USE OF EXTRUSION TECHNOLOGY AND FAT REPLACERS TO PRODUCE HIGH PROTEIN, LOW FAT CHEESE

by

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ABSTRACT

Use of Extrusion Technology and Fat Replacers to Produce High Protein, Low Fat Cheese

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This study investigated the use of extrusion technology and fat replacers to produce high protein, low fat Cheddar cheese. In chapter 3, four different fat replacers were tested at the highest concentration level of each, as recommended by the manufacturers for low fat cheese, to investigate the change in cheese texture and optimize extruder conditions. In addition, the press time/pressure combinations of the extruded cheeses were optimized. The fat replacers and extruder conditions that were effective in improving the texture of low fat cheese were then used in chapter 4.

In chapter 4, three fat replacers were used at three different concentrations (lowest, middle and highest) as recommended by the manufacturers for replacing fat in cheese. The fat replacers were microcrystalline cellulose (MCC 1) (0.125%, 1.06% and 2%), whey protein concentrate (WPC 2) (0.50%, 0.75% and 1%) and whey protein concentrate (WPC 1) (0.40%, 2.20% and 4%). These fat replacers were effective in improving the texture of low fat cheese as determined from the results of chapter 3. The extruded cheese samples with and without fat replacers were analyzed for texture at three different time periods (1 day, 1 week, and 1 month). None of the fat replacers used were effective in improving the texture of low fat cheese significantly.

Since none of the treatments statistically improved the texture of low fat cheese, in the next part of the study, extrusion alone and WPC 1 at the middle concentration were then used to produce low fat cheese with high protein content by blending low moisture aged Cheddar cheese and nonfat cheese. Extrusion of cheese blends with or without fat replacer yielded cheese with high protein level. It was concluded from the study that the fat replacers we used were not effective in improving the texture but extrusion of aged Cheddar cheese with nonfat cheese can yield high protein cheese.

(73 pages)

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LIST OF ABBREVIATIONS AND DEFINITIONS

Abbreviations

MCC1 - Micro crystalline cellulose (commercial name Nova Gel RCN 15)

MCC2 - Micro crystalline cellulose (commercial name Vitacel MCG)

WPC1 - Whey protein concentrate (commercial name Temp Pro)

WPC 2 - Whey protein concentrate (commercial name Simplesse 500)

Extr – Extruded

Lowest or Low - Lowest concentration of fat replacer recommended by manufacturers

Middle or Mid. - Middle concentration of fat replacer

Highest or High - Highest concentration of fat replacer recommended by manufacturers

AgB185 - Aggiano cheese: nonfat = 15:85 without fat replacer

AgB285 - Aggiano cheese: nonfat = 15:85 with fat replacer WPC 1 Mid.

AgB190- Aggiano cheese: nonfat = 10:90 without fat replacer

AgB290 - Aggiano cheese: nonfat = 10:90 with fat replacer WPC 1 Mid.

High Protein Cheese – Cheese containing 10g or more protein per 28g of serving size.

Definitions

MCC 1 Low, Mid and High concentrations are 2.0%, 1.06% and 0.125%. The low and high concentration was recommended by the manufacturers and middle concentration was calculated.

WPC 1 Low, Mid and High concentrations are 4.0%, 2.20% and 0.40%. The low and high concentration was recommended by the manufacturers and middle concentration was calculated.

WPC 2 Low, Mid and High concentrations are 1.0%, 0.75% and 0.50%. The low and high concentration was recommended by the manufacturers and middle concentration was calculated.

CHAPTER 1

GENERAL INTRODUCTION

With an increasing rate of obesity and consumer awareness about health, the efforts to make healthy foods are increasing daily. Reduction of calories or fat from the food products has been a concern for a long time for food developers. Fortification or addition of supplements to improve nutritional value of food products is also an acceptable way to attract customers. Cheese is one such food product where making it a low calorie, healthy food option has always been a challenge. The main factor in determining cheese quality has undoubtedly been its texture and consumers have found low fat cheese to be harder and unacceptable. A recent study has pointed out that consumers are not ready to trade texture for lower calories or lower fat percentage (Drake, 2009).

Considerable research has been performed to improve the texture of low fat cheese (Gwartney, Foegeding & Larik, 2002; Lawrence, Creamer & Gilles, 1987) and various fat replacers have been widely used in such studies (Kavas, Oysun, Kink & Uysal, 2004; McMahon, Payne, Fife & Oberg 1996). Application of starch while making cheese is also being studied widely by scientists, to give a satisfactory and healthy cheese. Different types of fat replacers are available, both carbohydrate based and protein based.

Apart from texture improvement, flavor is another aspect scientists have to be concerned about as full fat cheese is creamier and has richer flavor due to fat globules. Using fat replacers may mimic the texture to some extent but flavor will again be a challenge. This research serves as a step towards understanding how fat replacers can help improve the texture, and if the use of extrusion technology can help disrupt the protein matrix to positively affect cheese texture.

CHAPTER 2

LITERATURE REVIEW

Standard of identity, according to the United States Food and Drug Administration, to label any food as low fat states that it must contain no more than 3 grams fat per serving size (28 grams of cheese) (21 CFR, Part 101.62). For cheese to be labeled as low fat, it must have 6% or less fat per serving size. To label a cheese as high protein, it must contain 10 grams or more protein per serving size. There have been several studies regarding low fat cheese texture and methods to improve it (Banks, 2005; Drake & Swanson, 1995; Mistry, 2001).

2.1 Defects of Low fat Cheese Texture

Texture is one of the main factors in determining the quality of cheese (Gwartney, Foegeding & Larik, 2002). Fat plays an important role in flavor, texture and appearance of cheese. Removal or reduction of fat causes rubberiness, hardness, dryness, fracturablity, springiness and crumbliness in cheese (Adhikari, Heyman & Huff, 2003; Banks 2005). Texture defects may arise due to a change in the protein matrix with the removal of fat (Bryant, Ustunol & Steffe, 1995). The role of fat in the texture of full fat cheese is still under study, but it is hypothesized that it gives creamier and mouth-coating feel, and also imparts discontinuity to the protein matrix. Weak spots in the discontinuous protein matrix are created which improves the chewability of the cheese (Johnson, Kapoor, McMahon, McCoy & Narasimmon, 2009). The texture of the cheese is the result of intricate interactions of components: fat, moisture, protein, calcium and pH. The texture of low fat cheese is generally unacceptable because the reduction of fat in addition to necessary changes during cheese manufacture alters the ratio of these components. Fat forms cavities in the cheese and hence give it an open structure. In full fat or reduced fat cheese the protein matrix is more open and the spaces are occupied by fat globules. In low fat cheese the protein matrix is more compact (Aryana & Haque, 2001; McMahon, Payne, Fife & Oberg 1996; Rahimi, Khosrowshahi, Madadlou & Azaznia, 2007). The bitter flavor of nonfat or low fat cheese may be due to lack of butter fat.

2.2 Texture Modifications

Several methods to date have been used to modify and improve the texture of low fat cheese. Alteration of cheese milk processing conditions, modification in cheese making procedures (Banks, Brechany & Christie, 1989; Dabour, Kheadr, Benhamou, Fliss & LaPointe, 2006; Mistry, 2001) and the use of fat replacers has been explored. An increase in the moisture content has been suggested to improve the properties of low fat cheese (Rodriguez, 1998). Others have suggested that it is necessary to maintain the same moisture ratio in nonfat cheese as found in full fat cheese (Mistry, 2001). One of the approaches to maintain the same moisture ratio as found in full fat cheese is the use of fat replacers.

2.3 Alternate Milk Treatments

Studies have been carried out regarding usage of ultra-filtered milk for manufacturing low fat cheese (De Boer & Nooy, 1980; Drake & Swanson, 1995; McGregor & White, 1990). Low fat Cheddar and Mozzarella cheeses made from ultra-filtered milk were observed to have increased moisture content, but there was no texture improvement according to Drake and Swanson (1995). They also observed that homogenization of milk before cheese making can improve texture of low fat cheese when compared to low fat control cheese using Texture Profile Analysis (TPA). In other studies, homogenization of cream used in low fat cheese improved the texture, flavor and appearance, due to decreased size of fat globules, which were distributed evenly throughout the protein matrix (Metzger & Mistry, 1994, 1995). The temperature of pasteurization of milk used for cheese making also improved cheese texture when measured instrumentally, but sensory scoring and overall acceptability were less affected (Guinee, Auty & Fenelon, 2000).

Improvement in texture of low fat cheese and reduced fat Kashar cheese was seen when milk used for cheese making was pre-acidified (Fife, McMahon & Oberg, 1996; Merrill, Oberg & McMahon, 1994; Metzger, Barbano & Kindstedt, 2001). Calcium content of the cheese was seen to be increased when the fat content was decreased. It was observed that low fat Mozzarella had 50% more calcium than low moisture part skim mozzarella (Metzger, Barbano, Kindstedt & Guo 2001). However, acidifying the milk prior to cheese making reduced the final calcium content of low fat cheese. Calcium is important in protein cross linking and reducing calcium content results in a softer, less chewy cheese. Pre-acidification and type of acid used reduced the yield efficiency by

2.2% - 5.5%, as casein and fat loss in whey increased (Metzger, Barbano, Rudan & Kindstedt, 2001).

