of the milk (McMahon et al, 1996; Ma et al, 1997; Haque et al, 2007).

Homogenization of the cream portion of milk will increase moisture retention by denaturation of proteins and incorporation of whey proteins into the cheese, and has been shown to decrease firmness in low fat natural cheeses (Gilles and Lawrence, 1981; Metzger and Mistry, 1994).

It was hypothesized that creating a gel that can be emulsified into the fat during homogenization of cream will act as “filler” during curd formation and improve texture. The objective of this study was to screen which hydrocolloids are best suited to mimic the properties of fat in low fat cheese in small experimental batches, when they are homogenized with the cream portion of the cheese milk.

## **2.2 Materials and Methods**

### **2.2.1 Materials**

Pasteurized skim milk and pasteurized heavy whipping cream were purchased from a foodservice supplier in bulk. Alginate FD 155, Xanthan 80, Pectin XSS 100, and Carageenan CH 407 were obtained from Danisco USA Inc., (New century, KS) and Novagel RCN 15 was obtained from FMC Biopolymer, (Philadelphia, PA). Novagel RCN 15 is a mixture of microcrystalline cellulose and guar gum. Cultures Choozit M58 (selected strains of *Lactococcus lactis* ssp. *lactis*), Choozit Flavobac LF304 (*Lactobacillus casei*) and Flavogard 360 (selected strains of *Lactococcus lactis* ssp. *cremoris*), were sourced from Danisco USA Inc., (New Century, KS). Whey protein concentrate containing an elevated proportion of fat globule membrane, Avonlac 180, was obtained from Glanbia Nutritionals (Monroe, WI). The rennet (Chymax double strength fermentation rennet, 73863) and annatto color no. 70463 were acquired from Chr. Hansen (Milwaukee, WI). Lactic acid (85%) was added to reduce the milk pH and was USP/FCC grade a (Fisher Scientific, Fairlawn, NJ). Salt was purchased from Morton International Inc. (Chicago, IL).

### **2.2.2 Determining the concentration of hydrocolloids in the gel mix, and the mixing conditions**

The levels of hydrocolloids were determined initially based on the manufacturer's recommendation, literature review, and the principal investigator's experience. The hydrocolloids were weighed, dry mixed with whey protein concentrate, and added slowly to the pre weighed water in a glass jar container. A high shear mixer (Omni mixer model no: 17105, dial setting 4, rotor/stator tissue dismembrator blade configuration) was used

for blending the hydrocolloid mixture to observe any lumps in the mixture. The mixtures were made with both warm water and water at room temperature, and mixing time was varied, to identify the best conditions for obtaining a homogenous mass for each hydrocolloid. The mixtures were stored overnight at 4°C to observe if there was syneresis in the mixture. Viscosity was observed visually for each of the hydrocolloid mixtures for a gel like consistency with no syneresis after overnight storage.

### 2.2.3 Cheese making

The filler gel was prepared the day prior to cheese making a. The water temperature for Novagel, Carrageen, and low-fat control sample blending was 25° C and they were blended for 5 minutes. For xanthan gum, pectin and alginate, the water temperature was 40°C and mixing time was 7 minutes to completely blend the material. The formulas used for making the hydrocolloid blends are shown in Table 5.

Table 5. Hydrocolloid blend formulas used during lab scale trials

Blends	% Composition				
	Danisco Pectin XSS 100	Danisco Alginate FD155	Danisco Xanthan 80	FMC Novagel RCN 15	Danisco Carageenan CH 407
Water	99.34	99.56	99.58	98.95	99.25
Hydrocolloid	0.415	0.19	0.17	0.8	0.5
Avonlac 180	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100

Pasteurized cream in an amount necessary to standardize the cheese milk to 0.6% fat, 0.8 % hydrocolloid blend (as a percent of total cheese milk) and the amount of pasteurized skim milk necessary to produce a homogenized blend which would be 20%

of the total cheese milk were homogenized at 4° C temperature on a 2-stage homogenizer (Niro Panda, GEA, Hudson, WI) at 160 bar. The cheese making steps for lab scale production of a 10 kg (22 lb) batch are low fat cheese described below:

- Cream, filler gel and skim milk were combined and homogenized on a two-stage Niro Panda homogenizer at 160 bar (110 bar 1<sup>st</sup> stage, 50 bar 2<sup>nd</sup> stage). The quantity of the homogenized portion was 20% of the total cheese milk blend
- The homogenized cream/gel blend was added to remainder of the cheese milk
- Cheese milk was warmed to 31° C (88 F)
- Diluted (1:10) lactic acid solution was added to milk to adjust pH to 6.2
- Starter culture was added as Choozit M58:0.165g/ Kg of milk, Flavobac LF304:0.0516g / Kg of milk and Flavogard 360:0.019g/ Kg of milk
- Milk was agitated for 15 minutes
- Rennet was added at a rate of 0.096 ml/kg cheese milk
- Coagulum was cut in approximately 20 – 30 minutes
- Curd was allowed to heal for 5 minutes
- Curd was cooked with agitation and temperature was brought to 35.5° C (96 F) over 30 minutes (Titrable acidity (TA) target 0.135 – 0.14% lactic acid)
- The curd was held with stirring at 35.5° C (96 F) until a desired pH was reached (pH- 5.9 and TA approximately 0.18% lactic acid).
- Whey was drained and curd was dry stirred until pH 5.7 achieved (TA approx. 0.2% lactic acid)
- Salting was done in 3 equal stages, 5 minutes apart at a rate of 0.2% of the starting cheese milk

- Curd was hooped in custom one pound Wilson-style stainless steel cheese hoops overnight on vertical lever press with 8.83 Kg of weight on the lever (approximately 18.12 Kg weight on surface of the hoop)
- Cheese was pressed approximately 14 hours at room temperature, removed from hoops, vacuum packaged and stored at less than 4 ° C.

Low fat control was made with homogenized cream and skim, with no added whey protein concentrate or water. Full fat control was made with a stirred curd procedure with pH and TA targets designed to produce a good quality full fat cheese with the same cultures used for making low fat cheese.

The 0.8 Kg (1.76 lb) cheese block obtained after pressing was vacuum packaged for further evaluation. Samples of each type of cheese were taken for analysis at 0 and 1 month of ripening. The sample block was trimmed from all the sides before cut in half. The first half was analyzed for tests explained later in the section at 1 week of ripening. The second half was re- vacuum packed for analysis at one month age.

#### **2.2.4 Compositional analysis**

Vacuum Oven method (Method 18.10 A. *Class O*) for cheese was used as per Standard Methods for the Analysis of Dairy Products (Wehr, 2004). Gerber method (Method 18.8 D *Class O*) was used for determining the fat content of milk and cream. The Babcock method (Method 18.8 A. *Class O*) was used for cheese fat with 20% fat Paley bottles (Richardson, 1985). Protein was determined by measuring the total nitrogen using the Dumas method on a Leco nitrogen analyzer (Tru Spec N, Model No. 630-100-200, St. Joseph, MI) and converting it to protein content by multiplying with a factor of 6.38. The pH of the cheese samples was measured on an Acorn® pH 6 Meter (Oakton

Instruments, Vernon Hills, IL) with an Orion 8172BNWP Ross Sure-Flow pH electrode (Thermo Fisher Scientific, Inc., Waltham, MA). Yield percentage was calculated by dividing the weight of pressed curd to the weight of milk used.

### **2.2.5 Texture analysis**

Texture profile analysis (TPA) was conducted using a TA.XT 2i plus Texture Analyzer (Texture Technologies, Scarsdale, NY) at 0 and 1 month of aging using a two-bite compression test with TA-11 probe with 25 mm diameter and 35 mm tall acrylic cylinder. Samples were prepared immediately prior to analysis by cutting cheese samples with a no. 12 cork borer (15 mm diameter) and cutting to a length of 25 mm. Samples were compressed at 2.0 mm/second by 48% of the original height (12 mm) to pre-failure, and then compressed a second time after 6 seconds rest period by 75% (19 mm) of the original height which went past fracture in most samples.

### **2.2.6 Sensory analysis**

Sensory analysis was performed by untrained panelists (faculty, staff and students of University of Minnesota). Panelists were instructed to place coded cheese samples spatially on a 61 x 61 cm sheet of paper based on their perceived texture and flavor differences relative to each other. The locations of the samples were recorded, and the distance of each sample to the location the panelist placed the full-fat control (which was also presented blindly) was measured. Panelists were asked to record their comments in regards to flavor and texture for the sample groupings. Their comments were open-ended, and there was no prompting for the terms used.

### **2.2.7 Statistical analysis and sampling plan**

The experiment was replicated two times in a randomized block design. The compositional data analysis and texture data were analyzed using one-way ANOVA. All tests for TPA were conducted in triplicate. Differences between means were determined using Fisher's least significance difference (LSD) test at a 95 % confidence interval.

Statistical analysis for sensory was carried using a randomized complete block design which incorporated seven treatments, two replicates, nine panelists for blocking, and the distance was used as the response variable. Fisher's LSD test was used to determine whether statistically significant differences occurred between the means using XLSTAT<sup>®</sup> software (Addinsoft, 2010). The samples were analyzed at 0 and 1 month for sensory and texture evaluation.

## **2.3 Results and Discussion**

### **2.3.1 Compositional analysis**

The composition of the full fat and low fat cheeses are shown in Table 6. The fat and moisture contents of the low fat cheeses were not significantly different from each other. However, the moisture content of low fat control was directionally lower than the low fat treatment cheeses. Several authors who used fat replacers in cheese making reported an increase in moisture content of the treatments (Ma et al., 1997; Lobato Calleros et al., 2001; Sahan et al., 2008).

The pH of cheeses was significantly different between the samples. Full fat cheese was lower in moisture and had a lower pH than the low fat cheeses as intended by the use of the full fat cheese make procedure. No significant differences were found in protein and yield between the low fat treatments which indicates a consistent cheese make during duplication, and there were no issues with the ability to form and cut a coagulum.

Table 6. Chemical composition of stirred curd Cheddar cheeses with different hydrocolloids and with whey protein concentrate at 30 days of ripening

Parameters	Treatments						
	FFC <sup>1</sup>	LFC <sup>2</sup>	Alginate <sup>3</sup>	Carageenan <sup>4</sup>	Novagel <sup>5</sup>	Pectin <sup>6</sup>	Xanthan <sup>7</sup>
Fat (%)	34.30 <sup>a</sup>	6.30 <sup>b</sup>	5.80 <sup>b</sup>	5.70 <sup>b</sup>	5.70 <sup>b</sup>	5.70 <sup>b</sup>	5.60 <sup>b</sup>
pH	4.78 <sup>c</sup>	4.92 <sup>b</sup>	4.95 <sup>b</sup>	5.08 <sup>a</sup>	4.99 <sup>b</sup>	4.99 <sup>b</sup>	5.09 <sup>a</sup>
Moisture (%)	35.65 <sup>a</sup>	52.65 <sup>b</sup>	53.72 <sup>b</sup>	52.96 <sup>b</sup>	52.85 <sup>b</sup>	53.00 <sup>b</sup>	53.72 <sup>b</sup>
Protein (%)	24.41 <sup>a</sup>	31.73 <sup>b</sup>	30.33 <sup>b</sup>	31.09 <sup>b</sup>	30.24 <sup>b</sup>	30.90 <sup>b</sup>	31.17 <sup>b</sup>
Yield (%)	10.12 <sup>a</sup>	7.56 <sup>b</sup>	7.60 <sup>b</sup>	7.39 <sup>b</sup>	7.56 <sup>b</sup>	7.33 <sup>b</sup>	7.25 <sup>b</sup>

<sup>a-b</sup> Means within the same row with different superscript differ significantly (P<0.05)

<sup>1</sup> FFC: full fat control

<sup>2</sup> LFC: low fat control

<sup>3</sup> Alginate: low fat cheese made with sodium alginate (Danisco USA Inc., New Century, KS)

<sup>4</sup> Carrageenan: low fat cheese made with carrageenan (Danisco USA Inc., New Century, KS)

<sup>5</sup> Novagel: low fat cheese made with Novagel (FMC Biopolymer, Philadelphia, PA)

<sup>6</sup> pectin, low fat cheese made with pectin (Danisco USA Inc., New Century, KS)

<sup>7</sup> Xanthan: low fat cheese made with Xanthan gum (Danisco USA Inc., New Century, KS)

### 2.3.2 Texture

Texture profile analysis was performed in triplicate and a typical graph of low fat Cheddar cheese made with Xanthan Gum is shown in Figure 4.

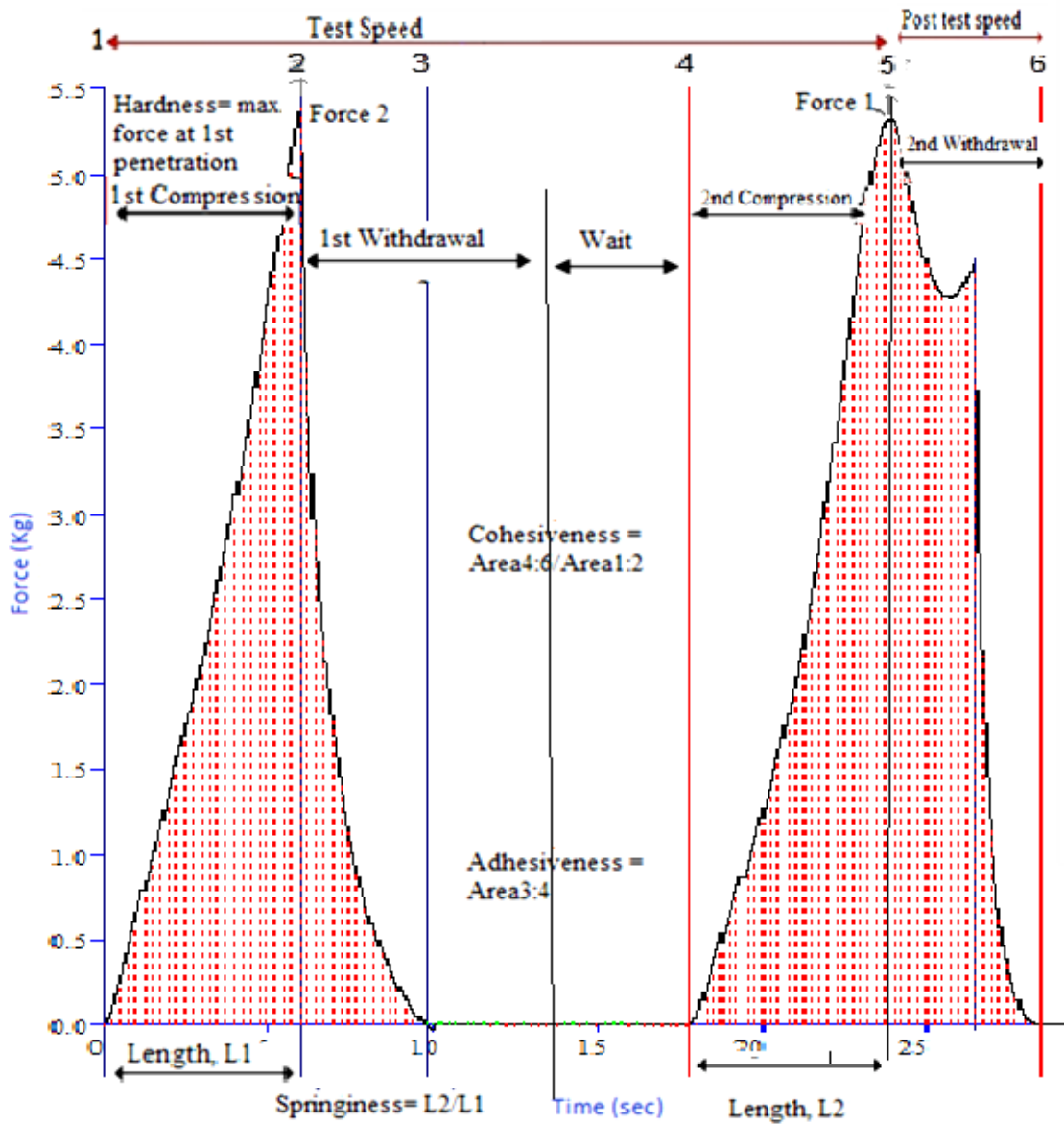


Figure 4. A typical graph, calculations and definitions for a TPA curve for low fat Cheddar cheese made with Xanthan Gum (Danisco USA Inc., New Century, KS)

Firmness is defined as the amount of force required to compress the sample (Brown et al., 2003). As shown in Table 7, the low fat cheeses were firmer than full fat control with the exception of the pectin treatment. In a study of low fat white brined cheese with and without fat replacers (Simplese® D100 and Novagel®NC-200) varied results for hardness on an instrumental texture analyzer were found when compared with full fat cheese showed (Romeih et al., 2002). They found that low fat cheese with Novagel was less hard than the full fat cheese and low fat cheese control was hardest of all. The reduction in fat increased firmness, springiness, cohesiveness and adhesiveness in this study. .

Springiness is the rate to which a compressed and deformed product recovered to its original condition after the first compression tests is removed (Civille and Szczesniak, 1973).

Cohesiveness is the ratio of force area at second bite to force area at the first bite. (How well the product withstands a second deformation relative to the deformation of the first compression!).

Adhesiveness is the amount of work required to overcome the cheese that adheres to the contact surfaces of the food tested and the testing equipment (Tunick, 2000).

Novagel and xanthan treatments were significantly springier than any other treatment. Liu et al., (2008) compared full fat and low fat cheese analogues with or without pectin gel for several TPA parameters such as hardness, springiness, cohesiveness and adhesiveness. Low fat cheese analogues with fat mimetics were springier than full fat cheese analogues in their study. The pectin treatment was more cohesive than any other treatments. Cohesiveness increased in almost all treatments with the exception of the

alginate treatment. This result is in agreement with authors (Bryant et al., 1995; Rudan et al., 1999; Kahyaoglu et al., 2005), who reported that as fat content decreases in cheese, cohesiveness increases. There was no difference found between the low-fat treatments in terms of adhesiveness.

Table 7. Summary of textural properties of cheeses with varied fat contents and influence of polysaccharides as determined by TA.XT analyzer at 30 days of ripening.

Parameters	Treatments						
	FFC <sup>1</sup>	LFC <sup>2</sup>	Alginate <sup>3</sup>	Carageenan <sup>4</sup>	Novagel <sup>5</sup>	Pectin <sup>6</sup>	Xanthan <sup>7</sup>
Firmness (g)	3361.04 <sup>c</sup>	6427.29 <sup>a</sup>	5473.61 <sup>ab</sup>	5395.61 <sup>ab</sup>	5546.81 <sup>ab</sup>	3368.26 <sup>c</sup>	4841.35 <sup>b</sup>
Springiness	1.05 <sup>c</sup>	1.35 <sup>ab</sup>	1.28 <sup>b</sup>	1.38 <sup>ab</sup>	1.43 <sup>a</sup>	1.39 <sup>ab</sup>	1.44 <sup>a</sup>
Cohesiveness	1.15 <sup>cd</sup>	1.26 <sup>cd</sup>	0.84 <sup>d</sup>	1.96 <sup>b</sup>	1.45 <sup>bc</sup>	2.67 <sup>a</sup>	1.63 <sup>bc</sup>
Adhesiveness (g.sec)	-59.36 <sup>b</sup>	-16.55 <sup>a</sup>	-23.57 <sup>a</sup>	-16.59 <sup>a</sup>	-23.23 <sup>a</sup>	-19.37 <sup>a</sup>	-9.37 <sup>a</sup>

<sup>a-b</sup> Means within the same row with different superscript letter differ significantly (P<0.05)

<sup>1</sup>FFC: full fat control

<sup>2</sup>LFC: low fat control

<sup>3</sup>Alginate: low fat cheese made with sodium alginate (Danisco USA Inc., New Century, KS)

<sup>4</sup>Carageenan: low fat cheese made with carrageenan (Danisco USA Inc., New Century, KS)

<sup>5</sup>Novagel: low fat cheese made with Novagel (FMC Biopolymer, Philadelphia, PA)

<sup>6</sup>pectin, low fat cheese made with pectin (Danisco USA Inc., New Century, KS)

<sup>7</sup>Xanthan: low fat cheese made with Xanthan gum (Danisco USA Inc., New Century, KS)

### 2.3.3 Sensory

Novagel was judged to be most similar to the full-fat control by panelists as shown in Table 8. The Novagel treatment was found to be statistically different from the low fat control in the distance measured to the placement of the full fat cheese sample. The next closest distance observed to full fat were the pectin and carrageenan treatments, but they were not significantly different from low fat control (Figure 5).

Table 8. Least square means distance in centimeters measured between the treatments and full fat control. Distance was measured from the full fat control to the treatments shown below. The distance for full fat is considered 0 and hence is not shown in the table below.

