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INFLUENCE OF TREATMENTS ON HYDRATED MILK PROTEIN CONCENTRATE PARTICLES AND RESULTING CAST FETA CHEESE CHARACTERISTICS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Ching-Jung Josephine Kuo, M.S.

The Ohio State University
2001

Dissertation Committee:
Professor W. James Harper, Adviser
Professor Grady W. Chism
Professor Michael E. Mangino
Professor Valente B. Alvarez

Approved by

W. James Harper
Adviser
Food Science and Nutrition Graduate Program
Milk protein concentrate (MPC) is a relatively new food ingredient which contains both casein and whey proteins. Due to its protein composition, MPC can provide various functional properties in a food system as well as improve the nutritional value of the food products. However, one of the most economically feasible applications of MPC is in the cheese-making industry, especially where the milk supply is limited.

Recombined milk fat and 56% MPC is used for cheese-making in some areas outside of the United States. MPC with protein content higher than 60% has been reported to be difficult to use in cheese-making, including cast Feta cheese production.

A hypothesis of shell formation during spray-drying of MPC particle that affected the properties of the MPC particles was proposed. The objectives of this study were to provide an understanding of the MPC particle variability related to the functionalities of the MPC in rennet coagulation and cheese-making, and to investigate means to improve the cheese-making properties of commercially available high protein content MPCs.

High protein content MPC samples tended to have a large particle size. All the MPC samples with protein content higher than 50% had a particle size larger than 20 um. Ultrasonication, heat, and shear by homogenization were some of the effective ways to
dissociate the particles. A combination of two treatments provided synergistic effects on dissociating particles.

Addition of CaCl₂ was needed for the MPC solution to form a rennet-induced gel. The gel strength was affected by the treatments of the MPC solution, as well as the added rennet and calcium concentrations. Generally, the correlation between the MPC hydrated particle size and the resulting rennet gel strength was negative.

There was no good correlation between the hydrated MPC particle size and the hardness of the resulting cheese. However, the MPC85 cheese was significantly softer than its MPC56 counterpart. The pH of the cheese had a large impact on the cheese texture. Addition of lactose into the MPC85 cheese milk reduced the pH of the cheese and improved cheese hardness. The ESEM examination of surface microstructure revealed differences between MPC cheese samples.
Dedicated to my parents and my brothers
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VITA

March 28, 1961 ..................................... Born – Tainan, Taiwan

1994 .................................................... M.S. Food Management
The Ohio State University

1996 .................................................... M.S. Food Science
The Ohio State University

1996 – present ................................. Graduate Research Associate
J.T. Parker Chair Laboratory
The Ohio State University
Columbus, Ohio

FIELD OF STUDY

Major field: Food Science and Nutrition
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CHAPTER 1

INTRODUCTION

Milk protein concentrate (MPC) is a relatively new protein-enriched dairy food ingredient, containing both whey protein and casein derived from the ultrafiltration of skim milk. The protein content of MPC can range from 40% to more than 85%. Due to its protein composition, MPC can provide various functional properties in a food system such as water binding and emulsification, as well as improve the nutritional value of the food products in which it is used. However, one of the most economically feasible applications of MPC is in the cheese making industry, especially where the milk supply is limited, to both make cheese from recombined milk and to serve as a milk extension for cheese in order to achieve higher yield without any major capital investment.

In many developing countries, fresh milk production is not sufficient to meet the demand for making dairy foods. Milk powder (skim and whole) is often used to make reconstituted or recombined milk, either as fluid milk or as ingredients for other dairy products for local consumption (IDF, 1990). Milk protein concentrate has the same advantages as milk powder in terms of handling and storage; however, the protein content
of MPC is much higher than milk powder. The cost of transportation and storage per protein unit, therefore, will be more economical than milk powder. The lower lactose content also results in a lower impact on the environment.

Using ingredients other than fresh milk to make cheese does not meet the current regulation in the United States (Codex Standard, A-6, 1978). However, this limitation may be eliminated in the near future. According to the recent proposed changes of Codex standards, MPC will be permitted as the sole milk ingredient in cheese manufacture (Draft Revised Codex Standard for Cheese, 1997).

Variation in the functionality of protein-enriched dairy ingredients has been reported, especially in the case of whey protein concentrates (WPC). Only one report has mentioned variation in the functionality of commercial MPCs (De Castro, 1999). The author found that there was a very wide range of difference in commercial MPC samples in respect to solubility and viscosity of hydrated products. This was noted both for products from different manufacturers and for products of different protein contents made by the same manufacturer.

The general hypothesis for this study was that the variability of the functionality of commercial MPC samples could be attributed to the characteristics of the hydrated particles. The smaller particles provide larger surface area than the larger particles do in the same volume content. Since most of the functionality of ingredients is related to the interaction on the surface of the hydrated particles, small particles might provide more areas for interaction than large particles, and result in better functional performance.
The objectives of this study were to provide an understanding of the basic reason for variability in MPC functional properties with special attention to rennet coagulation and cheese making, and to investigate means to improve the cheese making properties of commercially available high protein content (more than 70%) MPC.

The study was divided into three phases. (a) Investigation of the particle properties of hydrated MPC samples of various protein contents and from various sources, and the potential availability of micellar casein after hydration. (b) Evaluation of the rennetability and the characteristics of the rennet gel from selected MPCs. (c) Study of the factors affecting the properties of cast Feta cheese made from recombined MPC and milk fat.
LITERATURE REVIEW

2.1 Characteristics of Milk Protein Concentrate (MPC)

Milk protein concentrate is a relatively new dairy protein product, which is a source of milk solids and can improve the nutritional value of food products. MPC contains both caseins and whey proteins and could be a powerful functional food ingredient (Getler, et al., 1997).

Many terms have been used in literature to describe milk protein concentrates that are produced by ultrafiltration of skimmilk. For example, "retentate powder", "native milk protein concentrate", "ultrafiltered milk protein concentrate", "skim milk retentate powder", etc. were used by researchers cited by Novak (1991). Therefore, the International Dairy Federation (IDF) recommends using "milk protein concentrate (MPC)" for products made from membrane filtration of skim milk with protein contents.
from 50 to 85% and use “milk protein (MP)” for products with protein content is higher than 85%. The number that follows MPC and MP represents the protein content of the product (Novak, 1991).

2.1.1 Processing

Milk protein concentrate is produced by ultrafiltration (UF), or a combination of ultrafiltration and diafiltration (DF), of skim milk which is HTST or UHT treated, then evaporated and spray dried (Novak, 1996). During membrane filtration, lactose is removed with permeate. Therefore, protein content is higher and lactose content is lower in MPC than in skim milk powder. Because lactose and minerals in milk have different solubility at different temperatures and pH ranges, manipulating the processing temperature and the fluid pH during membrane filtration can achieve various levels of lactose and minerals in the final product (St-Gelais, et al., 1992). The general process flow-chart of MPC is shown in Figure 2.1. In general, the skim milk is ultrafiltered, diafiltered, further concentrated by evaporation and spray-dried. The hydration properties of the MPC are most affected by the drying step (De Castro, 1999).

The raw material quality, heat treatment, degree of concentration, and other parameters during evaporation and spray drying all have effects on the quality of the final MPC product. The temperature during ultrafiltration generally stays either above 50°C (Hot) or below 10°C (Cold) to control bacteria growth. Depending on the amount of heat treatment during processing, the solubility of MPC may decrease, if the storage
temperature is higher than 20°C (Novak, 1991). Heat treatment during processing of MPC affects its properties. El-Samragy and his co-workers (1993a) suggested the MPC produced by cold UF had a better solubility than that made by hot UF. Moreover, protein denaturation and Maillard reactions occurred with hot UF that could degrade the quality of product.

De Castro (1999) evaluated several commercial MPC samples and found a wide variation in functional properties such as solubility, viscosity, foaming, etc. She also showed that the outlet temperature of drying 70% MPC influenced the hydration characteristics of the MPC. Higher outlet temperatures caused a loss in the hydration properties of the MPC.

2.2 Heat-Induced Changes of Milk Proteins

Heat treatment changes milk proteins through denaturation of whey protein and can alter their functionality. This topic has been extensively reviewed by several researchers (Cherry, 1981; Kinsella, 1984; Wong, 1988; Phillips & Finley, 1989; Fox, 1992). The adsorption of denatured whey protein to casein micelles and formation of complexes between beta-lactoglobulin (β-Lg) and kappa-casein by hydrophobic interaction and disulfide linkages could occur after heat treatment (Park, et al., 1996). Alpha-lactalbumin (α-La), β-Lg, and caseins can form aggregates with themselves as well. At pH 6.7, heat treatment results in β-Lg interacting with kappa-casein at the
surface of casein micelles, which slightly increases that heat stability of milk protein. At a pH higher than 6.9, β-Lg forms soluble complexes with caseins more quickly, thus decreasing the heat stability of milk protein (Rattray & Jelen, 1997). In the presence of sugars, the denaturation of α-La and β-Lg is reduced (Kinsella, 1984).

Corredig and Dalgleish (1996) found that nearly all β-Lg reacted with k-casein in milk treated by indirect heating, but not in milk treated by direct heating. Denaturation of β-Lg and association of β-Lg and casein increased as the heating temperature of milk increased (Waungana, et al., 1997). The complex formed between β-Lg and k-casein during heat treatment retards rennet action, which is not favorable for cheese making (Kinsella, 1984). On the other hand, the β-Lg and k-casein complex increases the firmness and water binding capacity of protein gel which is desired during manufacture of yogurt. The solubility of protein decreases as heat denaturation increases. In most of food product applications, high solubility of protein is desired (Kinsella, 1984).

Besides denaturation of whey proteins and the formation of complexes between whey proteins and caseins due to heat treatment, Maillard reactions can occur between an amino group of milk proteins and the reducing group of lactose upon heat treatment. Color and flavor changes in milk powder under certain storage conditions have been reported by Matsuda, et al. (1991). Ennis and Mulvihill (1999) suggested that the variability in hydration characteristics of protein molecules could be modified by Maillard reaction.
2.3 Functional Properties of Milk Proteins

The functional properties of proteins have received considerable attention (Cherry, 1981; Kinsella & Soucie, 1989; Fox, 1992). The functional properties are dependent upon the structure of the protein, which relates to amino acid composition, charge distribution, size, shape, etc. Water binding, gelation, emulsification, and foaming are some of the functional properties of milk proteins that have been investigated and apply mostly to food products (Kinsella, 1984; Kilara, 1994). Water binding and gelation are related to hydrodynamic properties of proteins, whereas emulsification and foaming are related to surface properties of proteins (Damodaran, 1994).

The hydration property of a protein is its ability to bind or entrap water that is important in many functional food applications. The level of available water greatly affects the level of hydration of proteins. The degree of hydration is commonly related to relative humidity or water activity of the environment. For some proteins, the degree of hydration could be temperature dependent. Solubilization and swelling indicate high hydration values for Sodium-caseinate under high water activity conditions (Mulvihill, 1992). The factors affecting the hydration of protein have been reviewed by Dumoulin and Bimbenet (1998), Fennema, (1996), and Gregory (1998).
2.4 Particle Characteristics of Milk Powder

The particle size and structure of spray-dried whole milk powder are greatly affected by the type of drier used (Faldt & Sjoholm, 1996). Chen and Lloyd (1994) concluded that particle size of dried milk affected bulk density, flowability, dustiness, and dispersability in water. In order to reduce dustiness and increase dispersability, agglomeration was applied to milk powder to form larger particles. Agglomerated milk particles can be easily broken down by air pressure (Chen & Lloyd, 1994). Baldwin & Woodhams (1974) reported that heat induced aggregation decreased the dispersability of milk powder. On the other hand, large particle size due to decreases pressure of the atomizer during spray drying improved the solubility of milk powder (Baldwin & Woodhams, 1974). El-Shibiny and co-workers (1996) suggested that during diafiltration of skim milk colloidal calcium was partially removed from casein micelles and induced the formation of micelle aggregates. Thus, the size of casein micelles increased after diafiltration.

Kneifel and Seiler (1993) found that the wetting and rehydration rates of milk protein powder depended on the structure of the particle and the degree of stirring during mixing with water. The structure of spray-dried milk protein powder is porous and globular while the structure of drum-dried powder is irregular. Thus, spray-dried powder usually has better wettablity then drum-dried powder. Temperature, pH, and presence of
other ingredients, such as salts, can affect the performance of milk powder dispersability as well. The optimum temperature for dispersing milk powder was determined to be 60°C (Kneifel & Seiler, 1993).

McKenna et al. (1999) examined the microstructure of whole milk powder particles. They found that the slowly dissolving particles in cold water were intact milk powder particles whereas the sediment obtained from hot water testing was a continuous matrix with a large amount of small particles and some large particles. The particles from higher heat treatment milk powder had more hair-like structure on the casein micelles than from lower heat treatment milk powders. The heat stability of milk powder was affected by the adsorption of casein micelles on the surface of fat globules. Kalab (1993) examined the particles of spray-dried, drum-dried, and instantized milk powder by electron microscopy to reveal the differences in particle surface structure. It would be expected that the same factors that affect dry milk powder particles and their hydration would also apply to MPC. Further it would be expected that MPC would be more difficult to hydrate on the basis of its higher protein content.

2.5 Dissociating Agents

Dissociating agents affect the binding of protein molecules by interfering with hydrogen bonding, hydrophobic interaction, and the disulfide linkages. Their effectiveness is dependent on the concentration of the chemicals or the intensity of the physical treatments (Ali et al., 1980a & b; Cheftel et al., 1985; Nakai & Li-Chan, 1988).
2.5.1 Shear – ultrasonication and homogenization

Shear would be expected to assist in the hydration of MPC. Ultrasonication, one form of shear, could disrupt hydrophobic interactions, thus altering protein structure. Ultrasonication could induce both dissociation and formation of protein aggregates depending on the condition of the proteins (Nakai & Li-Chan, 1988).

Homogenization, another form of shear, could also be expected to affect the hydration of MPC. Most of the studies related to effects of homogenization have either focused on the particle size of fat globules (Zadow & Hardham, 1984; Kebary & Morris, 1989), or on the properties of resulting cheese (Vaitkus & Sauts, 1979; Abrahamsen & Holmen, 1981; Abd El-Salam & El-Shibiny, 1982). However, Snoeren et al. (1984) mentioned that homogenization pressure applied to milk concentrate affected the solubility of the resulting spray-dried whole milk.

2.5.2 Urea

Urea interferes with hydrogen bonds between water molecules and increases the solubility of apolar molecules in water. Urea was referred to as a “structure breaker” of water by some researchers (Nakai & Li-Chan, 1988). Urea decreases hydrophobic interaction, and in high concentration (4-8M), urea induces denaturation of proteins
(Cheftel et al., 1985). Although urea exists in the milk naturally, addition of urea into milk caused micellar casein to dissociate into the soluble phase to some extend (Ali et al., 1980a).

2.5.3 Sodium dodecyl sulfate (SDS)

Sodium dodecyl sulfate (SDS) is a surface-active agent. SDS, an ionic detergent, can associate with protein residues hydrophobically, and thus, is a very powerful denaturing agent. It interferes with hydrophobic interactions that maintain native protein structure and causes proteins to unfold. It then interacts with the unfolded protein hydrophobically (Cheftel et al., 1985).