2.4 Alterations in Procedures for Making Cheese

To mitigate some of the texture related defects of low fat cheese, several alterations in make procedures have been studied. Reducing cooking temperature and time, shorter stirring time, washing curd, and larger cut size are few examples of procedure alterations. These alterations principally increase milk nonfat solids (MNFS) (Banks, Brechany & Christie, 1989; Mistry, 2001). Lower cooking temperatures, higher pH and salting while making low fat Cheddar cheese allowed an increase in the moisture content and final cheese pH. Lower cooking temperature slowed down the rate of expulsion of whey from the cheese curd, while higher salting pH reduced the time needed for whey drainage (Dabour, Kheadr, Benhamou, Fliss, & LaPointe, 2006).

Fracturability of the cheese determined by TPA reduces when the moisture content of the cheese increases. Cohesiveness, on the other hand, decreases as the moisture content of the cheese is increased. Moisture content of the low fat or nonfat product should be slightly higher than its full fat counterpart to achieve similar texture (Emmons, Kalab, Larmond & Lowrie, 1980). Increasing the pH of curd milling affects the firmness and composition of low fat or reduced fat cheeses (Guinee, Auty & Fenelon, 2000). Although texture of low fat cheese can be improved by altering the make procedures, these improvements are not markedly different to generate satisfactory and acceptable texture.

2.5 Use of Fat Replacers

Another approach to improve the texture of low fat cheese is by using fat replacers. Fat replacers are classified either as mimetics or substitutes. A fat-based product with properties similar to natural fat with reduced calories is called a fat substitute, while carbohydrate or protein based products which mimic the properties of fat are called mimetics or fat replacers (Ma, Drake, Barbosa-Canovas & Swanson, 1997; Rodriguez, 1998). Fat replacers have been added to improve the texture and appearance of low fat and nonfat cheeses (Rahimi, Khosrowshahi, Madadlou & Azaznia, 2007). Microparticulated protein based and microparticulated carbohydrate based fat replacers are the two categories that have been recommended for use in cheese products (Romeih, Michaelidou, Biliaderis & Zerfiridis, 2002). By trapping moisture, the fat replacers, provide creamy and lubricated feel to the cheese but these cannot positively impact the flavor defects in cheese. There are many publications which discuss the use of fat replacers in cheese but only one reference estimated the amount of retained fat replacers when added to the milk prior to cheese making. They estimated 70% retention of the used amount of fat replacers (McMahon, Payne, Fife & Oberg 1996). The microstructure of low fat cheese with different fat replacers and full fat cheese were compared. Studies showed that low fat cheese with protein based fat replacers was less hard in texture than low fat cheese without fat replacers. The protein matrix seemed to be disrupted by fat replacer particles and hence there was a discontinuity in the matrix (McMahon, Payne, Fife & Oberg 1996). Other studies that have investigated the use of fat replacers in cheeses are given in Table 2.1.

2.6 Extrusion Technology

Extrusion technology involves the formation of an extrudate through continuous mixing, kneading and expulsion of moistened starchy and/or proteinaceous materials using a die (Burtea, 2001; Harper, 1981). Enough pressure is applied in an extruder to force a material through a die (Rauwendaal, 1998). Based on the desired type of extrudate, extruders can be classified into different kinds, but single and twin-screw cooking extruders are the most commonly used extruders in the food industry.

Over seven decades, extrusion technology has commonly been used in developing food products in the food industry. The first commercial application of single screw extruders to be commercialized was with the production of pasta from semolina in Italy during the mid-1930s (Huber, 2000; Rokey, 2000). Thereafter many applications of an extruder were used on a commercial scale: for example, expanded corn snack (Huber, 2000; Rokey, 2000), expanded pet food, meat extenders and meat analogs from textured vegetable protein. The high temperature short time heat treatment allows complete starch gelatinization (Huber, 2000), which make puffed characteristics possible. As the extrudate leaves the die, pressure is released, moisture is flashed off, and an exothermic post-die expansion transpires. Extruders help some process steps and increases efficiency and therefore reduce production cost and lead to commercial development of cooked extruded products (Riaz, 2000). The application of continuous extrusion technology is versatile, with high throughput, minimal cost and improved energy efficiency, which are all attractive traits to manufacturers. Research for the development of healthy functional snack foods using extrusion technology continues to be a major focus of academic and industry interests. There is a wide scope of extrusion technology application. Transformation of grains and high protein material into various snack foods is an example of thermoplastic extrusion technology (Camire & King, 1991; Huber, 2001). During extrusion, food is exposed to heat and shear stress allowing new starch and protein interactions. The molecules realign and interact to form matrices as a result of starch gelatinization and protein denaturation (Harper, 1981).

An extruder is comprised of an Archimedean flighted helical screw which rotate within a fixed metal barrel. Dry materials are added to the barrel via a feed hopper. The hopper maintains uniformity and continuity of the material added to the extruder, thus resulting in a homogenous product and preventing surging. An extruder can be configured for low, medium or high shear by the sequence of the screws and paddles in the barrel. The screws promote conveyance, heating, melting and mixing the material throughout the barrel (Rauwendaal, 1998). Screws ensure extrusion to be a continuous process and the design of screws is important, as the paddles on the screw control the flow and create shear and back pressure. Apart from screw and paddle sequence, the speed of co-rotating screws and the temperature during the extrusion significantly influence the texture of the final product. The rate of speed will influence the amount of input shear, residence time in the barrel and denaturation of protein.

Commonly used extruders make use of single or twin screws for the extrusion process. Twin screw extruders offer an advantage over single screw extruders as they have different degrees of screw meshing and direction of rotation. Also different varieties of materials can be processed using twin screw extruders. Low moisture materials can be extruded, eliminating the need for a preconditioning stage. The heating thermocouples are lined in the extruder barrel which can be monitored and controlled externally. Heat will result in protein denaturation, making room for new protein- protein interactions. As the mixing and heating increases throughout the barrel, it generates pressure at the die-end. When adequate pressure is generated to overcome the resistance of the die, the material is discharged. Sudden decrease of pressure and water vaporization from the extrudate results in an expanded or puffed product (Rauwendaal, 1998). The reproducibility of the product greatly depends on the ability to control extrusion parameters (Huber, 1991). The independent variables that control the quality attributes of the product are material feed rate, liquid feed rate, screw speed, screw and paddle configuration, die shape, and barrel temperature (Huber, 1991). Dependent variables include temperature within the barrel and exit temperature, residence time, barrel pressure and specific mechanical energy. To measure the final quality of the extrudate, final moisture content, extrudate expansion, texture, color and flavor, can be used as parameters (Huber, 1991).

An extruder can be configured for low, medium or high shear by the sequence of the screws and paddles in the barrel. The screws promote conveyance and the paddles interrupt the flow and create shear and back pressure. Twin screws are more functional and can be used at higher moisture level (>40%) than single screws which is limited to a low moisture level (<35%) (Walsh & Carpenter, 2008). Extruders at different shears are used to produce different food products. Apart from screw and paddle sequence, the speed of co-rotating screws and the temperature during the extrusion significantly influence the texture of the final product. High temperature will result in protein denaturation, making room for new protein- protein interactions. The rate of speed will influence the amount of input shear, residence time in the barrel and denaturation of protein.

2.7 Texture Analysis of Cheese

Texture is the first noticeable attribute that is influenced by fat reduction in cheese. Some of the values that are important for cheese texture like hardness, adhesiveness, cohesiveness and fracturability, can be calculated using Texture Profile Analysis (TPA). Due to fat reduction, hardness and springiness increases while adhesiveness and cohesiveness decreases. Using two bite mechanical compressions, the force-compression value is determined instrumentally, which simulates the first two bites taken during chewing (Bourne, 1978; Bourne & Comstock, 1981). Figure 2.1 shows the graph, simulating the first two bites. TPA uses various compressions such as 20% or 70% of the original height of the cheese sample. Compression levels can often vary between studies and give different results depending on how far the sample is compressed and the strain needed to cause fracture. There are various standard TPA terms which are defined in Table 2.2 (Bourne, 1978).

2.8 High Protein Foods

Today consumers are also looking for food products which can supply additional nutrients in their daily diet. This has increased demand in producing food products rich in protein or high protein foods. Cheese is one such product which is consumed almost daily in an American diet and increasing the protein content may increase consumer consumption. Cheese is considered high protein if it contains more than 10 g of protein per serving size of 28 g. High protein foods are associated with weight loss, increased satiety (Baba, Sawaya, Torbay, Habbal, Azar & Hashim, 1999; Brehm, Seeley, Daniels & D'Alessio, 2003) and improve cardiovascular risk factors (Kelemen, Kushi, Jacobs & Cerhan, 2005).

Today nearly 66% of the Americans are overweight and 33% are clinically obese with body mass index ≥ 30 kg/m^2 (Flegal, Carroll, Ogden & Johnson, 2002). In a study conducted by Hill & Blundell in 1986, it was observed that after consuming a high protein meal (31% of the total energy) the subjects expressed stronger feeling of fullness than the subjects who consumed a high carbohydrate meal (52% of the total energy). In a systematic review (Halton & Hu, 2004) of randomized studies on the effect of high protein diet on thermogenesis, satiety, body weight and weight loss, there was convincing evidence that higher protein diet increases satiety than lower protein diets. Also a higher protein diet helps in weight reduction and leads to a reduced subsequent energy intake. With increasing health concerns, we investigated the production of high protein low fat cheese in this study.