Treatments	Total sum of distance(cm)	Mean distance (cm)	Standard Error
Novagel <sup>1</sup>	522.50	29.03 <sup>b</sup>	2.63
pectin <sup>2</sup>	616.50	34.25 <sup>ab</sup>	4.25
Carrageenan <sup>3</sup>	618.00	34.33 <sup>ab</sup>	2.81
Alginate <sup>4</sup>	705.50	39.24 <sup>ab</sup>	3.25
Xanthan <sup>5</sup>	645.50	35.86 <sup>a</sup>	3.95
Low Fat control <sup>6</sup>	722.50	40.14 <sup>a</sup>	3.27

<sup>a-b</sup> Means within the same row with different superscript letter differ significantly (P<0.05)

<sup>1</sup>Novagel: low fat cheese made with Novagel (FMC Biopolymer, Philadelphia, PA)

<sup>2</sup>pectin, low fat cheese made with pectin (Danisco USA Inc., New Century, KS)

<sup>3</sup>Carrageenan: low fat cheese made with carrageenan (Danisco USA Inc., New Century, KS)

<sup>4</sup>Alginate: low fat cheese made with sodium alginate (Danisco USA Inc., New Century, KS)

<sup>5</sup> Xanthan: low fat cheese made with Xanthan gum (Danisco USA Inc., New Century, KS)

<sup>6</sup>LFC: low fat control

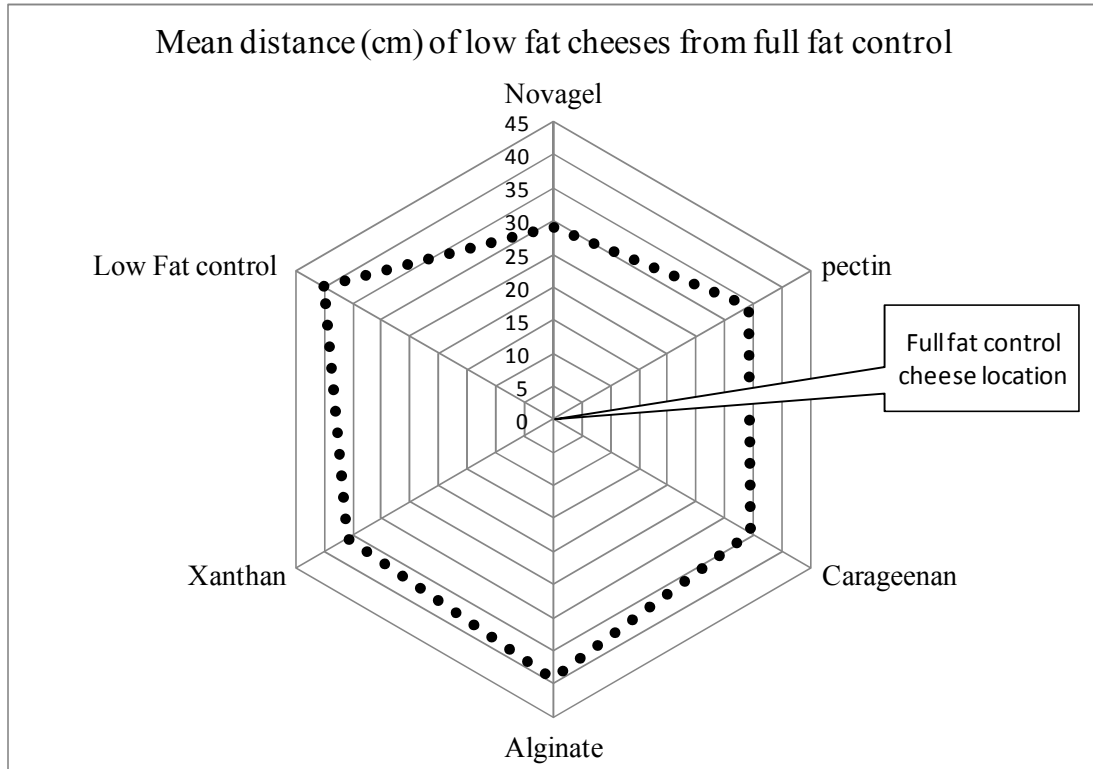


Figure 5. A radar chart showing the mean distance (in cm) of low fat cheeses with and without hydrocolloids from the full fat control cheese placed in the center. The mean distance is average of 18 panelists who evaluated cheese based on the textural and flavor characteristics of the cheese

Comments recorded for the Novagel treatment cheese were that it was less rubbery and less firm and it was moister than other low-fat cheeses. Several panelists commented that the pectin treatment had a mild and well rounded flavor and was slightly less rubbery than other low fat cheeses. Comments recorded for the carrageenan treatment cheese was that it was softer, had a clean taste, and had less cheese flavor. Comments recorded for the low fat control cheese included firm, rubbery, and bitter and unclear flavor.

## **2.4 Conclusions**

The Novagel treatment was judged to be the most similar to full fat control by sensory analysis, and was significantly different from low fat control. The pectin treatment was the least firm of the low fat cheeses as measured by TPA, and not significantly different from full fat control. Because of these reasons, it was decided to investigate the use of Novagel and pectin further by manufacturing pilot plant scale batches of low fat stirred curd Cheddar cheese and evaluating them throughout their shelf-life by chemical, sensory and physical analysis.

## CHAPTER 3

### 3.1 Introduction

The fat entrapped in the network formed by the casein proteins is responsible for much of the texture attributes of cheese (Banks, 2004). Low fat cheese does not inherit the properties of full fat cheese because of the lack of fat and results in a firm textured cheese predominated by the effects of the protein (Drake and Swanson, 1995).

Hydrocolloids have been evaluated in low fat cheese manufacture as a way to improve the textural characteristics, but they have not been homogenized into the cream portion of the milk (McMahon et al, 1996; Ma et al, 1997; Haque et al, 2007).

Homogenization of the cream portion of milk will increase moisture retention by denaturation of proteins and incorporation of whey proteins into the cheese, and has been shown to decrease firmness in low fat natural cheeses (Gilles and Lawrence, 1981; Metzger and Mistry, 1994). The screening and evaluation of hydrocolloids for use in low fat Cheddar cheese, was described in Chapter 2 and in Kumar and Schoenfuss (2010). Pectin and Novagel containing bench-top produced cheeses were the least firm in texture of all low fat cheese and were most similar in sensory parameters with full fat cheese respectively.

It was hypothesized that creating a gel that can be emulsified into the fat during homogenization of cream will act as “filler” during curd formation and improve texture. But the effect of the added hydrocolloids on aging also needs to be evaluated.

The aging process involves microbiological and biochemical changes that effect the flavor and texture. Proteolysis and the production of flavor compounds such as

organic acids are also typically evaluated during cheese aging to examine differences in treatments.

Organic acids in cheese are the result of hydrolysis of milk constituents during lipolysis and biochemical metabolism (Izco et al., 2002). The amount of organic acids present in cheese has been monitored for the activity of primary and secondary starter culture activity during the cheese ripening and has been studied in regards to flavor differences in cheese (Marsili et al., 1981). Ong and Shah (2009) measured the effect of elevated storage temperature and the addition of probiotic cultures, a mixed strain of *Bifidobacterium* and *Lactobacillus*, in proteolysis and flavor of Cheddar cheese. They reported an increase in soluble nitrogen and organic acids such as lactic and acetic acid lead to a significant increase in Cheddary flavor. The hydrolysis of  $\alpha$ s1-CN,  $\beta$ -CN and increase in lactic and acetic acid were correlated with the sour acid and vinegary type flavor.

The hypothesis for this study is that the addition of a hydrocolloid (pectin and Novagel) in a homogenized cream base to low fat cheese will disrupt the protein matrix and improve the texture of low fat cheese. The objectives to test this hypothesis include the production of pilot plant scale batches of cheese with either Novagel or pectin, and to evaluate the samples monthly for instrumental texture, proteolysis, and organic acid production. Sensory evaluation by descriptive sensory analysis of the aged cheese was also used to evaluate differences in treatments.

## **3.2 Materials and Methods**

### **3.2.1 Fat Replacers and Ingredients**

Raw milk was obtained from the University of Minnesota dairy, Saint Paul, MN. Pectin 100 was obtained from Danisco USA Inc., New century, KS and Novagel RCN 15 was obtained from FMC Biopolymer, Philadelphia, PA. Novagel RCN 15 is a mixture of microcrystalline cellulose and guar gum. The bacterial cultures Choozit M58 (selected strains of *Lactococcus lactis* ssp. *lactis*), Choozit Flavobac LF304 (*Lactobacillus casei*) and Flavogard 360 (selected strains of *Lactococcus lactis* ssp. *cremoris*), were sourced from Danisco USA Inc., (New Century, KS). Whey protein concentrate (WPC) (Avonlac 180) was obtained from Glanbia Nutritionals, Monroe, WI. The rennet (chymosin double strength, 73863) and annatto color no. 70463 were acquired from Chr. Hansen, Milwaukee, WI. Salt was purchased from Morton International Inc. (Chicago, IL).

### **3.2.2 Experimental Design**

In this study, two treatments and three controls were chosen as a part of the study design. The treatments were low fat cheese with pectin (LP) and low fat cheese with Novagel (LNG). Controls were full fat (FFC), low fat (LF) and low fat Cheddar with whey protein concentrate added (LFW). Both low fat cheeses were made with homogenization of the cream portion. These five cheese makes were duplicated and cheeses were analyzed monthly for four months starting at week one for chemical and physical properties that were expected to change during shelf-life. Cheeses were made in the pilot plant at the University of Minnesota, Saint Paul, MN in 1200 pound batches.

### 3.2.3 Cheese Making

The hydrocolloid filler gel was prepared by dry blending the hydrocolloids and WPC, then blending with water at room temperature and mixing with a high shear mixer for 5 - 7 minutes. The water temperature for Novagel, and low-fat control sample blending was 25° C and they were blended for 5 minutes. For pectin, the water temperature was 40° C and mixing time was 7 minutes to completely blend the material. Pectin was added at 0.415% and Novagel RCN 15 was added at the rate of 0.8% of the total cheese milk in the batch. Whey protein concentrate was added at 0.25% of the total cheese milk. The levels for hydrocolloids were determined based on the previous experiments as described in the previous chapter.

Pasteurized cream (approximately **0.84%** of total cheese milk) in an amount necessary to standardize the cheese milk to 0.6% fat, hydrocolloid blend (approximately **0.8%** of total cheese milk) and the amount of pasteurized skim milk (approximately **18.3%** of total cheese milk) necessary to produce a homogenized blend which would be 20% (0.84% + 0.8% + 18.3%) of the total cheese milk were homogenized at refrigerated temperature on a 2-stage homogenizer (Gaulin, Model no. 125 / 83 MF12A 8PSX) at 160 bars (110 bar - 1<sup>st</sup> stage, 50 bar - 2<sup>nd</sup> stage). The homogenized blend (Figure 6) was added to the rest of the skim milk, and cheese was produced using a modified low fat stirred curd procedure in 1200 pound batches.

Hydrocolloid + whey protein powder + water = hydrocolloid gel



Mixed with high shear mixer for 7 minutes



Hydrocolloid gel, pasteurized cream, pasteurized portion of skim milk (Homogenized, 2 stage pressure 110/50 bar at refrigerated temperature)



Homogenized blend (20%) added to the rest of skim milk (80%) in cheese vat

Figure 6. Flow diagram for making hydrocolloid gel and cheese milk

For both of the low fat controls, no pectin or Novagel was added. For LF, the homogenized blend was prepared only with cream and skim milk. For LFW, the homogenized blend was prepared using whey protein concentrate, cream and skim milk. The blends were added to the rest of the 80% pasteurized skim milk to make the cheese. Cheese milk was pre-acidified to a pH of 6.2 with a 10% solution of food grade lactic acid prior to culturing. The combination of three cultures (Choozit M58, Choozit Flavobac LF 304 and Flavogard 360) were added at 0.165g/Kg of milk, 0.0516g / Kg of milk and 0.019g / Kg of milk respectively. Cheese was prepared using a stirred curd method as depicted in appendix A.

The 9.072 Kg (20 lb) cheese block was cut into 0.453 Kg (1 lb) blocks after 7 days of storage at 4° C and vacuum packaged individually for sampling. Samples of each type of cheese were taken for analysis at 0 and each month of ripening. Random samples were taken by opening a different 0.453 Kg (1 lb) block of cheese in each sampling period.

### **3.2.4 Compositional Analysis**

Moisture in cheese was measured by vacuum oven (Method 18.10 A. *Class O*) as per Wehr (2004). The Babcock method (Method 15.083) was used for cheese fat with 20% fat Paley bottles as per standard methods (Wehr, 2004). Total Protein was determined by the Dumas method on a Leco Tru spec N nitrogen analyzer (Tru Spec N, Model No. 630-100-200, St. Joseph, MI). Total Ash in cheese was determined as per standard method no. 15.041(Wehr, 2004) (Appendix B). Salt was analyzed by atomic absorption spectroscopy (Kira, 2004).

### **3.2.5 Proteolysis**

The cheeses were sampled every month through 4 months of aging starting 1 week after the manufacturing day for the initial sample. For proteolysis, water soluble nitrogen was analyzed according to the method of Kuchroo and Fox (1982) with the following modifications. The cheese sample was grated, weighed and mixed with twice the sample weight of water in a Osterizer blender (Model no. 6640-022, Mexico) for 2 minutes at high speed (12000 rpm) and centrifuged (Beckman Coulter Model number GS-6R, Brea, CA) at 3000g for 30 minutes (Appendix C). After centrifugation, extract was filtered through Whatman no. 1 filter paper (Cat no. 1001 125, Fisher Scientific) and stored in 15ml centrifuge tubes at -20° C until samples are analyzed. The extract was analyzed for total nitrogen content in the sample by the Dumas method on a Leco Tru Spec N nitrogen analyzer (Model No. 630-100-200, Leco Corp. Inc, St. Joseph, MI).

### **3.2.6 Organic Acid Determination**

Eight organic acids were quantified throughout aging (oxalic, formic, citric, pyruvic, acetic, lactic, propionic and butyric). Sample preparation for determining organic acids in cheese was based on previous studies and is described below (Izco et al., 2002; Zeppa and Rolle, 2008). All standards were purchased from Sigma Aldrich. Separations were performed on a P/ACE™ MDQ capillary electrophoresis (Beckman Coulter, Inc., Brea, CA) with indirect UV detection at 230 nm. The Anion Analysis Kit (No: A53537, Beckman Coulter, Inc., Brea, CA) capillary, reagents and procedure were used for the analysis.

Sample was prepared by grinding 1 gram of cheese sample in 50 ml DD water for 1 min using Powergen 700 tissue homogenizer (Fisher Scientific, Netherlands) followed

by mixing for 15 minutes with a magnetic stirrer at room temperature. The mixture was centrifuged at 14000 g for 1 min and supernatant was filtered with a 0.45  $\mu\text{m}$  PVDF membrane disposable syringe filter (Millipore corp. Co., Cork, Ireland). The separations were carried out on 75  $\mu\text{m}$  ID bare fused silica capillary having 50 cm effective length. The sample was injected for 10 s at 3448 Pa (0.5 p.s.i.), at 30kV with reverse polarity and separation was performed at 25° C. An internal injection standard (sodium octanoate) was used for the quantification of the anions.

### **3.2.7 Texture Analysis**

Texture profile analysis (TPA) was conducted using a TA.XT 2i plus Texture Analyzer (Texture Technologies, Scarsdale, NY) at 0, 1, 2, 3 and 4 month of aging using a two-bite compression test with a 25 mm diameter and 35 mm tall acrylic cylinder probe. Samples were stored at 7 °C overnight and prepared immediately prior to analysis by cutting the cheese samples with a no. 12 cork borer (15 mm radius) and cutting to a length of 25 mm. Samples were compressed at 2.0 mm/second by 48% of the original height (12 mm) to pre-failure, and then compressed a second time after 6 seconds rest period by 75% (19 mm) of the original height which went past fracture in most samples.

### **3.2.8 Sensory Analysis**

An eleven member of trained descriptive panel evaluated the samples at the Sensory Center, University of Minnesota, Saint Paul, MN at 120 days of ripening. Panelists were presented three cubes (1.5  $\text{cm}^3$ ) of each cheese at room temperature (70 °F) in a random 3-digit coded plastic 4 oz cup with lid. Each panelist evaluated each sample by rating the intensity of the attributes on 20 point line scales labeled ‘none’ at the left end and ‘intense’ at the right end. Within a session serving orders were balanced for

order and carryover effects. Intensity ratings of flavor and taste were made on the standard citric acid scale; ratings of odors were made on the standard butanol scale; texture ratings were made on a 20 point scale anchored with references. References only pertained to the first 15 points of the flavor scale and to only the first 12 points of the aroma scales. Panelists were instructed to wear nose clips when evaluating the taste attributes.

### **3.2.9 Confocal laser scanning microscopy (CLSM)**

The microstructure of cheeses were imaged using a Nikon C1si hyperspectral confocal microscope (Nikon Instruments Inc., Melville, NY) at the University-wide Imaging Center, University of Minnesota, Saint Paul, MN at 8 months of ripening. Cheese samples were refrigerated until cut into sections 1 cm x 1 cm x 1mm using a clean, dry razor blade. Samples were then stained with Nile Red (561 nm excitation) for lipids, phospholipids and phosphoproteins, and Green Fast (488 nm excitation) for protein (Invitrogen life technologies, Carlsbad, CA). Labeling with Nile Red and Green Fast for fat and protein respectively can be observed as fat being red in color and protein colored green. Auto fluorescence of carbohydrates was captured using an excitation wavelength of 405 nm and the blue regions indicate the carbohydrates.

All images were collected using a 32 element, multianode photomultiplier tube (PMT) detector at the spectral bandwidth of 420-740 nm at 10 nm bins. Hyperspectral images were unmixed using a linear least-square algorithm (Larson, 2006). Z-series data were collected using a 0.9  $\mu\text{m}$  step size. Unless noted otherwise, the images present here are maximum intensity projections of the unmixed z-series.

### **3.2.10 Statistical Analysis**

The experiment was replicated two times in a randomized block design. The compositional data analysis and texture data were conducted using mixed models. All tests for TPA were conducted in triplicate. Differences between means were determined using Fishers' least significance difference (LSD) test at 95 % confidence interval. XLSTAT<sup>®</sup> (Addinsoft, USA, NY) was used for statistical analysis of the data, to determine differences between treatment means and correlation coefficients.

The sensory results were analyzed by SAS PROC GLM (version 9.1) to determine whether the samples differed in any of the specific attributes. The attribute intensity was the dependent variable; judge, sample, batch, sample\*batch were predictors. We selected alpha of 0.05 for testing for significant differences.

### **3.3 Results and Discussions**

#### **3.3.1 Compositional Analysis**

The process of making low fat Cheddar cheese by standardizing the fat content approximately to 0.6 % in milk, results in higher moisture and firmer texture than the full fat Cheddar. Several factors have been discussed in chapter 1.2 to reduce firmness in an effort to develop low fat cheese. The cheese matrix may be viewed as proteins forming the body building structure of the matrix, with water entrapped inside the matrix, and fat acting as inert filler (Le et al., 2011). With this principle, a filler gel was created by homogenizing selected hydrocolloids with the cream portion of cheese milk. This filler gel may mimic the properties of fat in the casein matrix, and prevent the continuous, dense network thereby creating a softer bodied cheese. The filler gel may entrap moisture in the matrix resulting in a higher moisture cheese. Compositional analysis of cheese was performed 1 week after manufacture, and then every month through 4 months of ripening. The chemical composition of the treatment cheeses is shown in Table 9. The low fat cheeses were different from full fat cheese in fat, pH, moisture, protein, ash, and yield. The target fat content in the low fat cheese to claim “low fat” should be less than 6 percent (21 CFR 101.62 (b)). There were no differences in fat found between the low fat cheeses. The fat contents of low fat cheeses were within 5.1 to 5.45 which met the low fat label claim as mentioned in the above code of federal regulations. This was necessary to compare the effect of treatments (different hydrocolloids) with the same fat content. The milk was standardized accordingly to target less than 6 % fat in the final product.

Moisture content for LNG and LP was significantly higher than low fat control at the end of week 1 and month 4 respectively. Most of the treatments during aging did not show any differences when compared with low fat control (Figure 7).

Romeih et al., (2002) did not find any increase in moisture when they used Simplese® D 100, a microparticulated whey protein concentrate and Novagel™ NC 200 in the making of low fat white brined cheese. However, other researchers who used fat replacers (Novagel™ RCN 15, used in this study) in low fat mozzarella cheeses found significantly higher moisture than the low fat control (McMahon et al., 1996; Rudan et al., 1999). In this study, the treatments did not show any increase in moisture, as it did not retain in the cheese matrix. An observation was made during cheese making that hydrocolloids settled at the bottom of the cheese vat and got drained with whey. Also, the amount of hydrocolloids used for making filler gel, was not enough for entrapping the hydrocolloids in the cheese matrix. This is in agreement with the lab scale trials, where the moisture of low fat cheese made with hydrocolloids was not significantly different from low fat control. None of the researchers have compared the effect of hydrocolloids on moisture of low fat Cheddar cheese containing less than 6% fat in finished cheese.