2.5.4 Beta-mercaptoethanol

Beta-mercaptoethanol (ME) is a reducing agent, which can cleave disulfide linkages and modify protein conformation (Cheftel et al., 1985). Beta-mercaptoethanol has been used in electrophoresis to show S-S interaction of milk proteins.
2.6 Use of Dry Milk Products

Milk powder and dried milk products, either from whole milk or skim milk, have been used to substitute fresh fluid milk in areas where the supply of fluid milk is not able to meet the demand. In areas where the transportation of fresh milk is difficult, dried milk products also play an important role to fulfill the need for dairy products. Milk powder is used as an ingredient in food products for its nutritional value or functional properties (Al-Tahiri, 1987). Using milk powder in cheese-making to improve yield has been practiced for some time. Low protein content milk protein concentrate has been used to make several types of cheese (Jensen et al., 1987; Novak, 1991).

When using dried milk products as ingredients, recombination and reconstitution are required. As cited in Al-Tahiri (1987), the Code of Principles concerning milk and milk products (FAO/WHO, 1973) gave clear definitions to these two terms.

"Reconstituted (product), is the milk product resulting from the addition of water to the dried or condensed form of (product) in the amount necessary to re-establish the specified water-solids ratio. Recombined (product), is the milk product resulting from the combining of milk fat and milk solid-not-fat in one or more of the various forms with or without water. This combination must be made so as to re-establish the product’s specified fat to solid-not-fat ratio and solid to water ratio.”
2.7 Rennet

Rennet is the common name for a group of proteolytic enzymes, including chymosin and pepsin, which will cause milk to clot. Traditionally, rennet is extracted from the fourth stomach of calves. Today, rennet can be extracted from the fourth stomach of bovine animals, produced by microorganisms, or derived from plant tissue (Brown & Ernstrom, 1988).

2.8 Rennet Induced Coagulation

Details of the action and mechanisms involved in rennet coagulation of milk have been well described by a number of researchers (Green et al., 1978; Dalgleish, 1979, Fox, 1987; McMahon et al., 1984; Robson & Dalgleish, 1984; Peri et al., 1990). The rennet-induced gel structure changes over time. Zoon et al., (1988a) noted that micelles started to fuse during aging of the gel, thus, increasing gel strength. They also concluded that the gel made from reconstituted skim milk had a similar time-dependent behavior as the gel made from fresh milk and suggested the interactions involved in a gel network are the same in both types of milk.
2.8.1 Factors affecting the rennet action and gel formation

The rates of hydrolysis and aggregation are affected by pH and temperature of the milk, concentration and type of enzyme, and concentration of casein and other components in the milk. The optimum pH for k-casein hydrolysis by chymosin is around pH 5.0 to 5.5. The optimum temperature of chymosin activity is 40°C (Dalglish, 1992).

Dalgleish (1986) reported the rate of rennet action was faster on small micelles than on large ones. Ford and Grandison (1986) reported that the rennet clotting time was slightly slower for large micelles than for small micelles, and the strength of gel from small micelles was higher than that from large micelles. The gel formed from smaller micelles was more compact and firmer. Although smaller casein micelles fused more extensively than the larger ones, the microstructures of rennet gels were similar regardless of the size of the casein micelles (Niki et al., 1994a).

Addition of CaCl₂ improves the rate of milk coagulation (Korolczuk & Maubois, 1988), thus the amount of rennet, or coagulant, used can be reduced. The use of calcium chloride is a common practice in commercial cheese-making (Green, 1977). Bringe and Kinsella (1986) indicated the concentration of CaCl₂ affected the rate of hydrolysis of the Phe₁₀₅-Met₁₀₆ bond. At CaCl₂ concentrations less than 10mM, the rates of hydrolysis and coagulation increased as CaCl₂ concentration increased. This is due to the increases in aggregatability of para-k-casein and the speed of the chymosin reaction.
A minimum concentration of calcium is needed for renneted milk to coagulate. Addition of calcium to milk increased the resulting gel strength. However, very high concentrations of calcium resulted in an increase in coagulation time. Inorganic phosphate, which exists as micellar calcium phosphate (MCP) in the micelle, is also important to rennet gel formation. Removal of MCP from milk increases the rennet gel time. Calcium ion is thought to alter the charge of casein micelles and phosphate is thought to alter the structure of casein micelles (Zoon et al., 1988c).

Although increasing milk concentration by ultrafiltration and increasing the rennet concentration both resulted in a decrease in gel time; the reported effects on gel strength are not consistent among researchers (Zoon et al., 1988a). Dejmek (1989) cited that after coagulation, the gel strength continuously increased up to 10 hours. The rennet gel strength was affected by protein concentration positively. A significant reduction of rennet used in cheese-making with ultrafiltration concentrated milk was reported by Green (1977).

Several studies have found homogenization, heat treatment, and other factors affect the characteristics of rennet coagulation of milk. Homogenization reduces rennet coagulation time, whey drainage rate of rennet curd, and curd strength (Robson & Dalgleish, 1984; Ghosh et al., 1994).

Singh and Fox (1986) reported that milk preheated at high temperature at pH 7.3 was not rennet coagulable, whereas milk without serum protein heated at same condition was readily coagulable by rennet. They indicated the denatured whey protein coated on the micelle and interfered with the rennet coagulation of the milk. Park et al. (1996) also
indicated that highly heated milk took longer to start coagulation than did regularly pasteurized milk. The deposit of denatured β-Lg on the surface of casein micelles due to high heat treatment interfered the accessibility of rennet; thus delaying coagulation. Ghosh et al. (1996) reported that the denatured whey proteins weakened the rennet curd strength, but decreased the syneresis of the curd.

2.9 Modified Milk in Cheese Making

Ultrafiltration (UF) and other methods, such as addition of concentrated milk and milk protein powder are commonly used by dairy industry in many countries, other than the United States, to standardize cheese milk, minimize seasonal variation of milk, and improve plant yield and efficiency (Hickey, 1993; Hickey & Versteeg, 1993; McMahon et al., 1993; Sharma et al., 1994).

Milk protein concentrates have been used in the manufacture of recombined cheese (Novak, 1991). Factors that need to be considered are the characteristics of milk protein concentrates and their influence on the rennet coagulation properties of the recombined milk.
2.9.1 Utilization of ultrafiltration

Ultrafiltration can be applied either to milk before adding starter and rennet or to cheese milk before curd formation (Rosenberg, 1995). McMahon et al. (1993) found ultrafiltration of cheese milk resulted in shorter coagulation time and a firmer gel. Ultrafiltration of milk was proven to be useful to reduce the amount of acid whey during fresh cheese production (Schkoda & Kessler, 1996). Hickey and Versteeg. (1993) found that ultrafiltrated milk at low concentration resulted in better curd size and texture of cottage cheese compared with those made from regular raw milk. Although several advantages of using ultrafiltration have been found in cheese making, it has not been successfully used in semi-hard and hard cheese due to negative effects on the texture of cheese after ripening (Anderson & Mistry, 1994; St-Gelais et al., 1995; Samuelsson et al., 1997).

2.9.2 Reconstituted / recombined milk

Reconstituted or recombined milk has been applied to cheese-making in many countries, other than the United States, where the fresh milk supply is not stable or sufficient (IDF, 1990; Jana & Thakar, 1996). Using recombined milk for cheese-making instead of fresh milk could alter the milk clotting time, curd strength, and whey drainage rate (Jensen & Nielsen, 1982). Conventional cheese making procedures have been successfully used to produce soft cheese from recombined milk (Kosikowski, 1958;
Bjerre, 1990; Jana & Thakar, 1996); however, modification of cheese-making procedures is needed for most of the cheese made from recombined milk (Abd El-Salam et al., 1981; Moneib et al., 1981; El-Safty & Ismail, 1982).

Flavor and texture are the most problematic areas for recombined milk cheese. El-Safty and Ismail (1982) found addition of free fatty acids improved flavor and accelerated ripening of Domiati cheese made from recombined skim milk powder mixed with raw milk. Abd El-Salam et al. (1981) suggested that the addition of buttermilk increased the yield and improved the flavor of pickled soft cheese made from high total solid (30%) recombined skim milk and anhydrous milk fat. Moneib et al. (1981) found high total solid reconstituted milk provided better properties in pickled soft cheese.

Recombined milk protein concentrate has been used in production of white cheese and fresh cheese (Gilles, 1984; Jana & Thakar, 1996; Mistry & Pulgar, 1996). Due to the difference of lactose and mineral contents and the heat treatment during processing, an adjustment of the procedure of cheese making from MPC is usually needed. Most of the milk protein concentrates used in the studies were produced on laboratory and pilot plant scales and the protein contents range from 60% to 80% (El-Samragy et al., 1993a & b; Mistry & Pulgar, 1996). The MPC was either used to enrich the protein content of cheese milk, or reconstituted into a much higher total solid solution than fresh milk when used to make cheese (Bjerre, 1990; Jana & Thakar, 1996). However, the higher protein content (80%) MPC was only used as an ingredient for making bulk starter rather than as an ingredient for cheese milk (Mistry & Pulgar, 1996).
Novak (1991) suggested that MPC is a good substitution for skim milk powder in cheese-making; and the resulting products would have consistent quality and high yield. The reconstituted cheese milk with a dry solid content equivalent to the resulting cheese would retain whey in the cheese. Feta was the first cheese to be made by recombined "cheese-powder" in which the lactose was converted into lactic acid before drying. Using recombined MPC to the same protein content as fresh milk to make cheese has not been evaluated.

2.10 Manufacture of Structure Feta Cheese and Cast Feta Cheese

Feta cheese is categorized as soft cheese because its moisture content is between 55 to 65% (Abd El-Salam, 1987). Lloyd and Ramshaw (1979) described the attributes of Bulgarian-style Feta cheese as: "smooth porcelain-like chalky-white exterior surface", "smooth, sealed, velvety but not fatty interior-cut surface", "soft, short but not crumbly body with few fermentation holes", "a piquant, fresh, acid and clean salty flavor", "pH 4.0-4.2, acidity about 300°T", "3.6% NaCl", and "moisture 49-50%".

2.10.1 Structure Feta cheese

Traditional Feta cheese is made from sheep's milk and is produced in the mountainous regions of Greece by shepherds. It is preserved and ripened in wooden barrels or tin boxes with brine for at least 2 months before being consumed (Anifantakis,
Some technological developments have been applied in the modern industrial manufacture of Feta cheese. Ultrafiltration of milk to reduce whey drainage is the biggest difference between the traditional method and the modern industrial process (Tamime & Kirkegaard, 1991). Recombined milk also has been used in Feta and other white cheeses’ manufacture for years in Egypt (Van Gennip, 1990).

The general steps of structure Feta cheese processing include: pasteurization of milk, homogenization of milk, cooling, culturing, renneting, coagulation, curd cutting, molding, pressing, acidification, cheese cutting, filling tins, salting, closing, and storage (Van Gennip, 1990; Tamime & Kirkegaard, 1991).

2.10.2 Cast Feta cheese

Cast Feta cheese has a closed texture, compared with structure Feta cheese, which has an open texture. The differences in processing procedures between structure Feta and Cast Feta are the steps after coagulation. When making Cast Feta, the curd is filled into tins directly to incubate, then brined and packed for storage (Van Gennip, 1990; Tamime & Kirkegaard, 1991). As a model system, cast Feta cheese could provide a means of evaluation of the effect of MPC on cheese characteristics as well as a model to determine the significant characteristics of MPC.
2.11 Factors Affecting Cheese Texture

Cheese texture is affected by milk composition, protein concentration, type of coagulant used, manufacturing process and the conditions used for ripening.

2.11.1 Components of cheese milk

Katsiari and Voutsinas (1994) reported that decreased fat content in cheese milk resulted in an increase of moisture content. A higher protein content in the low fat cheese milk increased the water-binding capacity thereby increasing moisture content. The higher protein content in low fat Feta also resulted in higher salt retention. The fat content did not affect the pH or acidity of the cheese significantly. The cheese yield decreased as the fat content decreased. The body and texture of very low fat cheese (from 1.5% fat milk) was hard and deteriorated while others improved after 180 days of storage. Strong acidity and high salt content are characteristics of Feta cheese flavor; therefore, the cheese made from 1.5% fat milk was still acceptable.

Steffi et al., (1999) reported the strength of rennet gel decreased by the addition of whey protein concentrate. The concentration of casein per unit volume was reduced by addition of whey protein concentrate (WPC) and resulted in less interaction between para-casein micelles thus resulting in a coarse network and weaker gel. The size of the holes in the rennet-induced casein network was about 10u. If the particle size was larger than 10 u, it disrupted the gel structure, whereas holes smaller than 10 u acted as "inert
filler”. “Fat globules and whey protein particles from WPI are acting in the casein network holes as “inert fillers” and their influence on gel strength can be reduced to a dilution effect.” (Steffl et al., 1999)

Schkoda et al., (1997) suggested the texture of the casein gel, that was made by acidification and rennet action, was not markedly affected by the mineral content of the solution. The serum holding capacity of casein gel could be increased by addition of lactose and minerals. However, rennet action reduced the serum holding capacity of the casein gel.

Abd El-Salam (1987) concluded that pickled soft cheese made from reconstituted or recombined milk had a relatively low moisture content. Increased total solids content of cheese milk resulted in higher moisture content in cheese. The protein matrix was affected by the pH of the cheese. Fresh Feta cheese had a pH around 4.5, which is close to the isoelectric point of casein; therefore, casein aggregates were more compact. The type of raw materials, fresh milk or dried milk powder, had effects on the microstructure of Feta type cheese. The difference of cheese structure was more evident after 2 months of storage. The microstructure of reconstituted milk cheese was looser, more porous; and less homogeneous compared with that from fresh milk cheese. The smooth body of ripened brine pickled cheese was the result of partial loss of calcium and continuous proteolysis of caseins (Abd El-Salam, 1987).
2.11.2 Homogenization and concentration of cheese milk

Cheese milk made from fresh milk is not usually homogenized. However, homogenization is needed when cheese milk is made from recombined milk and milk fat. Green et al. (1983) mentioned the cheese curd made from homogenized milk was not as firm and syneresed less compared with that made from non-homogenized milk. The resulting cheese retained more fat, thus had a soft, smooth and elastic texture.

Homogenization of milk reduced the curd firming rate while concentration of milk greatly increased the curd firming rate. The curd texture was coarser in concentrated milk and less coarse in homogenized milk. Homogenization resulted in a lower rate of whey loss and fat loss in whey when compared with concentration effects. The cheese made from concentrated milk was drier and more granular. The surface area of casein micelles was associated with fat in the homogenized milk that interfered with the formation of linkage between casein during curd fusion, which explained the weaker curd resulting from homogenized milk (Green et al., 1983).

Malin et al. (1993) concluded high homogenization pressure and high temperature during cooking retarded proteolysis. Kessler (1997) reported homogenization after heating of milk increased the stability of casein gel because the newly formed fat globules and the dissociated casein micelles were not covered by the denatured whey protein. The hydrophobic interaction in the acidic condition stabilized the casein gel network.
Samal et al., (1993) reported that homogenization of fresh or reconstituted milk and fresh or manufactured cream resulted in a decrease of whey exudation from Feta cheese during storage. The reduction of whey loss by homogenization was attributed to the change of size, surface area, or surface coating of fat globules. The casein coated fat globules became part of the casein matrix that retarded moisture loss.