2.9 Hypothesis and Objectives

The hypothesis of this study is that extrusion modification of low fat (high protein) Cheddar cheese alone or in combination with fat replacers can enhance the texture of the low fat cheese by decreasing the hardness and springiness, and increasing the cohesiveness. The first objective to test the hypothesis was to optimize the physiochemical (formulation, temperature and pressure) and configuration parameters (screw and paddle sequences) of the extruder to allow extrusion-modification of low fat cheese (6% fat) with or without fat replacers (choosing from WPC 1, MCC 1, WPC 2,) and continue until product characteristics were improved.

Secondly, low fat cheese (2% fat) was extruded with fat replacers, at three concentrations and the extruded cheeses were analyzed for texture at three different time periods i.e. 1day, 1 week and 1 month. Cheese was stored frozen and at refrigeration temperature for analysis. Additional parameters investigated were press pressure (0 to 60 psi) and time (15 min to hours) to allow the extruded cheese to knit.

In the second phase of second objective, extrusion technology was used to blend high fat aged cheese (Utah State University Aggiano) with nonfat cheese, to achieve high protein cheese, with the fat replacers WPC 1 and extrusion alone at two different mixture concentrations of cheese (90:10 and 85:15). Extruded cheeses were analyzed for moisture and nitrogen content at three different time periods i.e. 1 day, 1 week and 1 month. Nitrogen measurement was then used to estimate the amount of protein. Cheese samples were stored at refrigeration temperature.

Table 2.1
Some studies that have investigated the use of fat replacers in low fat cheeses

Cheese type	Fat replacers used individually	Functional changes compared to low or reduced fat
Low fat white-brined cheese[1]	0.7 or 1.4% Oat beta-glucan	Improved texture but lower flavor and color
Low fat fresh Kashar[2]	1% Simplesse 100 or 1% Dairy-Lo or 5% Raftiline HP	Simplesse and Raftiline improved the texture and sensory properties up to 60 days
Imitation Mozzarella cheese[3]	8-43 % dry basis Novelose 240 (fiber)	Decreased hardness
Low fat Iranian White Cheese[4]	0.75 % Gum Tragacanth	Improved texture, water binding, decreased hardness
Low fat Cheddar[5]	Beta-glucan Nutrim	Decreased hardness and sensory scores
Low fat white pickled cheese[6]	0.5% Simplesse 100 or 0.5% Dairy-Lo or 0.5% Perfectamyl or 0.4% Satiagel	Dairy-Lo and Satiagel were similar in texture to low fat sample
Low fat white brined cheese[7]	1% Simplesse 100 or 0.125 % NovaGel NC200	Improved texture, Simplesse also showed improved appearance
Low fat Cheddar cheese[8]	1% Dairy Lo or 1.5% Simplesse or 1.2% Stellar, or 0.2% NovaGel	Simplesse and NovaGel imparted discontinuity to the casein matrix
Low fat Mozzarella[9]	0.6% Simplesse or 0.6% Stellar or 2.5% Dairy-Low or 2.5% NovaGel	Cheeses with Stellar and Simplesse showed greater initial meltability but all cheeses showed the same meltability after 21 days.

References

[1] Volikakis, Biliaderis, Vamvakas & Zerfiridis, 2004.
[2] Koca & Metin, 2004
[3] Noronha, O'Riordan & O'Sullivan, 2007
[4] Rahimi, Khosrowshahi, Madadlou & Azaznia, 2007
[5] Konulkar, Inglett, Warner & Carriere, 2004
[6] Kavas, Oysun, Kink & Uysal, 2004
[7] Romeih, Michaelidou, Biliaderis & Zerfiridis, 2002
[8] Aryana & Haque, 2001
[9] McMahon, Payne, Fife & Oberg 1996

Table 2.2
Standard TPA parameters and their definitions (Bourne, 1982)

Hardness	The peak force of the first compression of the product during the first bite. The hardness typically occurs at the point of deepest compression for most products.
Fracturability	Fracturability point occurs where the plot has its first significant peak (where the force falls off) during the probe's first compression of the product.
Cohesiveness	Cohesiveness is how well the product withstands a second deformation under two bite tests relative to how it behaved under the first deformation.
Springiness	Springiness is physically springing back of the product after it has been deformed during the first compression.
Chewiness	Chewiness only applies for solid products and is calculated as Gumminess*Springiness. Chewiness is mutually exclusive with Gumminess since a product would not be both a solid and a semi-solid at the same time.
Gumminess	Gumminess only applies to semi-solid products and is Hardness *Cohesiveness Gumminess is mutually exclusive with Chewiness since a product would not be both a semi-solid and a solid at the same time
Resilience	Resilience is how well a product retains its original shape after the compression.

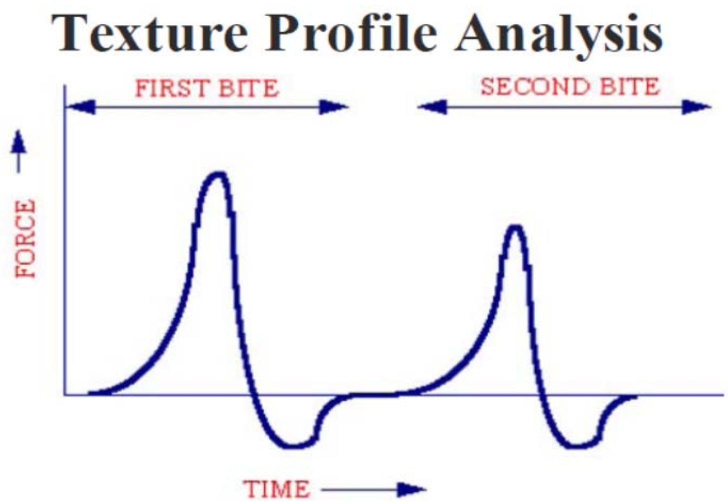

Fig. 2.1. Bite Simulation in Texture Profile Assay

CHAPTER 3

OPTIMIZATION OF EXTRUDER CONDITIONS AND SCREENING OF FAT REPLACERS BASED ON THEIR ABILITY TO AFFECT THE TEXTURE OF LOW FAT CHEESE

3.1 Introduction

There are many defects associated with texture of low fat cheese. It is too hard and chewy, and it is often disliked by the consumer despite having low calories or fat content. Replacing the fat globules with fat replacers give cheese an open structure which can help to overcome a rubbery defect. Fat replacers have been studied microscopically where the space they occupy in casein matrix is dependent on the size of microparticulation of fat replacer, size of fat replacer particles and the method that is used to infuse fat replacer in the casein matrix (McMahon, Payne, Fife & Oberg 1996).

In this chapter, a method to incorporate fat replacer in cheese was to add fat replacer to ground cheese and then use extrusion technology. Extrusion can also help improve overall texture by applying back pressure on the samples and giving a puffed texture. The extruder condition will also be important, as temperature should be below the melt temperature; but high enough to allow formation of new protein-protein interactions.

3.2 Materials and Methods

3.2.1 Fat Replacers and Cheese

Fat replacers used in this study were either microparticulated cellulose based or microparticulated protein based. The amount of fat replacers added to the cheese according to the manufacturer's recommendation for low fat cheese. Whey protein-based fat replacers Temp Pro^{TM} (WPC 1) and Simplesse 500^{TM} (WPC 2) were from Leprino Foods, Denver, CO, USA and CP Kelco U.S., Inc., Atlanta, GA, USA, respectively. The carbohydrate-based fat replacer NovaGel^{TM} RCN 15 (MCC1) was from FMC BioPolymer, Philadelphia, PA, USA. The amount of fat replacer used and their properties are mentioned in Table 3.1. The cheese used in this chapter was made in the Western Dairy Center, Utah State University. The composition of the cheese used was: moisture 54%, fat 6% and protein 34%. The cheese was comminuted using an Urschel comitrol processor (Fig 3.1) to test different particle sizes. The cheese and fat replacers were then sieved through US#4 size sieve to get even distribution of fat replacers before extruding.

3.2.2 Extrusion

The extruder used in this study was an APV Baker MPF 19TC (APV Baker, Inc. Grand Rapids, MI, USA) twin-screw extruder with a length/diameter barrel of 25:1. Auto tune temperature controller was used to control the temperature zones along the barrel. The extrusion parameters included the temperature of the barrel, pressure, exit temperature, torque, screw speed and feed rate. These parameters were optimized and were recorded before beginning the extrusion and during extrusion (Table 3.2).

The extruded samples were then pressed at three different pressures (recorded on gauge) (15 psi, 30 psi and 60 psi) for three different time periods (25 minutes, 45 minutes and 1 hour) to determine the pressure/time combination which will knit the extruded cheese together.

3.2.3 Texture Profile Analysis

The texture was analyzed using a TA-XT Plus texture analyzer (Stable Micro Systems, Godalming, Surrey, UK) equipped with a flat plunger at speed of 5 mm /sec and a 5 kg load cell. Cheese plugs (height 2.0 cm and diameter of 1.6 cm) were obtained using a stainless steel borer. The cheese samples were analyzed for 25% compression of the original height.

3.2.4 Statistical Analysis

The texture parameters were then analyzed by Statistical Analysis Software 9.0 (SAS Institute, Inc., Cary, NC) for analysis of variance (ANOVA) and were grouped to determine the significant differences in the parameters. Control cheese used in this study was 6% fat cheese.