Table 9. Chemical composition of low fat and full cheese.

% Composition <sup>6</sup>		Treatments			Control		SE <sup>7</sup>
		LP <sup>1</sup>	LNG <sup>2</sup>	LFW <sup>3</sup>	LFC <sup>4</sup>	FFC <sup>5</sup>	
Fat		5.45 <sup>b</sup>	5.1 <sup>b</sup>	5.1 <sup>b</sup>	5.4 <sup>b</sup>	36.5 <sup>a</sup>	0.34
Ash		3.90 <sup>b</sup>	4.10 <sup>ab</sup>	4.34 <sup>a</sup>	4.03 <sup>ab</sup>	2.76 <sup>c</sup>	0.11
Yield		6.71 <sup>b</sup>	6.67 <sup>b</sup>	6.36 <sup>b</sup>	6.37 <sup>b</sup>	10.12 <sup>a</sup>	0.27
Salt		1.48 <sup>a</sup>	1.60 <sup>a</sup>	1.72 <sup>a</sup>	1.70 <sup>a</sup>	1.72 <sup>a</sup>	0.15
pH	1 week	5.02 <sup>b</sup>	4.96 <sup>c</sup>	5.10 <sup>a</sup>	4.96 <sup>c</sup>	4.78 <sup>d</sup>	0.02
	1 mon	4.98 <sup>bc</sup>	5.02 <sup>ab</sup>	5.08 <sup>a</sup>	4.92 <sup>c</sup>	4.80 <sup>d</sup>	0.03
	2 mon	4.93 <sup>b</sup>	5.08 <sup>a</sup>	5.08 <sup>a</sup>	4.90 <sup>b</sup>	4.82 <sup>b</sup>	0.05
	3 mon	4.96 <sup>a</sup>	5.06 <sup>a</sup>	5.01 <sup>a</sup>	4.78 <sup>b</sup>	5.05 <sup>a</sup>	0.05
	4 mon	5.02 <sup>ab</sup>	5.01 <sup>ab</sup>	5.09 <sup>a</sup>	4.96 <sup>b</sup>	4.78 <sup>c</sup>	0.05
Moisture(%)	1 week	50.65 <sup>b</sup>	51.50 <sup>a</sup>	51.34 <sup>ab</sup>	51.03 <sup>ab</sup>	35.49 <sup>c</sup>	0.03
	1 mon	51.18 <sup>a</sup>	50.96 <sup>a</sup>	50.39 <sup>a</sup>	50.59 <sup>a</sup>	36.56 <sup>b</sup>	0.06
	2 mon	51.63 <sup>a</sup>	51.61 <sup>a</sup>	51.05 <sup>a</sup>	50.11 <sup>a</sup>	36.83 <sup>b</sup>	0.83
	3 mon	52.30 <sup>a</sup>	51.81 <sup>a</sup>	50.81 <sup>a</sup>	50.13 <sup>a</sup>	37.13 <sup>b</sup>	1.18
	4 mon	51.39 <sup>a</sup>	50.89 <sup>ab</sup>	50.72 <sup>ab</sup>	49.88 <sup>b</sup>	36.77 <sup>c</sup>	0.78
Protein(%)	1 week	37.48 <sup>ab</sup>	36.08 <sup>c</sup>	37.12 <sup>bc</sup>	38.72 <sup>a</sup>	24.39 <sup>d</sup>	0.54
	1 mon	36.11 <sup>a</sup>	35.14 <sup>a</sup>	35.66 <sup>a</sup>	36.23 <sup>a</sup>	24.68 <sup>b</sup>	0.60
	2 mon	37.28 <sup>a</sup>	36.43 <sup>a</sup>	36.93 <sup>a</sup>	37.36 <sup>a</sup>	25.10 <sup>b</sup>	1.08
	3 mon	38.06 <sup>a</sup>	37.03 <sup>a</sup>	37.04 <sup>a</sup>	37.58 <sup>a</sup>	25.44 <sup>b</sup>	1.12
	4 mon	38.23 <sup>a</sup>	37.50 <sup>a</sup>	37.62 <sup>a</sup>	37.44 <sup>a</sup>	25.12 <sup>b</sup>	0.82

<sup>a-d</sup> Means within the same row with a different superscript differ significantly ( $p < 0.05$ )

<sup>1</sup>LP: Low Fat with pectin

<sup>2</sup>LNG: Low Fat with Novagel

<sup>3</sup>LFW: Low Fat with Whey Protein Concentrate

<sup>4</sup>LF: Low Fat Control

<sup>5</sup>FFC: Full Fat Control

<sup>6</sup>% expressed as percent by weight on a wet basis. Fat, Ash, Yield and Salt were evaluated after 1 week of storage at 4° C. Moisture, pH and Protein were evaluated every month through 4 months of aging

<sup>7</sup>Largest standard error of all treatments is shown for each parameter

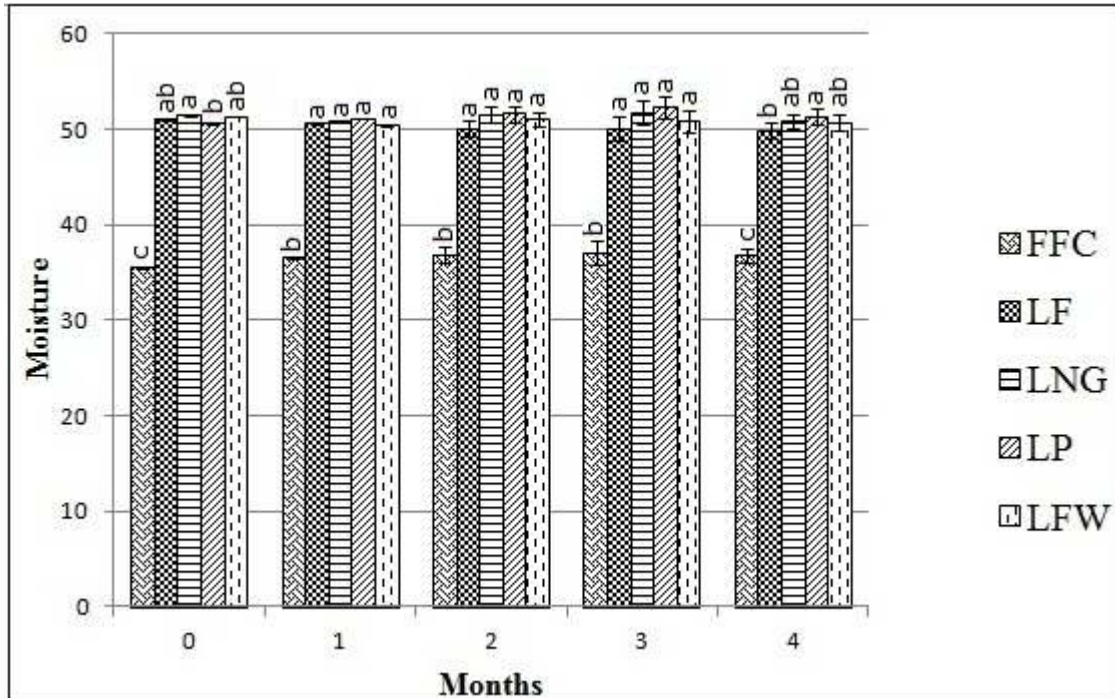


Figure 7. Effects of treatment and aging on moisture content of Cheddar cheeses made of full fat control (FFC); Low fat control (LFC); Low fat cheese with pectin addition (LP); low fat cheese with Novagel addition (LNG) and Low fat cheese with whey protein concentrate (LFW) over four months of aging. Time 0 months indicate one week after cheese make. Means within the same month with a different letter differ significantly ( $p < 0.05$ ).

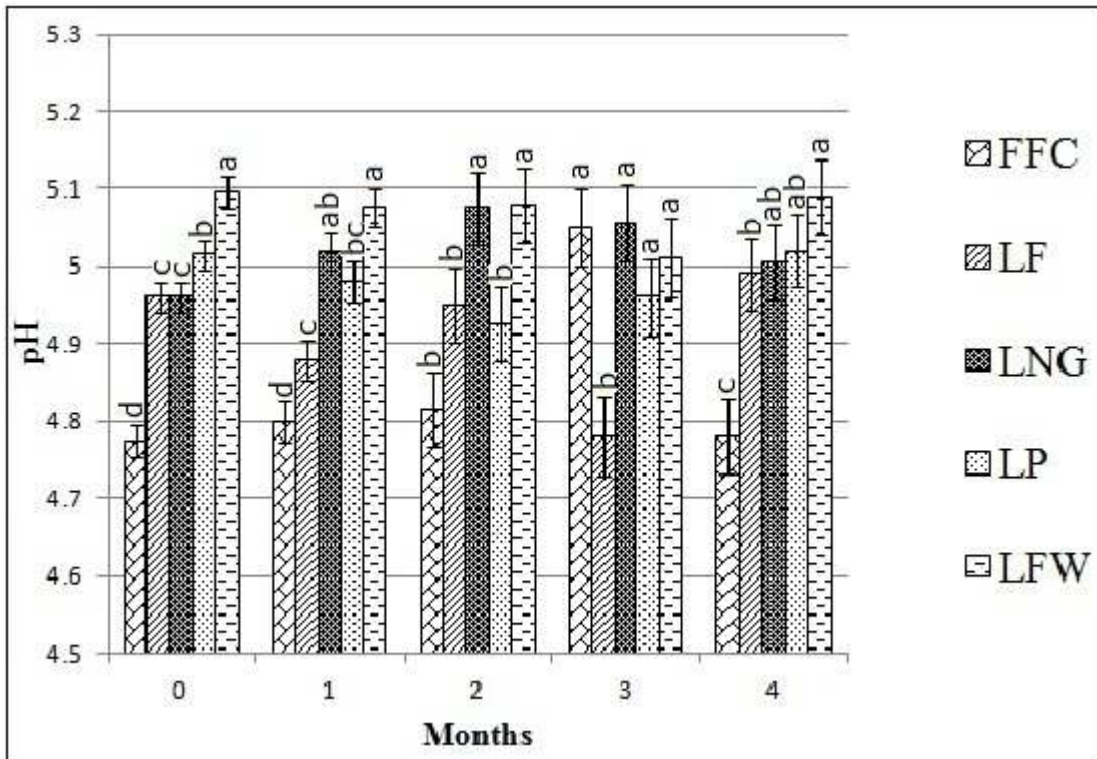


Figure 8. Effects of treatment and aging on pH of Cheddar cheeses made of full fat control (FFC); Low fat control (LFC); Low fat cheese with pectin addition (LP); low fat cheese with Novagel addition (LNG) and Low fat cheese with whey protein concentrate (LFW) over four months of aging. Time 0 months indicate one week after cheese make. Means within the same month with a different letter differ significantly ( $p < 0.05$ ).

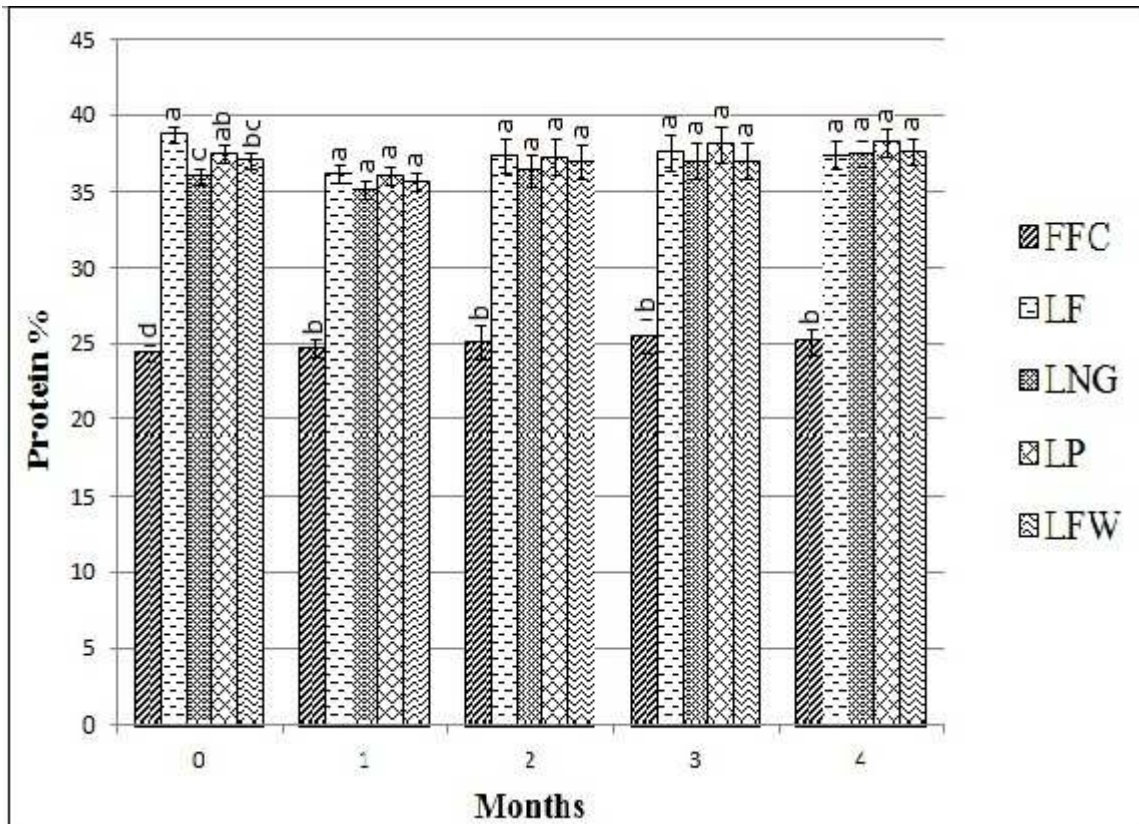


Figure 9. Effects of treatment and aging on protein content of Cheddar cheeses made of full fat control (FFC); Low fat control (LFC); Low fat cheese with pectin addition (LP); low fat cheese with Novagel addition (LNG) and Low fat cheese with whey protein concentrate (LFW) over four months of aging. Time 0 months indicate one week after cheese make. Means within the same month with a different letter differ significantly ( $p < 0.05$ ).

Total protein of treatment cheeses was higher than the full fat control due to the lower fat content in the treatment cheese (Figure 9). Several researchers have reported a dense matrix of protein in low fat cheeses (McMahon et al., 1996; Konuklar et al., 2004; Haque et al., 2007). The low fat cheese containing WPC had a higher pH than LFC throughout the ripening period (Figure 8). The typical pH ranges for a stirred curd type Cheddar cheese were listed from 5.10 to 5.45 for a premium quality grade (Scott, 1998). All of the cheeses, except LFW, had a lower pH value to be considered for a good quality young Cheddar cheese. This may be due to the higher inoculation rate of starter culture. The acid development during cheese making in this study was slower during the ripening period but accelerated in the later stages of cheese making. To account for this, salt was added in the curd at pH 5.7 to stop the acid development. In a typical milled curd Cheddar cheese make, salting is done at pH 5.5 (Shakeel-ur-Rehman et al., 2008). None of the authors have studied the effect of pH in low fat Cheddar type cheese with hydrocolloids as fat replacers. During the cheese make, pH was measured by taking a whey sample instead of cheese curd to determine the next stage of cheese make. It is advisable to measure the cheese curd pH to closely extrapolate the pH in finished cheese by wrapping the curd around the pH probe or by inserting the probe into the curd (Scott, 1998).

Salt content of all the cheeses ranged from 1.48 % to 1.72%. Several researchers have studied the effect of salt to moisture ratio in the cheese which affects finished cheese pH, controlling the growth of non starter lactic acid bacteria and proteolysis (Al Otaibi and Wilbey, 2004; Upreti and Metzger, 2007; Agarwal et al., 2008). Since the low fat cheeses had higher moisture than the full fat control, salt addition rate was increased

to target the similar salt to moisture ratio. In this study we were not able to achieve S/M ratio (3.2) in low fat cheeses as compared to full fat (4.6). Low S/M ratio in low fat cheeses may have affected the flavor profile and texture of the cheese.

An analysis of variance summary of pH, moisture, and protein was generated for treatments, month, and treatment-month interactions (Table 10). None of these parameters were significantly different for month and treatments interactions. However, treatments were different from each other over four months of ripening period. All low fat cheeses were different in moisture, protein, and pH from the full fat cheese. Between the low fat cheese treatments and control, these components did not significantly differ. None of the researchers have conducted an aging study for the compositional parameters in low fat Cheddar cheese with hydrocolloids having less than 6% fat. However, McMahon et al., (1996) who used fat replacers (Novagel™ RCN 15, used in this study) in low fat mozzarella cheeses, found significantly higher moisture than the low fat control.

Table 10. Analysis of variance summary (degree of freedom, sum of squares, mean squares, f value and probabilities) of compositional parameters during 120 days of ripening at 4° C

Compositional Parameters	Source	D F	Sum of squares	Mean squares	F	Pr > F
pH	Treatments	4	12340.163	3085.041	42.881	< 0.0001
	Month	4	818.772	204.693	2.845	0.051
	Month*Treatments	16	1154.331	72.146	1.003	0.491
Moisture	Treatments	4	3032.846	758.211	737.746	< 0.0001
	Month	4	4.816	1.204	1.171	0.332
	Month*Treatments	16	13.180	0.824	0.802	0.678
Protein	Treatments	4	1135.314	283.828	563.676	< 0.0001
	Month	4	14.970	3.742	7.432	0.001
	Month*Treatments	16	5.964	0.373	0.740	0.727

\*Statistically significant ( $P < 0.05$ )

### 3.3.2 Sensory Analysis

Sensory perception (flavor, texture, and appearance) is one of the most important criteria in determining consumer acceptance of the cheese. A typical full fat Cheddar cheese should possess the characteristics of good Cheddar flavor which may be described as clean, nutty flavor, cooked/milky, diacetyl, pleasantly acidic, and moderately aromatic (Patridge, 2009). In contrast, low-fat Cheddar cheese has many challenges including a change in consumer flavor perception. Reduction of almost 80% fat from full fat Cheddar cheese causes a reduction of buttery flavor notes, decrease in fatty acids compounds such as methyl ketones and hexanoic acids (Banks et al., 1989). Fat contributes to flavor development through lipolysis as free fatty acids serve as precursors for other flavor compounds (Collins et al., 2003).

Mean values for the texture and flavor attributes of cheeses are presented in Table 11. The sensory analysis was performed at 120 days of ripening. The full fat cheese had more overall flavor, milkier, sweet, salty, umami, diacetyl, cooked milk, and brothy flavors. Similar results for full fat cheeses have been summarized in a recent study done by Drake et al., (2010).

Table 11: Mean values (over all judges, batches, and sensory replicates; N=11) and F and p values (for treatments from the analysis of variance) of attributes that differed significantly among LF, FFC, LFW, LNG, and LP at 120 days of ripening.