2.11.3 Coagulants

Several types of coagulants are used in cheese making. The type of coagulants and the concentration of coagulant used in cheese making could have effects on cheese texture. Rennet is the major coagulant used in Feta cheese production. Alichanidis et al., (1984) concluded that the Feta cheese made by microbial coagulants were not significantly different from those made by calf rennet in cheese yield, flavor, texture, and acceptability of ripened cheeses. The pH of cheese ranged from 4.35 to 4.39 after 2 months of ripening. Wium and Qvist (1996) reported the texture of UF-Feta cheese was affected by the concentration of rennet added. The texture of Feta with a high rennet concentration was firm and gritty, whereas with a low rennet concentration was soft and sticky.
2.11.4 Brining during ripening of cheese

Brining is necessary for pickled cheese to become ripened. Noel (1996) mentioned the texture of cheese results from the structural organization of the major components during ripening. The pH and degree of proteolysis had major effects on cheese texture. Cheese takes up salt and loses water and some dry matter during brining. The properties of milk, the processing condition, and the brining condition all affected the amount of water retained in the cheese (Van Den Berg, 1993). Abd El-Salam, et al. (1993) stated that insufficient acidity, low storage temperature, and low salt content in brine would cause excessive softening of cheese during storage.

Pierre et al. (1999) reported that the total moisture content and proteolytic rate decreased during ripening while cheese texture became more compact after ripening. Cheese with low total solids had large empty cavities and formed continuous void spaces whereas cheese with high total solid was more compact. The water was in a dispersed phase in high solid cheese, but was in a continuous phase in low solid cheese. The growth of surface flora resulted in an increase in pH during ripening.

The salt, either dry or brine, used to pickle Feta is added after the cheese curd is formed. More time is needed for the salt to penetrate into the cheese block, thus decreased pH results in loss of moisture. During storage, the fat content increases, and pH and moisture content decrease. A high salt content brine results in lower moisture
content in Feta cheese compared with a low salt content brine. During storage, the pH of
Feta cheese does not change much and the structure of the casein aggregates remains
about the same (Abd El-Salam, 1987).

All the above studies are related to structural Feta. However, similar effects could
be anticipated on cast Feta cheese.

2.12 Physical Examination of Cheese Quality

Texture analysis and microstructure examination are two physical means of
evaluating the quality of cheese.

2.12.1 Texture analysis

Texture is one of the important attributes of cheese quality. Although the texture
of cheese is traditionally evaluated by sensory methodology, instrumental measurement of
texture has become more and more popular (Sohn, 1996). The results from instrumental
methods have been proven to have a good correlation with the results from sensory
methodology (Green et al., 1981; Zoon, 1996). Zoon (1996) mentioned that compression
testing is the most frequently used test for cheese texture.
Instruments for texture analysis, such as the Texture analyzer, TA-XT2, are used in research on cheese texture and the results are widely accepted (Brennan & Bourne, 1994; Drake et al., 1996; Lobato-Calleros et al., 1997; Kailasapathy, 1998; Suwonsichon & Peleg, 1999).

2.12.2 Microstructure

Structural images of food products including the development and changes during processing provide information for understanding the texture and sensory properties of food products, and, furthermore, facilitate the incorporation of new ingredients and development of new products (Kalab, 1993).

Electron microscopy provides a tangible image of the 3-dimensional structure of a subject. Scanning and transmission electron microscopy have been used in several studies of microstructure of casein micelles, rennet curd, and cheese (Green et al., 1981; Fichtali et al. 1990; Roefs et al., 1990; Kalab, 1993; Oberg et al., 1993; Anderson & Mistry, 1994). Confocal laser scanning microscopy (CLSM) has been used to examine the microstructure of cheese (Sohn, 1996). CLSM has the advantage of providing three-dimensional images of objects without fixation of the sample. It is a simpler alternative to observing cheese structure compared to electron microscopy.

Danilatos (1993) noted good vacuum is essential for the electron microscope to generate and propagate the electron beam. Environmental scanning electron microscopy (ESEM), a modified model of scanning electron microscopy, is a new development that
allows gas to exist in the specimen chamber. One of the advantages of ESEM is that sample preparation is not required. Therefore, the true surface structure of an object can be obtained (Danilatos, 1990). The ESEM is capable of examining fully hydrated samples and the hydration process as well. However, variation of relatively humidity in the ESEM chamber is limited (Prack, 1993).

The FEI Company (2000) described the Philips XL30 ESEM-FEG as the first scanning electron microscope with a Schottky Field Emission Source, which provides a stable, high brightness source for outstanding observation. The instrument uses water vapor to provide a wet mode and uses any type of gas to provide pressure. Since the sample does not need a conductive coating, the potential interference artifacts are eliminated.

Most studies using ESEM are for examining fiber, microorganisms, rock, and cement. Very few ESEM studies of food products have been published (Danilatos, 1991). Although several studies of microstructure of Feta cheese were cited by Abd El-Salam (1987), they were traditional Feta cheese samples, which have a different microstructure from cast Feta cheese structure. No previous studies of microstructure of cast Feta cheese have been reported.
De Castro (1999) hypothesized that there was a skin layer formed during the drying of MPC particles and the drier outlet temperature affected the hydration of the resulting MPC70 sample particles.

A preliminary test of particle sizes of dry and hydrated samples of several commercial MPC samples of differing protein content suggests that differences in hydrated samples exist. This preliminary test is summarized in Section 5.1.1. A further hypothesis of the skin formation during drying was made for this study.

In order to gain a better understanding of the characteristics of commercial MPC samples, a series of experiments were set up. These studies were divided into three phases – MPC particle characteristics, MPC rennet gel properties, and characteristics of cast Feta-type cheese made from commercial MPC samples.
3.1 Hypothesis

During the drying of MPC, the droplet of retentate forms a thin outer layer (shell). Diffusion of water causes migration of whey proteins and small micelles to the surface where the proteins become more concentrated. Heat induced disulfide interactions occur between whey proteins and between whey proteins and kappa-casein on the surface of small micelles, thus resulting in a decrease in the hydration properties of the dried particles. These reactions would be more pronounced with an increase in the protein content of the retentate, thus increasing the resistance of the shell during hydration. The proposed change of the droplet during drying is illustrated in Figure 3.1. Unless the surface of the particle can be hydrated or ruptured, the casein micelles within the dried particle will not be fully functional. This would be especially true in cheese making, where it is known that kappa-casein on the surface of the casein micelle must be available for rennet action and for proper curd formation.

3.2 Objectives

3.2.1 Phase 1 - particle characteristics

- To determine the particle characteristics of various commercial MPC samples of different manufacturers and of different protein contents.
• To determine the bonding forces of the MPC particle shells by applying various
dissociating treatments including chemicals, heat, and shear to the sample solution.

3.2.2 Phase 2 – Rennet gel properties

• To determine the rennetability of commercial MPC samples.
• To determine the effects of addition of CaCl₂ on the rennet gel made from MPC
  samples.
• To determine the effects on rennetability of various treatments that increase hydration
  of the commercial MPC sample solutions.

3.2.3 Phase 3 – Cast Feta-type cheese characteristics

• To determine the effects of using MPC samples of differing protein contents on the
  characteristics of cast Feta cheese
• To determine the age effects of MPC samples on the characteristics of the resulting cast
  Feta cheese.
• To determine the effects of various pretreatments of MPC solution on the resulting cast
  Feta cheese characteristics.
Figure 3.1: Changes of droplet and shell formation during drying
CHAPTER 4

PROCEDURES

4.1 Particle Hydration Characteristics

4.1.1 Particle characteristics of commercial milk protein concentrates samples

Twenty four commercial milk protein concentrate (MPC) samples, were obtained from ten different countries:

MPC40 : 40-49% protein - 3 samples (V - X)
MPC50 : 50-59% protein - 5 samples (Q - U)
MPC60-70 : 60-79% protein - 5 samples (L - P)
MPC80 : 80-89% protein - 11 samples (A - K)

Milk protein concentrates were made into a 10% protein solution with deionized water. The solution was hydrated at room temperature on a stirring plate stirred at a moderate speed for 1 hour. Samples were taken after hydration for particle size
measurement. The obscuration of the sampling solution was kept between 19 and 36% during measurement. No record was kept of the specific volume required to achieve this obscuration.

4.1.2 Factors affecting hydrated particle characteristics of MPC samples

To assess the ease of dissociating the large particles during hydration and to determine the holding force of the particle outer shell, some dissociating treatments were applied to the hydrated particles for a certain period of time in the Malvern instrument sample holder and the effects on the particle characteristics were examined.

4.1.2.1 Commercial MPC samples from various manufacturers and regions

The commercial MPC samples previously evaluated for particle size were evaluated further in respect to their dissociation by various treatments over 15 minutes. The dissociating treatments used included ultrasonication (US), and addition of urea (Urea), sodium dodecyl sulfate (SDS), and 2-mercaptoethanol (2ME). The particle size distribution was examined before and after treatment at 5-minute intervals of 15 minutes in total. The dissociating treatments were initialed in the Malvern sample holder after the sample had reached an obscuration between 24 to 34%. No record was kept of the specific volume required to achieve this obscuration.
4.1.2.2 Commercial MPC samples from a single manufacturer

In order to gain a better understanding of the factors affecting the dissociation of MPC particles, three MPC products with 56%, 70% and 85% protein were obtained from a single manufacturer. The composition information of these products, together with water activity and pH of a 10% protein solution after 1-hour room temperature hydration is in Table 4.1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Protein %</th>
<th>Fat %</th>
<th>Lactose %</th>
<th>Moisture %</th>
<th>Water activity</th>
<th>pH of solution</th>
</tr>
</thead>
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<tr>
<td>NZ56</td>
<td>56</td>
<td>1.2</td>
<td>31.0</td>
<td>3.8</td>
<td>0.28</td>
<td>6.6</td>
</tr>
<tr>
<td>NZ70</td>
<td>70</td>
<td>1.4</td>
<td>13.2</td>
<td>4.2</td>
<td>0.26</td>
<td>6.7</td>
</tr>
<tr>
<td>NZ85</td>
<td>81</td>
<td>1.9</td>
<td>3.9</td>
<td>3.9</td>
<td>0.18</td>
<td>6.8</td>
</tr>
</tbody>
</table>

Table 4.1: Composition and water activity of MPC NZ56, NZ70, and NZ85 samples, and pH of their 10% protein solutions

All samples were hydrated at room temperature for 1 hour before ultrasonication and the dissociating chemicals used previously applied. In addition, some sample solutions were heated to 60°C and held for 5 minutes (Heat), and some were applied sheare by a homogenizer after 1-hour hydration. Shear by the homogenizer was carried out at either 25°C (Homo-25) or 60°C (Homo-60). The shear pressure was 2000 psi using a laboratory scale bench-top homogenizer (Milko-tester MK II, A/S N. Foss
Electric, Hillerod, Denmark). These MPC56, 70 and 85 samples were also sent to the Malvern Analytical Laboratory (Southborough, MA) for dry powder particle size determination.

During hydrated particle size measurement, care was taken to set the initial obscuration in between 25% to 29%, which is well within the ideal 10% to 30% range for the instrument to obtain reliable measurements. A record was kept of the protein concentration in the Malvern sample holder when the target obscuration was achieved. Three concentrations (100, 200, and 1000 ppm) of urea, SDS and 2ME were applied to 1-hour hydrated MPC56 solutions in the sample beaker to determine the concentration effects on particle dissociation. Later, the lowest concentration (100 ppm) of those chemicals was added to both MPC70 and MPC85 solutions in the same procedure to minimize a rapid drop in obscuration. A record was also kept of the change in obscuration once the dissociating treatment was applied.

4.1.3 Solubility

The MPC samples were made into 10% protein solutions and hydrated at room temperature for 1 hour. The hydrated solution was centrifuged at 1000 x g for 10 minutes by a bench-top centrifuge (Marathon 13K/M Microcentrifuge, Fisher Scientific, Pittsburgh, PA). The supernatent and sediment were separated and dried in an oven at 70°C overnight. The solubility was calculated as follows:
Solubility (%) = \[
\frac{100 \times (\text{wet weight of supernatent} \times \% \text{ solid of supernatent})}{(\text{wet weight of supernatent} \times \% \text{ solid of supernatent}) + (\text{wet weight of sediment} \times \% \text{ solid of sediment})}
\]

4.2 Rennet Gel Properties

a. Materials

The MPC samples used in this section were selected from the MPC samples used in the previous section, but some of them were not from the same batches. The rennet used was a 1% dilution of a fermentation produced Chymosin (Chr. Hansen’s Inc.) The calcium chloride used was a 2M solution made from calcium chloride dihydrate (Fisher Scientific). A 5% lactic acid (Fisher Scientific) solution was used to adjust the pH of the MPC solution.

b. General procedure

The MPC sample was made into a 10% protein solution and hydrated with assigned conditions. The assigned treatment was applied to the solution at the end of hydration. The pH of the solution was adjusted to 6.4. The solution was then warmed to 30°C. Fifty ml of the warm solution was poured into a 50 ml beaker. The assigned amount of rennet and CaCl₂ were added into the 50 ml solution and the mixture was incubated in a 33°C waterbath until the gel was set. Three replicate gel samples were
made for each treatment combination. The particle size of the MPC solution was measured before pH adjustment. The gel set time was recorded by visual determination at 5 minutes intervals. The gel was stored in a refrigerator over night before the gel strength was determined by using a Texture Analyser, TA-XT2 (Texture Technologies Corp., USA).

4.2.1 Effects of rennet and calcium concentrations

Two milk protein concentrate samples, A85 and A70, were obtained from the same manufacturer. The information of the MPC samples is shown in Table 4.2.

<table>
<thead>
<tr>
<th>Sample</th>
<th>% protein</th>
<th>Outlet temperature - °C</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>A70</td>
<td>69</td>
<td>90</td>
<td>A</td>
</tr>
<tr>
<td>A85</td>
<td>82</td>
<td>90</td>
<td>A</td>
</tr>
</tbody>
</table>

Table 4.2: Information of MPC A70 and A85

Five hydration treatments were applied to the MPC solutions after 1-hour hydration at room temperature. The sample codes and the treatments are as follows:

1H : 1 hour hydration at room temperature
US : 1 hour hydration at room temperature and ultrasonicated for 5 minutes
H60 : 1 hour hydration at room temperature and heated at 60°C for 30 minutes
HUS: 1 hour hydration at room temperature, heated at 60°C for 30 minutes, and ultrasonicated for 5 minutes.

HO: 1 hour hydration at room temperature, heated at 60°C for 30 minutes, then stored overnight at 4°C.

After the hydration treatment, the 10% protein solution was diluted into a 3.5% protein solution before pH adjustment. Each combination of CaCl₂ and rennet was added into a 50ml MPC solution. The combination code and the amount of rennet and calcium chloride are as follow:

a: 150 ul rennet + 45 ul CaCl₂
b: 150 ul rennet + 90 ul CaCl₂
c: 250 ul rennet + 45 ul CaCl₂
d: 250 ul rennet + 90 ul CaCl₂

4.2.2 Effect of shear

To prove the proposed hypothesis that increased dissociation of MPC particles could provide more available casein micelles to improve rennet gel formation, the effects of shear of MPC solution was tested. Three commercial MPC samples with different protein contents (MPC56, MPC70, MPC85) were obtained from the same manufacturer. The composition of the MPC samples is shown in Table 4.3. All the MPC samples were made into 10% protein solutions and hydrated at room temperature for 1 hour. At the end of hydration, half of the solution was heated to 60°C, applied shear by using the lab scale
homogenizer, and cooled to 30°C before pH adjustment. The other half of the solution was warmed to 30°C before pH adjustment. The rennet gel was made directly from the 10% protein solution. Ninety micro-liters of 2M CaCl₂ and 150 ul of 1% rennet were added into 50 ml of MPC solution.