3.3 Results and Discussion

The extruder parameters were optimized and it was essential to maintain all the temperature zones ≤35°C, so that the cheese sample did not melt inside the barrel. The cheese was ground to different sizes and 2 mm was found to be most uniform and extrudable. After extrusion, samples were pressed for 25 minutes at 60 psi to knit particles together. This time/pressure combination was used in chapter 4 as well. The extruded samples and pressed samples are shown in Appendix A.

The TPA analysis was carried out at 25% compression of the original height and the data for parameters were recorded (Table 3.3). The four parameters that were recorded were hardness, cohesiveness, springiness, and chewiness. Adhesiveness was not recorded because some data points were missing when samples were tested.

Table 3.3 shows that cheese hardness was reduced by all extrusion treatments. Extrusion in combination with WPC 1 was significantly less hard than extrusion with MCC 1 or MCC 2. For the texture parameters of cohesiveness and springiness, all the samples were statistically not different than each other except WPC 1. WPC 1 was less cohesive and springy than all the other samples. For the texture parameter chewiness, WPC 1 and control extruded alone were not different than each other and also they were less chewy than all the other samples ($p < 0.05$). MCC 1 was less chewy than MCC 2 and control cheese but chewier ($p < 0.05$) than WPC 1 and control extruded cheese. The control cheese was chewier than all the other samples ($p < 0.05$).

3.4 Conclusion

The temperatures and other extruder parameters that were suitable for extruding cheese without changing the identity of sample were determined in this study. WPC 1 was observed to make the cheese gluey or watery. Regarding TPA, 25% compression was ideal for two bite simulation. After statistical analysis it was determined that extrusion alone and WPC 1 middle concentration were able to improve the texture of low fat cheese. Also MCC 1 was approaching the same texture as imparted by WPC 1. So for the next phase of study, MCC 1, WPC 1 and extrusion alone were identified as treatments. Since positive results were obtained by use of WPC 1, which is a protein-based fat replacer, another whey protein based fat replacer was used in later studies (WPC 2).

Table 3.1
Texture modifying ingredients

Product name	Composition	Amount Used	Source	Functionality
WPC 1	80% WPC	4%	Leprino Foods	Heat stable whey protein, stays fluid at retort temperatures, will not interact with casein, matrix interruption
MCC 1	Microcrystalline cellulose	2%	FMC BioPolymer	Gel particles interrupt casein structure by reacting with kappa casein to form a curd that can entrap moisture
MCC 2	Insoluble microcrystalline cellulose	2%	JR Rettenmaier	Matrix interruption, fat imitation

Table 3.2
Initial extruder parameters recorded before and during extrusion

Extruder Conditions	Start Up Conditions	Collection Conditions
Dry Feed (rpm)	400	800
Screw Speed	150	200
Set Temp °C /Recorded Temp. °C		
Zone 1	35/ 28	41
Zone 2	15/ 38	46
Zone 3	35/ 37	37
Zone 4	35/ 39	36
Zone 5	20/ 24	24
Melt Temp	30.4	45.1
Pressure psi	-110	-30
Torque %	35%	25%

Table 3.3
Means for texture parameters of low fat cheese with or without fat replacers

	Texture Parameter (S.I. Units)	Control not Extruded (6%)	Control Extruded (6%)	MCC 1 Extruded	MCC 2 Extruded	WPC 1 Extruded
Mean*	Hardness (N)	3271.5^{a}	927.0^{d}	1316.7^{c}	1660.4^{b}	1032.0^{d}
	Cohesiveness	0.91^{a}	0.89^{a}	0.90^{a}	0.90^{a}	0.83^{b}
	Springiness (mm)	0.95^{a}	0.95^{a}	0.93^{a}	0.93^{a}	0.89^{b}
	Chewiness (N*mm)	2822.7^{a}	779.1^{d}	1098.5^{c}	1403.4^{b}	764.2^{d}

*****values within a row sharing the same letter are not different ($p \leq 0.05$); ($n = 9$).

Fig 3.1 Urschel Comitrol Processor

CHAPTER 4

INVESTIGATING THE USE OF FAT REPLACERS AND EXTRUSION ON PRODUCTION OF LOW FAT, HIGH PROTEIN CHEESE

4.1 ABSTRACT

This study investigated the use of extrusion technology and fat replacers to improve the texture of low fat cheddar cheese. A twin screw extruder and three fat replacers [WPC 1 (4.0%, 2.20% and 0.40%), MCC 1 (2.0%, 1.06% and 0.125%) and WPC 2 (1.0%, 0.75% and 0.50%)] were used at three different concentrations (lowest, middle and highest) as recommended by the manufacturers for replacing fat in cheese. The extruded cheese samples with and without fat replacers were analyzed for texture at three different time periods (1 day, 1 week and 1 month). It was observed that none of the fat replacers were effective in improving the texture of low fat cheese and the time periods were statistically not significant. Amongst all the treatments, two of them: middle concentration (2.20%) of WPC 1 and extrusion of 2% fat (low fat) cheese were chosen for the next phase of the study. These two treatments were used to produce high protein, low fat cheese with the blend of low moisture aged cheddar cheese and nonfat cheese. The treatments used in this part of study successfully yielded high protein cheese.

4.2 INTRODUCTION

There is an increasing demand to produce low fat/reduced fat products to improve the nutritional value even though manufacturing low fat Cheddar cheese has always being a challenge with respect to texture and flavor. Fat plays an important role in flavor, texture and appearance of cheese. Removal or reduction of fat causes rubberiness, hardness, dryness, fracturability, springiness and crumbliness in cheese (Adhikari, 2003; Banks 2005). Texture defects may arise due to a change in protein matrix with the removal of fat (Bryant, Ustunol & Steffe, 1995). The Cheddar cheese matrix is formed by casein with fat globules entrapped (Lawrence, Creamer & Gilles, 1987; Prentice, Langley & Marshall, 1993). Several methods to date have been used to modify and improve the texture of low fat cheese. Alteration of cheese milk processing conditions, modification in cheese making procedures (Banks, Brechany & Christie, 1989; Dabour, Kheadr, Benhamou, Fliss & LaPointe, 2006; Mistry, 2001) and the use of fat replacers have been explored. Fat replacers have been used extensively to improve the texture and appearance of low fat and nonfat cheeses (Rahimi, Khosrowshahi, Madadlou & Azaznia, 2007). Microparticulated protein based and microparticulated carbohydrate based fat replacers are the two categories that have been recommended for use in cheese products (Romeih, Michaelidou, Biliaderis & Zerfiridis, 2002). The fat replacers, by trapping moisture, provide a creamy and lubricated feel to the cheese but these cannot positively impact the flavor defects in cheese.

Apart from low fat foods, consumers are also looking for food products which can supply additional nutrients in their daily diet. This has increased the demand for high protein foods. Cheese is one such product which is consumed almost daily in an American diet and increasing the protein content may increase consumer consumption. Cheese is considered high protein if it contains more than 10 g of protein per serving size of 28 g. High protein foods are associated with weight loss, increased satiety (Baba, Sawaya, Torbay, Habbal, Azar & Hashim, 1999; Brehm, Seeley, Daniels & D'Alessio, 2003) and improve cardiovascular risk factors (Kelemen, Kushi, Jacobs & Cerhan, 2005).

Today nearly 66% of the Americans are overweight and 33% are clinically obese with body mass index ≥ 30 kg/m^2 (Flegal, Carroll, Ogden & Johnson, 2002). With increasing health concerns, we investigated the production of high protein low fat cheese in this study.

Extruders at different shears are used to produce food products with different textures. Extruders can be configured for low, medium or high shear by the sequence of the screws and paddles in the barrel. The screws promote conveyance and the paddles interrupt the flow and create shear and back pressure. Twin screws are more functional and can be used at higher moisture level (>40%) than single screw extruders which are limited to low moisture levels (<35%) (Walsh and Carpenter, 2008). Apart from screw and paddle sequence, the speed of the co-rotating screws and the temperature during the extrusion significantly influence the texture of the final product. High temperature will result in protein denaturation, allowing for new protein-protein interactions. The rate of speed will influence the amount of input shear, residence time in the barrel and denaturation of protein. Studies have indicated that using fat replacers to improve texture of low fat cheese is effective (Table 2.1) but using extrusion technology to improve the texture of cheese has not been studied before. This present study investigated the hypothesis that extrusion modification of low fat Cheddar cheese alone or with fat replacers can improve the texture of low fat cheese and produce a high protein cheese.

4.3 MATERIALS AND METHODS

4.3.1 Fat Replacers and Cheeses

Whey protein-based fat replacers Temp Pro™ (WPC 1) and Simplesse 500™ (WPC 2) were from Leprino Foods, Denver, CO, USA and CP Kelco U.S., Inc., Atlanta, GA, USA, respectively. The microcrystalline cellulose (carbohydrate-based) fat replacer NovaGel™ RCN 15 (MCC1) was from FMC BioPolymer, Philadelphia, PA, USA.

The low fat and reduced fat cheddar cheeses (2, 6, and 13% fat) and full fat Aggiano and Old Juniper Cheddar cheeses were manufactured in Utah State University Western Dairy center and Dairy Products Laboratory. The nonfat cheese (0% fat) was donated by Dr. Lloyd E. Metzger at South Dakota State University.