Attribute	LF	FFC	LFW	LNG	LP	F Value	P Value
Overall aroma	7.6 <sup>b</sup>	9.0 <sup>a</sup>	8.3 <sup>ab</sup>	8.2 <sup>ab</sup>	8.2 <sup>ab</sup>	2.81	0.026
Diacetyl aroma	4.7 <sup>ab</sup>	5.4 <sup>a</sup>	4.7 <sup>ab</sup>	4.3 <sup>ab</sup>	4.1 <sup>b</sup>	2.68	0.033
Milky aroma	1.3 <sup>b</sup>	1.9 <sup>a</sup>	1.4 <sup>b</sup>	1.3 <sup>b</sup>	1.2 <sup>b</sup>	3.57	0.007
Brothy aroma	1.4 <sup>ab</sup>	1.8 <sup>a</sup>	0.8 <sup>b</sup>	1.6 <sup>a</sup>	1.4 <sup>ab</sup>	3.73	0.006
Sour dairy aroma	2.0 <sup>a</sup>	1.4 <sup>a</sup>	1.8 <sup>a</sup>	1.8 <sup>a</sup>	1.4 <sup>a</sup>	2.65	0.034
Salty	1.8 <sup>b</sup>	3.1 <sup>a</sup>	2.0 <sup>b</sup>	1.9 <sup>b</sup>	2.1 <sup>b</sup>	5.91	0.000
Sour	4.2 <sup>a</sup>	1.3 <sup>b</sup>	3.6 <sup>a</sup>	4.3 <sup>a</sup>	4.1 <sup>a</sup>	17.69	<.0001
Bitter	5.4 <sup>a</sup>	0.6 <sup>c</sup>	3.6 <sup>b</sup>	5.0 <sup>a</sup>	4.6 <sup>a</sup>	31.12	<.0001
Umami	0.4 <sup>b</sup>	1.2 <sup>a</sup>	0.6 <sup>b</sup>	0.4 <sup>b</sup>	0.5 <sup>b</sup>	6.96	<.0001
Diacetyl flavor	2.2 <sup>b</sup>	5.5 <sup>a</sup>	2.7 <sup>b</sup>	2.6 <sup>b</sup>	2.7 <sup>b</sup>	14.65	<.0001
Cooked flavor	1.3 <sup>a</sup>	2.1 <sup>a</sup>	1.2 <sup>a</sup>	1.2 <sup>a</sup>	1.1 <sup>a</sup>	2.48	0.0451
Metallic flavor	2.9 <sup>a</sup>	0.2 <sup>b</sup>	2.2 <sup>a</sup>	3.1 <sup>a</sup>	2.7 <sup>a</sup>	14.26	<.0001
Milky flavor	0.5 <sup>b</sup>	1.9 <sup>a</sup>	0.6 <sup>b</sup>	0.4 <sup>b</sup>	0.6 <sup>b</sup>	16.47	<.0001
Brothy flavor	0.8 <sup>b</sup>	2.2 <sup>a</sup>	0.7 <sup>b</sup>	0.9 <sup>b</sup>	0.9 <sup>b</sup>	11.27	<.0001
Fermented flavor	1.2 <sup>a</sup>	0.0 <sup>b</sup>	0.6 <sup>ab</sup>	0.9 <sup>ab</sup>	0.6 <sup>ab</sup>	3.12	0.0161
Sour dairy flavor	4.4 <sup>a</sup>	1.4 <sup>b</sup>	3.6 <sup>a</sup>	3.6 <sup>a</sup>	3.8 <sup>a</sup>	10.22	<.0001
Soapy flavor	1.8 <sup>a</sup>	0.2 <sup>b</sup>	1.2 <sup>a</sup>	1.6 <sup>a</sup>	1.5 <sup>a</sup>	16.92	<.0001
Astringency	1.7 <sup>a</sup>	0.3 <sup>b</sup>	0.9 <sup>ab</sup>	1.2 <sup>a</sup>	1.2 <sup>a</sup>	4.17	0.0029
Pungent	1.5 <sup>a</sup>	0.2 <sup>b</sup>	1.2 <sup>a</sup>	1.5 <sup>a</sup>	1.3 <sup>a</sup>	3.52	0.0084
Hand firmness	16.7 <sup>a</sup>	12.6 <sup>b</sup>	16.7 <sup>a</sup>	17.7 <sup>a</sup>	17.2 <sup>a</sup>	19.45	<.0001
Hand springiness	8.6 <sup>a</sup>	5.8 <sup>b</sup>	8.2 <sup>a</sup>	7.9 <sup>a</sup>	8.6 <sup>a</sup>	4.04	0.0036
Hand cohesiveness	14.4 <sup>a</sup>	11.5 <sup>b</sup>	15.5 <sup>a</sup>	14.7 <sup>a</sup>	15.7 <sup>a</sup>	8.71	<.0001
Hand slipperiness	2.8 <sup>b</sup>	11.3 <sup>a</sup>	3.3 <sup>b</sup>	2.6 <sup>b</sup>	2.8 <sup>b</sup>	175.55	<.0001
First bite firmness	12.7 <sup>a</sup>	4.6 <sup>b</sup>	12.5 <sup>a</sup>	12.4 <sup>a</sup>	12.8 <sup>a</sup>	116.78	<.0001
First bite stickiness	6.6 <sup>b</sup>	14.2 <sup>a</sup>	5.6 <sup>b</sup>	5.9 <sup>b</sup>	6.3 <sup>b</sup>	71.41	<.0001
First bite brittleness	7.5 <sup>a</sup>	1.9 <sup>c</sup>	5.9 <sup>b</sup>	7.2 <sup>ab</sup>	7.2 <sup>ab</sup>	34.58	<.0001
Firmness 5 chews	12.7 <sup>a</sup>	6.1 <sup>b</sup>	12.7 <sup>a</sup>	12.3 <sup>a</sup>	12.5 <sup>a</sup>	85.70	<.0001
Stickiness 5 chews	6.7 <sup>b</sup>	14.3 <sup>a</sup>	5.8 <sup>b</sup>	6.3 <sup>b</sup>	6.4 <sup>b</sup>	92.45	<.0001
Brittleness 5 chews	8.2 <sup>a</sup>	2.0 <sup>b</sup>	7.5 <sup>a</sup>	8.7 <sup>a</sup>	7.6 <sup>a</sup>	54.09	<.0001
Curdiness 5 chews	4.2 <sup>a</sup>	1.4 <sup>b</sup>	3.5 <sup>a</sup>	4.5 <sup>a</sup>	3.9 <sup>a</sup>	11.58	<.0001

Means within the same row with a different superscript differ significantly (p<0.05)  
Means followed by the same letter were not significantly different based on the Student-Newman-Keuls test.

The lower fat cheeses were more sour, bitter, metallic, unclean, soapy, pungent, and sour dairy flavors. LFW was found to be less bitter than the other low fat cheeses and was directionally higher in overall aroma. The low bitterness score and high overall aroma may be explained for higher salt content in LFW. Saint-Eve et al., (2009) studied the effect of reducing salt and fat content, on the impact of texture and sensory perceptions of flavored model cheeses. They found reduced fat cheese with 20% fat and 0.5 % salt was less intense than 1.5% salt model cheese. It was found to be less crumbly, less springy, and least firm than the lower salt content for in-mouth evaluation. Mistry and Kasperson (1998) investigated the impact of salt on the sensory characteristics of reduced fat cheddar cheeses (14-15 % fat) made with milled curd method (contrary to this study which was made with stirred curd method) and reported lower flavor intensity, acidity, and bitterness for cheese with 2.04% salt as compared to 1.3% salt. Also, the intensities of Cheddar cheese flavor, acidity, and bitterness were scored on an 11-point scale where as in this study, each panelist evaluated each sample by rating the intensity of the attributes on a 20 point line scale labeled 'none' at the left end and 'intense' at the right end.

LP and LNG have the same amount of whey protein concentrate, as LFW but panelists did not note any differences between pectin and Novagel treated cheeses. Panelists found LFW to be directionally less metallic, soapy, and sour than LP and LNG. Texture differences could be an explanation for differences in flavor. Aryana and Haque (2002) indicated less diacetyl flavor in low fat cheddar cheese containing Novagel compared to low fat cheddar control. Overall, they reported a decreasing trend in volatile compounds during ripening period for low fat cheddar cheese made with commercial fat

replacers. With the amount and type of fat replacers used, there were no significant differences found in the composition, which may also explain the similar sensory perceptions among the low fat cheeses.

Lauverjat et al., (2009) explained the food breakdown properties during mastication related to the release of flavor compounds in their study. The stronger protein-protein interactions in low fat cheeses may hinder the release of flavor compounds.

The textural differences (hardness, springiness, cohesiveness, chewiness) between the full fat and the reduced fat cheeses (14-21% fat) have been documented in a study with natural and processed cheese (Gwartney et al., 2002). They acknowledged that reduced-fat cheeses were higher in springiness, hardness, fracturability, waxiness, and chewiness.

The differences in sensory parameters between the cheeses have been shown with the help of a non parametric method, Principal Component Analysis (PCA). PCA is a standard tool for displaying the multivariate data table graphically by extracting relevant information from a complex data set differentiated by multiple variables (Foegeding and Drake, 2007). These techniques are often used by the researchers to differentiate the products by several sensory descriptors. Figure 10 shows PCA of control and treatment cheeses with component 1 having 88% of the total variation and component 2 with 6% of the total variation.

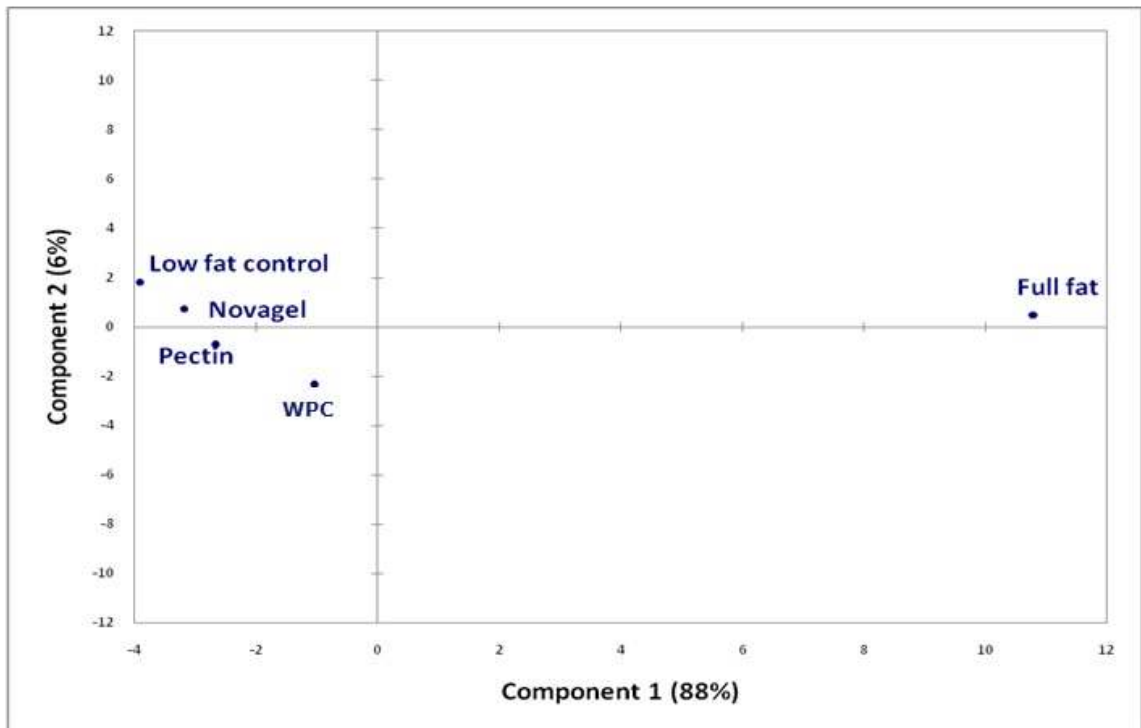


Figure 10. Principal component analysis of sensory parameters used to differentiate Cheddar cheeses made of full fat control (Full fat); Low fat control; Low fat cheese with pectin addition (Pectin); Low fat cheese with Novagel addition (Novagel) and Low fat cheese with whey protein concentrate (WPC) over four months of aging. The components 1 and 2 were differ by over all judges, batches, and sensory replicates; N=11 with the following attributes: overall aroma, milky aroma sweet, salty umami, diacetyl flavor, cooked flavor, milky flavor, brothy flavor, slipperiness, first bite stickiness, and stickiness, 2 chews.

The first component (horizontal axis) positively correlated ( $>0.9$ ) with the following attributes: overall aroma, milky aroma sweet, salty umami, diacetyl flavor, cooked flavor, milky flavor, brothy flavor, slipperiness, first bite stickiness, and stickiness 2 chews. The first component correlated negatively ( $< -0.9$ ) with sour, bitter, metallic flavor, unclean flavor, sour dairy flavor, soapy flavor, astringency, pungency, firmness, springiness, first bite firmness, first bite brittleness, firmness 5 chews, brittleness 5 chews, and curdiness 5 chews.

Component 2 (vertical axis) only accounted for 6% of the total variability with Judges, batches and sensory replicates. The low fat cheeses did not significantly distinguish among themselves but they were very different from the full fat cheese. Low fat control was farthest from the full fat control followed by LNG, LP, and LFW. We would have liked pectin and Novagel further away from low fat control, but it did show movement in the right direction.

During previous study at lab scale trials (Chapter 2), low fat cheese with Novagel was most similar to full fat control in sensory attributes. The pilot plant study at larger batch size did not reproduce a similar result for several reasons. The panelists were not same for the sensory testing for both lab scale and pilot scale trials.

In lab scale sensory analysis, panelists were not asked for descriptive analysis, rather they blindly measured the textural and flavor differences spatially on a piece of paper between the cheeses. In addition to this, hydrocolloids may be entrapped more during the lab scale trials as the cheese making vat (22 lbs trial batch) was much smaller in size as compared to pilot scale trials (1200 lb batch size).

During draining of whey in lab scale trials, we didn't see any hydrocolloid going away with the whey where as in pilot scale trials; we did observe hydrocolloid mixture settled at the bottom of tank, which got drained with the whey. Along with the differences in batch sizes, sensory evaluation was performed after one month of ripening in case of lab scale trials where as tasting panel was conducted after 4 months of aging in pilot plant trials.

### 3.3.3 Texture Analysis

Instrumental texture profile analysis is a common way to measure texture differences between cheese treatments (Bryant et al., 1995; Chevanan et al., 2006; Dabour et al., 2006; Liu et al., 2008). TPA tests were performed in triplicate and a typical graph is shown in Figure 11. A summary of analysis of variance is generated for understanding the interactions between the treatments and months over the ripening period (Table 12).

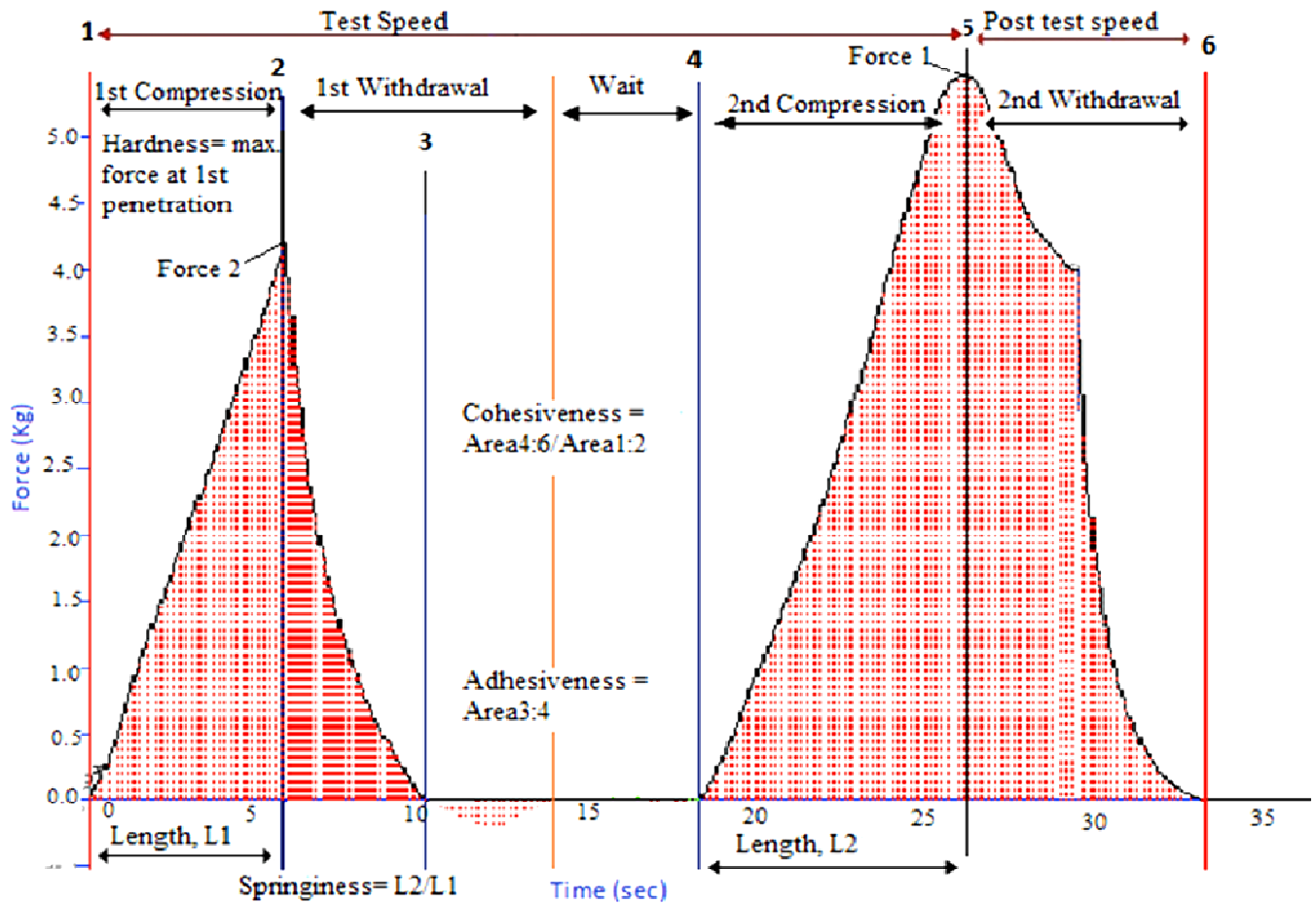


Figure 11. A typical graph, calculations and definitions for a TPA curve for Cheddar cheese made with Novagel (FMC Biopolymer, Philadelphia, PA)

Table 12. Analysis of variance summary (degree of freedom, sum of squares, mean squares and probabilities) of texture profile analysis parameters by TA XT plus analyzer during 120 days of ripening at 4° C

TPA Parameters	Source	DF	Sum of squares	Mean squares	F	Pr > F
Firmness	Treatments	4	12340.163	3085.041	42.881	< 0.0001
	Month	4	818.772	204.693	2.845	0.051
	Month*Treatments	16	1154.331	72.146	1.003	0.491
Springiness	Treatments	4	0.614	0.154	51.005	< 0.0001
	Month	4	0.082	0.020	6.792	0.001
	Month*Treatments	16	0.039	0.002	0.804	0.668
Cohesiveness	Treatments	4	7.109	1.777	14.718	< 0.0001
	Month	4	2.637	0.659	5.460	0.004
	Month*Treatments	16	3.416	0.213	1.768	0.114
Resilience	Treatments	4	0.447	0.112	52.479	< 0.0001
	Month	4	0.124	0.031	14.526	< 0.0001
	Month*Treatments	16	0.068	0.004	2.006	0.071

\*Statistically significant ( $P < 0.05$ )

The definition of the texture terms, and their calculations are described in Figure 8. Firmness is defined as the amount of force required to compress the sample (Brown et al., 2003). As shown in Table 13, the low fat cheeses were firmer than full fat control. Low fat cheeses suffered textural defects such as firmer, rubbery, dry, and grainy, which is less acceptable to consumers. Cheese with firm body offers resistance to pressure applied during mastication. These firm bodied cheeses may have short texture which breaks easily during bending a plug (Patridge, 2009). Addition of pectin and Novagel did

not reduce the firmness, but values were directionally lower than LFC initially (Figure 12). The firmness result contradicts with the lab scale trials as cheese made with pectin was not different when compared with the full fat. Liu et al., (2008) compared the full fat and low fat cheese analogues made with and without pectin gel in a lab scale environment. They stated that low fat cheese analogues made with pectin gel were more similar to full fat cheese analogues.

Springiness is the distance to which a compressed and deformed product recovers to its original height after the first compression force is removed (Civille and Szczesniak, 1973). In other terms, it indicates the rubbery texture experienced while biting the sample during eating (Adhikari et al., 2003). Springiness of cheeses increased with the reduction in fat. However, there are no detectable differences found in springiness between the low fat cheeses. These results correlate with the findings during lab scale trials of 10 Kg (22 pounds) batch size. Bryant et al., (1995) investigated the impact on hardness, springiness, adhesiveness, and cohesiveness of Cheddar cheese with varying fat contents from 34% to 13% in the finished cheese ripened for 4 months at 4 °C. They summarized an increase in springiness with a decrease in fat content. They followed a milled curd cheese making procedure with no addition of hydrocolloids.

Resilience is how well the product can regain its original height. Resilience is measured after the withdrawal of the first compression, before the waiting period starts (Figure 14).

There were no differences found in the resilience between the low fat cheeses. However, it did change between the months with a trend in reduction throughout aging (Table 12). The effect of proteolysis on the protein network may be the cause. Chevanan et al.,

(2006) studied the effect of calcium and phosphorus with salt to moisture ratio in full fat cheese. They reported a decrease in resilience over 4 months of aging and then an increase until the end of their study period (eight months).

Cohesiveness is the ratio of force area of the second bite to the force area of the first bite. It indicates how well the product withstands a second deformation relative to the deformation at first compression. Cohesiveness of low fat cheese was similar to springiness and increased with a decrease in fat (Figure 15). In descriptive terms, cohesiveness may also be explained as the degree to which sample deforms rather than fractures during first one to three bites using the molars (Drake et al., 1999). For an ideal Cheddar cheese, it should not fracture in first bite between the molars otherwise it is considered as weak and crumbly body. However, cheeses that were too firm to breakdown were also not desirable (Foegeding and Drake, 2007). Drake et al., (1999) reported decrease in cohesiveness in reduced fat processed cheese made with lecithin.

