APPLICATION OF FAT MIMETICS IN FETA CHEESE

A Thesis
Presented in Partial Fulfillment of the Requirements for
the Degree of Master of Science in the
Graduate school of The Ohio State University

By
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****

The Ohio State University
1997

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ABSTRACT

In the preliminary research functionality of six fat mimetics were tested, modified tapioca starch and lecithin were chosen to be used in the final research. According to the results of the final study, fat mimetics and fat levels were found to effect moisture, yield, fat, protein, and hardness but not pH and salt content of Feta cheese. At the used concentrations of mimetics, it was observed that fat reduced cheeses with modified tapioca starch had the highest moisture and lowest protein content. Relating to these chemical properties, they were less firm. The micrographs showed that they had a less dense structure with a smooth protein matrix. The structures were very similar to the full-fat control. They had a clean taste. Modified tapioca starch were found increase acceptability by Group 1. However, it decreased flavor score by Group 2. Increased acceptability might be a result of lubricity added by starch related to water retention. Decreased flavor scores might be a result of diluted flavor by increased water content. Lecithin did not bind water as much as starch did. But it helped in decreasing hardness. The microstructure of the reduced-fat cheese was very similar to the full-fat control. However low-fat cheese with lecithin was not very similar with its more rough matrix. Cheeses with lecithin were reported to have foreign flavors by some panelists.
They also had low flavor scores. Cheeses that contained a combination of starch and lecithin were more similar to cheeses with only lecithin. In addition, they did not have off-flavors. The combination cheeses had high texture and overall acceptability scores given by Group 1 and Group 2.

Use of a combination of modified tapioca starch and lecithin is recommended to be used in fat reduced Feta cheese as a result of this study. However, first shelf life should be studied in order to confirm that these mimetics do not cause off-flavors during the ripening period.
ACKNOWLEDGMENTS

I wish to thank my adviser Dr. Valente B. Alvarez for his intellectual support, encouragement, and enthusiasm which made this thesis possible, and for his patience in correcting both my stylistic and scientific errors.

Special thanks go to the other members of my advisory committee, Dr. James W. Harper and Dr. Michael E. Mangino for their valuable advice.

I am grateful to Drs. Aloisio J. Antunes, Fehmi Yazici and Karen Fligner for discussing with me various aspects of the thesis. I would like to thank to Albert McRoberts for his assistance in the pilot plant, without whose collaboration this project would have been impossible. I am indebted to my friends Bettsy Rice and Nikhil Prasad, for their assistance in lab work.

I also wish to thank to my mother, father and grandmother for their sincere support, love and constant prayers for my success.

Last but not the least, I would like to express the highest appreciation to Dilek Colak for her support and constant encouragement.
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Major Field: Food Science and Nutrition
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1. INTRODUCTION

Feta cheese is a soft white cheese ripened in brine. It has a salty and acidic taste. The fat content of Feta cheese is 20-25%. Traditional Feta cheese is made using sheep’s milk or a mixture of sheep’s and goat’s milk that imparts the characteristic flavor (Robinson and Tamime, 1991).

Reduced fat cheeses generally have some texture and flavor problems. Katsiari and Voutsinas (1994), reported that low-fat Feta cheese has more pronounced texture problems as compared to flavor problems. Protein dominated microstructure of low-fat cheese produces harder, more viscous and more elastic cheese. Low-fat cheeses have fewer fat globules within the protein matrix and the globules are usually smaller than in full-fat cheese (Mistry and Anderson, 1993).

Fat mimetics are compounds used to fully or partially replace fat in reduced fat foods. Fat mimetics are water soluble, polar compounds that function through binding additional water to improve texture. Very little reported information is available on use of commercial fat mimetics in cheeses.

Brummel and Lee (1990) studied the effects of adding carrageenan, pectin and guar gum to the cheese spreads. The researchers concluded that the texture of the spreads improved significantly, however, the dilute flavor of the reduced- and low-fat cheese spreads containing gums were less desirable as compared to the full-fat control. Studies of
the addition of denatured whey protein concentrate to low-fat processed cheese demonstrated that denatured whey protein improved the sensory characteristics of cheese (Salem et al., 1987).

Addition of lecithin improved texture of Cheddar cheese significantly (Drake et al., 1996b). Both sensory analysis and hardness data proved the similarity of texture to the control full-fat cheese. Yields of reduced-fat cheese increased consequently. However, it was reported that improvement of texture was accompanied by some foreign flavors described as soapy or petroleum-like. Sohn (1996) tested the use of 0.5% Lecithin in Swiss cheese and did not report any foreign flavor associated with the use of lecithin. Hardness of low-fat cheese containing lecithin was comparable to full-fat control.

In another study, Drake et al. (1996a) tested use of Dairy Lo™, Novagel™ and ALACO PALSTM in low-fat Cheddar cheese. The low-fat cheeses containing ALACO PALSTM, a protein based fat mimic, received the highest sensory scores for texture. However, it imparted a brownish hue to the cheese which was not desirable. Low-fat cheeses containing fat-mimetics were found to be less rubbery by the sensory panel. Bullens (1994) reported that the addition of Novagel™ to cheese milk improved moisture retention and texture of low-fat Cheddar cheese. However, the research also revealed that the increased moisture leads to a diluted flavor profile. It is proposed that microcrystalline cellulose particles in Novagel™ incorporated into the cheese milk acts as physical barrier between the casein network (Bullens et al., 1994).

Turin and Bonami (1995) studied the effects of addition of Simplesse™ to Caciotta cheese. The addition of Simplesse™ to the Caciotta cheese decreased rather than
increased available hydrophobic sites at the fat/water interface hence it did not fully substitute for fat in aroma and flavor binding. The cheeses were not sensory tested.

The objectives of this study were: 1) to determine the effects of fat reduction on composition, hardness, microstructure and sensory properties of Feta cheese, 2) to identify a fat mimetic compound to be used in the manufacture of fat reduced Feta cheese, 3) to evaluate the functionality of modified tapioca starch and lecithin individually and in combination as fat mimetics in fat reduced Feta cheese.
2. LITERATURE REVIEW

2.1. Feta Cheese

Feta is a soft white cheese manufactured from sheep’s milk or a mixture of
sheep’s and goat’s milk which originally appeared in the Balkans. Feta means ‘slice’ and
it derived from the traditional shape of the cheese blocks. Cheese which is kept in
cylindrical wooden kegs have the shape of watermelon slices (Anifantakis, 1991).

Feta cheese was only known in the Balkan countries for many centuries.
However, people who migrated from this region to USA, Australia, Canada and Europe
created new markets for Feta cheese and international trade was established for this
commodity (Efthymiou and Mattick; 1964, Horwood et al, 1981). Since the available
quantities of traditional Feta cheese were not sufficient to cover the demand, attempts
were made to produce domestic Feta cheese from cow’s milk. Nonetheless, it has been
difficult to duplicate the flavor of Feta cheese produced from sheep’s milk when using
cow’s milk. Cow’s milk Feta cheese is criticized by the customer who is accustomed to
the traditional product. The major points of criticism are dry and crumbly texture; and
acid, unnatural, and flat flavor (Abd el-Selam, 1987). Moreover, the color of the cheese
becomes yellowish increasing with aging caused by carotenes in cow’s milk. Sheep’s and
goat’s milk have trace amounts of these compounds (Swaisgold, 1985).
2.1.1 Milk

Sheep's and goat's milk greatly differ from cow's milk in composition (Table 2.1). The two milks have higher protein, fat and dry matter which affect the yield and properties of the resulting cheese. Sheep's and goat's milk fat is essential for the flavor formation in the ripe Feta cheese through lipolysis. These milk have considerable amounts of caproic, caprylic and capric acids which impart Feta cheese its typical piquant peppery flavor (Table 2.2). This flavor is not possible to produce with cow's milk. However, the use of lipase can help matching slightly rancid flavor of Feta cheese (Anifantakis, 1991).

<table>
<thead>
<tr>
<th></th>
<th>Fat</th>
<th>Protein</th>
<th>Lactose</th>
<th>Ash</th>
<th>Dry Matter</th>
<th>Water</th>
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<tr>
<td>Sheep</td>
<td>8.01</td>
<td>6.05</td>
<td>4.74</td>
<td>0.93</td>
<td>19.36</td>
<td>80.64</td>
</tr>
<tr>
<td>Goat</td>
<td>5.27</td>
<td>3.72</td>
<td>4.54</td>
<td>0.83</td>
<td>14.22</td>
<td>85.78</td>
</tr>
<tr>
<td>Cow</td>
<td>4.10</td>
<td>3.60</td>
<td>5.00</td>
<td>0.70</td>
<td>13.40</td>
<td>86.60</td>
</tr>
</tbody>
</table>

* Average of Vlahiko, Karagoiniko, Chiou, Attica and Ipiros breeds.
  
  b Average of Attica and Ipiros breeds.
  

Table 2.1 Average chemical composition (%w/w) of milk from Greek sheep and goat breeds and Western breed cows.
Fatty acids

<table>
<thead>
<tr>
<th></th>
<th>C4</th>
<th>C6</th>
<th>C8</th>
<th>C10</th>
<th>C12</th>
<th>C14</th>
<th>C16</th>
<th>C18</th>
<th>C18:1</th>
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<tr>
<td>Sheep</td>
<td>4.2</td>
<td>2.0</td>
<td>2.2</td>
<td>6.0</td>
<td>3.1</td>
<td>5.5</td>
<td>16.9</td>
<td>15.8</td>
<td>38.8</td>
</tr>
<tr>
<td>Goat</td>
<td>3.1</td>
<td>2.8</td>
<td>3.0</td>
<td>10.1</td>
<td>6.0</td>
<td>12.2</td>
<td>27.2</td>
<td>27.5</td>
<td>25.6</td>
</tr>
<tr>
<td>Cow</td>
<td>2.9</td>
<td>2.2</td>
<td>1.1</td>
<td>3.0</td>
<td>2.7</td>
<td>9.0</td>
<td>25.0</td>
<td>13.8</td>
<td>33.0</td>
</tr>
</tbody>
</table>

Based on Scott (1981)

Table 2.2 Fatty acid composition (%w/w) of sheep’s, goat’s and cow’s milk.

2.1.2 Methods of Manufacture

As stated earlier, either sheep’s milk or a mixture of sheep’s and goat’s milk should be used for traditional Feta cheese. The percentage of goat’s milk should not exceed 30% (Anifantakis, 1991). Otherwise a harder cheese with a stronger flavor would be produced. The fat content should be approximately 6.5-8%. Milk standardized to a casein to fat ratio of 0.7-0.8 yields cheese with most desirable characteristics (Pappas, 1994). Traditionally, milk was not pasteurized. This is still followed to some extent. However, pasteurization is an established practice in industrial scale production (Anifantakis, 1991; Scott, 1981). If the milk is pasteurized, usually calcium chloride is added to facilitate clotting (Walstra et al, 1987) and lipase is added to enhance the flavor (Efthymiou and Mattick, 1964).

Lactic acid bacteria are usually used as starter culture organisms. Tamime and Kirkegaard (1991) mentions use of *Lactococcus lactis* ssp. *lactis*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactococcus lactis* ssp. *cremoris*, *Streptococcus salivarus*
sse, Lactobacillus plantarum, Lactobacillus casei ssp. casei, Leuconostoc mesenteroides ssp. cremoris, Lactobacillus helveticus, and Lactobacillus acidophilus as starter cultures for Feta cheese. Most of these organisms are mesophilic, i.e., they are added to cheese milk at a temperature range of 32-34°C. The rate and ratio of addition is adjusted according to the organisms used. A ratio of 1:3 is suggested for Lactococcus lactis ssp. lactis and Lactobacillus delbrueckii ssp. bulgaricus, respectively (Abd el-Salam, 1987). It is also not a rare practice to add yogurt as starter culture due to its convenience and high availability (Anifantakis, 1986).

The rennet used for traditional production is obtained from the abomasa of lambs and kids fed exclusively on milk but not grass. Rennet paste is prepared from dried abomasa and ideally it should be used after determining its clotting activity (Anifantakis, 1991). When coagulation is complete, approximately 45-60 minutes, the curd is cut into small cubes with 2 cm wire knives and left for 5-10 min, which helps expel part of the whey. Thus, it makes the curd particles firmer with greater mechanical resistance against damage during transfer into the moulds. The molds are either stainless steel or plastic with small holes in them for easier drainage. They can be rectangular or round in shape. If the cheese is going to be ripened in wooden barrels, the round shaped moulds are preferred (Anifantakis, 1991). The curd is gradually transferred to the mold in order not to impede drainage. Filling moulds at once causes more whey to be retained in the cheese. After the molds are filled, they are transferred to inclined tables where the cheese is further drained for 2-3 hours. Then the moulds are turned upside down to facilitate
drainage and kept for another 2-3 hours. The drainage period is inversely related to
temperature (Scott, 1981).