4.3.2 Pre-extrusion Procedure

The cheddar cheeses (0% fat, and 2% fat), Old Juniper cheese and Aggiano cheese were comminuted using an Urschel comitrol processor, to a particle size of 2 mm and cheese particles were sieved through a standard US size # 4. Each cheese was vacuum sealed and stored at 4°C prior to extrusion. Fat replacers were added at the manufacture recommended highest, lowest and middle usage levels. Three fat replacers were used were WPC 1 (4.0%, 2.20% and 0.40%), MCC 1 (2.0%, 1.06% and 0.125%) and WPC 2 (1.0%, 0.75% and 0.50%). The fat replacers were sieved with the cheese (w/w) at each concentration to make sure an even distribution of fat replacers and the samples were vacuum sealed for extrusion.

4.3.3 Extrusion Procedures

The extruder used in this study was an APV Baker MPF 19TC (APV Baker, Inc. Grand Rapids, MI, USA) twin-screw extruder with a length/diameter barrel of 25:1. Auto tune temperature controller was used to control the temperature zones along the barrel. The temperature of all the five zones were maintained at 35°C and the exit temperature was kept below 37°C to eliminate any volatile flavor loss and extensive protein-protein cross linking. A KTron volumetric dispenser was used to introduce the comminuted cheese samples with twin auger screws at the speed of 700 rpm. Barrel screw speed was maintained at 200 rpm. To provide minimal amount of shear (depending on the sample type pressure which was between 0 and 30, a suitable screw and paddle configuration was sequenced. Replicate extrusions were performed keeping the above parameters constant. Extruded cheeses were collected in a mold and pressed at 60 psi for 25 minutes to knit the extruded cheeses and expel excess air.

Specific Mechanical Energy (SME):

Specific mechanical energy is the dissipated energy in the form of heat, expressed as per unit mass of the material. SME estimate provides a good indication of the work input from the motor into the extrusion and power needed to extrude a product to the final desired texture. And being independent of the scale, it is an advantage to upgrade any extrusion process. SME also has its effect on longitudinal expansion of the product. Onwulata, Mulvaney & Hsieh (1994) described SME as a linear combination of screw speed and moisture content of the samples. They also indicated that change in SME can be related to change in rheological properties of the product being extruded.

The SME was calculated as the product of three factors 1) normalized angular speed of the agitator, 2) the motor torque and 3) the motor power to mass flow rate ratio. The final unit of the SME is expressed in KJ/Kg. The mathematical expression the formula for SME is:

$$\boldsymbol{SME = \frac{N}{N0} \times \frac{Torque}{100} \times \frac{P}{mf}}$$

where:

N = rpm set during extrusion

N0 = Max. rpm of agitator

P = Power of the motor specific to extruder

mf = mass flow rate

For the extruder used in this study i.e. APV Baker M-19 twin-screw the following values was determined:

N0 = 500 (max rpm)

P = 2 kW (kilowatt)

4.3.4 Texture Profile Analysis (TPA), Protein and Moisture Determination

The texture was analyzed using a TA-XT Plus texture analyzer (Stable Micro Systems, Godalming, Surrey, UK) equipped with a flat plunger at speed of 5 mm /sec and a 2 kg load cell. Cheese plugs (height 2.0 cm and diameter of 1.6 cm) were obtained using a stainless steel borer. Samples were obtained at 4°C and analyzed at the same temperature. Samples were analyzed in triplicate using a two-bite test with a 25% compression of the original height of the samples. Several measurements were obtained, but the only measurements of interest were hardness, springiness and cohesiveness (self-adherence) at 25% compression level of the original height. The samples with the blend of Aggiano and nonfat cheddar cheese with and without fat replacers were analyzed for nitrogen (protein) by Utah State University Analytical Lab (USUAL). Two grams of freeze dried samples were analyzed by the Dumas method analyzing total nitrogen and converting to protein concentration. The moisture content of the cheese samples was determined by the SMART Turbo – Moisture Solids Analyzer (CEM Corporation, North Carolina, USA).

4.3.5 Experimental Design and Statistical Analysis

Three fat replacers (2 protein based and one carbohydrate based), a control (2% low fat cheese) and three texture analysis times (1day, 1 week and 1 month) were included in the study. Low fat cheese was extruded with all three fat replacers at three different concentrations and samples were analyzed for texture after each time point. Each fat replacer at each concentration was replicated and each time a random sample was chosen for extrusion (control or with fat replacer). After sample collection and pressing, each sample was assigned for TPA after 1 day, 1 week and 1 month analysis. The data was collected and analyzed using analysis of variance (ANOVA) using of the Statistical Analysis System and difference between means were determined using a least significant difference test. Significant differences were determined at $\alpha = 0.05$. From the analysis, the protein based fat replacer which performed well during extrusion was determined and that fat replacer was used with blends of Aggiano and nonfat cheese. To obtain low fat (high protein) cheese (Table 4.2), four treatments were designed which were 90:10 nonfat to Aggiano without any fat replacer (AgB190), 85:15 nonfat to Aggiano without any fat replacer (AgB185), 90:10 nonfat to Aggiano with WPC1 middle concentration (AgB290), 85:15 nonfat to Aggiano with WPC1 middle concentration (AgB285). The extrusion runs were randomized and the texture parameters analyzed were hardness, cohesiveness and springiness.

The extrusion conditions were kept constant as before, where temperature and pressure were controlled to avoid melting of cheese. Low fat cheese approaches the definition of a high protein product with approximately 9.5 g protein in a 28 oz serving, while nonfat cheese has over 10 g protein per serving.

4.4 RESULTS AND DISCUSSION

4.4.1 Texture Profile Analysis:

The texture profile analysis (TPA) of control cheese was carried out with control standard cheese. TPA results produced data for different textural parameters including hardness, cohesiveness and springiness and the result after analysis of variance of the three parameters are given in Appendix B.

Changes in each parameter over time are shown in Graphs 4.1, 4.2 and 4.3. ANOVA test was carried out on all the samples to identify significant differences between treatment, time and treatment & time interaction (Appendix B). To identify the samples which were statistically different than the low fat not extruded cheese, least significant difference was calculated for the three parameters: hardness, cohesiveness and springiness and the three different time periods: 1 day, 1 week and 1 month were pooled together as they were not significantly different. The data for the means obtained after TPA and LSD is recorded in Table 4.3.

Results from the ANOVA tables on three texture parameters: hardness, springiness and cohesiveness, shows that the three time periods were not significantly different and can be pooled for further analysis. Another parameter which was measured but not included in the analysis is adhesiveness. Adhesiveness is an empirical value and since the time between the compressions during two-bite test differs greatly than the situation in the mouth when chewing, and in this study adhesiveness reflected the instrumental value which is greater in magnitude than the real value so it was not considered in the study.

Results from Table 4.3 show that with respect to hardness only low fat extruded cheese was significantly different than low fat not extruded cheese. There were few samples like WPC 2 low and high concentrations and MCC 1 low concentration which had hardness values less than low fat not extruded cheese but like all others these samples they were statistically not different than low fat not extruded cheese. With respect to springiness, MCC 1 middle and high concentrations were significantly different than low fat extruded cheese, WPC 1 low, middle and high concentrations and WPC 2 middle and high concentrations. For cohesiveness there was no sample that was significantly different than low fat not extruded cheese.

After analyzing all the samples for texture and pooling the three time periods, it was decided that since there is no fat replacer that we tested was able to improve the texture of low fat not extruded cheese. In order to produce high protein cheese, extrusion alone and WPC 1 middle concentration was used in the next phase of the study. The selected treatments were then used to produce low fat cheese with blends of nonfat cheese and full fat cheese. The composition for low fat cheese was a blend of Aggiano cheese, which will impart textural and flavor properties to the resultant cheese and nonfat cheese (0%) for minimizing the fat level. These two cheeses, Aggiano and nonfat were mixed in two different proportions which were 15:85 and 10:90, respectively, and extruded alone without any fat replacer or with WPC 1 at the middle concentration. The texture analysis was performed for three time periods: 1 day, 1 week and 1 month. The figure 4.4, 4.5 and 4.6, shows the changes over time for the three texture parameters: hardness, springiness and cohesiveness. The effect of fat replacer, WPC1, used in this part of the study is compared with Aggiano and nonfat cheese blend without any fat replacer. It was determined statistically that there was no significant difference in the samples when comparing AgB185 to AgB285 and AgB190 to AgB290 for the texture parameters hardness and springiness. But for texture parameter cohesiveness, there was statistically significant difference when AgB190 is compared to AgB290, which means WPC1 had some effect on cohesiveness when these two blends were compared. Sensory test of these two cheese samples will be further able to indicate if consumers can notice the difference between the cohesiveness of AgB190 and AgB290 (Appendix B).

4.4.2 Protein and Moisture Content Analysis

The samples AgB185, AgB190, AgB285 and AgB290 were tested for moisture and crude protein. The results were analyzed and moisture content, protein per serving size and estimated fat percentage are mentioned in Table 4.4. As per definition of high protein cheese, protein content should be more than 10 g / 28g serving size.

It was observed in table 4.4 that moisture content of the cheese blends in all ratios was lower than the control cheeses manufactured at WDC, Utah State University (Table 4.1). The highest protein content was obtained by AgB290 and second highest protein content was obtained by AgB285. This indicates that whey protein based fat replacer did help in producing high protein cheese. McMahon, Payne, Fife & Oberg 1996, has reported that fat replacers have water holding ability and an increase in moisture level is observed when fat replacers are used in Mozzarella cheese. The protein amount per serving size was greater than 10 g which by definition meets the requirement of being marketed as low fat (high protein) cheese. We estimated fat percentage to range around 9.6% to 11.4% in the resultant cheese.