According to Foegeding and Drake (2007), it is not advisable to evaluate sensory and textural parameters in the same sessions. The cross interactions of flavor and texture may affect the liking scores. In this study, texture and flavor parameters were evaluated in the same sessions distributed between four separate sessions. This may explain the variability in the texture results obtained from sensory testing and instrumental testing.

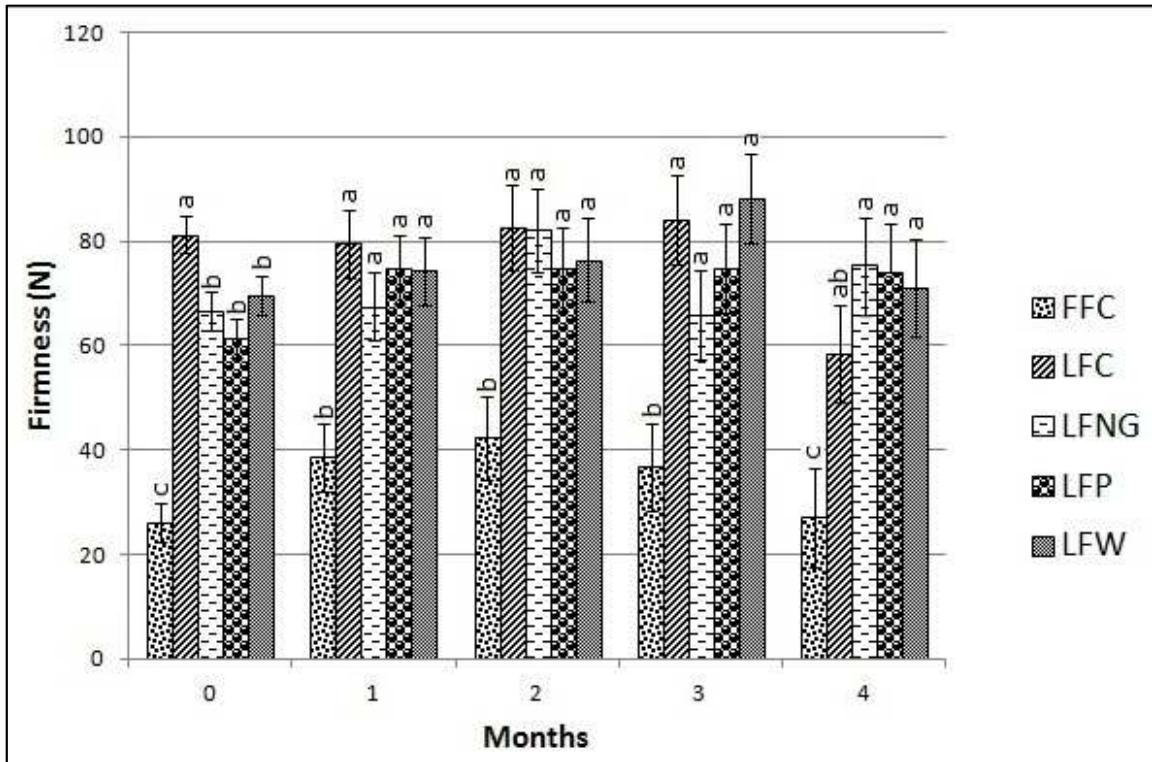


Figure 12. Texture parameter, Firmness of Cheddar cheeses made of full fat control (FFC); Low fat control (LFC); Low fat cheese with pectin addition (LP); low fat cheese with Novagel addition (LNG) and Low fat cheese with whey protein concentrate (LFW) over four months of aging. Time 0 months indicate one week after cheese make. Means within the same month with a different letter differ significantly ( $p < 0.05$ ).

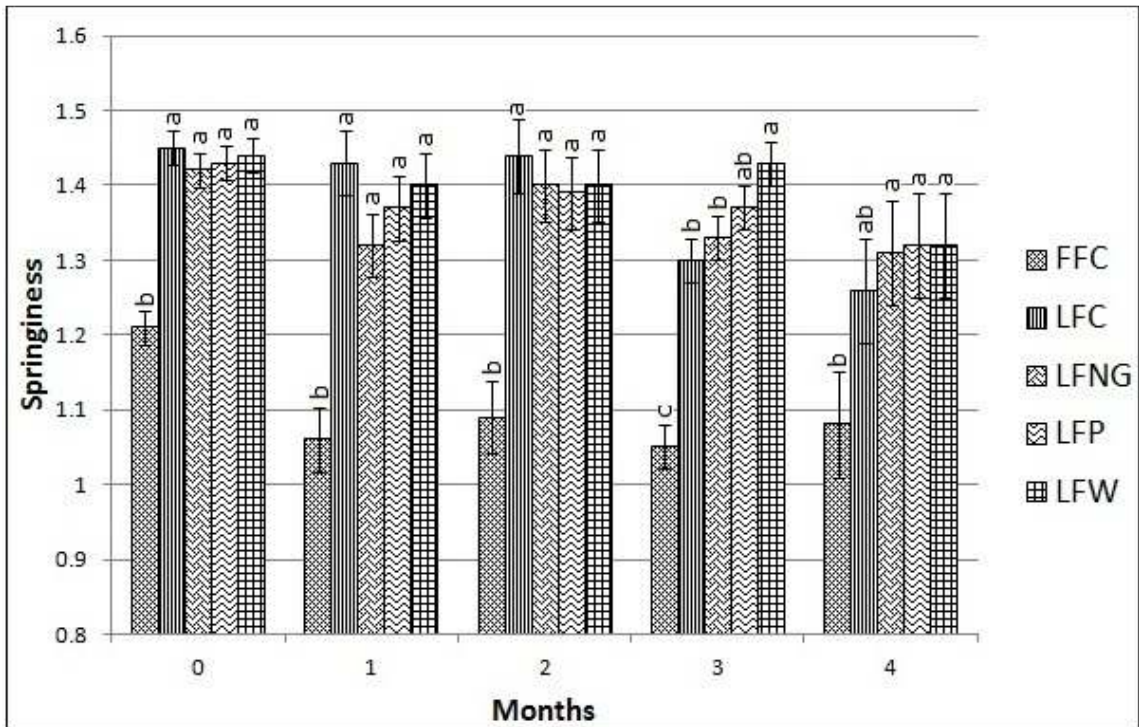


Figure 13. Texture parameter, Springiness of Cheddar cheeses made of full fat control (FFC); Low fat control (LFC); Low fat cheese with pectin addition (LP); low fat cheese with Novagel addition (LNG) and Low fat cheese with whey protein concentrate (LFW) over four months of aging. Time 0 months indicate one week after cheese make. Means within the same month with a different letter differ significantly ( $p < 0.05$ ).

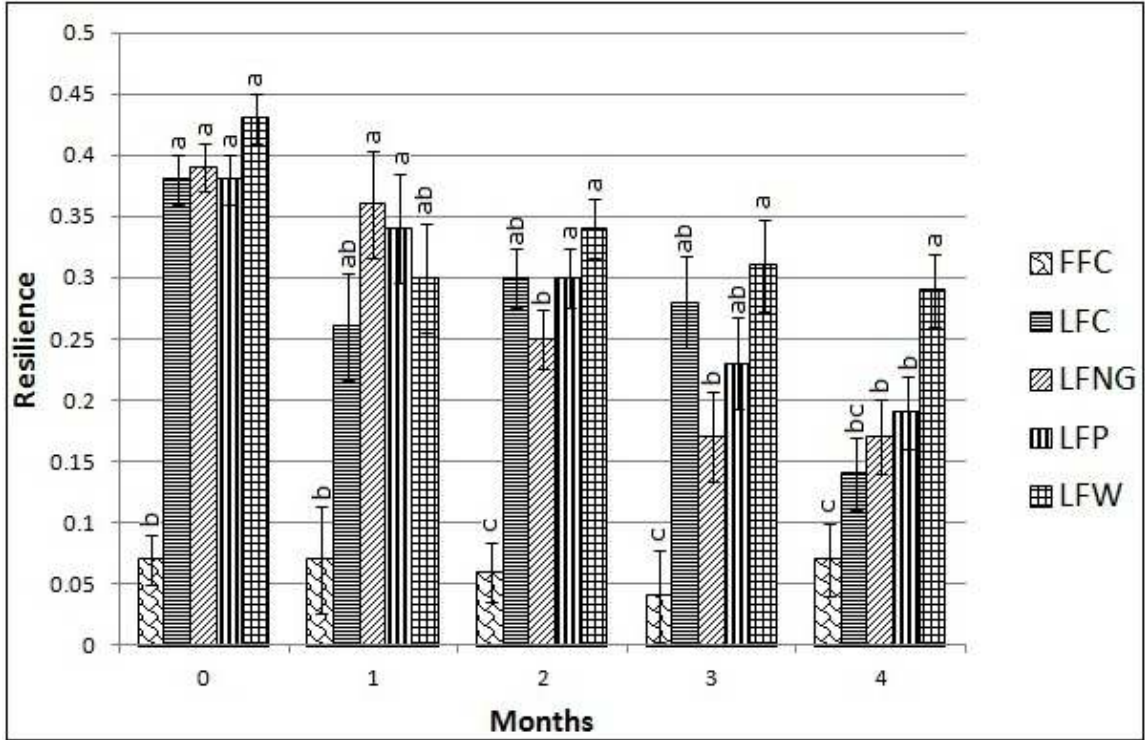


Figure 14. Texture parameter, Resilience of Cheddar cheeses made of full fat control (FFC); Low fat control (LFC); Low fat cheese with pectin addition (LP); low fat cheese with Novagel addition (LNG) and Low fat cheese with whey protein concentrate (LFW) over four months of aging. Time 0 months indicate one week after cheese make. Means within the same month with a different letter differ significantly ( $p < 0.05$ ).

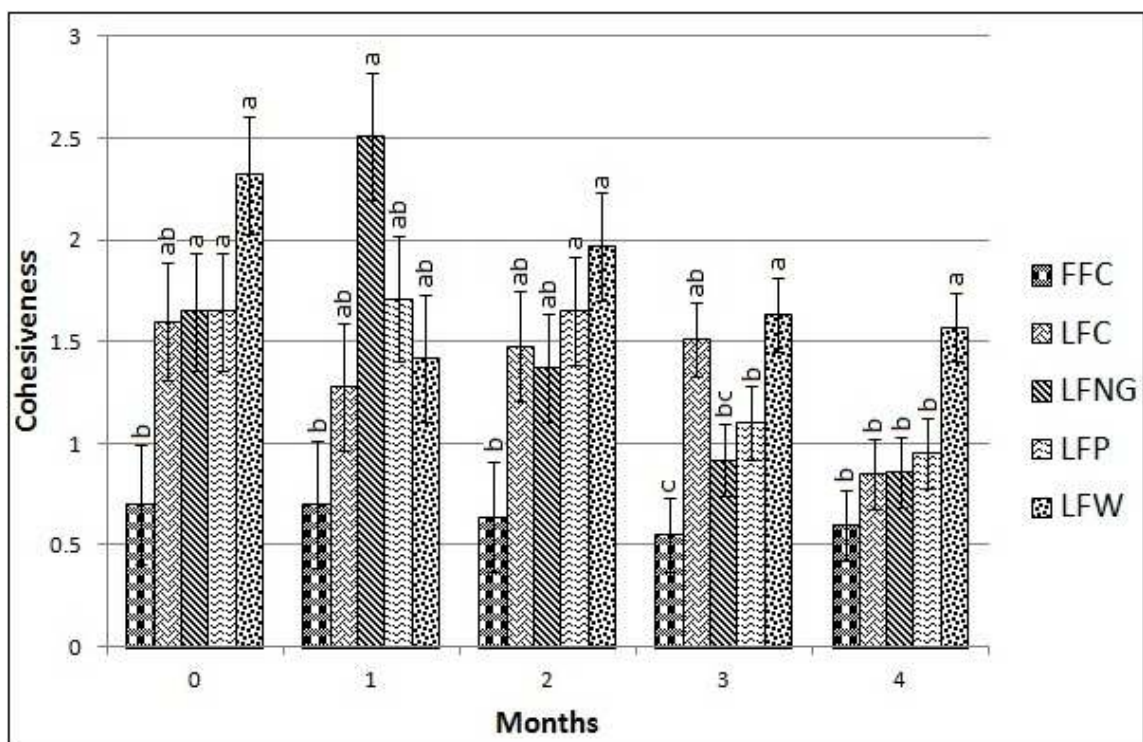


Figure 15. Texture parameter, Cohesiveness of Cheddar cheeses made of full fat control (FFC); Low fat control (LFC); Low fat cheese with pectin addition (LP); low fat cheese with Novagel addition (LNG) and Low fat cheese with whey protein concentrate (LFW) over four months of aging. Time 0 months indicate one week after cheese make. Means within the same month with a different letter differ significantly ( $p < 0.05$ ).

Table 13. Summary of measurement of textural properties of cheeses with varied fat contents and influence of polysaccharides as determined by TA XT analyzer every month till four months. Values are the means of replicates (N=3). Means are compared within each month.

Parameters with age		Treatments		Control			SE <sup>7</sup>
		LP <sup>1</sup>	LNG <sup>2</sup>	LFW <sup>3</sup>	LF <sup>4</sup>	FFC <sup>5</sup>	
Firmness (N)	1 Week	61.42 <sup>b</sup>	66.68 <sup>b</sup>	69.55 <sup>b</sup>	81.29 <sup>a</sup>	26.24 <sup>c</sup>	3.65
	1 mon	74.64 <sup>a</sup>	67.41 <sup>a</sup>	74.39 <sup>a</sup>	79.60 <sup>a</sup>	38.68 <sup>b</sup>	6.50
	2 mon	74.64 <sup>a</sup>	82.08 <sup>a</sup>	76.44 <sup>a</sup>	82.60 <sup>a</sup>	42.33 <sup>b</sup>	8.10
	3 mon	74.85 <sup>a</sup>	65.84 <sup>a</sup>	88.23 <sup>a</sup>	84.15 <sup>a</sup>	36.72 <sup>b</sup>	8.51
	4 mon	74.25 <sup>a</sup>	75.39 <sup>a</sup>	71.28 <sup>a</sup>	58.47 <sup>ab</sup>	27.06 <sup>c</sup>	9.32
Resilience	1 Week	0.38 <sup>a</sup>	0.39 <sup>a</sup>	0.43 <sup>a</sup>	0.38 <sup>a</sup>	0.07 <sup>b</sup>	0.02
	1 mon	0.34 <sup>a</sup>	0.36 <sup>a</sup>	0.30 <sup>ab</sup>	0.26 <sup>ab</sup>	0.07 <sup>b</sup>	0.04
	2 mon	0.30 <sup>a</sup>	0.25 <sup>b</sup>	0.34 <sup>a</sup>	0.3 <sup>ab</sup>	0.06 <sup>c</sup>	0.024
	3 mon	0.23 <sup>ab</sup>	0.17 <sup>b</sup>	0.31 <sup>a</sup>	0.28 <sup>ab</sup>	0.04 <sup>c</sup>	0.03
	4 mon	0.19 <sup>b</sup>	0.17 <sup>b</sup>	0.29 <sup>a</sup>	0.14 <sup>bc</sup>	0.07 <sup>c</sup>	0.037
Springiness	1 Week	1.43 <sup>a</sup>	1.42 <sup>a</sup>	1.44 <sup>a</sup>	1.45 <sup>a</sup>	1.21 <sup>b</sup>	0.023
	1 mon	1.37 <sup>a</sup>	1.32 <sup>a</sup>	1.40 <sup>a</sup>	1.43 <sup>a</sup>	1.06 <sup>b</sup>	0.043
	2 mon	1.39 <sup>a</sup>	1.40 <sup>a</sup>	1.40 <sup>a</sup>	1.44 <sup>a</sup>	1.09 <sup>b</sup>	0.049
	3 mon	1.37 <sup>ab</sup>	1.33 <sup>b</sup>	1.43 <sup>a</sup>	1.30 <sup>b</sup>	1.05 <sup>c</sup>	0.029
	4 mon	1.32 <sup>a</sup>	1.31 <sup>a</sup>	1.32 <sup>a</sup>	1.26 <sup>ab</sup>	1.08 <sup>b</sup>	0.07
Cohesiveness	1 Week	1.65 <sup>a</sup>	1.65 <sup>a</sup>	2.32 <sup>a</sup>	1.60 <sup>ab</sup>	0.7 <sup>b</sup>	0.29
	1 mon	1.71 <sup>ab</sup>	2.51 <sup>a</sup>	1.42 <sup>ab</sup>	1.28 <sup>ab</sup>	0.7 <sup>b</sup>	0.31
	2 mon	1.65 <sup>a</sup>	1.37 <sup>ab</sup>	1.97 <sup>a</sup>	1.48 <sup>ab</sup>	0.64 <sup>b</sup>	0.267
	3 mon	1.1 <sup>b</sup>	0.92 <sup>bc</sup>	1.63 <sup>a</sup>	1.51 <sup>ab</sup>	0.55 <sup>c</sup>	0.18
	4 mon	0.95 <sup>b</sup>	0.86 <sup>b</sup>	1.57 <sup>a</sup>	0.85 <sup>b</sup>	0.60 <sup>b</sup>	0.17
Adhesiveness	1 Week	-10.56 <sup>ab</sup>	-4.70 <sup>a</sup>	-12.87 <sup>ab</sup>	-26.60 <sup>b</sup>	0.26 <sup>a</sup>	5.8
	1 mon	-27.10 <sup>a</sup>	-23.49 <sup>a</sup>	-42.36 <sup>a</sup>	-14.23 <sup>a</sup>	-34.07 <sup>a</sup>	20.18
	2 mon	-12.66 <sup>a</sup>	-23.35 <sup>ab</sup>	-31.95 <sup>b</sup>	-13.61 <sup>ab</sup>	18.69 <sup>ab</sup>	6.8
	3 mon	-17.95 <sup>b</sup>	-24.68 <sup>a</sup>	-26.05 <sup>a</sup>	-30.20 <sup>a</sup>	-53.78 <sup>a</sup>	16.2
	4 mon	-46.19 <sup>a</sup>	-34.31 <sup>a</sup>	-36.59 <sup>a</sup>	-23.65 <sup>a</sup>	-27.85 <sup>a</sup>	15.9

Table Continued...

Item	Treatments		Control			SE <sup>7</sup>
	LP <sup>1</sup>	LNG <sup>2</sup>	LFW <sup>3</sup>	LF <sup>4</sup>	FFC <sup>5</sup>	
Gumminess(N) 1Week	101.02 <sup>b</sup>	110.01 <sup>b</sup>	159.7 <sup>a</sup>	129.57 <sup>ab</sup>	18.01 <sup>c</sup>	14.39
1 mon	124.51 <sup>ab</sup>	167.8 <sup>a</sup>	105.65 <sup>ab</sup>	102.08 <sup>ab</sup>	27.15 <sup>b</sup>	18.21
2 mon	124.08 <sup>a</sup>	113.03 <sup>a</sup>	152.52 <sup>a</sup>	122.67 <sup>a</sup>	26.58 <sup>b</sup>	19.51
3 mon	83.56 <sup>b</sup>	61.75 <sup>b</sup>	144.47 <sup>a</sup>	127.66 <sup>ab</sup>	20.06 <sup>c</sup>	13.42
4 mon	71.1 <sup>b</sup>	64.78 <sup>b</sup>	110.25 <sup>a</sup>	49.35 <sup>bc</sup>	16.05 <sup>c</sup>	12.85
Chewiness (N) 1Week	145.15 <sup>b</sup>	157.03 <sup>b</sup>	230.73 <sup>a</sup>	188.01 <sup>ab</sup>	22.03 <sup>c</sup>	11.02
1 mon	170.58 <sup>a</sup>	218.88 <sup>a</sup>	148.23 <sup>a</sup>	146.72 <sup>ab</sup>	28.53 <sup>b</sup>	15.74
2 mon	172.42 <sup>a</sup>	158.17 <sup>a</sup>	212.91 <sup>a</sup>	176.54 <sup>a</sup>	28.79 <sup>b</sup>	17.63
3 mon	115.8 <sup>b</sup>	82.23 <sup>bc</sup>	206.95 <sup>a</sup>	166.38 <sup>ab</sup>	21.01 <sup>c</sup>	11.75
4 mon	94.65 <sup>ab</sup>	85.31 <sup>ab</sup>	147.18 <sup>a</sup>	62.26 <sup>bc</sup>	17.41 <sup>c</sup>	8.01

<sup>a-c</sup> Means within a row with different superscripts differ ( $P < 0.05$ )

<sup>1</sup>LP: Low Fat with pectin

<sup>2</sup>LNG: Low Fat with Novagel

<sup>3</sup>LFW: Low Fat with Whey Protein Concentrate

<sup>4</sup>LF: Low Fat Control

<sup>5</sup>FFC: Full Fat Control

<sup>7</sup>Largest standard error of all treatments is shown for each parameter

### 3.3.4 Proteolysis

Proteolysis is one of the principal biochemical events during cheese ripening and leads to texture changes and flavor development. Enzymes from coagulant (e.g. chymosin), milk (e.g. plasmin), starter, non-starter or secondary cultures; all can play a role in protein breakdown over time (McSweeney, 2004). Assessment of proteolysis in cheese indicates the cheese maturity and quality (Fox et al., 2000). Several authors have developed wide range of specific and non specific techniques for determining proteolysis in Cheddar cheese (Guinee and McSweeney, 2006; Fox, 1989; Fox et al., 2000). One of the widely used non specific techniques is measuring water soluble nitrogen (WSN) which may be defined as the amount of nitrogen (in cheese) soluble in water. In this study, WSN was determined during cheese aging to examine differences between the treatments.