After the drainage period, the moulds are removed and the cheese is cut into
slices and dry salted using granular salt. Fine salt dissolves very fast therefore, moisture
of the cheese is drained very fast resulting a hard surface that delays drainage. To the
contrary, granular salt dissolves slowly which helps gradual drainage. During the period
of salting the cheeses blocks are turned upside down and additional salt is added every 12
hours. This procedure is repeated until the cheese contains 3-4% salt (Abd el-Salam,
1987; Kosikowski, 1982). Cheese blocks are further left on the cheese table until a slime
layer produced by bacteria and yeast forms. Slime formation is essential for the
characteristic flavor development of Feta cheese (Efthymiou, 1967).

Dry salting usually takes around 1-2 weeks depending on the ambient temperature
which should ideally be 16-18°C. Cooler temperatures require longer salting times. The
cheese blocks are then cleaned using water or brine with the help of a brush to remove
the impurities on the surface. After cleaning, cheese blocks are placed into the barrels
and 6-8% brine is added (Anifantakis, 1991). Flavor develops better in wooden barrels
than in tins. However, the filled barrels weigh approximately 50kg and are difficult to
handle. Modern industrial tins weigh approximately 17-18 kg and are easier to handle
and transport (Abd el-Salam, 1987).

Cheese is kept in brine at 14-18°C for 10-15 days until the pH reaches 4.4-4.6 and
the moisture is less than 56%. It is important to control the temperature to avoid
undesirable texture and flavor characteristics. For example, ambient temperatures
exceeding 20°C may lead to the formation of a large number of small eyes in the body of cheese (Anifantakis, 1991). A pH lower than 4.4 contributes an acidic taste and lower moisture content. Cheese with a pH higher than 4.6 has a limited shelf life. After the initial ripening at 14-18°C, the barrels/tins are transferred to cold rooms at 2-5°C. Cheese may be stored over a year at 2°C (Abd el-Salam, 1987; Anifantakis, 1986). Minimum ripening period is 1-2 months according to the literature (Scott, 1981; Anifantakis, 1991). During storage, brine should always cover the cheese blocks to avoid mold growth.
Sheep’s or a mixture of sheep’s and goat’s milk

\[ \downarrow \]

Standardization to 6.5-8% fat content

\[ \downarrow \]

Pasteurization

\[ \downarrow \]

Cooling to 32-34°C

\[ \downarrow \]

Addition of starter and CaCl₂

\[ \downarrow \]

Addition of rennet - coagulation in 45-60min

\[ \downarrow \]

Cutting curd with 2 cm knives

\[ \downarrow \]

Waiting for 5-10 min

\[ \downarrow \]

Transferring curd into the molds gradually

\[ \downarrow \]

Draining for 2-3 hr

\[ \downarrow \]

Turning upside down and keeping for another 2-3 hr

\[ \downarrow \]

Dry salting using granular salt at 16-18°C

\[ \downarrow \]

Turning molds upside down and salting again several times

\[ \downarrow \]

Cleaning cheese, placing cheese blocks into barrels/tins and adding 6-8% brine

\[ \downarrow \]

Keeping the containers of cheese at 14-18°C until pH=4.4-4.6 and moisture <56%

\[ \downarrow \]

Transfer to the cold rooms at 2-5°C

\[ \downarrow \]

Ripening 1-2 months minimum

Figure 2.1 Flow chart of Feta cheese manufacture
While traditional Feta cheese in Greece is produced using the procedure mentioned above, there are other methods used as well. Two major deviations from this procedure are in the molding and dry salting steps. In the molding step, pressure at the range of 1.3-1.9Pa, is applied instead of allowing natural drainage of whey. For this application, large moulds with the dimensions of 55x35x25cm are used (Kosikowski, 1982). The second major deviation is using brine with high concentrations of salt instead of dry salting. Brine concentrations of 16% to 23% are mentioned in the literature. After molding, fresh cheese blocks are usually kept at this concentration of brine for around 24 hr. Then, they are transferred to 6-14% brine (Scott, 1981; Kosikowski, 1982). Since the cheese tables cover quite a lot of space, this modification is often followed by the manufacturers outside Greece. In Greece, there are modern dairy plants that use space saving technologies as well as traditional producers who use cheese tables (Tamime and Kirkegaard, 1991).

There has been many attempts to make Feta cheese from cow’s milk (Efthymiou and Mattick, 1964; Zerfiridis and Kristoffersen, 1968; Mondal et al, 1989; Prasad, 1995). Their make-procedure is very similar to the described above.

2.1.3 Chemical Composition

In US, standard of identities of cheese are defined in 21 CFR, section 133. However, there is no definition for Feta cheese, yet. Greece, where Feta cheese is consumed most, set some standards Greek legislation classifies Feta cheese into four categories according to their chemical composition:
1. Excellent; moisture content less than 52.5% and fat content more than 22%

2. First quality; moisture content less than 56% and fat content more than 19%

3. Second quality; moisture content less than 56% and fat content more than 15%

4. Semiseparated; moisture content less than 56% and fat content more than 10%

(Anifantakis, 1986).

<table>
<thead>
<tr>
<th></th>
<th>Traditional sheep’s milk Feta*</th>
<th>Greek marketb</th>
<th>Cow’s milk Fetac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>55.16</td>
<td>54.34</td>
<td>48.42</td>
</tr>
<tr>
<td>Fat</td>
<td>23.66</td>
<td>22.72</td>
<td>25.30</td>
</tr>
<tr>
<td>Total Protein</td>
<td>17.26</td>
<td>17.59</td>
<td>16.90</td>
</tr>
<tr>
<td>Ash</td>
<td>1.23</td>
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<td>4.75</td>
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<tr>
<td>NaCl</td>
<td>2.80</td>
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<td>3.78</td>
</tr>
<tr>
<td>pH</td>
<td>4.36</td>
<td>4.39</td>
<td>5.07</td>
</tr>
</tbody>
</table>

* (Anifantakis, 1991)

b (Abd el-Salam, 1987)

c (Prasad, 1995)

Table 2.3 Chemical composition of Feta cheese

2.1.4 Changes during ripening

During ripening moisture content of Feta cheese decreases in order to establish osmotic equilibrium. This increases hardness of the cheese. Then, due to proteolysis protein matrix is partly break down. Thus, hardness of cheese decreases after initial increase. Protein content of the cheese decreases 2-3% again due to proteolysis.
Percentage of salt in cheese may increase slightly if dry salting is applied (Katsiari and Voutsinas, 1994a). However, it increases 3-4% if brining is applied without dry salting (Prasad, 1995).

Flavor formation takes place during ripening primarily due to lipolysis and proteolysis. A balanced combination of proteolysis products and free fatty acids is what imparts the characteristic flavor of Feta cheese (Vafopoulou et al, 1989). According to several workers, concentration of free fatty acids and their proportion to each other have a pronounced role in determining flavor in Feta cheese (Efthymiou, 1967; Siapantas, 1987; Harboe, 1994).

2.1.4.1 Proteolysis

Proteolysis is important for flavor development and texture of the cheese. It contributes flavor via formation of amino acids and peptides. Changes in texture occur due to partial breakdown of the casein network (Fox, 1989). In Feta cheese, proteolysis is mainly due to enzymes used for coagulation, proteinases produced by the microorganisms, slime produced during dry salting and indigenous milk proteinases. Traditionally, lamb and kid rennets obtained from abomas of young animals are used in the manufacture of Feta cheese (Anifantakis, 1991). That kind of rennet paste contain several enzymes besides chymosin (Abd el-Salam, 1987). Modern dairies use calf rennet, however it is a common practice to mix it with lamb and kid rennet to obtain a similar flavor profile to the traditional product (Tamime and Kirkegaard, 1991).

The rate of proteolysis and products of proteolysis can be observed by polyacrylamide gel electrophoresis (PAGE). While, this method was criticized as being
non-quantitative (Fox, 1989), onset of densitometers with high resolution and made quantitative analysis possible. Quantitative analysis with densitometers was conducted for low-fat cheese research by Turin and Bonomi, (1995) and Tunick et al (1993). The electrophoretic patterns of Feta cheese showed that $\alpha$ casein was more intensively degraded as compared to $\beta$ casein (Vafopoulou et al, 1989).

2.1.4.2 Lipolysis

The mildly rancid taste of Feta cheese is due to lipolysis. There are several sources of lipases that induce lipolysis in cheese such as milk itself, secondary flora, starter bacteria, and added lipases (Vindfeldt, 1993). The main source lipolytic enzymes in Feta cheese is suggested to be the slime which forms during dry salting and is washed of thereafter when cheese is placed in brine. Enzymes formed diffuses slowly into the cheese and hydrolyses milk fat producing a variety of fatty acids (Vafopoulou et al, 1989).

In Feta cheese, the major fatty acid residue is acetic acid (Efthymiou, 1967; Horwood et al, 1981) which is produced as a result of several fermentation pathways rather than lipolysis (Abd el-Salam, 1987). Acetic acid comprised 28-43% of fatty acids in mild Feta cheese of unknown age (Efthymiou, 1967) and 32% in cow’s milk Feta (Zerfiridis and Kristoffersen, 1968). Butyric (C4), caproic (C6) and caprylic (C8) acids which are produced through lipolysis are at lower levels as compared to acetic acid (Horwood et al, 1981). The overall flavor profile depends on a balanced combination of these fatty acids (Efthymiou, 1967).

Indigenous milk lipase is of little importance in cheese made from pasteurized milk. But thermo-resistant lipases from *Pseudomonas* and *Achromobacteria* can still
produce free fatty acids in cheese. Lactic acid bacteria have little lipolytic activity (Dulley and Grieve, 1974). Therefore they may not be the reason behind lipolysis.
2.2 Function of fat and effect of fat reduction in cheese

2.2.1 Reasons behind fat reduction

34 million of American adults between the ages of 20 and 74 are obese, with the high rates observed among the low-income and minority groups. While experts are not in complete agreement about the exact relationship between obesity and health, it is accepted that obesity is an independent risk factor for coronary heart disease, hypertension, colon cancer and adult onset diabetes (Kantor, 1990).

The implementation of U.S. Food and Drug Administration regulations concerning nutritional labeling of commercial food products has helped consumer to categorize foods by fundamental components such as fat, protein and carbohydrate. Fats can promote obesity because of their high caloric content. Fats contain around 9cal/g as compared to proteins and carbohydrates that contain 4cal/g. New labels also enabled the consumer to immediately see calories from fat and amount of saturated fat in the product (Honig, 1993). In addition to that, use of identifications such as “low-fat” and “reduced-fat” gives more information about the product. Low-fat means fat reduction of 50% or more, such that the product contains less than 3g of fat per reference amount which is 28g for cheese. Reduced-fat means fat content of the product is decreased by 25% or more from its original reference fat content (Drake and Swanson, 1995). Consumer awareness regarding sources and amount of fat in the product challenges food producers to develop modified versions of standard foods (Bullens, 1994). 93 million Americans consume low-calorie foods and beverages. Dairy products are only second to beverages
in terms of popularity. 70% of consumers polled in a study chose beverages and 65%
chose cheese, yogurt and sour cream (Barr, 1990).

Fat is a very functional component of cheese. It substantially contributes to
flavor, texture and appearance. Therefore, fat reduction is a very difficult task -level of
difficulty depending on the percent reduction- when it is aimed to generate products
similar to their full-fat counterparts. Low- and reduced-fat cheeses are usually identified
as bland, firm, rubbery and odd in color. Bitterness and off-flavors are also observed
(Degouy, 1993).

2.2.2 Flavor

Milk fat is a source and carrier of flavors in cheese (Olson and Johnson, 1990a).
Its principal attribute is high concentration of short chain fatty acids particularly butyric,
caproic, caprylic, capric and lauric acids with 4 to 12 carbons. Short chain fatty acids
make 21% of the fatty acid residues in milk fat triglycerides (Jeon,1994). Katsiari and
Voutsinas (1994a) report that the rate of lipolysis in Feta cheese decreases as the amount
of milk fat decreased.