4.4.3 Specific Mechanical Energy (SME)

Specific mechanical energy was calculated (Table 4.5) for the two treatments used to produce high protein cheese i.e. low fat extruded and WPC 1 middle concentration. The SME calculated for control extruded sample and WPC 1 middle concentration was found to be statistically different than each other. WPC 1 required less SME as compared to low fat extruded sample when screw speed was kept constant. According to Walsh & Wood (2010), with increase of fiber the viscosity of the sample increases and flow rate decreases. But it was observed during the experiment, WPC 1 made the cheese watery, (the reason for which is not determined) and that can be accounted for increased flow rate and hence lower viscosity, higher torque and lower SME compared to control. Onwulata, Mulvaney & Hsieh (1994), also observed that as moisture content increases, torque increases and higher screw speed or higher moisture can decrease the viscosity. Also they found out that with higher moisture content, SME decreases when screw speed is kept constant. Our results comply with theirs. The reason for high level of experimental error in this study is because the mass flow rate was controlled.

4.5 CONCLUSION

During the first half of the experiment, there was a control and three different fat replacers used at three different concentrations to determine if the extrusion technology and/or usage of fat replacers can improve the texture of low fat cheese. It was observed that none of the fat replacers were able to make a positive impact on the texture of the low fat cheese. Some of the treatments had lesser value for hardness than the control low fat but these values were statistically insignificant ($p \leq 0.05$). In the second half of the experiment the goal was to achieve high protein, low fat cheese. The cheeses used in this part of the study were nonfat cheese and Aggiano blended in two ratios: 90:10 and 85:15 and then extruded alone or with WPC 1 middle concentration. The reasons for selecting WPC 1 middle concentration were better extrudability and WPC being protein based fat replacer can add to overall protein amount in 28 g of serving size of cheese. The resultant cheese had more than 10g protein/28 g serving size and could be classified as high protein cheese but the textural properties still remain a challenge. Future work would be test the shelf life stability and sensory analysis of the extruded cheeses and some measures to improve the texture of low fat cheese.

Table 4.1
Proximate composition of cheeses produced for this study.

Cheese	Moisture (%)	Fat (%)	Protein (%)	Fat (g /28 g)	Protein (g /28g)
Aggiano	32	35	28.6	9.8	8
Low fat	54	6	34	1.68	9.52
Low fat	53	2	42.8	0.56	11.98
Nonfat	60	0.0	40	0.0	11.2

Table 4.2
Treatment designed with blends of Aggiano and low fat (2% fat) Cheddar cheese to obtain high protein cheese (>10g of protein per 28g of cheese).

Treatments	Full fat, aged Aggiano with fat replacer	Full fat, aged Aggiano (%)	Amt nonfat (%)	Estimated g protein /28 g	Estimated fat (g)	Treatment codes
Without fat replacer	15		85	10.1	5.35	AgB185
	10		90	10.27	3.57	AgB190
WPC 1 (middle concentration (2.20%))		15	85	10.25	5.35	AgB285
		10	90	10.37	3.57	AgB290

Table 4.3
Means ± standard error (n=9) of treatments[a] and texture parameters[b] of low fat (2% fat) extruded cheese formulated with 3 fat replacers (whey protein concentrate; WPC1 and WPC2, or microcrystalline cellulose; MCC1) at 3 levels (low, middle and high).

Treatments[a]	**Hardness[b]**	**Springiness[b]**	**Cohesiveness[b]**
Low fat Not Extr	1942.00 ± 0	0.93 ± 0.0	0.88 ± 0.0
Low fat Extr	2598.07 ± 513	0.94 ± 0.0	0.86 ± 0.0
WPC 1 Mid.	2373.54 ± 398	0.94 ± 0.0	0.90 ± 0.0
MCC 1 Mid.	2185.90 ± 193	0.92 ± 0.0	0.89 ± 0.0
WPC 2 Mid.	2110.95 ± 173	0.94 ± 0.0	0.91 ± 0.0
MCC 1 High	2093.69 ± 74	0.92 ± 0.0	0.89 ± 0.0
WPC 1 Low	2093.64 ± 228	0.94 ± 0.0	0.91 ± 0.0
WPC 1 High	1993.69 ± 72	0.94 ± 0.0	0.89 ± 0.0
WPC 2 Low	1828.94 ± 176	0.93 ± 0.0	0.91 ± 0.0
WPC 2 High	1735.03 ± 85	0.94 ± 0.0	0.90 ± 0.0
MCC 1 Low	1581.93 ± 166	0.93 ± 0.0	0.90 ± 0.0
$LSD_{0.05}$[c]	441	0.015	0.04

[a] Treatments are extruded cheese samples with fat replacers added in manufacturer's recommended amount.
[b] Three texture parameters were analyzed using Texture Profile Analysis : Hardness, Springiness and Cohesiveness.
[c] LSD = Least significant difference. Means within a column are significantly different ($p < 0.05$) if the difference between mean values is greater than LSD for that column.

Table 4.4
Moisture and protein concentration in the extruded cheese samples (All the combinations gave a high protein reduced fat cheese blend which is >10g protein /28g serving size and <13% fat.

Samples	Moisture content (%)	Protein (g) per serving size (28g)	Estimated Fat %
AgB190	43.80%	10.92g	9.66%
AgB290	37.12%	12.55g	11.35%
AgB185	44.80%	10.55g	9.67%
AgB285	41.73%	11.22g	10.62%

* The samples here are defined as: AgB185 = 85:15 nonfat to Aggiano without any fat replacer, AgB285 = 85:15 nonfat to Aggiano with WPC1 middle concentration (2.20%), AgB190 = 90:10 nonfat to Aggiano without any fat replacer, AgB290 = 90:10 nonfat to Aggiano with WPC1 middle concentration (2.20%)

Table 4.5
Specific Mechanical Energy calculations for the treatments used to produce high protein cheese

Samples	Mass flow rate (mf) g/min	Torque%	Mean SME calculated J/Kg *
Low fat extruded	58.36	20	206.5[a]
WPC 1 middle concentration (2.20%)	74.78	25	163.7[b]

*values sharing the same letter are not significantly different (p value ≤ 0.05).

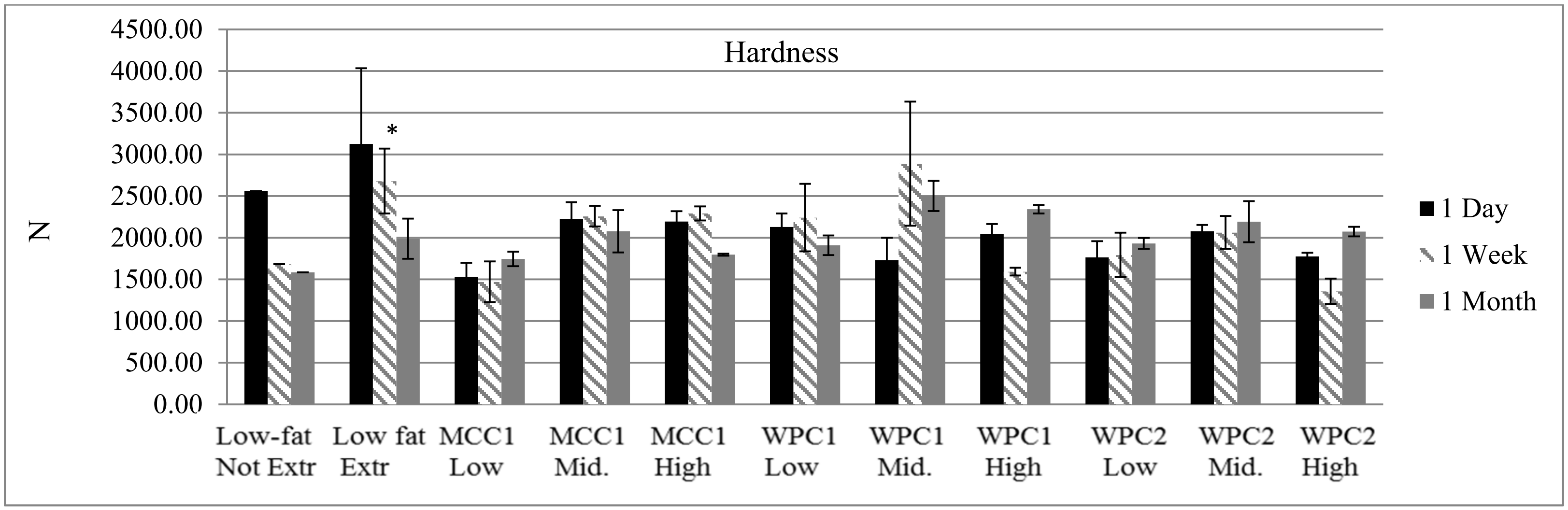

Fig.4.1 Hardness values (N or newton) of low fat (2% fat) extruded cheese formulated with three fat replacers (whey protein concentrate; WPC1 and WPC2, or microcrystalline cellulose; MCC1) at three levels (low, middle, and high) with their standard error.