The soluble nitrogen content of the cheese, expressed as the percent nitrogen in the extract, is shown in Table 14. All treatments showed an increase in water soluble nitrogen during aging (Figure 16), but it did not differ among the low fat cheese treatments. Guinee and McSweeney (2006) reported a decrease in water soluble nitrogen with decrease in fat level from 33% to 6% in Cheddar cheese at 225 days of ripening. However, in this study, no differences were found in WSN between the low fat and full fat cheese. This may suggest that level of fat is compensated by the higher amount of protein present in the low fat which indicated higher levels of WSN in low fat cheeses.

The increasing level of WSN during aging suggests the proteolytic activity by residual chymosin and by proteases in starter and non-starter bacteria. These enzymes are

responsible for increased WSN during ripening (Farkye, 1995). There were no differences in proteolysis between the low fat cheese treatments.

The rate and type of proteolysis is different among cheese varieties, one of the important factor is due to the diversity of starter and non starter organisms used in the cheese manufacturing (Fox, 1989).

Table 14. Age related changes in the water soluble nitrogen of the extracts for full fat and low fat cheeses at every month till four months of ripening. Water soluble extracts were analyzed for total nitrogen through combustion method. Values presented are the means from three replicates. Means are compared within each month.

Cheese Age	% WSN as a function of Total Nitrogen					SE <sup>7</sup>
	Treatments		Control			
	LP <sup>1</sup>	LNG <sup>2</sup>	LFW <sup>3</sup>	LF <sup>4</sup>	FFC <sup>5</sup>	
1 week	11.79 <sup>a</sup>	12.52 <sup>a</sup>	13.18 <sup>a</sup>	9.75 <sup>a</sup>	10.22 <sup>a</sup>	2.42
1 Month	12.91 <sup>a</sup>	15.21 <sup>a</sup>	16.71 <sup>a</sup>	12.98 <sup>a</sup>	13.17 <sup>a</sup>	1.46
2 Month	21.72 <sup>a</sup>	22.71 <sup>a</sup>	18.9 <sup>ab</sup>	15.83 <sup>b</sup>	21.04 <sup>ab</sup>	1.65
3 Month	20.79 <sup>a</sup>	22.2 <sup>a</sup>	21.22 <sup>a</sup>	22.62 <sup>a</sup>	21.91 <sup>a</sup>	1.71
4 Month	22.79 <sup>a</sup>	24.05 <sup>a</sup>	23.34 <sup>a</sup>	24.08 <sup>a</sup>	22.52 <sup>a</sup>	1.85

<sup>a-b</sup> Means within a row with different superscripts differ ( $P < 0.05$ )

<sup>1</sup>LNG: Low Fat with Novagel

<sup>2</sup>LFW: Low Fat with Whey Protein Concentrate

<sup>3</sup>LP: Low Fat with pectin

<sup>4</sup>FFC: Full Fat Control

<sup>5</sup>LF: Low Fat Control

<sup>7</sup>Largest standard error of all treatments is shown for each parameter

In the extraction method, water was used as a solvent. Although the same quantity of water and cheese was blended for each of the treatments, the cheese to water ratio,

which is dependent on the moisture content of cheese, will be different since the low fat cheeses had higher moisture than full fat. Figure 16 illustrates the proteolytic activity in treatment cheeses as a ratio of percent WSN to the total nitrogen (TN) in cheese.

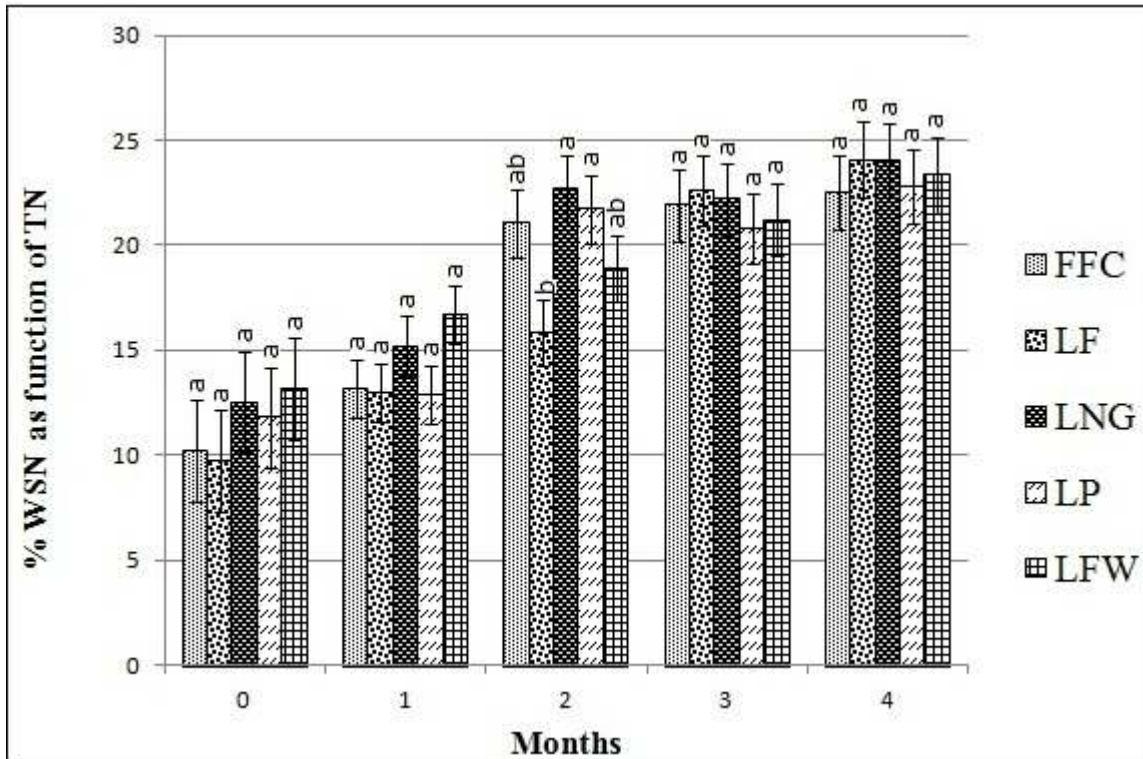


Figure 16. Effect of treatments on water soluble nitrogen (WSN) content of extracts: FFC - Full Fat Control; LF - Low Fat Control; LNG - Low Fat with Novagel; LP - Low Fat with pectin; and LFW - Low Fat with Whey Protein Concentrate over four months of ageing. Values are the means of replicates (N=3) calculated as % of total nitrogen (TN). Time 0 months indicate one week after cheese make. Means within the same month with a different letter differ significantly ( $p < 0.05$ ).

The maximum average of WSN for all the months was observed for LNG followed by other treatments in the order of LFW > LP > FFC > LF. This trend was not

correlated either with moisture content or protein content in the cheese (Table 9). Haque et al., (2007) reported a similar trend of increased total nitrogen in aqueous extracts for low fat cheddar cheese made with fat replacers. They used protein based fat replacer (Simplese and Dairy Lo); a carbohydrate based fat replacer (Stellar), and Novagel (used in this study) in the manufacture of low fat Cheddar cheese. In their study, the order of higher moisture content in treatment cheeses did not corresponded with higher total nitrogen in the aqueous extract.

The increase in %WSN / TN in the cheeses did not result in a decrease in firmness over time (Figure 12). However, there is a decrease in resilience, springiness, and cohesiveness of cheese with increasing proteolysis. A change in texture from casein degradation is one of the four contributions explained by Fox (1989) during proteolysis. No significant differences in textural characteristics and proteolysis (%WSN / TN), indicate similar biochemical changes occur during the ripening process. Sallamai et al., (2004) reported similar changes in textural parameters during ripening of Cheddar cheese treated with autolytic, proteolytic, and adjunct cultures.

Table 15. Degree of freedom, sum of squares, mean squares, F value and probabilities of age related changes (proteolysis) in low fat and full fat cheeses analyzed for water soluble nitrogen in the extract during 120 days of ripening at 4° C

Source	DF	Sum of squares	Mean squares	F	Pr > F
Treatments	4	23.638	5.909	1.711	0.187
Months	4	903.452	225.863	65.394	< 0.0001
Months * Treatments	16	58.935	3.683	1.066	0.440

\*Statistically significant ( $P < 0.05$ )

The rate of proteolysis in treatment and control cheeses were not different with varying fat contents (Table 15). The degree of proteolysis was significantly different within months as cheeses aged. The use of the same starter cultures for all treatments to observe the effect of hydrocolloids, could explain the continuity in proteolysis. Selection of different starter and adjunct cultures could have produced a cheese with different textural and sensory characteristics. To see the effect of different hydrocolloids in this study, we used the same set of the starter and adjunct cultures. Similar moisture content in cheeses could also have played a role for consistent extent of proteolysis.

### 3.3.5 Organic acids in cheese

Eight organic acids were analyzed based on the ones commonly found in Cheddar cheese (Izco et al., 2002; Zeppa and Rolle, 2008). The amount of organic acids present in cheese has been monitored for the activity of primary and secondary starter culture during the cheese ripening and has been studied in regards to flavor differences in cheese (Marsili et al., 1981).

An array of biochemical pathways yield several intermediate and end products of organic acids through these starter and non starter lactic acid bacteria (Lues and Botha, 1998). Evaluating these organic acids may provide invaluable information in determining the quality of a good Cheddar cheese. There are no said parameters for an ideal concentration of these organic acids but presence of these acids singly or in combination may have a positive and negative contribution to cheese flavor. The starter cultures used in the manufacture of cheese making produces several organic acids via pyruvate by utilizing available energy source (lactose) to equilibrate the redox balance (Leroy and De Vuyst, 2004). We hypothesized that organic acids content will be different in low fat cheeses treated with hydrocolloids. These starter cultures may metabolize these hydrocolloids to produce intermediate or end products of organic acids which may impact the flavor differences.

Analyses of these organic acids were performed by the regression equations derived from calibration curve runs of known concentrations of standards, as a ratio to a fixed concentration of an internal standard. Three levels were run in duplicate within the range of concentrations indicated in the literature. Those levels were used to form calibration curves and regression equations as shown in Table 16.

Table 16. Mean retention times, regression equations and coefficient of determination ( $R^2$ ) calculated with a standard solution for each of the compounds determined. Calibration curves were used in the analysis of organic acids. An internal standard (sodium octanoate) was used in the method.

Organic Acids	Mean retention times (min.)	Regression equation	$R^2$
Oxalic Acid	3.23	$y = 0.6679x - 0.0043$	1.0000
Formic Acid	3.54	$y = 1.1908x - 0.0062$	0.9946
Citric Acid	4.10	$y = 0.4507x + 0.0047$	0.9998
Pyruvic Acid	4.45	$y = 0.1201x + 0.0336$	0.3351
Acetic Acid	4.66	$y = 0.7666x + 0.0235$	0.9977
Lactic Acid	4.75	$y = 0.7624x + 0.3628$	0.9998
Propionic Acid	5.12	$y = 0.9484x + 0.2721$	0.9997
Butyric Acid	5.55	$y = 0.8871x - 0.0004$	0.9988

The organic acids were separated within the eight minutes of the run time. As per Table 16, the coefficient of determination ( $R^2$ ), for each organic acid was 0.994 (except for pyruvic acid, which was not quantified due to the low  $R^2$ ). The low coefficient of determination in pyruvic acid may have been caused by improper pipetting or mixing of the standard solution in the extract mixture.

The analysis of the means is shown in Table 17. Oxalic, propionic, and butyric acids were not detected in the cheeses under the conditions utilized in this method. Acetic, citric, formic, and lactic acids were easily identified and quantified in cheese samples and thus only these will be discussed in the later part of this section. As an example, an electropherogram of an extract from low fat cheese with Novagel is shown in figure 17 at one month of ripening.

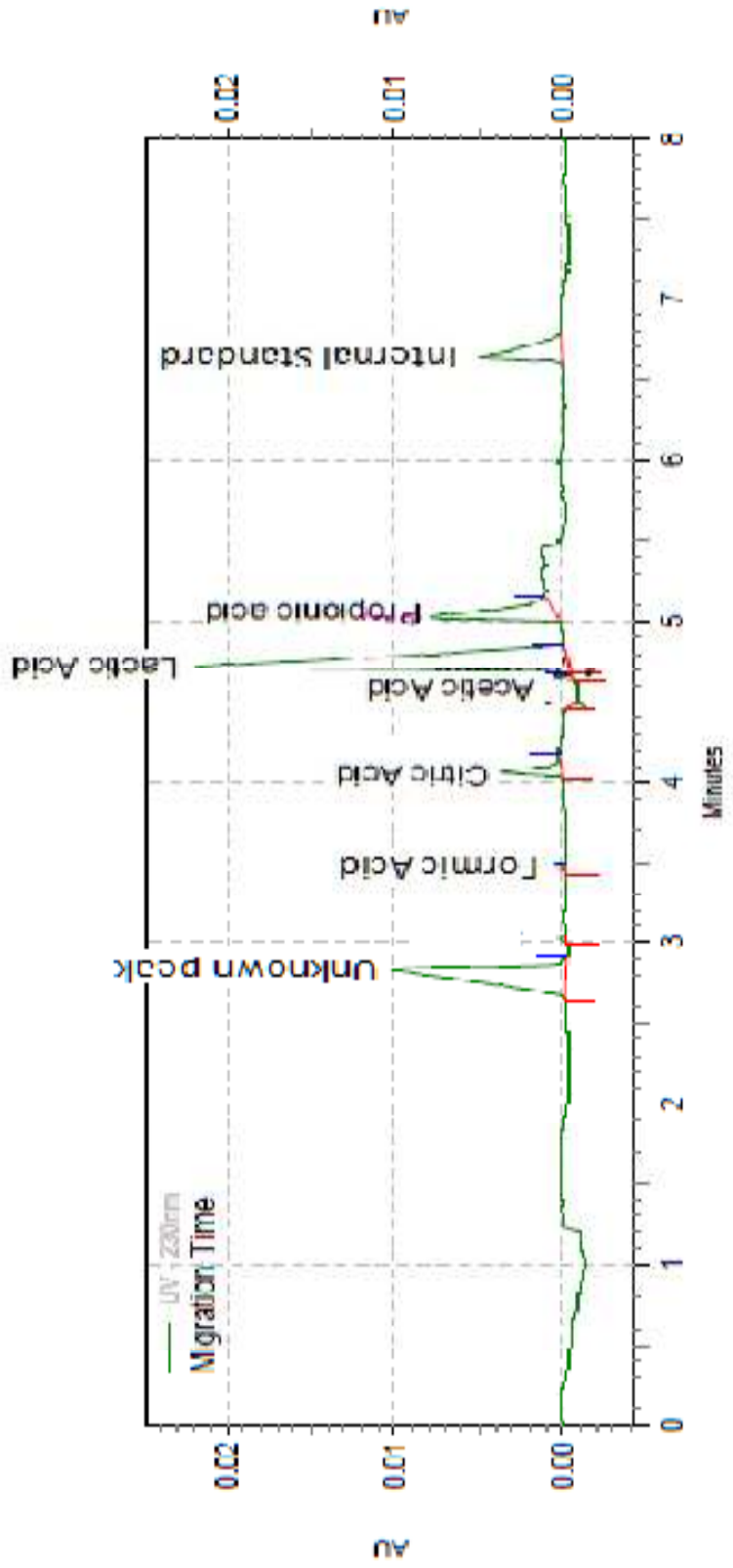


Figure 17. Electropherogram of the extract obtained from low fat cheese with Novagel at one month of ripening. Capillary electrophoresis, bare fused silica capillary; injection for 10 s at 0.5 p.s.i.; voltage -30kV; performed at 25° C; indirect UV detection at 230 nm

Table 17. Mean concentration expressed as mg/100g cheese for each organic acid in full fat and low fat cheeses determined each month through 120 days of ripening. Values are means of 2 samples with 2 injections per sample. Means are compared within each month.

Organic Acids		Treatments		Control			SE <sup>7</sup>
		LP <sup>1</sup>	LNG <sup>2</sup>	LFW <sup>3</sup>	LF <sup>4</sup>	FFC <sup>5</sup>	
Acetic Acid	1 week	0.20 <sup>a</sup>	0.17 <sup>a</sup>	0.34 <sup>a</sup>	0.15 <sup>a</sup>	0.13 <sup>a</sup>	0.104
	1 mon	0.12 <sup>b</sup>	0.15 <sup>ab</sup>	0.31 <sup>a</sup>	0.23 <sup>ab</sup>	0.10 <sup>b</sup>	0.067
	2 mon	0.19 <sup>ab</sup>	0.18 <sup>b</sup>	0.27 <sup>a</sup>	0.15 <sup>b</sup>	0.13 <sup>b</sup>	0.038
	3 mon	0.16 <sup>bc</sup>	0.19 <sup>abc</sup>	0.33 <sup>a</sup>	0.26 <sup>ab</sup>	0.06 <sup>c</sup>	0.041
	4 mon	0.12 <sup>a</sup>	0.21 <sup>a</sup>	0.20 <sup>a</sup>	0.22 <sup>a</sup>	0.08 <sup>a</sup>	0.047
Citric Acid	1 week	3.37 <sup>a</sup>	3.26 <sup>a</sup>	3.34 <sup>a</sup>	3.43 <sup>a</sup>	2.35 <sup>b</sup>	0.153
	1 mon	3.47 <sup>a</sup>	3.53 <sup>a</sup>	3.76 <sup>a</sup>	3.37 <sup>a</sup>	2.61 <sup>b</sup>	0.176
	2 mon	2.47 <sup>a</sup>	2.02 <sup>b</sup>	2.34 <sup>a</sup>	2.07 <sup>b</sup>	1.73 <sup>c</sup>	0.105
	3 mon	1.73 <sup>a</sup>	1.96 <sup>a</sup>	1.58 <sup>ab</sup>	1.76 <sup>a</sup>	1.14 <sup>b</sup>	0.225
	4 mon	2.23 <sup>a</sup>	2.20 <sup>a</sup>	1.89 <sup>a</sup>	2.51 <sup>a</sup>	1.59 <sup>a</sup>	0.400
Formic Acid	1 week	0.14 <sup>a</sup>	0.17 <sup>a</sup>	0.14 <sup>a</sup>	0.09 <sup>b</sup>	0.08 <sup>b</sup>	0.016
	1 mon	0.12 <sup>ab</sup>	0.07 <sup>d</sup>	0.12 <sup>a</sup>	0.01 <sup>bc</sup>	0.09 <sup>cd</sup>	0.008
	2 mon	0.12 <sup>a</sup>	0.12 <sup>a</sup>	0.12 <sup>a</sup>	0.10 <sup>a</sup>	0.10 <sup>a</sup>	0.01
	3 mon	0.10 <sup>a</sup>	0.12 <sup>a</sup>	0.12 <sup>a</sup>	0.10 <sup>a</sup>	0.09 <sup>a</sup>	0.033
	4 mon	0.11 <sup>b</sup>	0.26 <sup>a</sup>	0.11 <sup>b</sup>	0.11 <sup>b</sup>	0.06 <sup>c</sup>	0.007
Lactic Acid	1 week	25.50 <sup>a</sup>	24.63 <sup>ab</sup>	23.43 <sup>ab</sup>	22.73 <sup>b</sup>	16.82 <sup>c</sup>	0.911
	1 mon	24.31 <sup>a</sup>	23.12 <sup>a</sup>	23.94 <sup>a</sup>	22.92 <sup>a</sup>	17.01 <sup>a</sup>	1.245
	2 mon	25.91 <sup>a</sup>	23.48 <sup>ab</sup>	24.34 <sup>ab</sup>	21.71 <sup>bc</sup>	18.71 <sup>c</sup>	1.248
	3 mon	19.40 <sup>a</sup>	20.21 <sup>a</sup>	19.15 <sup>a</sup>	18.45 <sup>a</sup>	15.23 <sup>a</sup>	2.033
	4 mon	21.76 <sup>a</sup>	22.65 <sup>a</sup>	20.75 <sup>a</sup>	23.17 <sup>a</sup>	16.30 <sup>b</sup>	1.374

<sup>a-d</sup> Means within a row with different superscripts differ ( $P < 0.05$ )

<sup>1</sup>LP: Low Fat with pectin

<sup>2</sup>LNG: Low Fat with Novagel

<sup>3</sup>LFW: Low Fat with Whey Protein Concentrate

<sup>4</sup>LF: Low Fat Control

<sup>5</sup>FFC: Full Fat Control

<sup>7</sup>Largest standard error of all treatments is shown for each parameter

Table 18. Analysis of variance summary (degree of freedom, sum of squares, mean squares and probabilities) of organic acids analyzed during 120 days of ripening at 4° C

Organic Acids	Source	DF	Sum of squares	Mean squares	F	p Value*
Acetic Acid	Treatments	4	0.177	0.044	9.420	< 0.0001
	Month	4	0.009	0.002	0.488	0.744
	Month*Treatments	16	0.057	0.004	0.754	0.718
Citric Acid	Treatments	4	4.323	1.081	19.153	< 0.0001
	Month	4	21.998	5.500	97.468	< 0.0001
	Month*Treatments	16	1.284	0.080	1.422	0.209
Formic Acid	Treatments	4	0.023	0.006	17.147	< 0.0001
	Month	4	0.006	0.002	4.735	0.006
	Month*Treatments	16	0.037	0.002	7.053	< 0.0001
Lactic Acid	Treatments	4	279.514	69.878	35.037	< 0.0001
	Month	4	129.757	32.439	16.265	< 0.0001
	Month*Treatments	16	36.266	2.267	1.137	0.377

\*Statistically significant ( $P < 0.05$ )

As shown in Table 17, there were differences found in organic acid quantities between the treatments. LP has significantly higher amount of lactic acid than LF in 1 week and 2 months of ripening. FFC had lower lactic acid than all of the low fat cheeses in first week and fourth month of aging. Lactic acid was the most abundant organic acid found in all the cheeses. This is due to conversion of lactose into lactic acid by the starter organisms through metabolic pathways (Fox et al., 2000). This does not correlate with the initial pH of the full fat cheese, which was lower than most of the low fat cheeses (Table

9). Lactic acid concentration for all samples decreased at the end of third month with a slight increase by the end of fourth month (Figure 18).