Low-fat cheeses have higher moisture content. Replacing the fat with water
changes the microenvironment of the cheese which effects the kinetics of the system.
Change in bacterial growth -including that of the non-starter bacteria - and enzyme
activity might result in bitter, brothy flavor notes and excessive acidity (Olson and
Johnson, 1990b). It was suggested that increased moisture content further emphasize the
off-flavors (Degouy, 1993).
2.2.3 Texture

Fat reduction yielded harder cheese with higher cohesiveness and adhesiveness (Tunick et al, 1993; Katsiari and Voutsinas, 1994b; Banks et al, 1989). Mistry and Anderson (1993) concluded that increased firmness of low-fat cheese is a result of changed microstructure. Full-fat cheeses were characterized by a protein matrix interspersed with fat globules of varying shape and size. Low-fat cheeses had fewer fat globules which were usually smaller than in the full-fat cheese.

Feta and related cheeses have casein micelles identifiable and separate from each other rather than forming a continuous protein matrix (Glaser et al, 1979; Omar, 1985). They also exhibit increased hardness when fat content was reduced (Katsiari and Voutsinas, 1994a; El-Neshawy et al, 1988).
2.3 Low- and Reduced-Fat Cheese Technology

The demand for reduced- and low-fat cheeses increased dramatically during last years. However, the reduction of fat adversely affects both flavor and texture. Current research is focused on the improvement of the sensory properties of fat reduced cheeses. While achieving the lowest possible fat content is the objective, price constraints should also be taken into consideration. This is especially important in cheese manufacture where lowering fat content in the milk decreases the yields (Bullens, 1994). Three strategies are employed for the purpose of improving fat reduced cheeses: use of fat replacers, procedure modifications, use of adjunct cultures. So far, none of these methods were fully able to achieve replacing all functions of fat. Nonetheless, research using these three strategies managed to improve fat reduced cheeses considerably (Olson and Johnson, 1990a; Drake and Swanson, 1995).

2.3.1 Fat replacers

Fat replacers are grouped into two categories: fat mimetics and fat substitutes. Fat mimetics are mainly carbohydrates or proteins with polar and water-soluble nature. They primarily act through binding extra water that helps creating a sense of creaminess and lubricity similar to the full-fat products. However, they can not replace the nonpolar functional properties of fat such as flavor carrying capacity (Lucca and Tepper, 1994). On the other hand, fat substitutes are nonpolar compounds that have better capacity of imitating fat.
2.3.1.1 Fat mimetics

Fat mimetics are derived from proteins or carbohydrates that are currently approved by U.S. Food and Drug Administration. They are water-soluble compounds with polar groups. Other than improving texture, fat mimetics also increase yield of reduced- and low-fat cheeses (Olson and Johnson, 1990b). There are numerous commercially available fat mimetics which are reviewed in detail (Roller and Jones, 1996; Lucca and Tepper, 1994; Giese, 1996; Dziezak, 1989; Gorski, 1995; Haumann, 1986). A very comprehensive listing that consists of chemical composition, manufacturer, concentration used, special features and applications of almost 300 fat replacers is available as an appendix in *Handbook of Fat Replacers* (Roller and Jones, 1996).

Brummel and Lee (1990) studied the effects of adding carrageenan, pectin and guar gum to the cheese spreads and concluded that the texture of the spreads improved significantly however the dilute flavor of the reduced- and low-fat cheese spreads containing gums were less desirable as compared to the full-fat control. Studies of the addition of denatured whey protein concentrate to low-fat processed cheese demonstrated that denatured whey protein improved the sensory characteristics of cheese (Salem et al., 1987).

Addition of lecithin in the levels of 0.2 and 0.5% improved texture of Cheddar cheese significantly (Drake et al., 1996b). Both sensory analysis and hardness data proved the similarity of texture to the control full-fat cheese. The creation of a finely
dispersed network of fat globules with a concurrent increase in moisture could improve reduced fat cheese texture. Yields of reduced-fat cheese increased consequently. However, it was reported that improvement of texture was accompanied by some foreign flavors described as soapy or petroleum-like which were more notably perceived at 0.5% level. Sohn (1996) who also tested use of 0.5% Lecithin in Swiss cheese did not report any foreign flavor associated with the use of lecithin. Hardness of low-fat cheese containing lecithin was comparable to full-fat control. Cakmakci and Kurt (1993) also observed increased yield due to addition of lecithin at 0.05% level to Beyaz Peynir cheese. Sensory analysis was not performed.

In another study, Drake et al. (1996a) tested use of Dairy Lo™, Novagel™ and ALACO PALST™ in low-fat Cheddar cheese. The low-fat cheeses containing ALACO PALST™, a protein based fat mimetic, received the highest sensory scores for texture. However, it imparted a brownish hue to the cheese which was not desirable. Low-fat cheeses containing fat-mimetics were found to be less rubbery by the sensory panel. Scanning electron micrographs showed that cheeses containing fat mimetics exhibited a smoother protein matrix and a more finely dispersed fat network compared to low-fat cheese. Bullens (1994) reported that the addition of Novagel™ to cheese milk improved moisture retention and texture of low-fat Cheddar cheese. However, the research also revealed that the increased moisture leads to a diluted flavor profile. Incorporation of Lactobacillus casei as an adjunct culture did not help amending lacked Cheddar flavor. It is proposed that microcrystalline cellulose particles in Novagel™ incorporated into the cheese milk acts as physical barrier between the casein network (Bullens et al., 1994).
Turin and Bonami (1995) studied the effects of addition of Simplesse™ to Caciotta cheese. The addition of Simplesse™ to the Caciotta cheese decreased rather than increased available hydrophobic sites at the fat/water interface hence it did not fully substitute for fat in aroma and flavor binding. Proteolysis was monitored electrophoreically where enhanced proteolysis was observed for fat-substituted cheese. The cheeses were not sensory tested.

2.3.1.2 Fat Substitutes

Fat substitutes are compounds designed to replace fat on a weight-by-weight basis, usually having a similar chemical structure to fat but resistant to hydrolysis by digestive enzymes (Roller and Jones, 1996). They are either structured lipids or synthetic compounds that provide reduced caloric value or the remarkable advantage of contributing no calories. Olesra™ (marketed under the trade name Olean™) is the only true synthetic fat substitute which is approved by U.S. Food and Drug Administration for use in savory snacks, January 24, 1996. It is formed by reacting sugar with fatty acids. Because of the spatial configuration of these fatty acids around the inner core of the sugar molecule, it can not be digested (Akoh, 1996; Giese, 1996).

Sucrose polyesters with a variety of melting profiles and properties can be synthesized from different fatty acids. Drake et al. (1994) synthesized sucrose polyesters with milk-fat properties from milk-fat fatty acids and successfully incorporated in reduced- and low-fat natural Cheddar cheese. The hardness of cheeses containing sucrose polyesters were not significantly different from that of full-fat control cheeses. However, in every level of substitution the sensory panel were able to differentiate between the
control and the fat-replaced cheese. In addition, the color of the cheeses containing sucrose polyester were found to be different from the control by using color measurement devices. Only after a certain level of substitution, the difference was visible to the eye.

Consumption of sucrose polyesters must be in moderation to avoid possible side effects such as diarrhea. A patent has been obtained that describes use of polyol fatty acid esters and microfibrilated polysaccharides together to prevent laxative effects (Procter & Gamble, 1990). Another problem associated with sucrose polyesters is described as compensatory response. In a recent research by University of Leeds and Unilever Research Laboratium in The Netherlands, it was shown that the subjects whose fat consumption was reduced by 30%, eat significantly more in order to suppress the greater perception of hunger (Anon, 1996).

Esterified propoxylated glycerol by Arco Chemical Co. (Newtown Square, PA, USA) and dialkyl dihexadecylmalonate by Frito Lay Inc. (Dallas, TX, USA) are among synthetic fat substitutes that are under development (Roller and Jones, 1996). However, no non-caloric fat substitute has been approved to be used in cheese yet. On the contrary, some of the reduced-calorie fat substitutes are approved. One group of reduced-calorie fat substitutes that are currently available are the structured lipids. Structured lipids are triacylglycerols consisting of medium-chain and long-chain fatty acids. They provide less calories (5-7 kcal/g) as compared to regular triacylglycerols since they are digested and oxidized differently. Cheeses with flavor and texture that were comparable to those of full-fat control cheeses were successfully made from medium chain triacylglycerols
(Babayan and Rosenau, 1991). Caprenin™ (Procter & Gamble, Cincinnati, OH, USA) and Salatrim™ (Nabisco Foods Group, East Hanover, NJ, USA) are among commercially available structured lipids.

Fat substitutes can achieve replacing the texture and mouthfeel of fat. Since fat substitutes are non-polar, like fat, they exhibit the physical characteristics and flavor carrying capacity of fat also. Nevertheless, fat acts as flavor precursor in cheese (Jeon, 1994). Therefore, methods to provide flavor compounds originated from fat are needed to fully impart the functionality of fat. A method of imparting flavor by fat globules with elevated levels of flavor compounds was patented. According to this method, as little fat as 0.2% is enough to deliver flavor in a number of products including cheese (Singer et al., 1990).

2.3.2 Make-procedure changes

Procedure changes are the most economically efficient means of improving the quality of fat reduced cheeses. Most make-procedure changes aim to increase the moisture content. Increased moisture content provide a sense of creaminess and lubricity. Modifications, such as lowering the cook temperature, cooking for a shorter time and cold water washing increase moisture retention in the curd. Since fat reduced cheeses contain more moisture, bacteria added as starter culture can multiply to larger populations as compared to full-fat cheeses. This may lead to production of excess acidity and bitter flavors. Slow rate of acid development during processing is important for preventing increased rate of acid production. Procedure changes that serve controlling acid production are: using a small amount of lactic acid starter culture, using special
strains of cultures, decreasing ripening time and washing the curd (Olson and Johnson, 1990a)

Research for this purpose started as early as 1957. Irvine et al. studied the effects of decreasing the cook temperature and washing the curd to optimize the flavor of low-fat processed Cheddar cheese. Hargrove et al. (1996) recommended a combination of decreasing starter-ripening time and curd washing to improve the flavor of low-fat Cheddar cheese. Drake et al. (1995) compared several make-procedure changes including: decreased cook temperature, decreased ripe time, decreased starter, homogenization, and curd washing. They concluded that washing the curd with 35°C water for 5 minutes produced cheese with higher sensory quality as compared to low-fat counterparts. Other modifications did not have any significant effect.

In another study, it was concluded that reduced-fat Cheddar made using decreased cook temperature and decreased stir out time were comparable to full-fat cheese in sensory tests. However, when the fat was further reduced, low-fat cheddar cheese was firmer and exhibited low flavor levels which was made using decreased cook temperature and decreased stir out time (Banks et al., 1989). Elevation of pasteurization temperature in order to increase the denaturation of whey proteins and increase the water binding capacity in the cheese was recommended to improve the melting properties of mozzarella cheese (Merrill et al., 1994).

Homogenization of milk or cream is recommended for low-fat Cheddar and baby Swiss cheese (Metzger and Mistry, 1994; Sohn, 1996). Sohn concluded that homogenization of cream before standardization at 2500 psia resulted cheese with almost
the same hardness as full-fat cheese which is produced from unhomogenized milk. Drake et al. (1995) reported no improvement through the employment of homogenization of whole milk prior to standardization with skim milk. Homogenization enhances water entrapment and even distribution of fat globules throughout the protein matrix (Emmons et al., 1980; Jana and Upadhyay, 1992). Homogenization has a fat extending effect on fat reduced cheese.

The addition of milk solids was suggested as a way of increasing the buffering capacity within the cheese to control the acidity. Anderson et al. (1993) reported that the use of condensed skim milk with 15.40% total solids improved the flavor of reduced-fat Cheddar cheese.

Ultrafiltration of cheese milk was proposed to improve the flavor and texture of low-fat cheese by decreasing the amount of lactose available for the microorganisms and thus controlling the rate of acid development. It was also expected that whey proteins kept in the ultrafiltered milk would help binding extra water. McGregor and White (1990) reported no improvement of either flavor or texture through the use of ultrafiltration for low-fat Cheddar cheese although the moisture content was increased as expected.

2.3.3 Adjunct cultures

Development of bitterness in cheese considered to be due to the accumulation of bitter peptides during ripening. This might be the result of proteolytic or endopeptidolytic activity being too high as a result of higher moisture content, or the activities of other peptidases that are needed for the hydrolysis of bitter peptides being
too low (Ardo et al., 1989). Adjunct cultures can improve the flavor of fat reduced cheeses through increased proteolysis, specifically aminopepdidase activity, that reduces bitterness and increase the concentrations of desirable flavor peptides and precursors of flavor volatiles. (Thunell, 1994)

A heat treated culture of *Lactobacillus helveticus* is tested by Ardo et al. (1989). It was reported that bitterness was markedly reduced and flavor was enhanced as compared to the low-fat control in Swedish hard cheese. *Micrococcus* sp. LL3 not only increased proteolysis and produced intense flavor in reduced-fat Cheddar cheese also improved the texture (Lee et al., 1992). *Brevibacterium* species are also mentioned as adjunct cultures that improved flavor and texture (Weimer, 1992).