* The treatment which is different ($p < 0.05$) than all the other treatments when compared using ANOVA (refer Appendix B and Table 4.3)

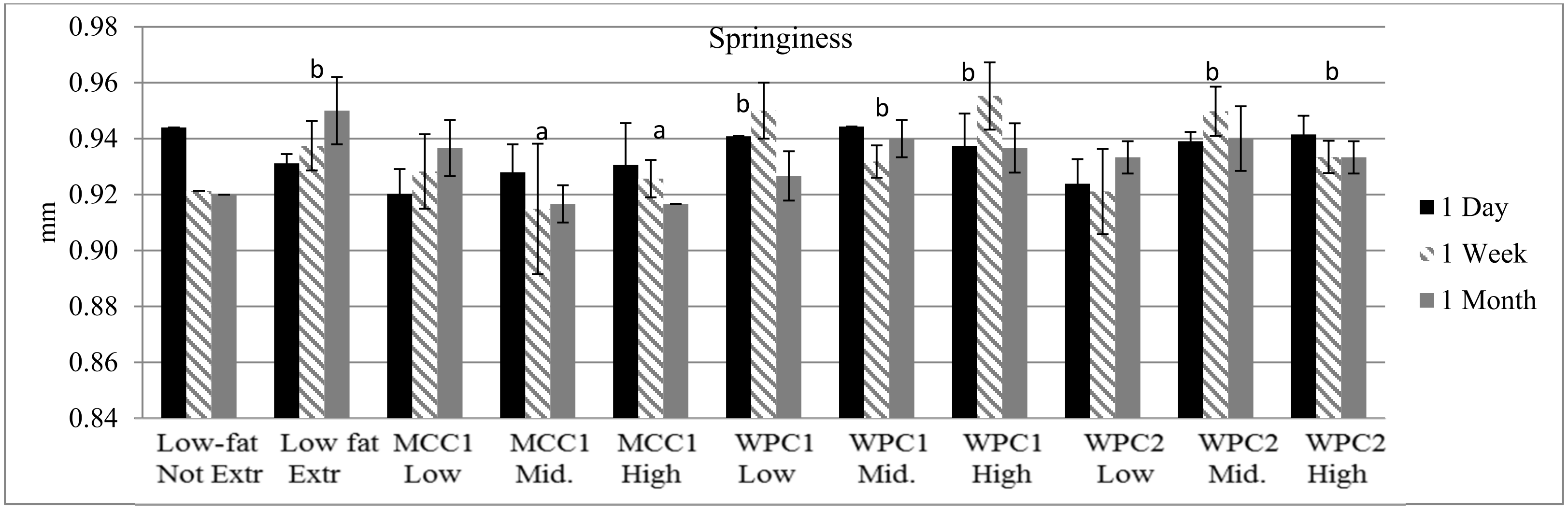

Fig.4.2 Springiness values (mm) of low fat (2% fat) extruded cheese formulated with three fat replacers (whey protein concentrate; WPC1 and WPC2, or microcrystalline cellulose; MCC1) at three levels (low, middle, and high) with their standard error.

* The treatments which are marked by the letter a are different ($p < 0.05$) than the treatments marked with letter b when compared using ANOVA (refer appendix B and Table 4.3)

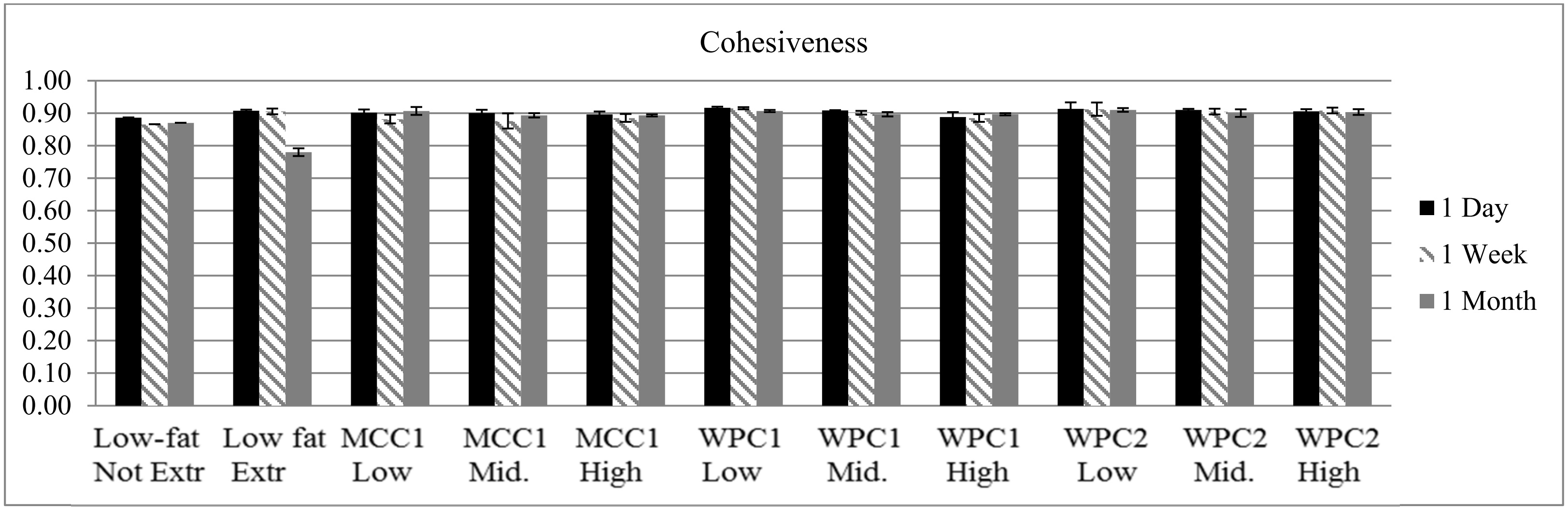

Fig.4.3 Cohesiveness values (unit less) of low fat (2% fat) extruded cheese formulated with three fat replacers (whey protein concentrate; WPC1 and WPC2, or microcrystalline cellulose; MCC1) at three levels (low, middle, and high) with their standard error.

There were no treatments which were different ($p<0.05$) than each other.

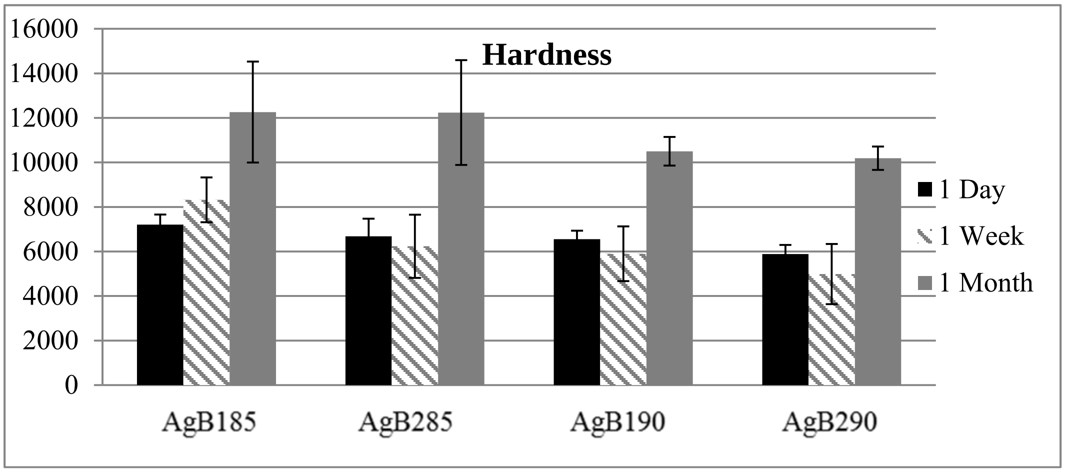

Fig 4.4 Hardness over time for the blend of Aggiano and nonfat cheese with standard deviation (refer Appendix C for t-test comparison)

* The samples in the x-axis are defined as: AgB185 = 85:15 nonfat to Aggiano without any fat replacer, AgB285 = 85:15 nonfat to Aggiano with WPC1 middle concentration (2.20%), AgB190 = 90:10 nonfat to Aggiano without any fat replacer, AgB290 = 90:10 nonfat to Aggiano with WPC1 middle concentration (2.20%)

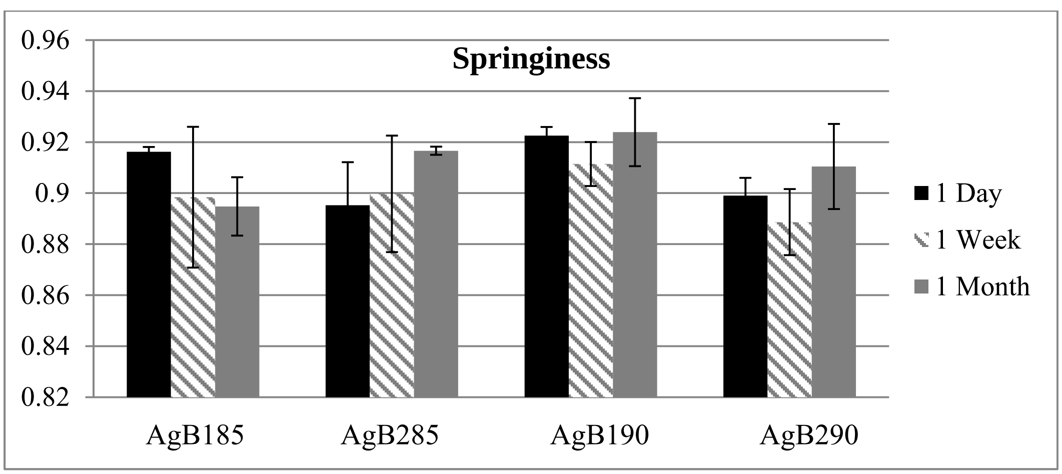

Fig 4.5 Springiness over time for the blend of Aggiano and nonfat cheese with standard deviations (refer Appendix C for t-test comparison)