<table>
<thead>
<tr>
<th>%</th>
<th>MPC 56 (56.0 - 58.0)</th>
<th>MPC 70 (68.5 - 71.0)</th>
<th>MPC 85 (80.0-83.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>56.0 (56.0 - 58.0)</td>
<td>70.0 (68.5 - 71.0)</td>
<td>81.0 (80.0-83.0)</td>
</tr>
<tr>
<td>Fat</td>
<td>1.2 (2.0 max)</td>
<td>1.4 (2.0 max)</td>
<td>1.9 (2.1 max)</td>
</tr>
<tr>
<td>Lactose</td>
<td>31.0</td>
<td>16.2</td>
<td>3.9 (5.5 max)</td>
</tr>
<tr>
<td>Ash</td>
<td>8.0 (8.5 max)</td>
<td>8.2 (10.0 max)</td>
<td>7.4 (8.5 max)</td>
</tr>
<tr>
<td>Calcium</td>
<td>1.8</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Moisture</td>
<td>3.8 (5.0 max)</td>
<td>4.2 (5.0 max)</td>
<td>3.9 (5.0 max)</td>
</tr>
</tbody>
</table>

Table 4.3: Manufacturer’s specifications of MPC56, 70, and 85 samples

4.3 Cast Feta Cheese Made from MPC Samples

4.3.1 Materials

The milk protein concentrates that were used in this phase of investigation were similar to those used in the section 4.2.2, but originated from different batches. The protein content determined by the Kjeldahl method was 56%, 69%, and 83% for MPC56, MPC70, and MPC85 respectively.
Additional ingredients that were used in cast Feta cheese making were as follows:

a. Butter - fat source

The butter addition to cheese milk was commercial unsalted sweet butter obtained from a local grocery store.

b. Calcium chloride - calcium source

Calcium Chloride Dihydrate obtained from Fisher Scientific (Fair Lawn, NJ) was used to make a 2M solution that was then added to the MPC cheese milk.

c. Starter Culture - fermentation source

The starter Cultures used were Direct Vat Set (DVS) cultures: YC-085, Thermophilic Lactic Culture; and R-604, Mesophilic Lactic Homofermentative Culture. The cultures were obtained from Chr. Hansen, Inc. (Milwaukee, WI).

d. Rennet - coagulant

The rennet used was recombinant Chymosin obtained from the Chr. Hansen, Inc. (Milwaukee, WI).

e. Salt - brine source

Regular table salt obtained from a local grocery store was used to pickle the fresh cast Feta cheese.
4.3.2 Formulation

The formula for the cheese milk is shown in Table 4.4. The cheese milk was prepared to contain 10% protein and 10% milk fat.

<table>
<thead>
<tr>
<th>Sample</th>
<th>MPC56</th>
<th>MPC70</th>
<th>MPC85</th>
<th>% by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPC</td>
<td>17.9</td>
<td>14.6</td>
<td>12.1</td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td>69.4</td>
<td>72.7</td>
<td>75.2</td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>12.7</td>
<td>12.7</td>
<td>12.7</td>
<td></td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Starter culture</td>
<td>2.2</td>
<td>2.2</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Rennet</td>
<td>0.0075</td>
<td>0.0075</td>
<td>0.0075</td>
<td></td>
</tr>
<tr>
<td>Salt</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.4: Formulation of cheese milk

4.3.3 General procedure

The cheese-making procedure is illustrated in Figure 4.1. The cheese container is a cylinder plastic cup with lid. The diameter and height of the container are both 70 mm. Each cup contained 150 grams of cheese milk. The particle sizes of MPC solution and cheese milk along with the pH, texture, moisture content, and surface microstructure of cheese were determined.
Figure 4.1: Flowchart of cast Feta cheese production
4.3.3.1 Effect of hydration time

The results of hydrated MPC particle characteristics and MPC rennet gel indicated the hydration treatment affected the dissociation of the particles and the strength of the rennet gel. Thus, hydration treatment of MPC solution could also affect the properties of resulting cast Feta cheese. Two batches of each MPC were used in this experiment. One was “New”- stored less than 6 months after production, and the other was “Old”- stored more than 12 months after production. Three hydration times — 1, 3, and 5 hours at room temperature, were applied to the 10% protein MPC solutions before the addition of butter and homogenization.

4.3.3.2 Effect of applying shear on MPC solution

The results from section 4.2.2 indicated that applying shear on MPC solution affected rennet gel strength. Thus, applying shear on MPC solution could also affect the properties of the resulting cast Feta cheese. The “New” batches of MPC 56, 70, and 85 used in section 4.3.3.1 were used in this section. After 1 hour of hydration, half of the solution was heated to 60°C and applied shear before addition of butter and further homogenization. The other half of the solution was heated to 60°C, had butter added, and was homogenized. The particle size of the solution was measured before heating and after homogenization.
4.3.3.3 Effect of lactose addition to MPC85 sample

A soft gel like texture and high pH of MPC85 cheese seen in sections 4.3.3.1 and 4.3.3.2 suggested that there might not be enough lactose in the MPC85 sample for a starter culture to grow and to lower the pH of the cheese to a desirable level. In order to resolve this suspicion, various concentrations of lactose were added into MPC85 cheese milk and the effects on pH and texture of the resulting cheeses were determined. The MPC85 used in section 4.3.3.2 was used in this section. The sample codes and variable are shown in Table 4.5.

<table>
<thead>
<tr>
<th>Code</th>
<th>85-0</th>
<th>85-5</th>
<th>85-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose %</td>
<td>0</td>
<td>5</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 4.5: Sample code and percentage of lactose addition to cheese milk

4.4 Data Collection

4.4.1 Particle size

The particle size was determined by using the Mastersizer Microplus (Micro-P, Malvern Instruments Limited, UK). A polydisperse analysis model was used. The standard system presentation (50HD), refractive index of 1.53 and absorptive index of
0.10, was selected to determine the particle size distribution. The range of particle size detection of this instrument is from 0.05 μm to 550 μm. The valid range of obscuration for this specific instrument is 5% to 50%, and, ideally, between 10 to 30%. The volume median diameter, $D(v, 0.5)$, and specific surface area (SSA) were recorded to represent the sample and compare with others.

4.4.2 Rennet gel strength

The gel strength was measured by using a Texture Analyser, TA-XT2 (Texture Technologies Corp., USA) with Stable MicroSystem (SMS, England). A 60-degree cone acrylic probe was used and maximum compression force (g) was recorded as the gel strength. The testing speed was set at 1 mm/sec and the testing distance was 15 mm. No fracture of the gel occurred.

4.4.3 Cast Feta cheese characteristics

a. pH of the cheese

The pH of the cheese was measured by using a pH meter (Accumat® pH meter 25, Fisher Scientific, Fair Lawn, NJ) with probe set on the top of the cheese, immediately following texture measurement.
b. Moisture content of the cheese

A sample was taken from the center of each cheese after 14 days of cold storage. Moisture content was determined by weight lost in a 70°C oven overnight.

c. Texture of cheese

The cheese texture was measured in the same way as indicated in section 4.4.2 for rennet gel strength. The cheese samples were taken on the first day, the 7th day and the 14th day after the cheese was made. Each sample, taken from the container without cutting, was measured at 3 different points on the top portion and 3 different points on the bottom portion. The average and standard deviation of the six measurements were calculated and used to compare with other samples.

d. Surface microstructure of cheese

An environmental scanning electronic microscope with Field Emission Gun (XL-30 ESEM - FEG, Philips), set to 4.5 Torr, wet, and 15.0 kV was used to examine the surface structure of the cheese at 800 times magnitude. The sample was taken from the center of each cheese after 14 days of cold storage and placed on a specimen holder without further sample preparation.
4.5 Data Analysis

Data was analyzed by one-way ANOVA, Tukey's comparison, and Pearson correlation at a significance level of 0.05, if necessary. Computer statistical analysis software, Minitab (Version 13), was used to perform the statistical analysis.
CHAPTER 5

RESULTS AND DISCUSSION

Commercial MPC samples of varying protein concentration from different manufacturers were evaluated for their hydration particle characteristics, ability to form rennet gels, and application in the manufacturer of cast Feta cheese. Factors affecting these properties also were investigated. The results of this investigation are summarized into several sections in this chapter.

5.1 Particle Hydration Characteristics

The particle hydration properties of a wide range of commercial MPC samples were investigated. The dry powder particle size of some samples were measured and compared with their 1-hour hydrated counterparts. In order to gain knowledge regarding differences between hydrated samples and the causes of these differences, several factors including dissociating chemicals, heat, and shear, which can affect hydrated particle
characteristics, were applied to the samples. The volume median diameter, D(v, 0.5), which divides the sample population into two equal parts, is used to represent particle size throughout the text.

5.1.1 Preliminary experiment

Three commercial MPC samples, MPC70A, MPC80B and MPC85A, were obtained from 2 sources. The MPC samples were made into 10% protein solutions and hydrated at room temperature for 1 hour. These powder samples were sent to Malvern Analytical Laboratories for dry powder particle size determination. The particle sizes, D(v, 0.5) and specific surface area (SSA), of the dry and 1-hour hydrated samples are shown in Table 5.1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>MPC70A</th>
<th>MPC80B</th>
<th>MPC85A</th>
</tr>
</thead>
<tbody>
<tr>
<td>um D(v, 0.5)</td>
<td>D(v, 0.5)</td>
<td>D(v, 0.5)</td>
<td></td>
</tr>
<tr>
<td>Dry</td>
<td>43.40</td>
<td>64.80</td>
<td>58.15</td>
</tr>
<tr>
<td>10%</td>
<td>64.71</td>
<td>39.52</td>
<td>78.63</td>
</tr>
<tr>
<td>m²/g</td>
<td>SSA</td>
<td>SSA</td>
<td>SSA</td>
</tr>
<tr>
<td>Dry</td>
<td>0.1896</td>
<td>0.1552</td>
<td>0.1715</td>
</tr>
<tr>
<td>10%</td>
<td>0.1653</td>
<td>0.6049</td>
<td>0.1521</td>
</tr>
</tbody>
</table>

Table 5.1: D(v, 0.5) in um and SSA in m²/g of dry powder and 10% protein 1-hour hydrated solution of MPC70A, MPC80B, and MPC85A samples

51
Differences in average particle sizes were observed between dry and hydrated samples and among different protein content samples. The hydrated particles of MPC70A and MPC85A both appeared to be larger than their dry powder counterparts, indicating uptake of water and swelling but no dissociation of the particles. However, the hydrated particles of MPC80B were smaller than its dry powder counterparts, suggesting some particles were dissociated during hydration. The hydration characteristics of the powders was not related to protein content, but was related to factory source. The particle changes due to hydration are most likely related to the processing parameters of the samples.

5.1.2 Particle size characteristics of commercial milk protein concentrate (MPC) samples from different sources

The hydrated particle size of several commercial MPC samples of various protein contents from different sources were measured. A summary of the range, and average for D(v, 0.5) and specific surface area (SSA) of the 24 commercial MPCs of different protein contents hydrated for 1 hour are in Tables 5.2 and 5.3 respectively. Detailed information for each individual sample is in Appendix A, Table A.1. The particle size distribution of each group of MPCs is shown in Appendix A, Figures A.1, A.2, A.7, A.12, and A.17.
<table>
<thead>
<tr>
<th>MPC protein %</th>
<th># of Sample</th>
<th>Range (um)</th>
<th>Average</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>40-49</td>
<td>3</td>
<td>0.35-0.43</td>
<td>0.39</td>
<td>0.033</td>
</tr>
<tr>
<td>50-59</td>
<td>5</td>
<td>19.64-94.50</td>
<td>52.16</td>
<td>26.379</td>
</tr>
<tr>
<td>60-79</td>
<td>5</td>
<td>27.37-99.20</td>
<td>61.61</td>
<td>24.630</td>
</tr>
<tr>
<td>80-89</td>
<td>11</td>
<td>23.75-85.93</td>
<td>57.08</td>
<td>20.582</td>
</tr>
</tbody>
</table>

SD – standard deviation

Table 5.2: Initial D(v, 0.5) in um of 1-hour hydrated MPC samples

<table>
<thead>
<tr>
<th>MPC protein %</th>
<th># of Sample</th>
<th>Range (m²/g)</th>
<th>Average</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>40-49</td>
<td>3</td>
<td>13.3651 - 16.4310</td>
<td>15.2962</td>
<td>1.4755</td>
</tr>
<tr>
<td>50-59</td>
<td>5</td>
<td>0.1997 - 4.5645</td>
<td>1.1801</td>
<td>1.7988</td>
</tr>
<tr>
<td>60-79</td>
<td>5</td>
<td>0.1450 - 3.4952</td>
<td>0.8424</td>
<td>1.4499</td>
</tr>
<tr>
<td>80-89</td>
<td>11</td>
<td>0.1938 - 2.1318</td>
<td>0.4021</td>
<td>0.5551</td>
</tr>
</tbody>
</table>

Table 5.3: Initial SSA in m²/g of 1-hour hydrated MPC samples

The particle size distribution among hydrated commercial MPC samples was highly variable between samples except for those having a protein content of less than 50%. The samples in the MPC40 group all had particle sizes less than 1 um and a narrow distribution curve among the majority of the particles (Figure A.1). This indicated that the MPC40 particles after 1 hour of hydration at room temperature existed almost entirely in a micellar form. For MPC samples with greater than 50% protein, the particle size was always greater than 20 um; which suggests limited dissociation of the particles to release micelles. This is supported by the particle size distribution curves in Appendix A,
Figures A.2, A.7, A.12, and A.17. Although the average particle size of the 50-59, 60-79, and 80-89% MPCs were similar, the standard deviation was extremely large (Table 5.2). The very wide variability in particle size agrees with the large differences in commercial MPC functionality reported by De Castro (1999).

The specific surface area (SSA) generally decreased with increasing protein content (Table 5.3). However, this trend is not statistically significant because of the large deviation in SSA of samples of the same protein content.

5.1.3 Factors affecting hydrated MPC particle characteristics

Several factors can be expected to contribute to the very broad differences in hydration properties of the MPC samples, which were produced under different processing conditions. The retentate concentration before spray-drying, the inlet and outlet temperature of the spray drier and the storage conditions of the MPC samples after manufacture could alter the hydration properties of the MPC samples. Interactions that occur at the surface (shell) of the particle during drying and storage could include hydrophobic interactions, hydrogen bonding, disulfide linkage, and polymerization due to Maillard reaction products. To determine the nature of such interactions, several dissociating chemicals - urea, beta-mercaptoethanol, and SDS, as well as ultrasonication were applied to the hydrated samples. Both chemical and ultrasonication treatments were applied into the Malvern sample holder with 500 ml of distilled water as the carrier medium. Therefore, the concentrations of the MPC protein and the additional chemicals
in the sample holder were very diluted. The effects of the treatments were determined by changes in particle size and would help to prove the proposed hypothesis that shell formation of MPC particles during drying is affected by the protein concentration of the retentate.