A number of adjunct cultures are commercially available from some major culture houses. Christian Hansen’s Laboratorium offers two DVS adjunct cultures, CR-210 and CR-213, which are non acid producers with high aminopepdidolytic activity (Vindfeldt, 1993). An optimum salt content of 1.8% and use of adjunct culture, CR-213, were reported to enhance the flavor of low-fat cheddar cheese (Banks et al., 1993). However, low-fat cheeses were significantly different from full-fat cheese in terms of texture.

Glutatione is a naturally occurring peptide in milk which is found to increase ripening rate significantly in Cheddar cheese. Some strains have the ability to readily transport glutatione into their cells. These strains are suggested to be better flavor producers. More research is needed to determine their flavor enhancing potential and mechanism (Thunell, 1994).
3. MATERIALS AND METHODS

3.1. Fat mimetics and fat levels

3.1.1. Preliminary research

Among all food products, cheese is one of the most challenging to produce a low-fat version (Drake and Swanson, 1995). Fat mimetics used for preliminary research were chosen among commercially available fat mimetics which were suggested by their manufacturers to be used in low-fat cheese. Some fat mimetics were eliminated at the very beginning such as whey protein concentrates which would not be retained in the casein matrix and tend to be in the whey phase. Some other fat mimetics required high heat treatment to be fully activated which Feta cheese manufacturing procedure does not involve.

Six fat mimetics were tested in the preliminary study (Table 3.1). N-Lite™ CL was obtained from National Starch and Chemical Co. (NJ, USA). It contains modified tapioca starch. It is recommended for dairy systems where high degree of lubricity and long term stability desired. It can be used in systems where temperature of 65°C or higher achieved. TrimChoice®-OC and Instant Stellar® were obtained from A.E. Staley Manufacturing Co., (IL, USA). TrimChoice®-OC is hydrolyzed oat and corn flour. Its main advantage is containing beta-glucan soluble fiber. It is produced by enzyme
treatment. Instant Stellar® is produced by controlled acid hydrolysis. It is not chemically modified. Yelkinol® P PE-040 (ADM Co., IL, USA) is composed of lecithins. It is 97% phospholipid. Simplesse® 100 (Kelco, Co., CA, USA) is microparticulated whey protein. It has deformable spheroidal particles that act as fracture points in the protein matrix, similar to the fat globules they replace. Novagel® NC-200 (FMC Co., NJ, USA) is composed of microcrystalline cellulose and gums that help dispersion. Similar to Simplesse®, it functions through physically imitating fat globules.

<table>
<thead>
<tr>
<th>Commercial Name</th>
<th>Composition</th>
<th>(%) Usage Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Lite™ CL</td>
<td>Modified Tapioca Starch</td>
<td>3</td>
</tr>
<tr>
<td>TrimChoice®-OC</td>
<td>Hydrolized Oat and Corn Flour</td>
<td>1.5</td>
</tr>
<tr>
<td>Instant Stellar®</td>
<td>Modified Corn Starch</td>
<td>1.5</td>
</tr>
<tr>
<td>Yelkinol® P PE-040</td>
<td>Lecithin</td>
<td>0.4</td>
</tr>
<tr>
<td>Simplesse® 100</td>
<td>Microparticulated Whey Protein</td>
<td>3</td>
</tr>
<tr>
<td>Novagel® NC-200</td>
<td>Microcrystalline cellulose, Carrageenan, Guar Gum</td>
<td>0.125</td>
</tr>
</tbody>
</table>

Table 3.1 Commercial name, composition and usage level of fat mimetics used for preliminary research.

All fat mimetics were used at the suggested levels by their manufacturers. Cheese was manufactured from milk that contains 1.5% milk fat. Also cheese without fat mimetics from milk at the same fat level was made. Control cheese was manufactured with milk that contains 3.25% fat without the addition of any fat mimetic. All cheese were prepared in duplicates.
3.1.2. Final research

Among the fat mimetics tested in the preliminary research, modified tapioca starch and lecithin were selected to be used in final research. The basis of this selection was explained in results and discussion part. All cheeses were manufactured in duplicates. Modified tapioca starch was added at 1% level and lecithin was added 0.2% level. Cheese with a combination of these fat mimetics were also manufactured with the addition of 0.5% starch and 0.1% lecithin.

<table>
<thead>
<tr>
<th>Fat Levels (in milk)</th>
<th>w/o fat mimic</th>
<th>Modified Tapioca starch (1%)</th>
<th>Modified Tapioca starch (0.5%) + Lecithin (0.1%)</th>
<th>Lecithin (0.3%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.25%</td>
<td>F</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.20%</td>
<td>R</td>
<td>TR</td>
<td>CR</td>
<td>LR</td>
</tr>
<tr>
<td>1.65%</td>
<td>L</td>
<td>TL</td>
<td>CL</td>
<td>LL</td>
</tr>
</tbody>
</table>

F: Full-fat, with no fat mimetics  
R: Reduced-fat, with no fat mimetic  
TR: Reduced-fat, modified tapioca starch  
L: Low-fat, with no fat mimetics  
TL: Low-fat, modified tapioca starch  
CR: Reduced-fat, modified tapioca starch + lecithin  
CL: Low-fat, modified tapioca starch + lecithin  
LR: Reduced-fat, with lecithin  
LL: Low-fat, with lecithin

Table 3.2 Cheeses prepared for final research.

In this part of the study, cheese was prepared from milk at three different fat levels. Control cheese (F) was prepared from whole milk with 3.25% fat. The fat level in whole milk was decreased 33% for a final fat content of 2.20%. Cheese prepared from this milk is called reduced-fat (R). The fat level in the milk was further reduced 50% for
a final fat content of 1.65%. The resulting cheese was identified as low-fat (L). The cheeses produced in this study, The cheeses were labeled as full-fat, reduced-fat and low-fat for categorization purposes only and may or may not satisfy the legal definitions of these terms.

3.2. Cheese making

Samples of Feta cheese were produced following method reported by Prasad (1995) with some modifications. Whey was not employed for preparing brine in the present research. This modification was done to discourage mold and yeast growth which might take place due to the rich nutrient concentration of whey. Another modification involves increasing coagulation time 30 min to 60 min. This modification was made since rennet with different activity was used.

3.2.1. Equipment cleaning and sanitation

Cheese vats, knives, moulds and all other appliances were washed, rinsed and then sanitized using 100 ppm chlorine solution. The solution was prepared using a commercial hypochloride solution, Dibac® (Diversity Corp., MI USA). It was sprayed through an air hose. Hands were washed and sanitized before contacting milk, curd or cheese.

3.2.2. Standardization

Fresh milk was supplied from the dairy farm of The Ohio State University. Milk was processed at the pilot plant of Department of Food Science and Technology, The Ohio State University. It was transferred to the pilot plant in milk cans which hold approximately 80 liters of milk at approximately 5 C.
Milk was separated into cream and skim milk by using an Alfa-Laval, Model 29-AE/60 separator. Separation process yielded skim milk with 0.01% milk fat. Amount of fat in the cream changed depending on the speed of rotation and separation time. Fat content of skim milk and cream was determined using Babcock Method (Marshall, 1992). Then, the milk was standardized to the desired fat content. After standardization, fat content was determined again to make sure that desired fat level was attained. If the level of fat was less than or more than intended, the error was corrected by adding cream or skim milk, respectively. After this correction, fat content was checked again. Then, amount of fat mimetic needed was calculated and added slowly to the milk accompanied with vigorous mixing until complete dispersion.

3.2.3. Homogenization and Pasteurization

Standardized milk was preheated to 60°C using plate heat exchangers and passed through homogenizer (Haenni, Co., Stuttgart, Germany) which was operated at 100 kg (one stage). Milk was pasteurized at 72°C for 30 sec followed homogenization. Pasteurized milk was cooled to 20-30°C and collected into clean and sanitized milk cans before it was transferred to cheese vats.

3.2.4. Curdling

Four vats of cheese were produced with 25 liter of milk each. Milk was weighed into sanitized containers and transferred to 22x22x50cm³ cheese vats. Temperature of steam heated vats were manually adjusted to 33°C. As soon as the temperature of milk was raised to 32°C, 0.015% (w/w) starter culture was added. Starter culture was composed of Lactococcus lactis ssp. lactis and Lactococcus lactis ssp. cremoris (Frozen
DVS R604-Chr. Hansen Inc.). 45 min of ripening time was allowed before the addition of rennet (Chymogen, Chr. Hansen Inc.) at a rate of 0.02%. Calf and kid lipases (Chr. Hansen Inc.) were also incorporated at this step at a rate of 0.0025% each. Titratable acidity and pH were measured frequently to monitor rate of acid development.

### 3.2.5. Curd cutting

After the curd reached desired consistency, curd was cut with 0.06 cm knives first with the horizontal knife then with the vertical one. Attention was given not to disturb the curd extensively and cut with a steady and continuous motion. After cutting, it was allowed to heal for 10-15 min in order to increase its mechanical strength against rupturing during transferring to the molds.

### 3.2.6. Molding

The curd was transferred to moulds (30x15x15 cm³) which were covered with cheese cloth. More curd was added to the moulds as whey drained out. Transferring to the moulds was completed in 1 hr. Moulds were turned upside down once every hour, in order to facilitate drainage. After 5 hr drainage at ambient temperature, the moulds were transferred to incubator at 22°C for 24 hr.

### 3.2.7. Brining

At the end of incubation, fresh cheese blocks were taken out of the moulds and cheese clothes. The blocks were cut into two parts, each of them having the approximate dimensions of 7x15x15cm³. The cheese was placed in 20 liter containers and 18% brine (for preliminary research) / 15% brine (for final research) was added to a level that it
covers the cheese blocks. pH of the brine was adjusted to 4.7 using 5% lactic acid solution. The containers were refrigerated at 5°C during the ripening period of 45 days.
Milk
Cow’s milk from OSU Dairy Farm

Standardization
3.25, 2.20 and 1.65% fat in the milk

Adding the fat mimetic

Homogenization
100 kg/cm² at 60°C

Pasteurization
at 72°C for 30 sec

Transfer milk to the vats. Adjust temperature to 32°C

Inoculation
Add DVS R604 starter culture at rate of 0.015%

Rennet and Lipase Addition
After 45 min coagulation with 0.02% rennet
Add calf and kid lipase 0.0025% each

Cutting
After about 60 minutes curd coagulated and was cut into 1 cm³ cubes

Healing
Leave for 10-15 minutes at 32°C

Molding
Transfer curd into perforated molds and turn molds every hour 5 times
Incubate for 34 hr at 22°C

Cut cheese into blocks of 7x15x15 cm³

Brining
Transfer to brine with 18% or 15% salt and adjust pH to 4.7

Ripening
Ripen for 45 days at 5°C

Figure 3.1 Flow chart for manufacture of Feta cheese
3.3. Analysis

3.3.1. Sample Collection and Preparation

Samples were collected and prepared just before the analyses. The outside sides of cheese blocks were cut about one cm and discarded. A slice of cheese was taken and grated. In all analyses, grated cheese were used except for texture analysis, scanning electron microscopy and sensory analysis. All analysis were done in triplicate unless otherwise is stated. The cheese samples were approximately 45 day old during analysis.

3.3.2. Chemical Analysis

Chemical analysis was conducted according to the standard methods described by Marshall (1992). The fat content was determined by Babcock method. Protein content was determined by micro-Kjeldahl method using Kjeltech™ Auto Sampler System (Tecator, Perstorp Analytical Co., Sweden). Sodium chloride content was determined using Volhard method. Moisture content was determined by heating to constant weight. pH was measured by a Fisher Accumet® pH meter (Model 630, Fisher Scientific, PA, USA).

3.3.3. Texture Analysis

Hardness of the samples were measured in Newton by using a Texture Analyzer® TA-XT2 (Texture Technologies Corp., NY, USA). After discarding 1cm thick slabs from all dimensions, 4x4x2 cm³ samples were cut carefully from cheese blocks. The cheese cubes were wrapped in polyethylene film and kept in refrigerator for 30 min so that all samples will equilibrate to 5°C. A cylindrical probe with a diameter of 1.0 cm and height of 3.1 cm was used to obtain a plot of force vs. time with 200 point per second data
acquisition rate. The probe moved 0.5 cm/s speed and penetrated the sample 1 cm, i.e., 50% compression was applied. Hardness is the maximum force needed to penetrate the sample. Texture profile analysis could not be performed on Feta cheese due to its crumbly nature.