*The samples in the x-axis are defined as: AgB185 = 85:15 nonfat to Aggiano without any fat replacer, AgB285 = 85:15 nonfat to Aggiano with WPC1 middle concentration (2.20%), AgB190 = 90:10 nonfat to Aggiano without any fat replacer, AgB290 = 90:10 nonfat to Aggiano with WPC1 middle concentration (2.20%)

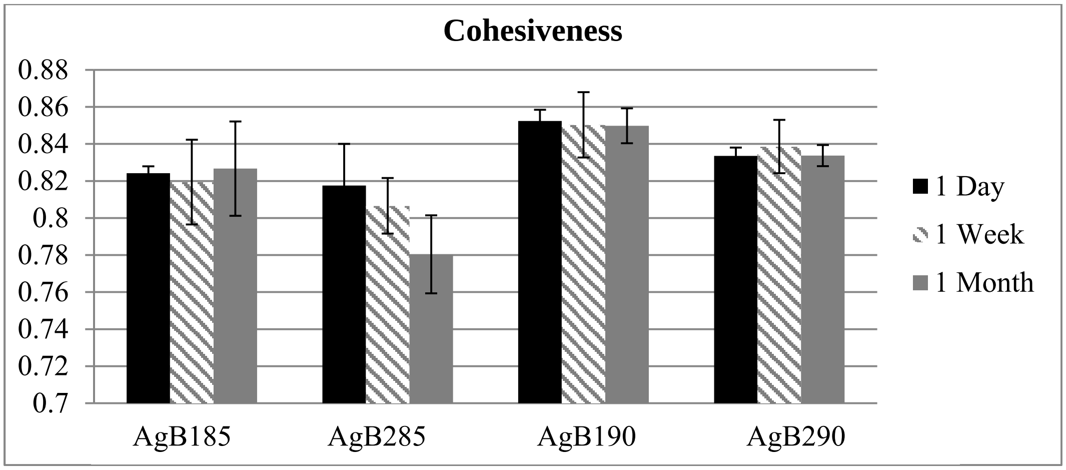

Fig 4.6 Cohesiveness over time for the blend of Aggiano and nonfat cheese with standard deviations
* The samples in the x-axis are defined as: AgB185 = 85:15 nonfat to Aggiano without any fat replacer, AgB285 = 85:15 nonfat to Aggiano with WPC1 middle concentration (2.20%) AgB190 = 90:10 nonfat to Aggiano without any fat replacer, AgB290 = 90:10 nonfat to Aggiano with WPC1 middle concentration (2.20%)

CHAPTER 5

GENERAL SUMMARY

In this study three fat replacers were initially extruded with low fat cheese. From these samples low fat extruded cheese and WPC 1 (whey protein concentrate) were two treatments which were significantly different than control cheese and exhibited positive effect on the texture using TPA analysis. MCC1 (microcrystalline cellulose) was also close in exhibiting texture improvements; hence these two fat replacers were then used in chapter 4. The extruder parameters were determined and optimized. The sample press pressure/time combination was determined. The extruded samples were pressed for 25 minutes at 60 psi, allowing enough time for cheese to knit back together.

In chapter 4, WPC 2 was added as an additional treatment. WPC 2 is a protein based fat replacer and since WPC 1 being protein based showed positive effect on texture, another protein based fat replacer was added to compare the two. The goal of this part of the study was to have low fat cheese with improved texture and blend nonfat and full fat cheese to yield high protein cheese.

The three fat replacers were added to low fat cheese (2% fat) in lowest and highest concentration as recommended by manufacturers and a middle concentration was also tested. TPA was carried out for three time intervals: 1 day, 1 week and 1 month. After statistical analysis it was determined that time was not significantly different for all the texture parameters tested. And for treatments, only low fat extruded cheese sample was statistically different than low fat not extruded cheese with respect to hardness. All the other treatments were statistically not significant with respect to hardness, springiness and cohesiveness.

In the second part of chapter 4, WPC 1 middle concentration and extrusions alone were chosen as the treatments to manufacture high protein cheese using extrusion. Since none of the fat replacers were able to make a positive impact on the texture of low fat cheese, so WPC 1 was picked as the fat replacer for next phase because it is a protein based fat replacer and was easily extrudable than other samples. Two blends of nonfat (0% fat) and full fat aged Aggiano cheeses were made: 85:15 and 90:10, respectively. Four treatments were designed with two blends and a fat replacer: AgB185, AgB190, AgB285 and AgB290. Extruder parameters and time/pressure combination of press were kept similar to the previous analysis. TPA was carried out at three different time periods: 1 day, 1 week and 1 month. The goal of this part of the study was to produce a high protein cheese which we were able to do for all the four above mentioned treatments.

The null hypothesis of the study was to see if extrusion alone or in combination with fat replacers can improve the texture of low fat cheese. The null hypothesis was rejected; the fat replacers were not able to improve the texture of low fat cheese. Future work in this research would include shelf stability, use of other available fat replacers and sensory analysis of extruded cheese products.

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APPENDICES

APPENDIX A

EXTRUDER AND EXTRUDED SAMPLES

Fig.A1 Extruder and a close view of the five temperature zones.

Fig. A2 Extruded samples before and after pressing and vacuum sealed.

Appendix B

Table B.1 ANOVA Table for Hardness

Hardness STATISTICA summary of all effects; design:						
Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
TRTMT	10	731738.5	66	218537.1	3.348349	0.0014225
TIME	2	80609.6	66	218537.1	0.36886	0.6929388
TRTMT*TIME	20	405045.5	66	218537.1	1.85344	0.0322084

Table B.2 ANOVA Table for Springiness

Springiness STATISTICA summary of all effects; design:						
Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
TRTMT	10	0.0005885	66	0.0002687	2.190226	0.0290997
TIME	2	0.0000616	66	0.0002687	0.229323	0.7957023
TRTMT*TIME	20	0.0002194	66	0.0002687	0.816541	0.6853943

Table B.3 ANOVA Table for Cohesiveness

Cohesiveness STATISTICA summary of all effects; design:						
Effect	df Effect	MS Effect	df Error	MS Error	F	p-level
TRTMT	10	0.002005	66	0.001601	1.252366	0.275654
TIME	2	0.002294	66	0.001601	1.432808	0.245963
TRTMT*TIME	20	0.001578	66	0.001601	0.985868	0.489821

B. 4 Paired t- test result for Hardness

t-Test: Paired Two Sample (AgB185 AND AgB285)

	AgB185	*AgB285*
Mean	9261.54	8384.558
Variance	7056617	11197431
Observations	3	3
Pearson Correlation	0.961479	
Hypothesized Mean Difference	0	
df	2	
t Stat	1.409911	
P(T<=t) two-tail*	0.293971	
t Critical two-tail	4.302653	

t-Test: Paired Two Sample (AgB190 and AgB290)

	AgB190	*AgB290*
Mean	7652.095	7018.549
Variance	6197747	7746693
Observations	3	3
Pearson Correlation	0.999554	
Hypothesized Mean Difference	0	
df	2	
t Stat	3.608371	
P(T<=t) two-tail*	0.068954	
t Critical two-tail	4.302653	

* If the p value for the two-tail paired t-test is <0.05 then the treatments are not significantly different than each other.

B.5 Paired t- test result for Springiness

t-Test: Paired Two Sample (AgB185 and AgB285)

	AgB185	*AgB285*
Mean	0.90314	0.903865
Variance	0.000132	0.000127
Observations	3	3
Pearson Correlation	-0.77077	
Hypothesized Mean Difference	0	
df	2	
t Stat	-0.05865	
P(T<=t) two-tail*	0.958565	
t Critical two-tail	4.302653	

t-Test: Paired Two Sample (AgB190 and AgB290)

	AgB190	*AgB290*
Mean	0.919295	0.903156
Variance	4.7E-05	3.99E-05
Observations	3	3
Pearson Correlation	0.520929	
Hypothesized Mean Difference	0	
df	2	
t Stat	4.323364	
P(T<=t) two-tail*	0.051447	
t Critical two-tail	4.302653	

* If the p value for the two-tail paired t-test is <0.05 then the treatments are not significantly different than each other.

B.6 Paired t- test result for Cohesiveness

t-Test: Paired Two Sample (AgB185 and AgB285)

	AgB185	*AgB285*
Mean	0.823436	0.801499
Variance	1.35E-05	0.000365
Observations	3	3
Pearson Correlation	-0.54266	
Hypothesized Mean Difference	0	
df	2	
t Stat	1.782151	
P(T<=t) two-tail*	0.21667	
t Critical two-tail	4.302653	

t-Test: Paired Two Sample for Means (AgB190 and AgB290)

	AgB190	*AgB290*
Mean	0.850828	0.835275
Variance	1.89E-06	8.26E-06
Observations	3	3
Pearson Correlation	-0.35401	
Hypothesized Mean Difference	0	
df	2	
t Stat	7.486434	
P(T<=t) two-tail*	0.017379	
t Critical two-tail	4.302653	

* If the p value for the two-tail paired t-test is <0.05 then the treatments are not significantly different than each other.