Acetic acid was not significantly different in the treatments at 1 week of aging. High standard error may explain the inconsistency in the results at 1 week of aging (Figure 19). LFW has directionally higher acetic acid during the ripening period. The metabolism of lactic acid may convert it in acetic acid resulting in higher levels. Enzymes such as lactic dehydrogenase and pyruvate-formate lyase are responsible for converting lactose to lactic acid and eventually to acetic acid via pyruvate metabolism (Fox et al., 2000).

The acetic acid level decreased in LFW over four months of ripening period. Acetic acid in Cheddar cheese is considered as a contributing factor for cheese flavor, although not necessarily preferable (Singh et al., 2003). Higher acetic acid in LFW might contribute for directionally higher overall aroma than low fat control cheese.

Low fat cheese with Novagel generally contained a higher amount of formic acid than other cheeses, and full fat cheese generally had the least (Figure 20). All of the cheeses remained fairly constant in formic acid content till the end of the ripening period, except Novagel, which showed an abrupt increase in the last month. An analysis of variance summary also represented a significant difference in month and treatments interactions for formic acid (Table 18).

The reason for the variability of the formic acid results for this may be due to degradation of cellulose to formic acid (Atalla and Isogai, 2005). Novagel mainly consists of microcrystalline cellulose which may breakdown during the ripening period. This microcrystalline cellulose forms glucose by acid hydrolysis which is further

degraded to hydroxymethylfurfural, levulinic acid, and formic acid (Atalla and Isogai, 2005).

Citric acid showed a decreasing trend for the first three months of ripening but there was an increase in the fourth month as illustrated in Figure 20. All the low fat cheese treatments had a higher concentration of citric acid than control for the first 3 months of ripening. The starter organism used in the manufacture of low fat cheese, *Lactococcus lactis* ssp. *cremoris*, may metabolize citrate to flavor compound such as diacetyl during early stages of ripening. In later stages of ripening, when there is a sufficient increase in non-starter lactic acid bacteria, the mesophilic starter culture may catabolizes citrate to ethanol, acetate, and formate (Fox et al., 2000).

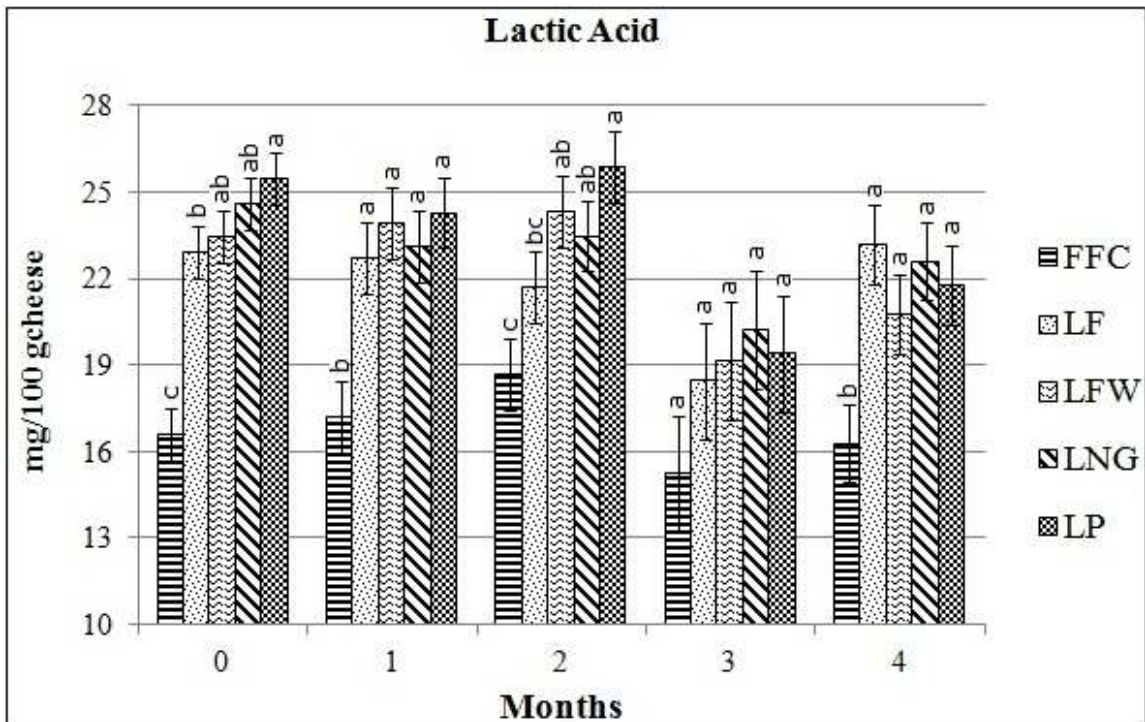


Figure 18. Lactic acid content of Cheddar cheeses made of full fat control (FFC); Low fat control (LFC); Low fat cheese with pectin addition (LP); low fat cheese with Novagel addition (LNG) and Low fat cheese with whey protein concentrate (LFW) over four months of aging. Time 0 months indicate one week after cheese make. Means within the same month with a different letter differ significantly ( $p < 0.05$ ).

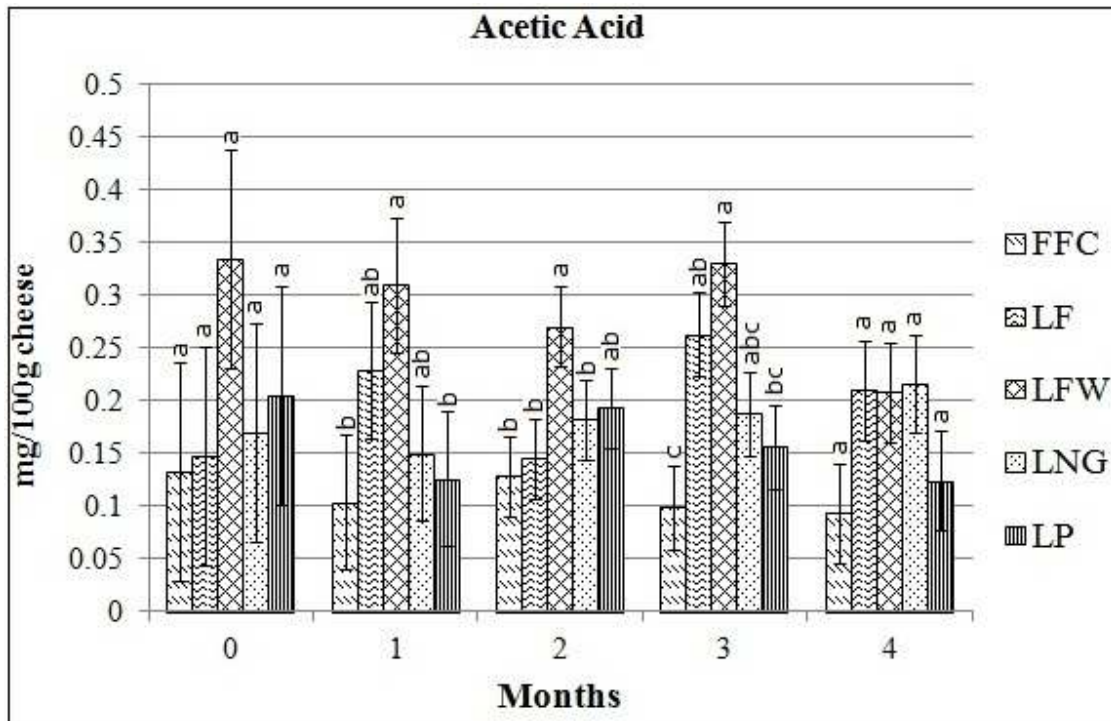


Figure 19. Acetic acid content of Cheddar cheeses made of full fat control (FFC); Low fat control (LFC); Low fat cheese with pectin addition (LP); low fat cheese with Novagel addition (LNG) and Low fat cheese with whey protein concentrate (LFW) over four months of aging. Time 0 months indicate one week after cheese make. Means within the same month with a different letter differ significantly ( $p < 0.05$ ).

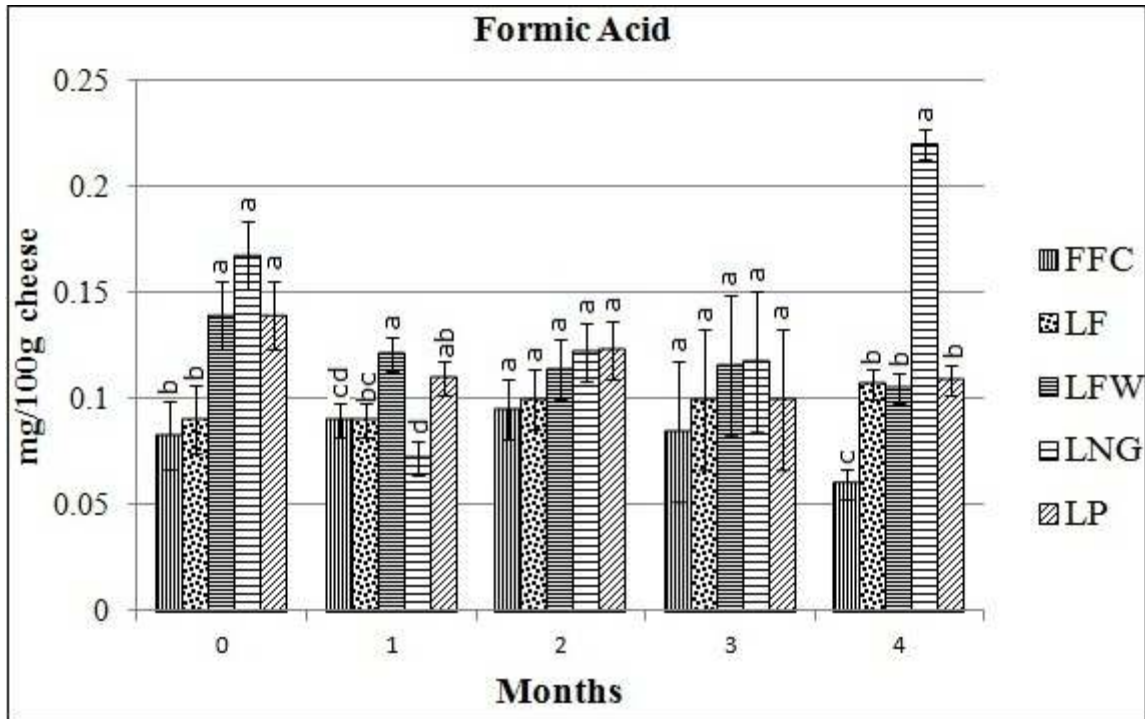


Figure 20. Formic acid content of Cheddar cheeses made of full fat control (FFC); Low fat control (LFC); Low fat cheese with pectin addition (LP); low fat cheese with Novagel addition (LNG) and Low fat cheese with whey protein concentrate (LFW) over four months of aging. Time 0 months indicate one week after cheese make. Means within the same month with a different letter differ significantly ( $p < 0.05$ ).

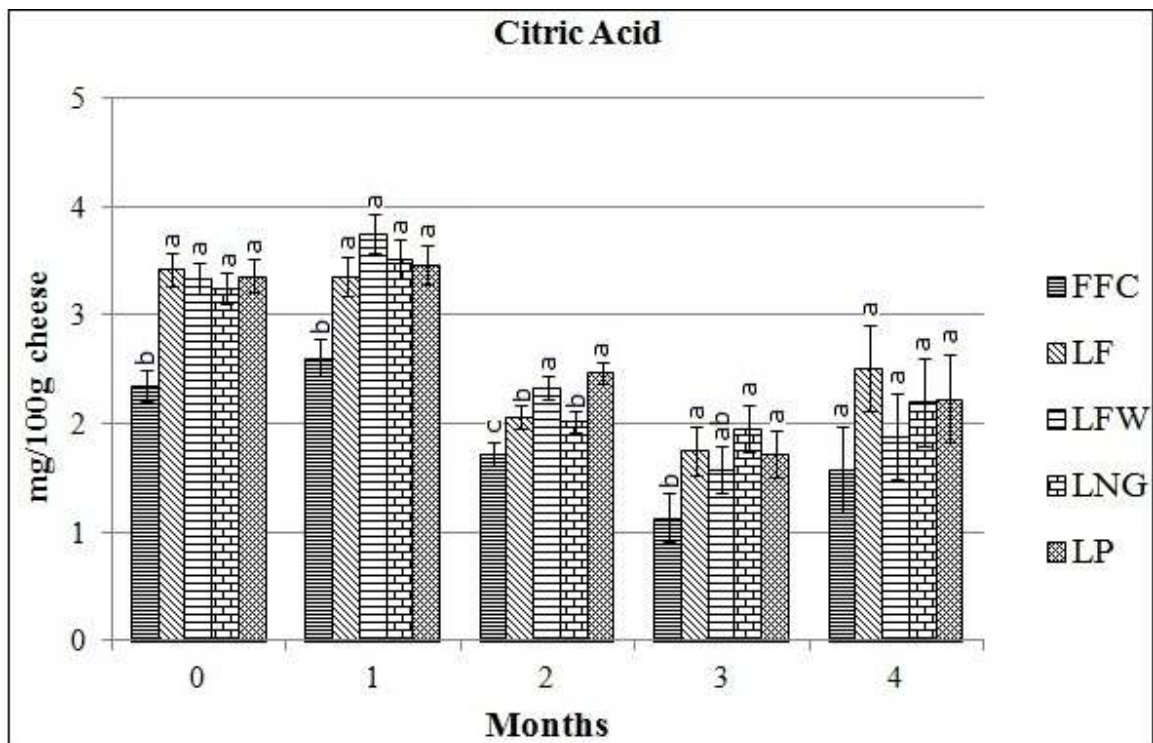


Figure 21. Citric acid content of Cheddar cheeses made of full fat control (FFC); Low fat control (LFC); Low fat cheese with pectin addition (LP); low fat cheese with Novagel addition (LNG) and Low fat cheese with whey protein concentrate (LFW) over four months of aging. Time 0 months indicate one week after cheese make. Means within the same month with a different letter differ significantly ( $p < 0.05$ ).

### **3.3.6 Microstructure of cheese by confocal laser scanning microscopy**

Confocal Laser Scanning Microscope (CLSM) is widely used in studying the distribution pattern of fat and protein in dairy products. Carbohydrate based fat replacers are used in the manufacture of low fat cheeses to overcome the quality defects (Rodriguez, 1998). The objective of performing confocal laser microscopy is to locate any hydrocolloids present in the cheese and to notice any differences in the fat globule size and its distribution. As explained in the cheese making section 3.2.3, the hydrocolloids were homogenized with cream portion of cheese milk. Homogenization increases the surface area of the fat globules, and an increased amount of denatured casein and whey proteins associated with the surface of the fat, which increases the amount of water they bind (Malin et al., 1995). Smaller fat globules have greater surface area and hence quantity of milk fat globule membrane per unit mass of fat is greater than large fat globules which impact the flavor differences in cheese (O'Mahony et al., 2005).

CLSM has several advantages over other conventional methods as it has the ability to image the sample optically in “Z” section to avoid any damage on the surface of the sample. It also facilitates easy identification of different components by fluorescent labeling and a better resolution (Everett and Auty, 2008).

Emmons et al., (1980) investigated the microstructure of cheese made from whole un-homogenized milk and homogenized low fat milk. They revealed that full fat cheese had larger and irregular fat globules clustered together in a sponge like structure where as low fat cheeses had small, circular and more compact structure due to dominated protein matrix.

Full fat cheese and low fat cheese, with or without inclusion of hydrocolloids, were studied by CLSM. Cheeses were imaged in triplicate and exhibited similar structures; hence only a representative sample of each treatment is shown in Figure 22.

The microstructure of full fat cheese (Figure 22.A) clearly showed larger fat globules, differences in globule size, and more overall fat content. The fat particles are close to each other with a few clumping. This is in agreement with Rogers et al., (2010) who examined the CLSM images of cheese with varying fat contents (33% to 3%). They observed that fat globules adhered to each other and had irregular shapes in higher fat (33% and 28%) cheeses where as lower fat cheeses had more spherical shape and dispersed throughout the protein matrix.

In low fat cheeses, the fat is uniformly distributed with no evidence of aggregation. Low fat control cheese (LF), without any addition of hydrocolloid but homogenized, shows fat globule much smaller in size than full fat. The particles are evenly distributed in the protein network. As seen in Figure 22.B, reduction in fat resulted in denser regions of protein with small darker holes in the matrix. Metzger and Mistry (1995) examined the microstructure of cheese with scanning electron microscope and determined the fat globule size and distribution by analyzing the milk, whey, and cream individually under light microscope. They summarized that homogenization of the cream portion used to manufacture reduced fat Cheddar cheese resulted in small fat globules that were evenly dispersed in the protein matrix.

Distribution of fat globules within the protein matrix in the low fat cheeses containing hydrocolloids showed differences in other microstructural characteristics.