5.1.3.1 Commercial MPC samples from various manufacturers and regions

The percent change in $D(v, 0.5)$ after 5 minutes of ultrasonication and treatments with urea (1200 ppm), and beta-mercaptoethanol (1120 ppm) are summarized in Table 5.4. The $D(v, 0.5)$ after 15 minutes of treatments and the percentage change after 5 minutes of treatments for individual samples are shown in Appendix A, Tables A.2 and A.3.

Use of SDS at a concentration equal to 500ppm in the sample solution resulted in a rapid drop in obscuration. The obscuration of some samples dropped below 5% within 5 minutes after the addition of SDS, thus, the particle size data was not reliable. This suggests that SDS not only penetrated the outer shell of the particle, but also dissociated the casein micelles in the interior. Therefore, this data is not considered to be reliable and is not shown here (See Appendix A, Table A.2). All other treatments, with a few exceptions, showed variable dissociation of the large particles. Most of the change occurred in the first 5 minutes of treatment.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Range</th>
<th>Average</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPC50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultrasonication</td>
<td>-3 to -98</td>
<td>-51</td>
<td>41.6</td>
</tr>
<tr>
<td>Urea</td>
<td>-1 to -36</td>
<td>-16</td>
<td>15.1</td>
</tr>
<tr>
<td>2-ME</td>
<td>8 to -96</td>
<td>-31 (-41*)</td>
<td>41.0</td>
</tr>
<tr>
<td>MPC60-70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultrasonication</td>
<td>-32 to -98</td>
<td>-62</td>
<td>25.2</td>
</tr>
<tr>
<td>Urea</td>
<td>-7 to -27</td>
<td>-16</td>
<td>9.6</td>
</tr>
<tr>
<td>2-ME</td>
<td>-9 to -99</td>
<td>-35</td>
<td>36.3</td>
</tr>
<tr>
<td>MPC80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultrasonication</td>
<td>59 to -98</td>
<td>-28 (-57*)</td>
<td>67.2</td>
</tr>
<tr>
<td>Urea</td>
<td>8 to -41</td>
<td>-22 (-24*)</td>
<td>21.1</td>
</tr>
<tr>
<td>2-ME</td>
<td>-24 to -83</td>
<td>-44</td>
<td>27.4</td>
</tr>
<tr>
<td>MPC85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultrasonication</td>
<td>-29 to -67</td>
<td>-41</td>
<td>13.1</td>
</tr>
<tr>
<td>Urea</td>
<td>-4 to -31</td>
<td>-11</td>
<td>9.1</td>
</tr>
<tr>
<td>2-ME</td>
<td>-7 to -36</td>
<td>-18</td>
<td>10.1</td>
</tr>
</tbody>
</table>

* Average omitting single sample that showed an increase in particle size

Table 5.4: Percent change in D(v, 0.5) of MPC samples after 5 minutes of treatments

Overall, the changes in particle size ranged from -3 to -98% for ultrasonication, from 8 to -41% for urea, and from 8 to -99% for beta-mercaptoethanol. The effects were highly variable between treatments and between samples for a given treatment. In general, the treatments in order of decreasing effectiveness in dissociation of hydrated particles were ultrasonication, beta-mercaptoethanol, and urea.
However, beta-mercaptoethanol seemed to be less effective for dissociating MPC85 than for the other MPCs. This might be expected, since more interactions could occur during drying and storage with the higher protein content MPC samples. Due to a fairly large standard deviation the effects of the treatments were not significantly different. There was no apparent relationship between the effect of the dissociating agent and protein content for these samples.

Because of the extreme variability in the dissociation of MPC particles after 1 hour of hydration at room temperature, it is very difficult to interpret the results. The data does suggest that there is a very great difference in the surface characteristics such as resistance to dissociation of the MPC particles from different sources. Disulfide bonds do appear to be involved, to a varying degree, in resistance of the particle to hydration. The differences observed could be related to both processing differences and to differences in the age of the samples.

5.1.3.2 Commercial MPC samples from a single manufacturer

Because of the extreme variability in the hydration characteristics of MPC from difference sources, attention was directed to MPC samples of differing protein contents from a single manufacturer.
5.1.3.2.1 Comparison of particle size of dry and hydrated MPC samples

Three MPC samples of different protein contents were obtained from the same manufacturer. The particle sizes of the dry powder sample and of the hydrated particles were determined. The method of the particle size determination was the same as stated previously. The $D(v, 0.5)$ and SSA of the dry and hydrated MPC56, 70, and 85 samples are shown in Table 5.5.

<table>
<thead>
<tr>
<th>Sample</th>
<th>MPC56</th>
<th>MPC70</th>
<th>MPC85</th>
<th>MPC56</th>
<th>MPC70</th>
<th>MPC85</th>
</tr>
</thead>
<tbody>
<tr>
<td>um D(v, 0.5)</td>
<td>D(v, 0.5)</td>
<td>D(v, 0.5)</td>
<td>m²/g</td>
<td>SSA</td>
<td>SSA</td>
<td>SSA</td>
</tr>
<tr>
<td>Dry</td>
<td>47.65</td>
<td>69.99</td>
<td>49.39</td>
<td>0.1989</td>
<td>0.1323</td>
<td>0.2019</td>
</tr>
<tr>
<td>10%</td>
<td>43.13</td>
<td>63.29</td>
<td>67.46</td>
<td>1.1760</td>
<td>0.1544</td>
<td>0.1821</td>
</tr>
</tbody>
</table>

Table 5.5: $D(v, 0.5)$ in um and SSA in m²/g of dry powder and 10% protein 1-hour hydrated solutions of MPC56, 70, and 85 samples from the same manufacturer

The dry powder MPC56 and MPC70 samples both had a larger particle size than their hydrated counterparts, whereas the hydrated MPC85 sample had a larger particle size than the dry powder sample. The results suggest that the hydrated MPC56 and MPC70 particles were partially dissociated. On the other hand, the hydrated MPC85 particles were just swelling due to water uptake without dissociation. The particle size
changes due to hydration were consistent with the changes in SSA. An increase in SSA of the hydrated MPC56 and MPC70 samples indicated an increase of small particles. A slight decrease in SSA of the hydrated MPC85 indicated an increase in particle size.

5.1.3.2.2 Effect of various concentrations of dissociating chemicals

During particle size measurement, the 1-hour hydrated sample solution was added into the instrument sample holder until the obscuration rose to approximately 25%. The protein concentration required to achieve this obscuration was approximately 600 ppm for MPC56. The dissociating chemical was added into the holder after the initial particle size was measured and mixed for 5 minutes before another particle size measurement was taken.

The effects of various concentrations (100, 200, and 1000 ppm) of the dissociating chemicals on the particle size of MPC56 were determined. The D(v, 0.5) and SSA of hydrated particles, the beginning and final obscurations, the volume of MPC solution required to achieve the initial obscuration, and the volume percent of particles less than 1 micron are in Table 5.6.

Addition of dissociating chemicals had a small, but significant effect on reducing the particle size of the 1-hour hydrated NZ56 in all concentrations. The effects of addition of 100 and 200 ppm of urea and of 2-mercaptoethanol were similar. Both urea and 2-mercaptoethanol seem to be less affective at 1000 ppm than at 100 or 200 ppm. However, 2-mercaptoethanol was more effective on dissociating particles than urea at the
same concentration. At 100 ppm, SDS had a similar effect as urea. At 300 ppm, the SDS effect was similar to the 2-mercaptoethanol effect at 200 ppm. With addition of 1000 ppm SDS, obscuration of the sample solution became too low to have a reliable measurement.

<table>
<thead>
<tr>
<th></th>
<th>D(v, 0.5) um</th>
<th>SSA M²/g</th>
<th>&lt; 1 um Vol %</th>
<th>Obscuration Initial %</th>
<th>Obscuration Final %</th>
<th>MPC added Vol - ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>44.86</td>
<td>1.0630</td>
<td>4.27</td>
<td>26.0</td>
<td></td>
<td>2.8</td>
</tr>
<tr>
<td>Urea-100</td>
<td>34.37</td>
<td>1.3713</td>
<td>5.22</td>
<td>26.2</td>
<td>23.3</td>
<td>3.0</td>
</tr>
<tr>
<td>2ME-100</td>
<td>28.06</td>
<td>2.1006</td>
<td>8.58</td>
<td>26.1</td>
<td>21.5</td>
<td>3.1</td>
</tr>
<tr>
<td>SDS-100</td>
<td>34.90</td>
<td>1.3740</td>
<td>5.23</td>
<td>25.9</td>
<td>22.9</td>
<td>3.0</td>
</tr>
<tr>
<td>Urea-200</td>
<td>32.99</td>
<td>1.4768</td>
<td>5.84</td>
<td>26.3</td>
<td>22.4</td>
<td>2.8</td>
</tr>
<tr>
<td>2ME-200</td>
<td>27.95</td>
<td>2.0983</td>
<td>8.50</td>
<td>25.3</td>
<td>20.6</td>
<td>3.0</td>
</tr>
<tr>
<td>SDS-300</td>
<td>23.87</td>
<td>2.9946</td>
<td>7.43</td>
<td>26.3</td>
<td>14.1</td>
<td>3.1</td>
</tr>
<tr>
<td>Urea-1000</td>
<td>39.58</td>
<td>1.0729</td>
<td>4.06</td>
<td>26.1</td>
<td>23.0</td>
<td>2.1</td>
</tr>
<tr>
<td>2ME-1000</td>
<td>33.68</td>
<td>1.4309</td>
<td>5.80</td>
<td>26.9</td>
<td>20.9</td>
<td>2.3</td>
</tr>
<tr>
<td>SDS-1000</td>
<td>33.68</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.2*</td>
</tr>
</tbody>
</table>

* Obscuration was too low to have a reliable measurement

Table 5.6: Effects of various concentrations (100, 200, and 1000 ppm) of dissociating chemicals on 1-hour hydrated MPC NZ56 sample
Based on the results with MPC56, 100 ppm of each dissociating chemical was tested on the 1-hour hydrated MPC70 and MPC85 samples by the same procedure. In addition to the chemical dissociation treatments, 5 minutes of ultrasonication was also applied by the built-in device of the Malvern instrument to the samples in the sample beaker.

The average $D(v, 0.5)$ of untreated and variously treated NZ56, 70, and 85 samples are compared in Figure 5.1. The protein concentrates in the sample beaker were approximately 300 ppm for both MPC70 and MPC85 samples and 800 ppm for MPC56 sample. The particle sizes of all three untreated 1-hour hydrated MPC samples were larger than 1 um which means the majority of the particles were not dissociated. In any case, the average particle size of hydrated NZ85 was the largest and hydrated NZ56 was the smallest among all samples. Ultrasonication had a significant effect on reducing particle size. Addition of 100 ppm of chemicals had a milder, but still significant effect on reducing particle size. Beta-mercaptoethanol had a relatively stronger effect than urea and SDS at 100 ppm concentration. The difference of effects between 100 ppm of urea and SDS was not significant.
The average particle size of NZ56 was 44.86 μm initially. Ultrasonication had a greater effect on reducing particle size compared to urea, SDS, and 2-mercaptoethanol. The obscuration of the sample solution dropped to almost half of the initial value after 5 minutes of ultrasonication. On the other hand, the obscuration did not change more than 5% after 5 minutes of mixing with dissociating chemicals at 100 ppm. Urea affected the particles slightly. SDS had a similar effect as urea at 100 ppm. Beta-mercaptoethanol had a greater effect than both urea and SDS at 100 ppm concentration. However, the effect was still mild (Figure A.22).
The initial particle size of NZ70 was 64.02 um. Five minutes of ultrasonication dropped the obscuration more than 10% and increased the micellar particles from 0% to 2% of the volume; but the D(v, 0.5) after ultrasonication was not significantly different from those after urea, SDS and 2- mercaptoethanol treatments (Figure A.23).

The average particle size of NZ85 was 70.26 um initially. Neither the dissociating chemicals nor ultrasonication were able to dissociate the particles into micellar form. The obscuration did not change more than 5% in all cases where dissociating agents were applied (Figure A.24).

5.1.3.2.3 Effect of heat and shear treatments

Further attention was given to the effect of heat treatment at 60°C for 30 minutes after 1-hour room temperature hydration. This was based on the fact that the optimum hydration temperature for milk powder is 60°C (Kneifel & Seiler, 1993). Also, since ultrasonication of the protein solution had a greater effect on particle dissociation, attention was directed to shear treatment by a homogenizer to the MPC solution at both 25°C and 60°C.

The average D(v, 0.5) of the heat-treated and sheared NZ56, 70, and 85 samples are compared in Table 5.7. Except for the sheared-at-60°C (Homo-60) samples, the particle sizes were significantly different among those three samples.
<table>
<thead>
<tr>
<th>D(v, 0.5) - um</th>
<th>Untreated</th>
<th>Heat</th>
<th>Homo-25</th>
<th>Homo-60</th>
</tr>
</thead>
<tbody>
<tr>
<td>NZ56</td>
<td>44.86</td>
<td>0.37</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>NZ70</td>
<td>64.02</td>
<td>0.47</td>
<td>0.38</td>
<td>0.35</td>
</tr>
<tr>
<td>NZ85</td>
<td>70.26</td>
<td>14.96</td>
<td>0.57</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Table 5.7: Average D(v, 0.5) of 1-hour hydrated MPC NZ56, 70, and 85 after heat treatment and shear treatment at 25°C (Homo-25) and 60°C (Homo-60)

The average D(v, 0.5) of the heat-treated and sheared NZ56 samples were similar and all were less than 1 um. Heat treatment reduced more than 75% of the NZ56 particles to micellar form whereas all the NZ56 particles became micelles after shear treatment. In the case of the NZ70 sample, the average D(v, 0.5) of heat-treated and sheared samples were all less than 1 um. However, heat treatment reduced less than 60% of the particles into micellar form whereas shear treatment at 25°C and 60°C reduced more than 79% and 88% of the particles into micellar form respectively. The average D(v, 0.5) of the heat-treated NZ85 was 14.96 um and sheared samples were both less than 1 um. After heat treatment, only 24% of the NZ85 particles were dissociated to micelles. Shear treatment at 25°C reduced more than half of the NZ85 particles to micellar range. However, when sheared at 60°C, more than 88% of the NZ85 particles were reduced to micellar form.
The effects of heat treatment were more pronounced for low protein MPC samples. The majority of the particles were in micellar form after shear treatment. However, shear treatment at 25°C was not as efficient as at 60°C in terms of dissociating particles. Heat treatment and shear treatment had a much greater effect on reducing particle size when compared with chemical and ultrasonication treatments.