3.3.4. Polyacrylamide gel electrophoresis

Electrophoresis was run according to a method described by Davis (1964) which was modified by Antunes (1996). Samples were prepared by dissolving defatted cheese in stacking gel buffer in a way that there would be 36 mg of protein in 3 ml of stacking gel buffer. Samples were kept in water bath at 37°C for 1 hr and 45 min after adding β-mercaptoethanol. Once prepared the samples were kept in freezer.

The running gel and stacking gel solutions contained 9.0% and 3.1% acrylamide, respectively. After the gels were formed, 5 µl sample was applied to each well. Therefore, 60µg of protein were injected to each of the wells. α- and β-caseins (Sigma Chemical, Co.) were applied as standards. Gels were run using Biorad® Mini Protean II System (Biorad Laboratories, Inc., CA, USA) at 220 V where current intensity were adjusted to 60 amp/gel. Gels were stained with Coomassie blue in 40%(v/v) methanol and 10%(v/v) acetic acid for 1 hr. Destaining in 4:1:5 methanol : acetic acid : water followed staining with several changes.

Gels were analyzed quantitatively by using a Biorad model GS-700 Imaging Densitometer interfaced with a computer running Molecular Analyst® software, version 1.4.1. (Biorad Laboratories, CA, USA). After the gels were scanned, optical density vs.
distance was plotted where area under the curve represents the amount of a certain protein fraction.

![Graph showing β-casein and α-casein peaks](image)

Figure 3.2. Graph obtained from Molecular Analyst® software.

3.3.5. Sensory analysis

Organoleptic evaluation of cheese samples were conducted by two different panels. First panel (Group 1) was composed of four members experienced with tasting American dairy products and familiar with Feta cheese. The second panel (Group 2) was formed by sixteen members that consumes routinely Feta type cheese produced from cow’s milk. Cheeses were evaluated at approximately 45th day of ripening. According to IDF (1987), samples for sensory analysis should be taken at the earliest suitable age. Minimum ripening time for Feta cheese is between 1-2 months (Mondal et al, 1989; Anfantakis, 1986). Panel members evaluated cheese for flavor, texture and overall acceptance by using a nine point structured scale, with 1 being the worst and 9 being the
best quality. Panel members were also instructed to report any defects in flavor (e.g. acid, bitter, flat, foreign, rancid, and salty) and texture (gritty, hard, pasty and soft). These terms for defects were chosen from IDF (1987) sensory guide for cheese. A copy of sensory test sheet is provided in Appendix C. The panelists were required to evaluate five samples at a time. They were provided with water and unsalted crackers to clean their palates between samples. The samples which were given three digit random numbers presented as 0.7x3x4 cm³ pieces.

3.3.6. Scanning Electron Microscopy

Microstructure of cheeses were examined by using scanning electron microscopy. Method described by Drake et al (1996b) was used to prepare the samples. Cheeses were cut into 4-5 mm cubes and fixed overnight in 2.8% glutaraldehyde solution. After fixation, cheese samples were washed with distilled water for 10 min three times, 30 min and 60 min; and dehydrated in a graded (20%, 40%, 60%, 80%, 95%, 100%) ethanol series for a time of 2 hr each. The samples then defatted three 15 min changes of chloroform. After this point samples were always kept refrigerated totally covered with ethanol until they were freeze fractured in liquid nitrogen. Freeze fractured samples were critical point dried in a Pelco-CPD-2 critical point drier (Ted Pella, Co., Redding, CA, USA). The samples were mounted on stubs and sputter coated with 30 nm gold-palladium. Scanning electron micrographs were taken with a Jeol, JSM-820, scanning electron microscope using Polaroid type 55 film (Polaroid Co.).
3.4. Statistical Design and Data Analysis

A 3x4 unbalanced factorial design was employed. Data analysis was conducted using statistical analysis software Minitab® Release 11.1. General Linear Model was used in order to see if fat level and fat mimetic type cause any difference (p<0.05) in means of response variables: moisture, yield, fat, protein, pH, salt, hardness and sensory properties. If there was a difference, Fisher's least significant difference (LSD) comparison test was applied in order to see which means were different.
4. RESULTS AND DISCUSSION

4.1. Preliminary research

Low-fat cheese without the addition of any replacer was significantly different from full-fat control in hardness, protein and moisture content (p<0.05) (Table 4.1). The hardness of cheese containing modified tapioca starch, hydrolyzed oat flour and corn flour, and lecithin were not significantly different from full-fat control.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Hardness (N)</th>
<th>% Moisture</th>
<th>% Protein</th>
<th>%Fat</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full-fat control</td>
<td>59.56a</td>
<td>57.39a</td>
<td>14.64a</td>
<td>20.6a</td>
<td>4.50a</td>
</tr>
<tr>
<td>Low-fat</td>
<td>93.44b</td>
<td>60.93b</td>
<td>18.43b</td>
<td>10.5b</td>
<td>4.59b</td>
</tr>
<tr>
<td>Modified Tapioca Starch</td>
<td>49.58c</td>
<td>63.02d</td>
<td>13.65c</td>
<td>8.08c</td>
<td>4.55c</td>
</tr>
<tr>
<td>Hydrolized Oat and Corn Flour</td>
<td>60.62a</td>
<td>62.23a</td>
<td>17.16a</td>
<td>8.25ad</td>
<td>4.59a</td>
</tr>
<tr>
<td>Modified Corn Starch</td>
<td>22.93c</td>
<td>65.96c</td>
<td>13.66c</td>
<td>7.08d</td>
<td>4.63d</td>
</tr>
<tr>
<td>Lecithin</td>
<td>61.70a</td>
<td>58.62ac</td>
<td>17.04c</td>
<td>11.25b</td>
<td>4.55a</td>
</tr>
<tr>
<td>Microcrystalline Cellulose, Carrageenan, Guar Gum</td>
<td>81.50a</td>
<td>59.94bc</td>
<td>17.36bc</td>
<td>10.58b</td>
<td>4.51a</td>
</tr>
<tr>
<td>Microparticulated Whey Protein</td>
<td>93.42b</td>
<td>58.39bc</td>
<td>20.69d</td>
<td>8.58c</td>
<td>4.62a</td>
</tr>
</tbody>
</table>

a,b,c,d: Different letters following means in a column represent significant differences (p<0.05).

Table 4.1 Effects of fat mimetics on hardness and chemical properties of Feta cheese. Cheese samples were produced in duplicate and analysis were performed in triplicate.
Cheese with starch based mimetics had higher moisture than the rest of the samples (Table 4.1). Modified tapioca starch and modified corn starch had the lowest protein content which may be a result of the dilution effect of their high moisture content. Their fat content is also low which may also be caused by the same factor.

The use of microcrystalline cellulose or microparticulated whey protein did not produce a cheese significantly softer than the low-fat cheese without the addition of any mimetic (Table 4.1). The sample with microparticulated whey protein had the highest protein content which is a result of its being a protein based fat mimetic. Lecithin imparted softness to the cheese acting as a "fat extender", it helped better distribution of the fat in the low-fat product. Lecithin having hydrophobic and hydrophilic sites is able to interact with fat, protein and moisture. Drake et. al. (1996) reported that they observed round web like strands that may help mimicking fat in low-fat Cheddar where lecithin was used as a fat mimetic.

The cheeses were tasted by a in-house panel but no sensory analysis was performed. The samples with high moisture content had generally better mouth coating characteristics except the one that contains modified corn starch which was gritty. This might be due to the particle size. Particles having a diameter greater than 8μm have a gritty mouthfeel (Degouy, 1993). The cheese with modified tapioca starch was better in terms of mouthfeel than the one that contained hydrolyzed oat and corn flour.

Based on the preliminary results, modified tapioca starch and lecithin were selected for the final study.
4.2. Final research

4.2.1. Effect of fat level and fat mimetics on compositional properties

4.2.1.1 Moisture

Results shown in Figure 4.1 indicate that as the fat content decreased moisture content of the cheese increased significantly (p<0.05) in all treatments except samples containing starch. Katsiari and Voutsinas (1994a) studied Feta cheese from milk at four different levels and measured fat, moisture and other physicochemical changes. The authors found that the moisture content of Feta cheese was inversely related to the fat content of cheese milk. Effect of fat reduction was the same on other cheese varieties as well such as Cheddar (Bullens et al, 1994), processed cheese (Brummel and Lee, 1990), and Cephalotyre cheeses (El-Neshawy et al, 1986). Reduced and low-fat cheese samples prepared with tapioca starch were not significantly different. This may be due to high water absorption capacity of starch (Tustaguzov, 1985) that masked the effect of fat on moisture.

Fat mimicetic type significantly effected moisture content of the cheese (Figure 4.1). Cheese samples containing modified tapioca starch had the highest moisture content of about 67% (Figure, 4.1). Starches increase moisture content entrapping water as a result of gelatinization (Hermansson, 1985). Additionally, tapioca starch is known for producing very smooth gels which makes it suitable as a fat mimetic (Anon, 1993). Cheese products made with lecithin and a combination of lecithin and tapioca starch were not significantly different in moisture content (Figure 4.1). Lecithin was tested in
reduced-fat Cheddar cheese. It was found to increase moisture content (Drake et al, 1996b). Lecithin may aid in incorporation of water in reduced fat cheeses.

![Moisture content of full-fat, reduced-fat and low-fat Feta cheese samples with tapioca starch, lecithin and their combination. Error bars represents standard deviations. Cheese samples were produced in duplicate and analysis were performed in triplicate.]

F: Full-fat, with no fat mimetics  
R: Reduced-fat, with no fat mimetic  
L: Low-fat, with no fat mimetics  
TL: Low-fat, modified tapioca starch  
TR: Reduced-fat, modified tapioca starch  
CR: Reduced-fat, modified tapioca starch + lecithin  
CL: Low-fat, modified tapioca starch + lecithin  
LR: Reduced-fat, with lecithin  
LL: Low-fat, with lecithin

4.2.1.2 Yield

Yield was calculated on wet basis. The fat level affected yield values significantly (p<0.05) (Figure 4.2). Yield of low-fat cheese was significantly lower from full-fat and reduced-fat cheeses. However, yield of reduced-fat cheese was not significantly different from full-fat cheese. This might be due to some losses of curd during transferring to the
molds. An overall reduction in the cheese yield is inevitable in the production of cheese from milk of low fat content, since the sum of the casein and fat content of the milk are the principal components which determine cheese yield. (Bullens, 1994). Accordingly, lowest yield values belongs to low and reduced-fat cheeses 10.79 and 11.26%, respectively. Yields per total solids showed the same trend that supported the effect of fat on yield.

![Graph showing fat contents of full-fat, reduced-fat and low-fat Feta cheeses samples with tapioca starch, lecithin and their combination. Error bars represents standard deviations. Cheese samples were produced in duplicate and analysis were performed in triplicate.](image)

F: Full-fat, with no fat mimetics
L: Low-fat, with no fat mimetics
TR: Reduced-fat, modified tapioca starch
CR: Reduced-fat, modified tapioca starch + lecithin
LR: Reduced-fat, with lecithin
R: Reduced-fat, with no fat mimetic
TL: Low-fat, modified tapioca starch
CL: Low-fat, modified tapioca starch + lecithin
LL: Low-fat, with lecithin

Figure 4.2 Fat contents of full-fat, reduced-fat and low-fat Feta cheeses samples with tapioca starch, lecithin and their combination. Error bars represents standard deviations. Cheese samples were produced in duplicate and analysis were performed in triplicate.
Fat mimetics had significant effect on yield values (Figure 4.3). Addition of modified tapioca starch resulted highest yield of 14.47%. At this time, the increase yield may be dependent on moisture retaining. Yields of cheeses with lecithin and combination of lecithin and starch were also higher than the cheeses that contain no fat mimic but not significantly. Lecithin was found to increase yield by increasing moisture retention in Cheddar cheese using at 0.2 and 0.5% level (Drake et al, 1996b). Cakmakci and Kurt (1993) observed increased yield due to the addition of lecithin at 0.05% level to Beyaz Peynir cheese.
Figure 4.3 Yields of full-fat, reduced-fat and low-fat Feta cheese samples with tapioca starch, lecithin and their combination. Error bars represents standard deviations. Cheese samples were produced in duplicate and analysis were performed once.