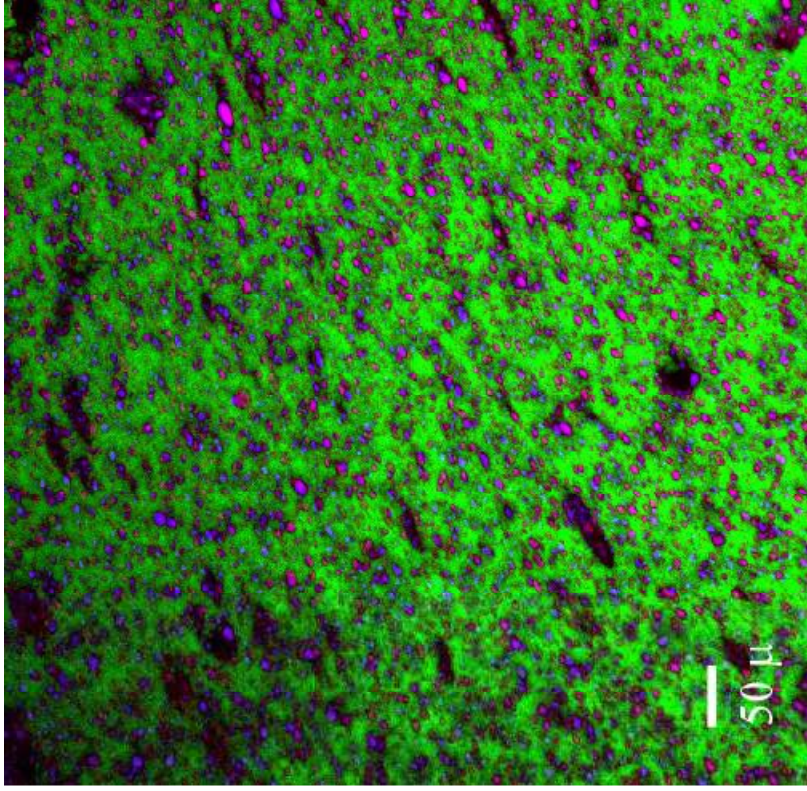
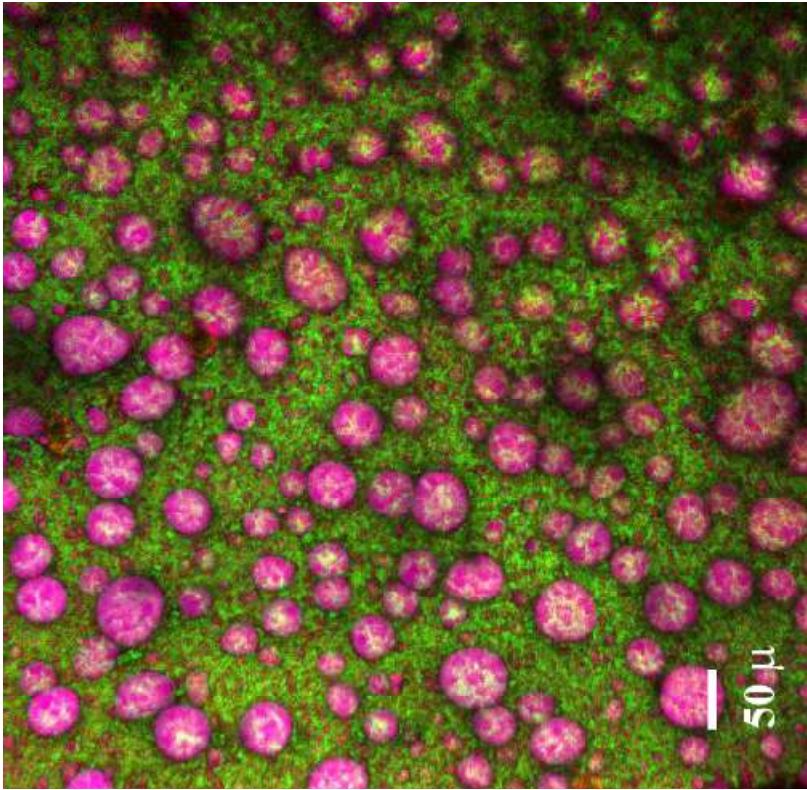
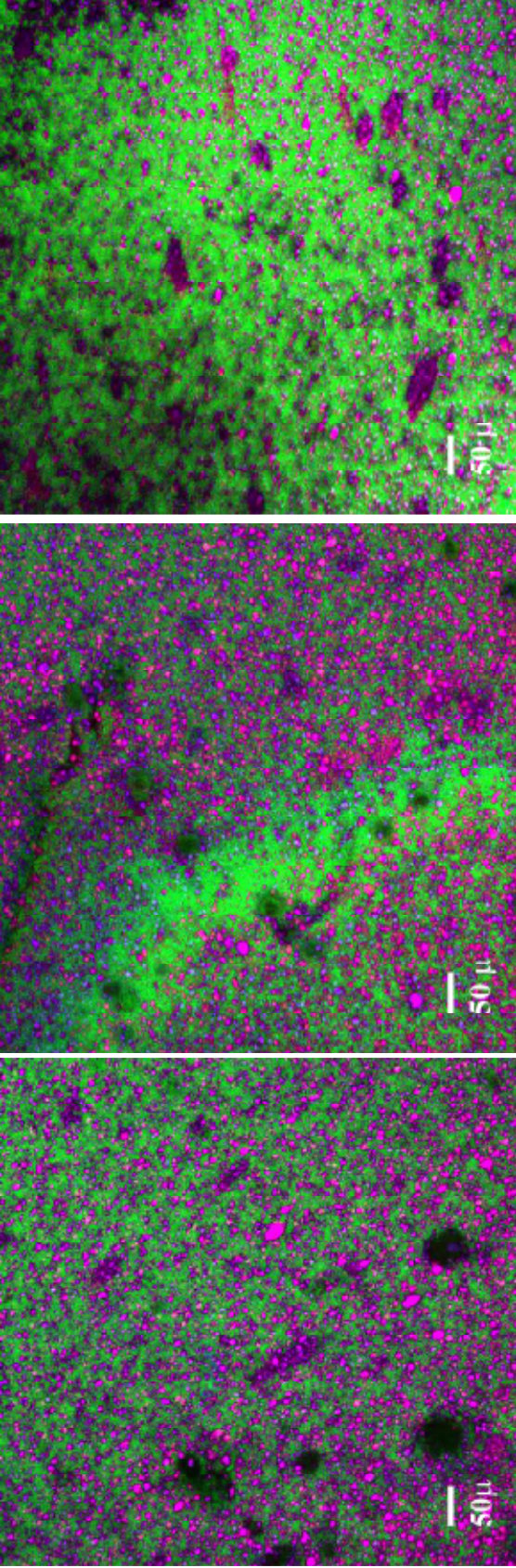


Figure 22. Confocal micrographs of treated cheese: A= Full fat cheese (33% fat) with no homogenization of fat; B = low fat control cheese with no hydrocolloids but homogenized cream portion. Scale bars are 50  $\mu$ ; Magnification 20X; Resolution 0.804pixels per micron.



**C**

**D**

**E**

Figure 23. Confocal micrographs of low fat treatment cheeses homogenized with cream and hydrocolloids together; C= low fat containing Novagel; D = low fat control with whey protein concentrate. Scale bars are 50  $\mu$ ;

Low fat cheese containing Novagel and Pectin were shown in Figure 23.C and 23.D respectively. Numerous fat and carbohydrates particles can be observed in both LNG and LP cheeses as compared to LF and LFW, predominantly higher in cheese containing pectin. Larger dark holes, as seen in LNG, may indicate gas holes or mechanical openings. The LP cheese (Figure 23.D) had fat particles coalesced with the carbohydrate. Lobato Calleros et al. (2001) observed that low fat cheese made with pectin had irregular fat globules and observed calcium pectate particles in the microstructure of low fat cheese made with low methoxyl pectin. They attributed this to the interaction of calcium and pectin which they added as a fat replacer in their study.

Konuklar et al., (2004) studied the effect of Nutrim; a  $\beta$ -glucan based fat replacer, in the microstructure of low fat Cheddar cheese through scanning electron microscope. They observed a homogenous size distribution of fat globules with a fracture in continuous protein matrix. They concluded that disruption of continuous protein matrix represented a spongy character similar to full fat cheese.

Aryana and Haque (2002) investigated the effect of fat replacers on the microstructure of low fat Cheddar cheese via scanning electron microscope. They found low fat cheese made with Novagel (a hydrocolloid, also used in this study) and Simplese (protein based fat replacer) exhibited the presence of larger structure within the protein matrix thus breaking the continuity of the matrix. Several authors have suggested that breaking the continuity of protein matrix imparts softness in the texture of low fat cheese (Banks et al., 1989; Johnson and Chen, 1995; Lobato Calleros et al., 2001; Konuklar et al., 2004).

LFW had a compact and continuous protein network (Figure 23.E) similar to LF. The dense protein matrix in low and reduced fat cheeses is responsible for firm and rubbery texture. The panelists in the sensory study have confirmed similar results. There were no significant differences found for hand firmness, hand springiness, and hand cohesiveness between low fat cheese made with whey protein concentrate and low fat control.

### 3.4 Conclusion

When making full fat and low fat cheeses with and without fat replacers, we observed several differences in sensory and chemical flavor attributes. In lab scale batches, Novagel samples were judged by panelists as most similar to the full fat control sample, and pectin had the most desirable texture when measured by TPA. However, these positive results were not detected when the same formulas were produced in pilot scale trials. There are several reasons to explain this inconsistency Hydrocolloid may have been better retained in the lab scale trials in comparison with pilot scale. Lab scale batches were stirred by hand, not mechanically as in the pilot scale batches. There could have been greater losses of hydrocolloid to the whey, but this difference was not quantified. The addition of different proportions of each hydrocolloid, and quantification of the hydrocolloid retained in the cheese would be recommended for further studies.

The pH of low fat and full fat cheeses in both the trials ranged from 4.9 to 5.05 which is considered low for a regular Cheddar cheese. Low pH in full fat and low fat cheese may also contribute to low sensory scores. None of the treatments showed differences in age related proteolysis compared to LF and FFC. Microstructural examination through confocal microscopy indicated that pectin and Novagel were most likely retained in LP and LNG cheeses respectively by homogenizing with the cream portion of the cheese milk, and there were interesting differences in the appearance of the fat in the network. These differences did not translate into differences in the sensory texture results, however. There were differences found in the organic acids in different treatments, and these differences have been shown to be responsible for differences in pH and sensory quality of cheeses in other studies (Drake et al., 2010). However, in this

study, these differences did not equate to significant differences between the cheeses when evaluated by the trained sensory panel. The textural parameter, resilience was significantly higher for LFW and directionally higher for LP than other control and treatments. Materials with a low modulus of elasticity and high stress represent good resilience. A lower the resilience would have represented the full fat texture. Several textural parameters like springiness, resilience and gumminess decreased during aging but the texture did not approach the desired full fat texture.

## CHAPTER 4

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## Appendixes

### Appendix A. Steps for making low fat Cheddar cheese

#### **DAY 1. Milk Receiving and Processing**

1. Milk is received, skimmed and pasteurized on first day of the week.

Pasteurization of skim milk is done through HTST pasteurizer.



2. Cream can be pasteurized in can at 90° C / 194° F for 15 seconds and cooled to 40° F immediately.



3. Analyze the fat content of skim milk and cream for standardization.



4. Plug in the values in excel sheet for amounts to be weighed to hit the fat target.

#### **DAY 2. Filler gel preparation / Cheese making**

Hydrocolloid mixing has to be done first thing in the morning (6:00am) prior to cheese making (starts at 8:00am).

5. Calculate quantity of hydrocolloid, WPC and water to be mixed.

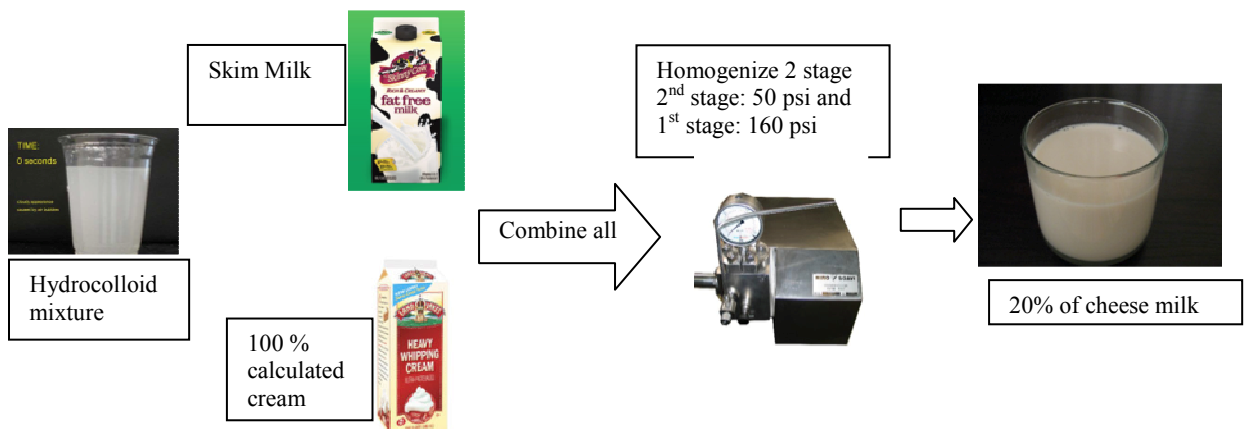
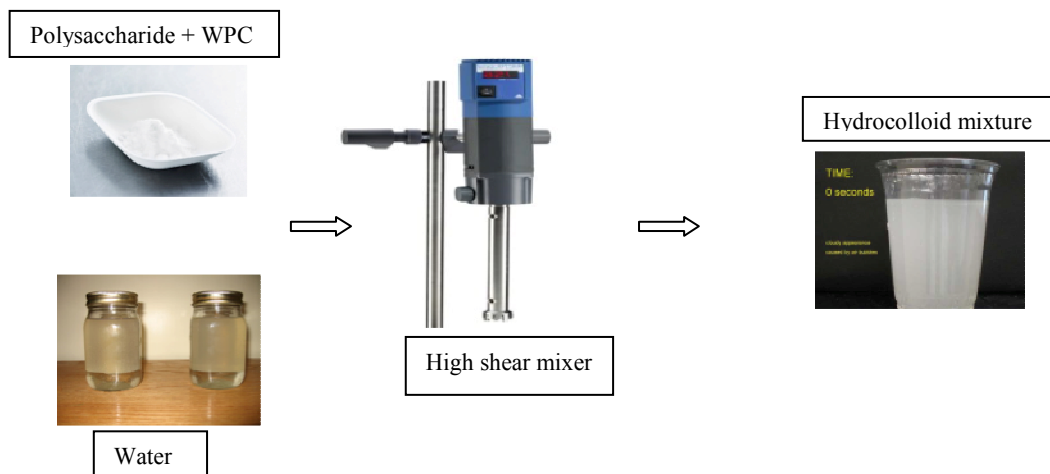


- Dissolve chosen hydrocolloid and WPC in water slowly while mixing with a high shear blender in a bucket. Make sure it dissolves completely and homogenous.

Slow down the rate of addition of hydrocolloid, if it gets lumpy.

(Take cold or warm water depending on type of hydrocolloid)

*CHALLENGE – Mixing of hydrocolloid (~14.62 lbs for 1200 lbs of milk) Solution – Use of another lab scale impulse mixer with high shear mixer can be used. A wide mouth plastic bucket will give extra space to use it with the high shear mixer.*



7. Blend calculated cream, part of skim milk and (10% of cheese milk) hydrocolloid mixture in homogenizer (Cold homogenization). The quantity calculated must be 20% more than the requirement to incorporate losses during homogenization.

*CHALLENGE – Mixing of filler gel ~ 283lbs. (skim milk, cream, hydrocolloid mix before homogenization). During phase I, presence of gel particles was observed if it was not mixed properly with high shear mixer before homogenization. Mixing prior to homo and in single container will provide homogenous mixing.*

Homo pressure - 2<sup>nd</sup> stage: 50 psi and 1<sup>st</sup> stage: 160 psi



8. Transfer rest of the pasteurized skim milk from tank to the cheese vat. Mix filler gel in cheese vat and keep the agitator running for homogenous mixing.



9. Analyze the fat (Babcock method) for desired value according to file during heating period.



10. **Standardization:** Adjust the fat content if necessary either by skim milk or cream. Check and record the pH in cheese making form.



11. **Preacidification:** Pre-acidify the content with diluted USP/FCC grade only lactic acid (1:10) to drop the pH to 6.2. Stir the cheese milk continuously during acid addition. (Check and record pH and TA)



12. Heat the contents to 88 ° F



13. **Starter Culture addition.** Add starter culture when milk reaches 88 F while the agitators are running. Keep stirring at slow speed for 15 min. (Check and record pH and TA)

*Check pH: Time dependent, agitate slowly for **15 minutes**). This is time dependent, not the drop in pH dependent which is done differently in normal FF cheese make procedure.*



14. **Color addition:** Add annatto color, 5 min after the addition of culture during stirring.



15. **Rennet addition** (2x chymosin: 0.044 ml/lb): Dilute the rennet in 100 ml of water in a plastic graduated cylinder. (**Do not sanitize the cylinder: It will inactivate the enzyme**) (Check and record pH and TA)



16. Stir the content for 1 min at slow speed. Stop the agitator and cover the vat.



17. **Coagulation** (Observe time: ~15-25 min): Check for firmness of the coagulum. Sanitize the cheese knife and have it ready 20 min ahead of cutting time.



18. **Cutting of curd:** (Check and record pH and TA). Cut the curd using horizontal knife first to form sheets, and then use the vertical knife lengthwise in the vat to

form strips. With the vertical wire knife cut the curd across the width of the vat to  
form cubes



19. **Healing:** Leave the curd for 5 minutes for healing. Gently scrape curd particles stuck to the sides the sides of the vat. Begin gentle agitation for 5 min.



20. **Cooking:** Begin heating while continuing the gentle agitation to bring temperature of the curd and whey up from 88 to 96°F in 30 min. (Check and record pH and TA). Make sure there rate of acidification is slow to avoid setting the outside of the curd particle. Moisture will not leave as well if the curd is heated too quickly.



21. Hold at 96 F for cook-out and check pH and TA for next step of draining. (Draining pH 5.9). Stir the contents constantly to prevent matting, but not aggressively. Check pH and TA every 20 min.



22. **Draining:** At the end of the stir, stop agitation and allow curd to settle down to the bottom of the vat for one minute. Place a strainer and bucket over valve of vat with a screen in the vat and start drawing whey.



23. End draining: Drain all the whey within 10-15 min while agitating very gently. PH of the whey at this point should be around 5.8- 5.85.



24. **Dry Stirring:** Dry stir the curd gently to reach pH 5.7. Pull all the curd from the corners into the center of the vat. Check and record pH during stirring.



25. **Salting** (0.2% of the weight of starting cheese milk). Divide the total quantity of salt to be added into three equal portions. Add each one of them at an interval of 5 mins.



26. Spray the first lot of salt while stirring the curd. Pull all the curd from the corners into the center of the vat for homogenous salting.



27. Wait for 5 min and repeat the above step for all three lots of salt to be added.



28. **Hooping:** Stir the salted curd for 10 min and start hooping into 20 lbs. sanitized hoops. Sanitize hoops and cheese cloth (dip in sanitizer bath for 5 mins) ahead of time and (check and record pH and TA)



29. Pressing of curd: Pressure 20 - 40 PSI for 8 hrs



**DAY 3. Vacuum packaging**

30. Vacuum package 20 lb. cheese blocks and store at 40 F. Make sure that the seal is good by not getting cheese or grease in the sealing area.

**Later - Vacuum packaging**

31. Cut the blocks into 1 lb. blocks after cheese has aged enough to allow cutting and salt has equilibrated (1 week). Label the blocks and store at 40 F.

## **Appendix B: Ashing of Cheese**

A. Preparation of Aqua regia solution: Mix three parts of HCL with one part of HNO<sub>3</sub> and dilute 1:1 with LG water. Example: For making 1 liter of aqua regia solution, we need 375 ml of HCL with 125 ml of HNO<sub>3</sub> mixed with 500 ml of LG water.

B. Preparation of Crucibles:

- Heat crucibles over low flame for few minutes and apply marking ink while crucible is warm. Let it dry.
- Submerge crucibles in aqua regia solution very carefully and soak it for several hours. (Overnight soaking is desirable)



Eye protectors and proper gloves are necessary to wear while handling aqua regia solution.

- Remove crucibles from aqua regia solution, rinse RO water
- Dry crucibles in atmospheric oven at 100° C for approximately 1 hr or until they are dry
- When dry , ignite crucible in muffle furnace to dull redness (can leave overnight)
- Cool to room temperature and place in desiccators until ready to use
- Crucibles are prepared in same way every time as it is described above after each use

C. Procedure:

- Weigh and on the analytical balance record exactly an amount of grated cheese (3 - 5 grams) in a prepared, pre-weighed crucible (record weight).

- Dry the samples in an atmospheric oven at 100° C for 1 hr.
- Transfer the crucibles to the hood and hold over a Bunsen burner, and carbonize slowly.
- Remove from the burner if fat ignites.
- Ignite cautiously to avoid spattering and remove crucible while fat is burning.
- Caution should be taken during carbonization so that none of its part becomes white.
- When flame ceases, place the samples in the muffle furnace.
- Draw a picture of the orientation of the crucibles in the furnace in case the labels disappear
- Incinerate sample at 550° C (overnight) until free from carbon and a light grey or white ash remains.
- Turn off the furnace and allow the samples to cool. Do not open the furnace door; otherwise it may break the fire protection of furnace.
- Check for drop in temperature with infra red thermometer after approx. 8 hrs.
- Remove the samples from the furnace (temperature approximately 100° C) and immediately transfer to the desiccators in the orientation they were in the furnace
- Weigh the sample and record the readings.
- Calculate %Ash =  $\text{Weight of residue} * 100 / \text{weight of sample}$ .

**Appendix C: Procedure for preparation of water-soluble nitrogen extract of cheese**

1. Grate cheese sample.
2. Weigh 40 g of sample in Osterizer grinder; add twice sample weight of water.
3. Grind the sample in Osterizer mixture at ~ 20 °C for 2 min at high speed. The settings will be at high and grind.
4. Weigh (approx. 40 grams) the mixture in 50 ml falcon tubes.
5. Warm to 40 °C and hold for 1 h.
6. Centrifuge at 3,000 x g for 30 min.
7. Filter supernatant through what man filter paper no. 1 and weigh the extract again.
8. Record the weight of extract.
9. Check the pH of the extract and record it.
10. This procedure will extract ~ 70 % of the water soluble nitrogen; if more complete extraction is required, steps 2-6 may be repeated.
11. Analyze for % Nitrogen in Leco Nitrogen Analyzer.