5.1.4 Solubility of MPC samples

Sediment was noticed in some 1-hour hydration MPC85 solutions after sitting for some time without stirring. Therefore, the solubility of the 1-hour hydration MPC56, 70, and 85, the same samples as used in section 5.1.3.2.3, were tested. The solubility of the MPC samples is shown in Table 5.8. The solubility of the 1-hour hydrated MPC56, 70, and 85 were 91.5%, 56%, and 33% respectively. The solubility decreased as the MPC protein content increased. The solubility had a negative correlation (-0.942) with particle size. This indicated the hard-to-dissociate particles from high protein MPC samples caused a decrease in solubility. The solubility of all three sheared 1-hour hydrated MPC solutions were almost 100%.
### Table 5.8: Average solubility (%) of 1-hour hydrated MPC56, 70 and 85 samples

<table>
<thead>
<tr>
<th>%</th>
<th>MPC56</th>
<th>MPC70</th>
<th>MPC85</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>91.48</td>
<td>56.12</td>
<td>32.88</td>
</tr>
<tr>
<td>SD*</td>
<td>0.31</td>
<td>1.52</td>
<td>0.52</td>
</tr>
</tbody>
</table>

* Standard deviation

5.2 Rennet Gel Properties

Previous industrial reports have indicated that MPC56 forms good rennet gels, whereas MPC70 and MPC85 do not form good rennet gels. Thus, attention was directed to determining the effects of high protein MPC samples on the time for rennet coagulation and rennet gel strength. Also, various treatments to improve rennet gel hardness were evaluated.

Preliminary evaluation suggested that 10% solutions of MPC85 samples hydrated at room temperature for 1 hour either did not form a rennet gel, or formed very weak gels in relation to milk. Addition of extra rennet or calcium chloride gave little improvement in gel formation. In contrast, MPC56 gave rennet gel strength similar to that of milk.
5.2.1 Effects of rennet and calcium concentrations

The effects of various concentrations of rennet and calcium chloride on the strengths of rennet gels made from MPC70 and MPC85 samples under the different hydration conditions are outlined in this section.

The strengths of rennet gels made from the MPC70 sample at various conditions are shown in Figure 5.2. The gels made from 1-hour hydration MPC (1H) were very weak at the lowest concentration of rennet and calcium, but improved as the rennet and calcium concentrations increased. The gels made from ultrasonicated (US) and heat-treated (H60) MPC solutions reacted to the increases of rennet and calcium concentrations in a similar way as the 1-hour hydrated MPC gels did, but in different magnitudes. All of them were more sensitive to an increase in rennet than in calcium concentration.

Although the strength of US gel at the lowest rennet and calcium concentration was not different from that of the 1H gel, the gel strength became significantly higher than those of the 1H counterparts as the rennet or calcium concentrations increased. The strength of H60 gel was higher than both 1H and US gels at the lowest rennet and calcium concentrations. The gel strength of H60 gel was higher than that of US gel at higher calcium concentrations, but not at higher rennet concentrations.

The gels made from both heated and overnight cold hydration (HO) and heated and ultrasonicated (HUS) MPC solutions had higher gel strength at the lowest rennet and calcium concentrations compared to the gels made from solutions treated with the other
three treatments (1-hour hydration - 1H, ultrasonication - US, and heat – H60). However, the gels reacted to the increase of rennet and calcium in very different patterns. The gels made from HO solution were more sensitive to an increase in calcium than they were to an increase in rennet concentration. Increased rennet concentration did not improve the strength of HO gel when compared to the H60 counterparts. On the other hand, the gels made from HUS solutions seemed to be more sensitive to an increase in rennet than to an increase in calcium concentration. However, neither rennet or calcium concentration

Figure 5.2: Hardness of rennet gel made from MPCA70 after various hydration treatments with various combinations of rennet and CaCl$_2$ addition
increases gave a great improvement to HUS gel strength. Overall, the treatments that improved the particle hydration also improved the rennet gel strength. Also, the highest concentration combination of rennet and calcium gave the highest gel strength.

Data for rennet gel strength using MPC85 is shown in Figure 5.3. The gels made from the 1-hour hydration MPC85 solutions were very weak. And, unlike MPC70 gels, the MPC85 gel strength was not improved by increasing either rennet or calcium concentration. All other treatments gave a higher gel strength at the lowest rennet and calcium concentration when compared to the 1H gel. The strength of the US gel was improved by increasing either rennet or calcium concentration, but not both.

Increasing either rennet or calcium concentration improved H60 gel strength, and the highest rennet and calcium concentration combination gave a significantly higher gel strength than the other combinations. For both HO and HUS MPC85 gels, only the highest rennet and calcium concentration combination gave significant improvement to gel strength. Also, gel strength was significantly higher than that of the H60 counterpart. The overall correlation between particle size (Appendix A, Table A.4) and gel strength is shown in Table 5.9. The correlation is negative and significant. This suggests that the smaller particles in the solution resulted in higher gel strength.
a: 150 ul rennet + 45 ul CaCl₂  

b: 150 ul rennet + 90 ul CaCl₂  

c: 250 ul rennet + 45 ul CaCl₂  

d: 250 ul rennet + 90 ul CaCl₂  

1H – 1-hour hydration at 25°C  

US – 1-hour hydration at 25°C, then ultrasonicated for 5 min.  

H60 – 1-hour hydration at 25°C, then heated to 60°C for 30 min.  

HO – 1-hour hydration 25°C, then heated to 60°C for 30 min. and stored at 4°C overnight  

HUS – 1-hour hydration at 25°C, then heated to 60°C for 30 min. and ultrasonicated for 5 min.  

Figure 5.3: Hardness of rennet gel made from MPC A85 after various hydration treatments with various combinations of rennet and CaCl₂ addition.

<table>
<thead>
<tr>
<th>Sample</th>
<th>A70</th>
<th>A85</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>-0.617</td>
<td>-0.706</td>
</tr>
<tr>
<td>P-value</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 5.9: Pearson correlation between D(v, 0.5) and rennet gel strength, of MPC A70 and A85 samples.
Hydration condition and treatments, as well as levels of rennet and calcium added, had different effects on gel strength. The effects of hydration condition were not consistent across the samples. In general, the gel strength increased as the amount of rennet and calcium increased. However, increasing calcium concentration was not always as effective as increasing rennet concentration in terms of improving gel strength. In a fixed volume, smaller particles provided more surface area for rennet action and provided more joints with calcium. The small particles formed a dense gel network and increased the gel strength.

5.2.2 Effect of shear treatment

Since shear treatment was shown to reduce particle size and showed a high presentation of available micellar casein, attention was directed to show the effect of the process on rennet gel strength for MPC56, 70, and 85 samples made by a single manufacturer. All the 10% protein solutions were hydrated at room temperature for 1 hour before shear treatment. After 1-hour hydration, half of the solution was heated to 60°C and sheared by a homogenizer. All the solutions were warmed or cooled to 30°C before their pH was adjusted to 6.4. One hundred and fifty micro-liters of a 1% rennet and 90 ul of 2M CaCl₂ was added into 50 ml of MPC solution. In all cases, the gel set time was less than 30 minutes.
The hardness of the rennet gel, the gel set time and the particle size of each MPC sample is shown in Table 5.10 and Figure 5.4. Although the hardness of the sheared MPC gels was higher than that of the un-sheared ones, most of them were not statistically significant. The hardness of the MPC56 and the MPC70 gels were not significantly different. However, the MPC85 gel was significantly softer than those of the MPC56 and the MPC70 counterparts. The sheared MPC85 gel was significantly harder than the un-sheared MPC85 gel. The gel set time was not different between sheared and un-sheared samples. Without addition of CaCl₂, the gel set time was 10 to 40 minutes longer than with CaCl₂ addition. The gel strength of the un-sheared samples was softer than that of the sheared samples.

The overall correlation between gel hardness and D(v, 0.5) and SSA of the hydrated MPC samples is shown in Table 5.11. In the case of un-sheared MPC samples, the negative correlation between gel hardness and D(v, 0.5) was significant. The smaller the hydrated particles, the harder the resulting gels. Since the particle sizes of sheared MPC samples were similar, the correlation between particle size and gel hardness was not significant. The correlation between gel hardness and SSA of hydrated MPC samples was not significant, which suggests that D(v, 0.5) is a better parameter for gel strength.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Gel hardness gram</th>
<th>Gel set time min</th>
<th>D(v, 0.5) um</th>
<th>SSA m²/gram</th>
<th>Obscuration %</th>
<th>MPC added ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPC56</td>
<td>152.5a</td>
<td>30</td>
<td>43.64</td>
<td>1.2901</td>
<td>25.9</td>
<td>3.4</td>
</tr>
<tr>
<td>MPC56Homo</td>
<td>167.3a</td>
<td>30</td>
<td>0.36</td>
<td>16.4015</td>
<td>26.2</td>
<td>7.3</td>
</tr>
<tr>
<td>MPC70</td>
<td>152.8a</td>
<td>25</td>
<td>53.18</td>
<td>0.3636</td>
<td>26.5</td>
<td>1.5</td>
</tr>
<tr>
<td>MPC70Homo</td>
<td>168.5a</td>
<td>25</td>
<td>0.35</td>
<td>16.7138</td>
<td>25.5</td>
<td>8.9</td>
</tr>
<tr>
<td>MPC85</td>
<td>84.5b</td>
<td>25</td>
<td>65.34</td>
<td>0.1704</td>
<td>27.7</td>
<td>1.4</td>
</tr>
<tr>
<td>MPC85Homo</td>
<td>111.7c</td>
<td>25</td>
<td>0.35</td>
<td>16.9142</td>
<td>25.3</td>
<td>8.1</td>
</tr>
</tbody>
</table>

a Homo represents the sheared sample  
b Numbers with the same letter are not significantly different

Table 5.10: Hardness of the rennet gels made from the MPC56, 70, and 85 samples and their corresponding particle size

Figure 5.4: Hardness in gram of the rennet gels made from the un-sheared and sheared (Homo) MPC 56, 70, and 85 samples (90 ul 2M CaCl₂ and 150 ul 1% rennet per 50 ml MPC solution)
<table>
<thead>
<tr>
<th>Sample</th>
<th>D(v, 0.5)</th>
<th>SSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P-value</td>
</tr>
<tr>
<td>Without shear</td>
<td>-0.845</td>
<td>0.004</td>
</tr>
<tr>
<td>With shear</td>
<td>-0.005</td>
<td>0.990</td>
</tr>
<tr>
<td>Both</td>
<td>-0.408</td>
<td>0.093</td>
</tr>
</tbody>
</table>

Table 5.11: Pearson correlation between rennet gel hardness and D(v, 0.5), and SSA

5.3 Influence of MPC on Cast Feta Cheese Characteristics

Low protein MPC has been used to make cast Feta cheese and other types of hard cheeses in industry. However, using high protein MPC has resulted in some quality problems with the cheese products. In order to understand the causes of the problems and investigate ways of improvement, a series of experiments were conducted using MPC as the sole protein source to make cast Feta cheese. The results from previous particle and rennet gel experiments showed relationships between hydration treatments and particle dissociation as well as particle dissociation and rennet gel strength. From this, particle dissociation can be expected to have an impact on resulting cast Feta cheese texture. The investigation in this phase included the effects of hydration time (1-, 3-, and 5-hour) of MPC, the MPC age effects (New and Old), the homogenization effects, and the effects of additional lactose (0, 5, and 12%) in high protein MPC. All the MPC samples were obtained from the same manufacturer.
5.3.1 Effect of hydration time

The $D(v, 0.5)$ of hydrated MPC samples before the addition of butter, shown in Figure 5.5, ranged from 33.53 to 96.20 um. The $D(v, 0.5)$ of the cheese milk, hydrated MPC solution with butter and homogenized ranged from 0.83 to 1.20 um, depending on the MPC sample and the hydration time. In general, particle size of the MPC solution decreased as hydration time increased. The particle sizes of new samples were smaller than their old counterparts. The hydration effects were more pronounced in MPC solution for high protein content MPC samples, but not in cheese milk. There is no correlation between hydrated MPC particle size and cheese milk particle size as well as between MPC protein content and cheese milk particle size. The particle size distribution curves of all the MPC solutions are shown in Appendix B, Figures B.1 through B.6.

The pH values of all the cheese samples are shown in Table 5.12. The pH of the cheese samples changed over time during storage. Some samples changed more than others. The pH's of cheese at Day 1 ranged from 4.41 to 5.79, and at Day 14 ranged from 4.48 to 5.78. All the pH's of MPC 56 and MPC 70 cheeses were lower than 5 on the 14th day. In contrast, pH's of MPC 85 cheeses were higher than 5 on the 14th day.
Figure 5.5: $D(v, 0.5)$ of the “New” and “Old” MPC56, 70, and 85 solutions at various hydration times (1, 3, and 5 hours)

The pH’s of the old MPC cheeses tended to be lower than that of the new MPC cheeses at Day 1 when compared with the same protein content MPC. However, the pH’s of most of the old MPC cheeses did not decrease much after 14 days of storage, and pH’s of some samples increased after storage. The dropping of pH during storage was more consistent for new MPC 56 and new MPC 70 cheeses. The hydration time of the MPC solution and ages of the MPC samples had effects on pH of some cheeses, but not others. The p values of the pH in each category are shown in Table 5.13.
<table>
<thead>
<tr>
<th>Sample</th>
<th>N56</th>
<th>N70</th>
<th>N85</th>
<th>O56</th>
<th>O70</th>
<th>O85</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydration time</td>
<td>0.000</td>
<td>0.661</td>
<td>0.261</td>
<td>0.001</td>
<td>0.000</td>
<td>0.861</td>
</tr>
<tr>
<td>Storage day</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.065</td>
<td>0.000</td>
</tr>
</tbody>
</table>

N – new MPC sample  O – old MPC sample  56 – MPC56  70 – MPC70  85 – MPC85  1H – 1-hour hydration  3H – 3-hour hydration  5H – 5-hour hydration

Table 5.12: pH of the cast Feta cheese made from the "New" and "Old" MPC samples at various hydration times and storage days

Table 5.13: P value of hydration time and storage day effects on the pH of the cast Feta cheese made from the “New” and “Old” MPC samples
Moisture content of the cheese was affected by MPC addition, with cheese made from milk with higher MPC addition showing a higher moisture content. The moisture content of the cast Feta cheese made from the “New” and “Old” MPC samples, shown in Figure 5.6, ranged from 59.2% to 63.3%. The moisture contents of the MPC 56 cheese samples were at the low end and the MPC85 cheese samples were at the high end. The moisture content was not significantly different between the old and new MPC cheese samples of the same protein content.