4.2.1.3 Protein

The fat level affected protein content significantly (p<0.05) (Figure 4.4). Decreasing fat level resulted increase in protein content (Figure 4.4). Removing fat increased the concentration of other components. Low and reduced-fat cheeses significantly had the highest protein contents 18.25 and 18.70% respectively.
Fat mimetic type had significant effect on protein content (Figure 4.4) which may be related to their moisture keeping effect. Cheeses with modified tapioca starch, with the highest moisture content, also had the lowest protein content though not significant. Increased firmness and rubberiness of low-fat cheeses are attributed to the undisrupted, continuous protein matrix (Olson and Johnson, 1990b). Therefore increased protein concentration may be related to the firmness of low-fat cheeses. Increased protein content was reported by workers who work on low-fat cheese (Katsiari and Voutsinas, 1994a; El-Neshawy) Fat mimetics, decrease protein percent by binding extra water (Drake, 1996a).
Figure 4.4 Protein contents of full-fat, reduced-fat and low-fat Feta cheese samples with tapioca starch, lecithin and their combination. Error bars represents standard deviations. Cheese samples were produced in duplicate and analysis were performed in triplicate.

4.2.1.4 pH

pH of the cheeses were not significantly affected neither by the fat level nor the fat mimetic used (Figure 4.5). This finding was in agreement with other research made on low-fat Feta (Katsiari and Voutsinas, 1994a), Kefalograviera (Katsiari and Voutsinas, 1994b) cheeses where the effect of fat level was not found to be significant on pH.
Samples with high moisture content were expected to have lower pH related to higher growth rate of lactic acid bacteria. However, genus *Lactococcus* is known for its salt sensitivity which would slow down its growth. (Fox, 1987).

![Graph showing pH levels of different types of cheese samples.](image)

**F**: Full-fat, with no fat mimetics  
**R**: Reduced-fat, with no fat mimetic  
**L**: Low-fat, with no fat mimetics  
**TL**: Low-fat, modified tapioca starch  
**CR**: Reduced-fat, modified tapioca starch + lecithin  
**CL**: Low-fat, modified tapioca starch + lecithin  
**LR**: Reduced-fat, with lecithin  
**LL**: Low-fat, with lecithin

Figure 4.5 pH of full-fat, reduced-fat and low-fat Feta cheeses samples with tapioca starch, lecithin and their combination. Error bars represents standard deviations. Cheese samples were produced in duplicate and analysis were performed once.

### 4.2.1.5 Salt

Salt content of the samples were not affected significantly from fat level or the fat mimetic used (Figure 4.6). The salt level of cheeses were around 5.95-6.20%. That
finding was in agreement with the work of Katsiari and Voutsinas (1994a) where they studied the effect of fat reduction on the salt content of cheeses. However, there was an insignificant increase in salt as the fat level decreased which is observed in cheeses without fat mimetics and cheeses with lecithin. This might be linked to increased protein content of low-fat cheeses which might contribute more binding sites for the salt (McGregor and White, 1990).
Figure 4.6 Salt contents of full-fat, reduced-fat and low-fat Feta cheeses samples with tapioca starch, lecithin and their combination. Error bars represent standard deviations. Cheese samples were produced in duplicate and analysis were performed in triplicate.

4.2.2 Hardness

Hardness of cheeses were significantly effected by the fat level (Figure 4.7). Decreasing fat level increased hardness significantly. Low-fat cheese without fat mimetics was the firmest with a hardness value of 73.88N. Emmons et al (1980) demonstrated that reduced-fat Cheddar cheese was considerably firmer than full-fat cheese. This difference was attributed to the presence of a denser matrix, which must be

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cut or deformed in texture assessments. Similar results were reported for many cheese varieties: Feta cheese, (Katsiari and Voutsinas, 1994a); Kefalograviera, (Katsiari and Voutsinas, 1994b); Cheddar, (Bullens, 1994); Domiati cheese (El-Neshawy et al, 1988).

Fat mimetics significantly affected hardness (Figure 7.7). Modified tapioca starch produced the softest cheese. Lecithin decreased the firmness. Drake et al (1996b) reported decrease in hardness by the use of lecithin in Cheddar cheese. Lecithin weakens the structures created by proteins through hydration (Haumann, 1992). Mimetics impart softness by entrapping moisture (Lucca and Tepper, 1994). Cheese containing tapioca starch had the lowest hardness value around 26-31N. Relative amounts of water, protein, and fat are the dominant factors affecting cheese hardness (Olson and Johnson, 1990b). Milk protein contributes to hardness and milk fat provides softness to the cheese (Mistry and Anderson, 1993). Therefore, cheeses with highest moisture content and lowest protein content would be expected to be a softest cheese.
Figure 4.7 Hardness of full-fat, reduced-fat and low-fat Feta cheese samples with tapioca starch, lecithin and their combination. Error bars represents standard deviations. Cheese samples were produced in duplicate and analysis were performed in triplicate.
4.2.3. Microstructure

Three distinct structures were observed: casein micelles, protein aggregates and voids. Separate casein micelles and protein aggregates were observed rather than a continuous matrix of protein. No spherical shaped spaces were observed that could be assumed to be left from fat globules during defating. Therefore, it was not possible to collect any information about the size and the intensity of the fat globules. Modified tapioca starch and lecithin were not observed as separate identities. They might be intermixed with protein aggregates. Starch might be embedded in the protein matrix and can be observed as an integral part of the matrix provided heating was applied during processing (Alvarez et al., 1991).

Micrographs of full-fat control showed a network composed of small, individual casein micelles. The voids were evenly distributed and the network was uniform (Figure 4.8). As the fat level decreased, roughness of protein matrix increased and the protein matrix became discontinuous (Figure 4.9). In reduced-fat and low-fat cheeses, protein aggregates were observed rather than casein micelles (Figure 4.10).

Micrographs of reduced- and low-fat cheeses with modified tapioca starch showed similar structure to full-fat control. They had a uniform protein network and the casein micelle size is similar to the full-fat control. (Figures 4.16 and 4.11).

Reduced-fat cheese with combination of modified tapioca starch and lecithin had a more smooth matrix than reduced-fat cheese without fat mimetic (Figure 4.12). However, it was not quite similar to the full-fat control. The protein matrix was denser with less void spaces. Low-fat cheese with modified tapioca starch (Figure 4.13) had
more rough protein matrix with more protein aggregates as compared to reduced-fat cheese with the same fat mimetic combination. These observations were the same for reduced-fat and low-fat cheese with lecithin (Figure 4.14 and Figure 4.15). The fine structure of reduced and low-fat cheeses with modified tapioca starch as compared to other cheeses with fat mimetics can be attributed to its their high moisture content. As the concentration of moisture increased, concentration of the protein matrix decreased.

Researchers who worked on low-fat cheese observed that there were less fat globules distributed in the protein matrix which were smaller in size. The greater the fat reduction, the larger the influence of protein on cheese structure. (Mistry and Anderson, 1993; Kalab, 1979). The increase in hardness associated with fat reduction may be highly related to microstructure. Fat globules entrapped in the protein matrix act as fracture points therefore decrease hardness. Protein in cheese is known for its stickiness, and as a reason for the rubbery and elastic nature of low-fat cheeses (Degouy, 1993).

In the present research, it was observed that the casein micelles aggregated more when the fat was reduced. The fat may act as an obstacle against aggregation. Fat mimetics impart softness to low-fat cheeses through moisture retention. The entrapped moisture in the gel of tapioca starch and water bound by lecithin may show similar functionality. To produce a low-fat cheese that has acceptable texture depends on imitating structural properties of fat (Mistry and Anderson, 1993).
Figure 4.8 Scanning electron micrograph of full-fat Feta cheese at x6,500 magnification. C: casein, P: protein aggregate, V: void.
Figure 4.9 Scanning electron micrograph of reduced-fat Feta cheese (R) at x6,500 magnification. B: bacteria.
Figure 4.10 Scanning electron micrograph of low-fat Feta cheese (L) at x6,500 magnification.
Figure 4.11 Scanning electron micrograph of reduced-fat Feta cheese with modified tapioca starch (TR) at x6,500 magnification.
Figure 4.12 Scanning electron micrograph of low-fat Feta cheese (TL) with modified tapioca starch at x6,500 magnification.
Figure 4.13 Scanning electron micrograph of reduced-fat Feta cheese (CR) with modified tapioca starch and lecithin at x6,500 magnification.
Figure 4.14 Scanning electron micrograph of low-fat Feta cheese (CL) with modified tapioca starch and lecithin at x6,500 magnification.
Figure 4.15 Scanning electron micrograph of reduced-fat Feta cheese (LR) with lecithin at x6,500 magnification.
Figure 4.16 Scanning electron micrograph of low-fat Feta cheese (LL) with lecithin at x6,500 magnification.
4.2.4. Sensory analysis

4.2.4.1 Flavor

Organoleptic evaluation of cheese samples were conducted by two different panels. First panel (Group 1) was composed of four members experienced with tasting American dairy products and familiar with Feta cheese. The second panel (Group 2) was formed by sixteen members that consumes routinely Feta type cheese produced from cow’s milk.

In the evaluation of reduced-fat cheeses, Group 1 gave the highest score to the flavor of reduced-fat Feta cheese without the addition of fat mimetics than others. However, it was significantly (p<0.05) different only from the reduced-fat cheese with lecithin (Table 4.2). Addition of lecithin adversely affected the flavor of low-fat cheese. Group 2 gave the highest score to the flavor of full-fat control. The difference was significant with all cheeses with the exception of the reduced-fat cheese that contained a combination of modified tapioca starch and lecithin.
Reduced-fat

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full-fat, with no fat mimetics</td>
<td>$5.50^{ab} \pm 1.73$</td>
<td>$6.75^a \pm 1.61$</td>
</tr>
<tr>
<td>Reduced-fat, with no fat mimic</td>
<td>$6.75^{bc} \pm 0.96$</td>
<td>$5.81^{bc} \pm 1.64$</td>
</tr>
<tr>
<td>Reduced-fat, modified tapioca starch</td>
<td>$6.25^{c} \pm 0.50$</td>
<td>$5.38^{c} \pm 1.45$</td>
</tr>
<tr>
<td>Reduced-fat, modified tapioca starch + lecithin</td>
<td>$5.50^{ab} \pm 1.00$</td>
<td>$6.31^{ab} \pm 1.62$</td>
</tr>
<tr>
<td>Reduced-fat, with lecithin</td>
<td>$4.75^{bc} \pm 1.26$</td>
<td>$5.31^{c} \pm 2.18$</td>
</tr>
</tbody>
</table>

\(a, b, c\) Means in the same column bearing a common superscript do not differ significantly (p< 0.05). Standard deviations are given after “\(\pm\)”.

Table 4.2 Results of sensory analysis of reduced-fat cheeses for flavor. The experiment was not replicated.

In the evaluation of low-fat cheeses, Group 1 judged that cheese with modified tapioca starch was significantly better than low-fat cheese without replacer and low-fat cheese with lecithin (Table 4.3). As in the reduced-fat cheeses, lecithin again received the lowest score. Group 2 judged that full-fat control had better flavor than others except the cheese that contains a combination of modified tapioca starch and lecithin. This might be a result of flavor dilution. Brummel and Lee (1990) reported that using hydrocolloids in process cheese spreads caused dilution of flavor.

All cheeses were criticized for being salty. Bitter or other kind of off-flavors were not noted very occasionally and was not specific to a certain fat level or mimetic. Cheeses with lecithin were more frequently criticized for having foreign flavors.
Milk fat is recognized to be a very important component influencing cheese flavor (Olson and Johnson, 1990). Cheeses with lower fat have usually less flavorful than full-fat products. However, the effect of reduction on cheese flavor depends on the type of cheese (Drake and Swanson, 1995).

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full-fat, with no fat mimetics</td>
<td>$5.00^{abc} \pm 0.82$</td>
<td>$6.73^a \pm 1.45$</td>
</tr>
<tr>
<td>Low-fat, with no fat mimetics</td>
<td>$3.75^a \pm 1.50$</td>
<td>$5.56^b \pm 1.46$</td>
</tr>
<tr>
<td>Low-fat, modified tapioca starch</td>
<td>$6.00^a \pm 1.83$</td>
<td>$5.31^b \pm 1.35$</td>
</tr>
<tr>
<td>Low-fat, modified tapioca starch + lecithin</td>
<td>$5.50^{ab} \pm 1.29$</td>
<td>$5.88^{ab} \pm 1.36$</td>
</tr>
<tr>
<td>Low-fat, with lecithin</td>
<td>$4.25^{bc} \pm 1.71$</td>
<td>$5.54^b \pm 1.98$</td>
</tr>
</tbody>
</table>

^a, b, c Means in the same column bearing a common superscript do not differ significantly ($p < 0.05$). Standard deviations are given after “±”.

Table 4.3 Results of sensory analysis of low-fat cheeses for flavor. The experiment was not replicated.

4.2.4.2 Texture

Group 1 did not find any significant difference between full-fat control and reduced-fat cheeses (Table 4.4). Group 2 scored low-fat cheese without fat mimetics lower than all other cheeses. Reduced-fat cheeses that contain fat mimetics and full-fat control were not significantly different.
<table>
<thead>
<tr>
<th>Reduced-fat</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full-fat, with no fat mimetics</td>
<td>5.50±1.29</td>
<td>6.81±1.42</td>
</tr>
<tr>
<td>Reduced-fat, with no fat mimic</td>
<td>6.75±1.89</td>
<td>5.50±1.83</td>
</tr>
<tr>
<td>Reduced-fat, modified tapioca starch</td>
<td>5.75±0.50</td>
<td>6.63±1.78</td>
</tr>
<tr>
<td>Reduced-fat, modified tapioca starch + lecithin</td>
<td>6.25±1.71</td>
<td>7.06±1.61</td>
</tr>
<tr>
<td>Reduced-fat, with lecithin</td>
<td>6.25±0.96</td>
<td>6.81±1.83</td>
</tr>
</tbody>
</table>

Means in the same column bearing a common superscript do not differ significantly (p< 0.05). Standard deviations are given after "±".

Table 4.4 Results of sensory analysis of reduced-fat cheeses for texture. The experiment was not replicated.

Group 1 did not determine any difference between low-fat cheeses and the full-fat control (Table 4.5). Group 2 gave the lowest score to low-fat cheese without mimic.

Full-fat control and the low-fat cheeses that contain lecithin and a combination of modified tapioca starch and lecithin were not significantly different from each other.

Lecithin and its combination with starch improved the texture.
<table>
<thead>
<tr>
<th>Low-fat</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full-fat, with no fat mimetics</td>
<td>5.00±2.83</td>
<td>7.38±1.02</td>
</tr>
<tr>
<td>Low-fat, with no fat mimetics</td>
<td>5.00±2.16</td>
<td>4.81±0.98</td>
</tr>
<tr>
<td>Low-fat, modified tapioca starch</td>
<td>6.50±1.00</td>
<td>6.13±1.82</td>
</tr>
<tr>
<td>Low-fat, modified tapioca starch + lecithin</td>
<td>6.50±1.73</td>
<td>6.63±1.20</td>
</tr>
<tr>
<td>Low-fat, with lecithin</td>
<td>5.50±1.91</td>
<td>6.69±1.85</td>
</tr>
</tbody>
</table>

*a, b, c* Means in the same column bearing a common superscript do not differ significantly (p< 0.05). Standard deviations are given after "±".

Table 4.5 Results of sensory analysis of low-fat cheeses for texture. The experiment was not replicated.

### 4.2.4.3 Overall Acceptability

Group 1 judged that there was no difference between the acceptability of low-fat cheeses and the full-fat control (Table 4.6). Lecithin had significantly lower score than the reduced-fat cheese without the addition of fat mimetics that received the highest score. Group 2 did not detect any difference in overall acceptability of reduced-fat cheeses and the full-fat control. This result suggested that the use of fat-replacers were not necessary at this level. The results obtained from Group 1 are parallel to the results of Mondal et al. (1989) who investigated effects of manufacturing parameters on cow’s milk Feta cheese. One of the production parameters evaluated in this study was the fat content in the milk. 2, 3.5, and 5% fat in the milk was tested. The cheeses were evaluated by a trained panel with 7 members that evaluated the samples or their flavor and texture.
According to this study, fat in milk had no significant effect on cheese flavor and texture.

In fact, the best cheese chosen by panelists were produced from milk with 2% fat. The results of Mondal et al. (1989) are parallel to the results obtained from the Group 1.

<table>
<thead>
<tr>
<th>Reduced-fat</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full-fat, with no fat mimetics</td>
<td>6.00&lt;sup&gt;a&lt;/sup&gt;±0.82</td>
<td>6.69&lt;sup&gt;a&lt;/sup&gt;±1.66</td>
</tr>
<tr>
<td>Reduced-fat, with no fat mimetic</td>
<td>6.75&lt;sup&gt;a&lt;/sup&gt;±1.89</td>
<td>5.94&lt;sup&gt;a&lt;/sup&gt;±2.02</td>
</tr>
<tr>
<td>Reduced-fat, modified tapioca starch</td>
<td>6.00&lt;sup&gt;a&lt;/sup&gt;±0.82</td>
<td>6.19&lt;sup&gt;a&lt;/sup&gt;±2.17</td>
</tr>
<tr>
<td>Reduced-fat, modified tapioca starch + lecithin</td>
<td>5.75&lt;sup&gt;a&lt;/sup&gt;±0.50</td>
<td>6.50&lt;sup&gt;a&lt;/sup&gt;±2.03</td>
</tr>
<tr>
<td>Reduced-fat, with lecithin</td>
<td>5.25&lt;sup&gt;a&lt;/sup&gt;±0.96</td>
<td>6.06&lt;sup&gt;a&lt;/sup&gt;±2.21</td>
</tr>
</tbody>
</table>

<sup>a, b, c</sup> Means in the same column bearing a common superscript do not differ significantly (p< 0.05). Standard deviations are given after "±".

Table 4.6 Results of sensory analysis of reduced-fat cheeses for overall acceptability. The experiment was not replicated.

Group 1 scored low-fat cheese without any fat mimetic and low-fat cheese with lecithin lowest (Table 4.7). Low-fat cheese containing modified tapioca starch was rated significantly higher than them. Group 2 scored full-fat control the highest and did not find any significant difference the full-fat control and the low-fat cheese that contained a combination of modified tapioca starch and lecithin. Katsiari and Voutsinas (1994a) evaluated manufacturing of traditional Feta cheese from milk containing 6, 4.5, 3, and 1.5% fat. The samples were prepared from sheep's milk and evaluated by a 5 member experienced but not trained panel. According to this research, there was a significant
relation between the overall score of the cheese and its fat level. The significant
difference only existed between the cheeses that were made from milk containing 6 and
1.5% fat. It was concluded that good low-fat Feta cheese can be made with acceptable
flavor, body and texture can be made from milk containing 1.5% fat. Findings of Katsiari
and Voutsinas (1994a) are in agreement with the results obtained from the first panel.

<table>
<thead>
<tr>
<th>Low-fat</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full-fat, with no fat mimetics</td>
<td>4.50bc±0.58</td>
<td>6.75a±1.39</td>
</tr>
<tr>
<td>Low-fat, with no fat mimetics</td>
<td>4.00c±1.41</td>
<td>5.69b±1.58</td>
</tr>
<tr>
<td>Low-fat, modified tapioca starch</td>
<td>6.50a±0.58</td>
<td>5.50b±1.46</td>
</tr>
<tr>
<td>Low-fat, modified tapioca starch + lecithin</td>
<td>5.50ab±1.29</td>
<td>6.25ab±1.44</td>
</tr>
<tr>
<td>Low-fat, with lecithin</td>
<td>4.00c±1.15</td>
<td>5.86b±1.63</td>
</tr>
</tbody>
</table>

\[ \text{a, b, c \: Means in the same column bearing a common superscript do not differ significantly (p< 0.05). Standard deviations are given after \"±\".} \]

Table 4.7 Results of sensory analysis of low-fat cheeses for overall acceptability. The experiment was not replicated.
4.2.5. Electrophoresis

Protein patterns from Feta cheese were evaluated. Bands of α casein, β casein were clearly identified. Figure 4. 22 shows protein bands of cheese extracts at 1st and 45th days of ripening. Similar observations were reported by Vafopoulou et al (1989) who studied accelerated ripening of sheep’s milk Feta cheese.

The protein bands at the 1st and 45th days were very similar. However, the bands at the 45th day were less intense in color. Protein bands with lower molecular weights were observed after 45 days of ripening. The loss of band intensity may be caused by slight proteolysis. Proteolysis in Feta cheese is mainly due to enzymes used for coagulation, proteinases produced by the microorganisms, and indigenous milk proteinases (Anifantalis, 1991) Proteolysis is important for flavor development and texture of the cheese. Proteolysis contributes flavor via formation of amino acids and peptides. Texture changes due to partial breakdown of the casein network (Fox, 1989).
Figure 4.17 Polyacrylamide gel electrophoretogram of extracted proteins of Feta cheese with modified tapioca starch. 1: $\alpha$ casein, 2: $\beta$ casein, 3: Reduced-fat cheese at the 1st day, 4: Reduced-fat cheese at the 45th day, 5: Low-fat cheese at the 1st day, 6: Low-fat cheese at the 45th day.
Optical densities of α and β casein in full-fat cheese, reduced-fat cheese, and low-fat cheese at day 45 are shown in Table 4.8. As fat level decreased, band intensity decreased. This might be a result of increased moisture content of the low-fat cheeses since it is known that increased moisture content promotes proteolysis in cheese due to increased growth rate of the bacteria (Fox, 1989). This result is in agreement with the findings of Turin and Bonomi (1995) who found that lowering fat content in Cacciotta cheese increased the extent of proteolysis. Rest of the data did not show strong trends. Electrophoresis was not found to be an efficient method of protein quantitation.
<table>
<thead>
<tr>
<th>(OD x mm)</th>
<th>α casein</th>
<th></th>
<th>β casein</th>
<th></th>
<th>α casein + β casein</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 45</td>
<td>Day 1</td>
<td>Day 45</td>
<td>Day 1</td>
<td>Day 45</td>
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<tr>
<td>F</td>
<td>1.0</td>
<td>0.6</td>
<td>1.4</td>
<td>1.4</td>
<td>2.4</td>
<td>2.0</td>
</tr>
<tr>
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<td>0.9</td>
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<td>1.1</td>
<td>2.3</td>
<td>1.7</td>
</tr>
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<td>L</td>
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<td>1.4</td>
<td>1.2</td>
<td>2.5</td>
<td>1.5</td>
</tr>
<tr>
<td>TR</td>
<td>0.8</td>
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<td>0.8</td>
<td>1.0</td>
<td>1.7</td>
<td>1.5</td>
</tr>
<tr>
<td>TL</td>
<td>1.2</td>
<td>0.2</td>
<td>1.2</td>
<td>1.0</td>
<td>2.5</td>
<td>1.3</td>
</tr>
<tr>
<td>CR</td>
<td>1.7</td>
<td>0.6</td>
<td>1.6</td>
<td>1.3</td>
<td>3.3</td>
<td>1.9</td>
</tr>
<tr>
<td>CL</td>
<td>1.8</td>
<td>0.9</td>
<td>1.7</td>
<td>1.4</td>
<td>3.3</td>
<td>2.4</td>
</tr>
<tr>
<td>LR</td>
<td>1.4</td>
<td>0.4</td>
<td>1.2</td>
<td>1.1</td>
<td>2.6</td>
<td>1.6</td>
</tr>
<tr>
<td>LL</td>
<td>1.2</td>
<td>0.4</td>
<td>1.1</td>
<td>1.1</td>
<td>2.4</td>
<td>1.6</td>
</tr>
</tbody>
</table>

F: Full-fat, with no fat mimetics  
R: Reduced-fat, with no fat mimetic  
L: Low-fat, with no fat mimetics  
TR: Reduced-fat, modified tapioca starch  
TL: Low-fat, modified tapioca starch  
CR: Reduced-fat, modified tapioca starch + lecithin  
CL: Low-fat, modified tapioca starch + lecithin  
LR: Reduced-fat, with lecithin  
LL: Low-fat, with lecithin

Table 4.8 Relative areas under the optical density vs. distance curves of scanned gels. The experiment was not replicated.
5. CONCLUSIONS

In the preliminary research functionality of six fat mimetics were tested. Cheese with starch based mimetics had higher moisture than the rest of the samples therefore they had more creamy mouthfeel except the one that contains modified corn starch which was gritty. The use of microcrystalline cellulose or microparticulated whey protein did not produce a cheese significantly softer than the low-fat cheese without the addition of any mimetic. Lecithin imparted softness to the cheese acting as a “fat extender”, it helped better distribution of the fat in the low-fat product. Lecithin having hydrophobic and hydrophilic sites is able to interact with fat, protein and moisture. Therefore modified tapioca starch and lecithin were chosen to be used in the final research.