The hardness values of cheeses made from new and old MPC samples at various hydration times are shown in Figures 5.7 through 5.10. In general, the age of the MPC sample and hydration time did not show effects on the cheese samples at Day 1. There were MPC age and hydration time effects on cheese hardness at Day 7 and Day 14, but the large deviation made the effects insignificant. The hardness of both new and old MPC cheese samples increased dramatically from Day 1 to Day 7, then decreased or stayed about the same from Day 7 to Day 14. Overall, the MPC85 cheese was much softer than the MPC56 and the MPC70 cheeses in any case. The hardness of the MPC cheese was not affected by the age of the MPC sample and the hydration time; but was significantly affected by the day of storage. The p-values of samples in each category are shown in Table 5.14.
Figure 5.6: Moisture content of the cast Feta cheese made from the "New" and "Old" 1-hour hydrated MPC56, 70, and 85 samples

Figure 5.7: Hardness of the cast Feta cheese made from the "New" and "Old" MPC56 at various hydration times (1, 3, and 5 hours) and storage days (1, 7, and 14 days)
Figure 5.8: Hardness of the cast Feta cheese made from the “New” and “Old” MPC70 at various hydration times (1, 3, and 5 hours) and storage days (1, 7, and 14 days)

Figure 5.9: Hardness of the cast Feta cheese made from the “New” and “Old” MPC85 at various hydration times (1, 3, and 5 hours) and storage days (1, 7, and 14 days)
Figure: 5.10: Hardness of the cast Feta cheese made from the “New” MPC56, 70, and 85 at various hydration times (1, 3, and 5 hours) and storage days (1, 7, and 14 days) (a); hardness of the cast Feta cheese made from the “Old” MPC56, 70, and 85 at various hydration times (1, 3, and 5 hours) and storage days (1, 7, and 14 days) (b)
<table>
<thead>
<tr>
<th>Sample</th>
<th>N56</th>
<th>N70</th>
<th>N85</th>
<th>O56</th>
<th>O70</th>
<th>O85</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydration time</td>
<td>0.177</td>
<td>0.005</td>
<td>0.204</td>
<td>0.731</td>
<td>0.162</td>
<td>0.442</td>
</tr>
<tr>
<td>Storage day</td>
<td>0.000</td>
<td>0.000</td>
<td>0.031</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

N – new MPC sample
O – old MPC sample
56 – MPC56
70 – MPC70
85 – MPC85

Table 5.14: P value of hydration time and storage day effects on hardness of the cast Feta cheese made from the “New” and “Old” MPC56, 70, and 85 samples

The correlation between particle size of the MPC solution, particle size of the cheese milk and hardness value of the cheese is shown in Tables 5.15.a and 5.15.b. The correlation between particle size of the “New” MPC 1-hour hydration solution and hardness of the resulting cheese was negative but not significant. The correlations of the “New” 3-hour and 5-hour hydration samples were positive with the 5-hour hydration sample data being significant. On the other hand, the correlations between particle size of the “Old” MPC solution and hardness value of the resulting cheese were negative; and almost all of them were not significant.

The correlations between D(v, 0.5) of the “New” MPC 1-hour and 5-hour hydration cheese milk and hardness value of the resulting cheese were negative; but only some of them were significant. The correlations of the “New” 3-hour hydration samples were positive, but not significant. On the other hand, almost all the correlations between particle size of the “Old” MPC cheese milk and hardness value of the resulting cheese were positive with a few being significant. Overall, the correlation between D(v, 0.5) and cheese hardness was not consistent.
<table>
<thead>
<tr>
<th></th>
<th>New-1H</th>
<th>New-3H</th>
<th>New-5H</th>
<th>Old-1H</th>
<th>Old-3H</th>
<th>Old-5H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>-0.701</td>
<td>0.104</td>
<td>0.899</td>
<td>-0.733</td>
<td>-0.720</td>
<td>-0.811</td>
</tr>
<tr>
<td>P value</td>
<td>0.121</td>
<td>0.844</td>
<td>0.015</td>
<td>0.097</td>
<td>0.107</td>
<td>0.050</td>
</tr>
<tr>
<td>Day 7</td>
<td>-0.713</td>
<td>0.197</td>
<td>0.919</td>
<td>-0.773</td>
<td>-0.730</td>
<td>-0.735</td>
</tr>
<tr>
<td>P value</td>
<td>0.111</td>
<td>0.708</td>
<td>0.010</td>
<td>0.072</td>
<td>0.100</td>
<td>0.096</td>
</tr>
<tr>
<td>Day 14</td>
<td>-0.418</td>
<td>0.555</td>
<td>0.841</td>
<td>-0.783</td>
<td>-0.695</td>
<td>-0.821</td>
</tr>
<tr>
<td>P value</td>
<td>0.410</td>
<td>0.253</td>
<td>0.036</td>
<td>0.066</td>
<td>0.125</td>
<td>0.045</td>
</tr>
</tbody>
</table>

Table 5.15: Pearson correlation between D(v, 0.5) of MPC solution and hardness of the cast Feta cheese made from the “New” and “Old” MPC samples (a); Pearson correlation between D(v, 0.5) of MPC cheese milk and hardness of the cast Feta cheese made from the “New” and “Old” MPC samples (b)

The overall correlation of pH, moisture content, and cheese hardness is shown in Table 5.16. The pH and the moisture content of the cheese had negative correlation with the hardness of the cheese. The pH effect was significant for both the “New” and “Old” samples; but the moisture content effect was only significant for the “Old” samples. In general, low pH and low moisture content of the cheese resulted in harder texture.
Table 5.16: Pearson correlation between pH, moisture content, and hardness of the cast Feta cheese made from the “New” and “Old” MPC samples

All MPC56 cheeses examined by ESEM had a continuous amorphous lumpy appearance. The cheese made from the old MPC56 sample had more defined lumps than the ones made from the new MPC56 sample. The 1-hour hydrated cheese had some apparent small dark spots. The size of the dark spots was larger in cheese made from the new MPC56 sample than the ones in cheese made from the old counterpart (Figures. 5.11 and 5.12). The cheese having more defined lumps seems to have higher hardness and the cheese having larger dark spots seems to have lower hardness when relating appearance to texture.

All MPC70 cheeses examined by ESEM had a continuous amorphous lumpy appearance. Except the 5 hour hydration cheese made from old MPC70 sample, all others had dark spots. The 1-hour hydrated old MPC70 cheese had more large spots. The 1-hour hydrated new MPC70 cheese had the smallest and not so well defined dark spots. The 5-hour hydrated old MPC70 cheese had the most defined continuous lumps (Figures. 5.13 and 5.14.). When relating appearance to texture, the cheese with the most defined lumps had the highest hardness and the cheese with larger dark spots had the lowest hardness.
All the MPC85 cheese examined by ESEM had dark spots and many of them were large. Some of the dark spots were big enough to break the continuous appearance of the cheese samples. The 5-hour hydrated new and old MPC85 cheeses had many more dark spaces than the 1-hour hydrated counterparts. The 1-hour hydrated old MPC85 cheese had a more continuous lumpy appearance followed by the 1-hour hydrated new MPC85 cheese. The appearance of the 5-hour hydrated MPC85 cheese was more like a sponge; which had a lot of large voided spaces. The cheese made from both the new and old samples at 1-hour hydration had less voided spaces and more continuous phases (Figures 5.15 and 5.16). Even when appearance was different, the texture of the cheese was not much different from each other.
Figure 5.11: Surface structure of the cast Feta cheese made from the 1-hour hydrated "New" MPC56 sample (a); surface structure of the cast Feta cheese made from the 5-hour hydrated "New" MPC56 sample (b)
Figure 5.12: Surface structure of the cast Feta cheese made from the 1-hour hydrated "Old" MPC56 sample (a); surface structure of the cast Feta cheese made from the 5-hour hydrated "Old" MPC56 sample (b)
Figure 5.13: Surface structure of the cast Feta cheese made from the 1-hour hydrated “New” MPC70 sample (a); surface structure of the cast Feta cheese made from the 5-hour hydrated “New” MPC70 sample (b)
Figure 5.14: Surface structure of the cast Feta cheese made from the 1-hour hydrated "Old" MPC70 sample (a); surface structure of the cast Feta cheese made from the 5-hour hydrated "Old" MPC70 sample (b)
Figure 5.15: Surface structure of the cast Feta cheese made from the 1-hour hydrated "New" MPC85 sample (a); surface structure of the cast Feta cheese made from the 5-hour hydrated "New" MPC85 sample (b)
Figure 5.16: Surface structure of the cast Feta cheese made from the 1-hour hydrated “Old” MPC85 sample (a); surface structure of the cast Feta cheese made from the 5-hour hydrated “Old” MPC85 sample (b)
5.3.2 Effect of shear treatment on MPC solution

The average \( D(v, 0.5) \) of the MPC solutions and cheese milk are shown in Figures 5.17 and 5.18 respectively. The particle sizes of un-sheared and sheared MPC solutions were quite different. The particle size of un-sheared MPC solution increased as the protein content of the MPC sample increased being consistent with previous results. After shear treatment, the particle size did not follow the rule. The particles of cheese milk from the sheared MPC solution were not different from that of their un-sheared counterparts. The particle size distribution curves of all the MPC solutions and cheese milk are shown in Appendix B, Figures B.7 through B.9.

Figure 5.17: \( D(v, 0.5) \) of the MPC56, 70, and 85 solutions without (Un-Homo) and with (Homo) shear treatment
Figure 5.18: $D(v, 0.5)$ of the cheese milk made from the MPC56, 70, and 85 solutions without (Un-Homo) and with (Homo) shear treatment.

The pH values of the cheese samples are shown in Table 5.17. The pH of most of the cheese decreased as the storage day increased. However, none of the pH’s were less than 5. There is no significant difference in the pH of the cheeses made from un-sheared and sheared MPC solutions.

The moisture contents of the cheese made from un-sheared and sheared MPC solutions are shown in Figure 5.19. The moisture content of the cheese made from sheared MPC solution tended to be lower than its un-sheared counterparts, although it was not significant. The moisture contents of MPC70 and MPC85 cheeses were
significantly higher than that of MPC56 cheeses. However, the moisture contents of all the cheese samples were higher than the standard moisture content (55 to 65%) of Feta cheese.

<table>
<thead>
<tr>
<th>Sample</th>
<th>56-Unsheared</th>
<th>56-Sheared</th>
<th>70-Unsheared</th>
<th>70-Sheared</th>
<th>85-Unsheared</th>
<th>85-Sheared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>5.52</td>
<td>5.74</td>
<td>5.71</td>
<td>5.72</td>
<td>5.95</td>
<td>5.73</td>
</tr>
<tr>
<td>Day 7</td>
<td>5.46</td>
<td>5.55</td>
<td>5.48</td>
<td>5.55</td>
<td>5.84</td>
<td>5.88</td>
</tr>
<tr>
<td>Day 14</td>
<td>4.69</td>
<td>5.33</td>
<td>5.46</td>
<td>5.47</td>
<td>5.81</td>
<td>5.91</td>
</tr>
</tbody>
</table>

Table 5.17: pH of the cast Feta cheese made from the un-sheared and sheared MPC56, 70, and 85 solutions

Figure 5.19: Moisture content of the cast Feta cheese made from the un-sheared (UnH) and sheared (Homo) MPC56, 70, and 85 solutions
All the cheeses were soft and had a gel like smooth appearance at Day 1. The MPC56 cheeses became firm and dry at Day 7, and became dryer and crumbly at Day 14. The MPC70 cheeses became dry and firm at Day 7. By Day 14, the MPC70 cheeses developed a softer layer on top, about 0.5 mm in thickness, while the rest of the portion stayed firm, dry, and somewhat crumbly. The MPC85 cheeses stayed gel like smooth and had a moist appearance throughout the 14 days of storage. There was no obvious difference between the cheeses made from un-homogenized MPC solution and its homogenized counterpart.

The hardness values of the cheeses are shown in Figure 5.20. There was no significant difference between the cheeses made from un-homogenized and homogenized MPC solutions, except the MPC85 cheeses at Day 14. The hardness of the MPC56 and MPC70 cheeses increased dramatically from Day 1 to Day 7, and changed somewhat from Day 7 to Day 14. Since the texture of the cheese became crumbly, the standard deviation became larger from Day 1 to Day 7 and Day 7 to Day 14.

The hardness of MPC85 cheese increased moderately from Day 1 to Day 7, and changed slightly from Day 7 to Day 14. The texture of the MPC85 cheese stayed gel-like, elastic, and smooth, the standard deviation being able to stay relatively small for all three measurements. It is worth notice that the effect of shear treatment on the hardness of the cheese became significant at Day 14. Overall, the cheese hardness increased as the storage day increased and decreased as the MPC protein content increased. The hardness of the MPC56 and MPC70 cheeses were not affected by shear treatment.
Figure 5.20: Hardness of the cast Feta cheese made from the un-sheared (Un-Homo) and sheared (Homo) MPC56, 70, 85 solutions at various storage days (1, 7, and 14 days)

The correlations between particle size of the MPC solution and cheese milk and the hardness of the resulting cheese are shown in Table 5.18. The correlation between particle size of the un-sheared MPC solution and the hardness of the resulting cheese was negative, but for the sheared samples it was positive. However, none of them was significant. The correlation between particle size of the cheese milk and the hardness of the resulting cheese was negative for both un-sheared and sheared samples. In general, the smaller particles in the cheese milk resulted in harder cheese. This trend was consistent with results from section 5.3.1. This correlation suggests that particle
size of the solution after shear treatment, similar to those of the prolong hydration time (3-hour, and 5-hour) solutions, was not a good indicator of hardness of the resulting cheese.

<table>
<thead>
<tr>
<th>Sample Treatment</th>
<th>MPC Solution - Hardness</th>
<th>Cheese Milk - Hardness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>-0.604</td>
<td>-0.728</td>
</tr>
<tr>
<td>P value</td>
<td>0.204</td>
<td>0.101</td>
</tr>
<tr>
<td>Day 7</td>
<td>-0.731</td>
<td>-0.822</td>
</tr>
<tr>
<td>P value</td>
<td>0.099</td>
<td>0.045</td>
</tr>
<tr>
<td>Day 14</td>
<td>-0.544</td>
<td>-0.646</td>
</tr>
<tr>
<td>P value</td>
<td>0.264</td>
<td>0.166</td>
</tr>
</tbody>
</table>

Table 5.18: Pearson correlation between \( D(v, 0.5) \) of MPC solution and cheese milk and hardness of the cast Feta cheese made from the un-sheared (Un-Homo) and sheared (Homo) MPC solutions.

The correlations between pH, moisture content, and hardness of the cheese are shown in Table 5.19. The pH and the moisture content both had a negative correlation with the hardness of the cheese. The pH effect was significant for both "un-sheared" and "sheared" samples; but the moisture content effect was not significant for both samples.

In general, low pH and low moisture content of the cheese resulted in harder texture that was consistent with the results from section 5.3.1.
<table>
<thead>
<tr>
<th>Sample</th>
<th>pH - Hardness</th>
<th>Moisture % - Hardness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Un-Homo</td>
<td>Homo</td>
</tr>
<tr>
<td>R</td>
<td>-0.717</td>
<td>-0.835</td>
</tr>
<tr>
<td>P value</td>
<td>0.030</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Table 5.19: Pearson correlation between pH, moisture content and hardness of the cast Feta cheese made from the un-sheared (Un-Homo) and sheared (Homo) MPC solutions

The surface images of cheese samples caught by ESEM are shown in Figures 5.21 to 5.23. The surface structure of the MPC56 and MPC70 cheeses was very similar. They all appeared to be lumpy, compact, and continuous aggregates. However, it seems that the cheese from the sheared MPC56 solution had more and larger dark spots than its un-sheared counterpart. On the other hand, the MPC85 cheese had a gel-like smooth appearance; therefore, there was not much structure shown by the ESEM. Similar to the MPC56 cheeses, the sheared MPC85 cheese showed more and larger dark spots than the un-sheared one.
Figure 5.21: Surface structure of the cast Feta cheese made from the un-sheared 1-hour hydrated MPC56 solution (a); surface structure of the cast Feta cheese made from the sheared 1-hour hydrated MPC56 solution (b)
Figure 5.22: Surface structure of the cast Feta cheese made from the un-sheared 1-hour hydrated MPC70 solution (a); the surface structure of the cast Feta cheese made from the sheared 1-hour hydrated MPC70 solution (b)
Figure 5.23: Surface structure of the cast Feta cheese made from the un-sheared 1-hour hydrated MPC85 solution (a); surface structure of the cast Feta cheese made from the sheared 1-hour hydrated MPC85 solution (b)
5.3.3 Effect of lactose addition to MPC85 sample

The results of the previous two sections, 5.3.1 and 5.3.2 showed that the pH had major effects on cheese hardness. Since the pH of MPC85 cheese stayed relatively high even after 14 days of storage, the insufficient lactose content in MPC85 became a concern. In this section, various amounts of lactose were added into MPC85 and the resulting cheese was evaluated.