According to the results of the final study, fat mimetics and fat levels were found to effect moisture, yield, fat, protein, and hardness but not pH and salt content of Feta cheese. At the used concentrations of mimetics, it was observed that fat reduced cheeses with modified tapioca starch had the highest moisture and lowest protein content. Relating to these chemical properties, they were less firm. The micrographs showed that they had a less dense structure with a smooth protein matrix. The structures were very similar to the full-fat control. They had a clean taste. Modified tapioca starch were found increase acceptability by Group 1. However, it decreased flavor score by Group 2.
Increased acceptability might be a result of lubricity added by starch related to water retention. Decreased flavor scores might be a result of diluted flavor by increased water content. Lecithin did not bind water as much as starch did. But it helped in decreasing hardness. The microstructure of the reduced-fat cheese was very similar to the full-fat control. However, low-fat cheese with lecithin was not very similar with its more rough matrix. Cheeses with lecithin were reported to have foreign flavors by some panelists. They also had low flavor scores. Cheeses that contained a combination of starch and lecithin were more similar to cheeses with only lecithin. In addition, they did not have off-flavors. The combination cheeses had high texture and overall acceptability scores given by Group 1 and Group 2.

Use of a combination of modified tapioca starch and lecithin is recommended to be used in fat reduced Feta cheese as a result of this study. However, first shelf life should be studied in order to confirm that these mimetics do not cause off-flavors during the ripening period.
Appendix A: Raw Data
<table>
<thead>
<tr>
<th>% Fat</th>
<th>% Moisture</th>
<th>% Protein</th>
<th>% Salt</th>
<th>Hardness (N)</th>
<th>pH</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>19.96</td>
<td>57.44</td>
<td>15.357</td>
<td>6.11</td>
<td>43.49</td>
<td>4.8</td>
</tr>
<tr>
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<td>14.295</td>
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</tr>
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<td></td>
</tr>
<tr>
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<tr>
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<tr>
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</tr>
<tr>
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<td>52.64</td>
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</tr>
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<td>18.651</td>
<td>5.89</td>
<td>68.1</td>
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<tr>
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<td>58.77</td>
<td>17.518</td>
<td>6.54</td>
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<td>68.41</td>
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<td>78.63</td>
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<td>5.92</td>
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<tr>
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<td>19.83</td>
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<td>5.87</td>
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<td>27.94</td>
<td>4.87</td>
</tr>
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<td>12.851</td>
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<td>67.59</td>
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<td>6.46</td>
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<td>67.44</td>
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<tr>
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<td>68.75</td>
<td>12.888</td>
<td>6.49</td>
<td>24.89</td>
<td></td>
</tr>
</tbody>
</table>

F: Full-fat, with no fat mimetics  
R: Reduced-fat, with no fat mimetic  
L: Low-fat, with no fat mimetics  
TR: Reduced-fat, modified tapioca starch

Table A.1 Values represent raw data. Each sample was produced in duplicate and the analysis were done in triplicate except pH and yield that were evaluated once on each duplicate sample.
<table>
<thead>
<tr>
<th></th>
<th>% Fat</th>
<th>% Moisture</th>
<th>% Protein</th>
<th>% Salt</th>
<th>Hardness (N)</th>
<th>pH</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL</td>
<td>9.31</td>
<td>68.28</td>
<td>15.519</td>
<td>5.91</td>
<td>31.21</td>
<td>4.74</td>
<td>13.98</td>
</tr>
<tr>
<td></td>
<td>9.51</td>
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<tr>
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<td>14.172</td>
<td>5.98</td>
<td>57.19</td>
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<td>10.96</td>
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<td>14.705</td>
<td>6.53</td>
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<td>14.629</td>
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<td>32.63</td>
<td>4.96</td>
<td>13.41</td>
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<td>13.724</td>
<td>5.92</td>
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<td>6.52</td>
<td>34.94</td>
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<td></td>
</tr>
<tr>
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<td>13.96</td>
<td>61.36</td>
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</tr>
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<td>LL</td>
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<td>4.85</td>
<td>12.71</td>
</tr>
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<td>63.34</td>
<td>13.599</td>
<td>6.23</td>
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<td>12.5</td>
</tr>
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<td>11.78</td>
<td>62.87</td>
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<tr>
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<td>15.978</td>
<td>5.92</td>
<td>35.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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<td>63.44</td>
<td>15.897</td>
<td>6.11</td>
<td>45.12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TL: Low-fat, modified tapioca starch  
CR: Reduced-fat, modified tapioca starch + lecithin  
CL: Low-fat, modified tapioca starch + lecithin  
LR: Reduced-fat, with lecithin  
LL: Low-fat, with lecithin

Table A.2 Values represent raw data. Samples produced in duplicates and analysis done in triplicate except pH and yield that were evaluated once on each duplicate sample.
Appendix B: Statistics
<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Seq SS</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>2</td>
<td>223.42</td>
<td>93.52</td>
<td>46.76</td>
<td>40.98</td>
<td>0.000</td>
</tr>
<tr>
<td>Mimetic</td>
<td>3</td>
<td>397.58</td>
<td>397.58</td>
<td>132.53</td>
<td>116.15</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
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<td>54.77</td>
<td>54.77</td>
<td>1.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

Table B.2 Statistical analysis of moisture data according with general linear model

<table>
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<tr>
<th></th>
<th>Full-fat</th>
<th>Reduced-fat</th>
<th>Low-fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Means</td>
<td>56.782</td>
<td>61.571</td>
<td>63.528</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>c</td>
</tr>
</tbody>
</table>

Table B.3 Summary of Fisher’s Least Significant Difference test on moisture content of Feta cheeses produced from milk with different fat contents. Means that bear the same letter are not significantly different (p<0.05).

<table>
<thead>
<tr>
<th></th>
<th>No mimetic</th>
<th>Starch</th>
<th>Starch + Lecithin</th>
<th>Lecithin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>59.080</td>
<td>67.357</td>
<td>60.427</td>
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<td>a</td>
<td>b</td>
<td>c</td>
<td>d</td>
</tr>
</tbody>
</table>

Table B.4 Summary of Fisher’s Least Significant Difference test on moisture content of Feta cheeses produced from milk with different fat mimetics. Means that bear the same letter are not significantly different (p<0.05).
Table B.5 Statistical analysis of yield data according with general linear model

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Seq SS</th>
<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>2</td>
<td>3.5197</td>
<td>11.7488</td>
<td>5.8744</td>
<td>99.49</td>
<td>0.000</td>
</tr>
<tr>
<td>Mimetic</td>
<td>3</td>
<td>20.2076</td>
<td>20.2076</td>
<td>6.7359</td>
<td>114.08</td>
<td>0.000</td>
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<tr>
<td>Error</td>
<td>12</td>
<td>0.7085</td>
<td>0.7085</td>
<td>0.0590</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>24.4358</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Table B.6 Summary of Fisher's Least Significant Difference test on yield of Feta cheeses produced from milk with different fat contents. Means that bear the same letter are not significantly different (p<0.05).

<table>
<thead>
<tr>
<th></th>
<th>Full-fat</th>
<th>Reduced-fat</th>
<th>Low-fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Means</td>
<td>13.82</td>
<td>13.00</td>
<td>12.42</td>
</tr>
<tr>
<td></td>
<td>a</td>
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<td>b</td>
</tr>
</tbody>
</table>

Table B.7 Summary of Fisher's Least Significant Difference test on yield of Feta cheeses produced from milk with different fat mimetics. Means that bear the same letter are not significantly different (p<0.05).

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<th>No mimetic</th>
<th>Starch</th>
<th>Starch + Lecithin</th>
<th>Lecithin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
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<td>14.19</td>
<td>12.77</td>
<td>12.87</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Source</td>
<td>DF</td>
<td>Seq SS</td>
<td>Adj SS</td>
<td>Adj MS</td>
</tr>
<tr>
<td>--------</td>
<td>----</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Fat</td>
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<td>4.889</td>
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<td>158.683</td>
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<tr>
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<td>48.243</td>
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<td>Total</td>
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Table B.8 Statistical analysis of protein data according with general linear model

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<th>Reduced-fat</th>
<th>Low-fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Means</td>
<td>14.83</td>
<td>15.36</td>
<td>15.77</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>ab</td>
<td>b</td>
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Table B.9 Summary of Fisher’s Least Significant Difference test on protein content of Feta cheeses produced from milk with different fat contents. Means that bear the same letter are not significantly different (p<0.05).

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<th>Lecithin</th>
</tr>
</thead>
<tbody>
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<td>15.47</td>
<td>14.77</td>
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<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>d</td>
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</table>

Table B.10 Summary of Fisher’s Least Significant Difference test on protein content of Feta cheeses produced from milk with different fat mimetics. Means that bear the same letter are not significantly different (p<0.05).
Table B.11 Statistical analysis of fat content data according with general linear model

<table>
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<th>Adj SS</th>
<th>Adj MS</th>
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<th>P</th>
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<tr>
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<td>85.77</td>
<td>28.59</td>
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<td>26.52</td>
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Table B.12 Summary of Fisher’s Least Significant Difference test on fat content of Feta cheeses produced from milk with different fat contents. Means that bear the same letter are not significantly different (p<0.05).

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<th>Low-fat</th>
</tr>
</thead>
<tbody>
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<td>10.97</td>
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<tr>
<td></td>
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<td>b</td>
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</tr>
</tbody>
</table>

Table B.13 Summary of Fisher’s Least Significant Difference test on fat content of Feta cheeses produced from milk with different fat mimetics. Means that bear the same letter are not significantly different (p<0.05).

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<tr>
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<th>Lecithin</th>
</tr>
</thead>
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<td>12.18</td>
</tr>
<tr>
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<td>Adj SS</td>
<td>Adj MS</td>
</tr>
<tr>
<td>-----------</td>
<td>----</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Fat</td>
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</tr>
<tr>
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<td>0.0190</td>
<td>0.0063</td>
</tr>
<tr>
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<td>0.0491</td>
<td>0.0040</td>
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Table B.14 Statistical analysis of pH data according with general linear model

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<th>P</th>
</tr>
</thead>
<tbody>
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<td>Fat</td>
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<td>0.1141</td>
<td>1.52</td>
<td>0.228</td>
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<td>0.0087</td>
<td>0.12</td>
<td>0.950</td>
</tr>
<tr>
<td>Error</td>
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<td>3.5940</td>
<td>3.5940</td>
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<tr>
<td>Total</td>
<td>53</td>
<td>3.9023</td>
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<td></td>
</tr>
</tbody>
</table>

Table B.15 Statistical analysis of salt content data according with general linear model

<table>
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<th>Adj SS</th>
<th>Adj MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
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<td>565.7</td>
<td>2264.8</td>
<td>1132.4</td>
<td>33.89</td>
<td>0.000</td>
</tr>
<tr>
<td>Mimetic</td>
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<td>11016.6</td>
<td>36.72</td>
<td>109.90</td>
<td>0.000</td>
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<tr>
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<td></td>
</tr>
</tbody>
</table>

Table B.16 Statistical analysis of fat content data according with general linear model
<table>
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<tr>
<th></th>
<th>Full-fat</th>
<th>Reduced-fat</th>
<th>Low-fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Means</td>
<td>47.60</td>
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<td>73.89</td>
</tr>
<tr>
<td>a</td>
<td>b</td>
<td>c</td>
<td></td>
</tr>
</tbody>
</table>

Table B.17 Summary of Fisher’s Least Significant Difference test on hardness of Feta cheeses produced from milk with different fat contents. Means that bear the same letter are not significantly different (p<0.05).

<table>
<thead>
<tr>
<th></th>
<th>No mimetic</th>
<th>Starch</th>
<th>Starch + Lecithin</th>
<th>Lecithin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>61.69</td>
<td>28.95</td>
<td>41.09</td>
<td>35.50</td>
</tr>
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<td>a</td>
<td>b</td>
<td>ab</td>
<td>b</td>
<td></td>
</tr>
</tbody>
</table>

Table B.18 Statistical analysis of hardness data according with general linear model
Appendix C: Sensory Score Sheet
Instructions:
You will receive five samples to evaluate for sensory characteristics. Please indicate the defects, if any, associated with the sample.

FLAVOR

243
1 2 3 4 5 6 7 8 9
very poor fair good very good
Any defects? acid☐, bitter☐, flat☐, foreign☐, rancid☐, salty☐, other☐, what?

907
1 2 3 4 5 6 7 8 9
very poor fair good very good
Any defects? acid☐, bitter☐, flat☐, foreign☐, rancid☐, salty☐, other☐, what?

634
1 2 3 4 5 6 7 8 9
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Any defects? acid☐, bitter☐, flat☐, foreign☐, rancid☐, salty☐, other☐, what?

316
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Any defects? acid☐, bitter☐, flat☐, foreign☐, rancid☐, salty☐, other☐, what?

981
1 2 3 4 5 6 7 8 9
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Any defects? acid☐, bitter☐, flat☐, foreign☐, rancid☐, salty☐, other☐, what?
TEXTURE

243

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Any defects? gritty, hard, pasty, soft, other, what?

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OVERALL ACCEPTABILITY

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316
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918
1 2 3 4 5 6 7 8 9
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LIST OF REFERENCES