The average $D(v, 0.5)$ of the hydrated MPC85 ranged from 46.9 to 63.9 um. The particle size tended to increase as lactose addition increased (Table 5.20). The increase in particle size was more pronounced from 5% to 12% than from 0% to 5% lactose added samples. The particle size distribution curves are shown in Appendix B, Figure B.10.

<table>
<thead>
<tr>
<th>Average</th>
<th>$D(v, 0.5)$ - um</th>
<th>SSA - m$^2$/g</th>
<th>Obscuration %</th>
<th>MPC used - ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>85-12</td>
<td>63.86</td>
<td>0.178</td>
<td>26.8</td>
<td>1.0</td>
</tr>
<tr>
<td>85-5</td>
<td>51.88</td>
<td>0.364</td>
<td>26.2</td>
<td>1.3</td>
</tr>
<tr>
<td>85-0</td>
<td>46.90</td>
<td>0.374</td>
<td>27.0</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Table 5.20: Particle size measurements of the MPC85 solutions with additional 0, 5, and 12% lactose

The pH of the cheese, shown in Table 5.21, decreased as lactose addition and storage time increased. However, the differences in pH between 5% and 12% lactose added samples were not significant. The pH never went below 5. On the 14$^{th}$ day, the...
pH of highest lactose content cheese was 5.15. Cheese without addition of lactose had a pH of 5.74. The decrease of pH was more pronounced between Day 7 and Day 14 than between Day 1 and Day 7.

<table>
<thead>
<tr>
<th>Lactose %</th>
<th>85-0%</th>
<th>85-5%</th>
<th>85-12%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>6.05</td>
<td>5.74</td>
<td>5.59</td>
</tr>
<tr>
<td>Day 7</td>
<td>5.93</td>
<td>5.61</td>
<td>5.50</td>
</tr>
<tr>
<td>Day 14</td>
<td>5.74</td>
<td>5.24</td>
<td>5.15</td>
</tr>
</tbody>
</table>

Table 5.21: pH of the cast Feta cheese made from the MPC85 cheese milk with additional 0, 5, and 12% lactose at various storage days

The moisture content of the cheese, shown in Table 5.22, ranged from 71% to 73%. The moisture contents of all cheese samples were higher than the standard of cast Feta cheese (55-65%). However, results were similar to those of section 5.3.2. It is interesting to notice that even the moisture content of the 0% lactose added cheese sample was similar to the 12% lactose added sample, the texture of the former sample was more similar to 5% lactose sample than to the later sample.

The 12% lactose added cheese was firm at Day 1, and became drier, more crumbly, and developed a gel like top at Day 7. The 0% and 5% lactose added cheeses were soft, elastic, and gel like at Day 1. The 0% lactose added cheese stayed soft and elastic after Day 14 whereas 5% lactose added cheese became firmer. However, at Day 14 the 5% lactose added cheese formed a gel like top about 0.5 cm in thickness and a
The hardness values of the cheeses are shown in Figure 5.24. The 12% lactose added cheese had the highest hardness. The hardness of cheese decreased as the percent of lactose addition decreased. However, the hardness of the cheese did not change much throughout the storage time. As the storage time increased, the texture of the cheese deviated more due to the development of the gel top and the semi-liquid layer. Deviation increased as the lactose content and storage time increased. At Day 1, the hardness values of the cheeses made with different lactose addition were significantly different from each other. At Day 14, as the deviation increased, the differences in hardness among samples became insignificant. Overall, the hardness of 12% lactose added cheese was much higher than the other two samples.
Figure 5.24: Hardness of the cast Feta cheese made from the MPC85 cheese milk with additional 0, 5, and 12% lactose

The correlation between D (v, 0.5) of hydrated MPC and hardness of cheese was positive. The correlation between hardness and pH of cheese was negative, but not significant (Table 5.23). The correlation between moisture content and hardness of cheese was negative. The positive correlation between particle size and hardness of the cheese was not consistent with previous results. Lower pH and lower moisture content related to higher hardness were consistent with previous results.
Table 5.23: Pearson correlation between D(v, 0.5) of MPC solution, pH, moisture content and hardness of the cast Feta cheese made from the MPC85 cheese milk with additional lactose

The surface images of the MPC85 cheese samples caught by ESEM are shown in Figures 5.25 throughout 5.27. The 0% lactose added sample did not have many features since it had a smooth gel-like texture. However, small dark spots were seen throughout. The 5% lactose added sample showed a lumpy appearance that was similar to what had been seen from earlier MPC56 and MPC70 cheeses. Many small dark spots also appeared. The 12% lactose added sample showed a well defined lumpy appearance and the lumps were much bigger than the 5% lactose added sample. However, the big lump seen seems to be an aggregate formed by many small lumps fused together. The obvious differences in surface structure of the cheese were well related to the differences in texture.

Addition of lactose to the MPC85 solution affected the particle size of the hydrated particles, the pH, the moisture content, and the hardness of the resulting cast Feta cheese. The particle size and hardness of the resulting cheese increased and the pH of resulting cheese decreased as the percent lactose addition increased. However, the changes of resulting cheese moisture contents were not consistent with the changes in lactose addition. Addition of more than 10% lactose resulted in a much firmer and drier
cheese texture that was closer to the MPC56 cheese texture. Lactose addition up to 5% did not seem to be enough to change the gel-like texture. The surface images caught by ESEM showed an interesting contrast but were not consistent with the expectations for texture.

Figure 5.25: Surface structure of the cast Feta cheese made from the 1-hour hydrated MPC85 sample without additional lactose
Figure 5.26: Surface structure of the cast Feta cheese made from the 1-hour hydrated MPC85 sample with additional 5% lactose

Figure 5.27: Surface structure of the cast Feta cheese made from the 1-hour hydrated MPC85 sample with additional 12% lactose
5.4 Discussion

5.4.1 Particle characteristics

The results of the preliminary experiment (section 5.1.1) indicate the particle changes during hydration are related more to the sources of the samples and less to the protein content of the MPC samples. Samples from different manufacturers are most likely to go through different processing procedures. The processing temperature, including the warm up temperature before filtration, the temperature during filtration to produce retentate, the inlet and outlet temperatures of the spray drier, as well as the storage temperature of the finished MPC, are all major factors that can affect the functionality of the product (Novak, 1991; El-Samragy et al., 1993a; De Castro, 1999). Therefore, it is no surprise that the MPC samples from different manufacturers behaved differently during hydration. A reduction in particle size after hydration is due to dissociation of particles whereas an increase in particle size after hydration is due to water uptake by the outer shell and swelling without dissociation of the particles. The effects of processing temperature were further supported by the results of section 5.1.2. These results showed a huge deviation of hydrated particle size within the same protein content MPC sample group containing samples from different sources.
5.4.2 Effect of Dissociating treatments on particles

Chemicals and shear can interfere with the protein-protein interaction of the hydrated particles. Although urea is able to interfere with hydrophobic interaction in water solution, the concentration was too dilute to be effective in this experiment. The effects of addition of SDS could indicate hydrophobic interaction was involved in particle shell formation in resisting dissociation during hydration. The particle changes after addition of 2-mercaptoethanol might indicate the involvement of disulfide linkages in the particle shell. Furthermore, the particle changes after ultrasonication application could indicate the existence of hydrogen bonding. The magnitude of the changes depends upon the degree of these interactions. The degree of interaction is a result of the processing.

The results of using the MPC samples from the same manufacturer of different protein contents (section 5.1.3.2) suggest that besides the processing temperature, the protein contents of the samples also play a role in the different hydration behaviors of the particles. More interactions occurred during processing and storage for high protein MPC samples because more proteins were available for interaction. Thus, the shells of high protein content particles were more resistant to dissociation.

Since the inlet and outlet drying temperatures of NZ56, NZ70, and NZ85 are similar; the protein, and possibly lactose, concentrations in the retentate were the factors that have major effects on the particle structure. According to the hypothesis, the NZ56 retentate had lower protein and higher lactose contents that could form a more porous particle shell structure. The porous structure allows the water to penetrate into particles.
more easily and increase the rate of dissociation. On the other hand, the NZ85 retentate had high protein and low lactose contents that could form a much stronger shell with a dense structure during drying due to more protein interactions and formation of disulfide linkages.

To interfere with the bonding force of the particle shell, energy input is necessary. Shear treatment by a homogenizer providing the highest energy input gave the highest particle size reduction. Heating the solution to 60°C also provides a considerable amount of energy to the system. However, the degree of heating should be carefully controlled. Since some of the milk proteins are heat sensitive, further denaturation of the protein could occur under high heat treatment and reduce protein solubility. The results of solubility test suggest that the MPC56 sample had less denatured proteins, due to the protective effect from high lactose content.

The reduction of particle size after each treatment could be a sign that the particle shells were ruptured. A combination of the treatments, such as heat and ultrasonication might be able to achieve higher reduction in particle size of high protein content particles.

5.4.3 Rennet gel made from MPC sample

All the treatments after 1-hour hydration reduced particle size. This indicated that all treatments ruptured more or less particles and released more casein micelles available for rennet action. In the case of the MPCA70 sample, there were some casein micelles

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available even in the 1-hour hydration solution. Within a certain range of rennet and calcium concentration, as the rennet and calcium concentrations increased, the gel strength increased.

In the case of MPCA85, the particles in the 1-hour hydrated solution were only swelling due to water uptake, and the number of casein micelles did not increase. Thus, increase in rennet and calcium concentrations showed no effects on the resulting gel strength. As the average particle size decreased due to treatments, the more casein micelles were available, and the concentration effects of rennet and calcium became more pronounced. Therefore, with the combination of the highest level of rennet and calcium concentrations and most available casein micelles (HUS d gel), the gel strength showed a dramatic increase.

The effects of hydration treatments on particle size were consistent with the results from section, 5.1. However, the effects of hydration treatments on the gel strength were not consistent across the MPC samples. Cold storage of milk, which changes the equilibrium of colloidal and soluble calcium, results in weaker rennet gels (Ali et al., 1980b; Zoon et al., 1988a). This is contrary to the results of the higher gel strengths of the overnight cold hydration (HO) MPC gels. Zoon et al., (1988a) stated the natural composition of milk and the hydration condition of reconstituted milk could affect its gelling behavior. The difference in particle behavior between milk and reconstituted MPC solution might be able to explain the disagreement of results.
The effects of rennet concentration in literature are not consistent. The results of this experiment, gel strength increased as the rennet content increased, agreed with some of the researchers cited by Zoon and co-workers (1988a). However, some MPC samples were more sensitive to the addition of calcium than to the addition of rennet. The differences in the concentration effects of calcium and rennet on the resulting rennet gel strength are supported by several other researchers (Bringe & Kinsella, 1986; Korolczuk & Maubois, 1988; Zoon et al., 1988c).

The strong negative correlation between particle size and gel strength further confirmed the proposed shell formation phenomenon that the rigid particle shell of high protein MPC restricted the release of casein micelles.

Shear treatment had effects on rennet gel strength, although it was not significant on the MPC56 and MPC70 samples. The rennet gel strength of the sheared MPC85 sample tended to be higher than the un-sheared sample due to the increase of available micellar casein. The behavior of MPC56 and 70 samples are consistent with Brunner’s (1974) report that homogenization of skim milk does not significantly change rennet curd characteristics.

The result of no differences between the sheared (smaller particles) and the un-sheared (larger particles) samples also was not comparable to other researchers’ reports that rennet action was faster on smaller casein micelles (Dalgleish, 1986) and the gel set time was slightly shorter for smaller micelles (Ford & Grandison, 1986). This is perhaps because in this experiment the gel set time was monitored by the visual test instead of an instrument, the gel set time could not be differentiated precisely. Moreover, the focus in
this experiment was on the amount of available micellar casein, not the size of the casein micelles. The negative correlation between particle size and gel strength, smaller particle size resulted in higher gel strength, was supported by Niki and co-workers (1994a&b).

Overall, the results of this phase of investigation support the hypothesis that applying shear to the MPC solution might make more micellar casein available for rennet action resulting in a harder rennet gel. The effect was more pronounced in the high protein content MPC samples.

5.4.4 Characteristics of the cast Feta cheese made from MPC sample

Particle size decreasing as hydration time increases is consistent with the results of De Castro (1999). The particle size of the higher protein content MPC sample tending to be larger than the lower protein content MPC is consistent with the results from section 5.1. However, this is not necessarily true when the hydration time is longer than 1 hour. Since the particle sizes of almost all the “Old” samples were significantly larger than their “New” counterparts, this suggests that some forms of polymerization or aggregation were in progress during storage.

The difference in particle size of the “Old” and the “New” samples became greater as protein content of the MPC increased. The continuous Maillard reaction during storage (Matsuda et al., 1991) might contribute to the formation of larger aggregates in the old samples. Al-Tahiri, (1987) also suggested that the polymerization
of particles makes solubility decrease as the age of the milk powder increases. After addition of butter and homogenization, the particle sizes of the cheese milk were not different.

The particle size increasing as lactose addition increased suggested lactose in the solution could help water absorption by the MPC particles. Particles swelled more as more water was absorbed by the particles.

The cast Feta cheese curd is coagulated by the combination of rennet action and low pH. The strong negative correlation between pH and texture of the cheese showed that the pH of the cheese had a major effect on the texture. According to Lucey and Fox (1993), cheese resulting from low pH curds tends to be crumbly and cheese resulting from high pH curds tends to be more elastic. Abd El-Salam (1987) suggested that the cheese texture would be more compact if the pH of cheese was close to the isoelectric point of casein. Abd El-Salam et al. (1993) also stated insufficient acidity causes defects in cheese texture. The pH's of the MPC56 and MPC85 cheeses were so different that the textures of the cheeses could not be similar. The MPC56 sample had higher lactose content, thus the MPC56 cheese had a lower pH after 14 days of storage. The high pH of the MPC 85 cheese after 14 days of storage could be the result of insufficient lactose content and protein degradation in the cheese.

The moisture content of a standard Feta cheese is in between 55% and 65% (Abd El-Salam, 1987). Only the cheese samples made in section 5.3.1 met the standard. The cheese samples made in sections 5.3.2 and 5.3.3 had much higher moisture contents. However, the moisture content of the MPC85 cheese was always higher than the MPC56
cheese. The high moisture content in the MPC85 cheese could be explained by the use of more water in the reconstituted MPC85 cheese milk and the better water binding properties of the MPC85 sample than the MPC56 sample. The high moisture content might be one of the factors that contributed to the soft texture of the MPC 85 cheeses (Pierre et al., 1999). The higher moisture content of the 5% lactose added MPC85 cheese sample might be a result of the semi-liquid layer formation after 14 days of storage.

Abd El-Salam (1987) states that the high total solid content in the cheese milk helps retain moisture and results in higher moisture content cheese. The results from this experiment were contrary to this statement. The MPC85 cheese milk had the lowest total solid content because the MPC85 sample contains less lactose and less MPC85 powder was used in the cheese milk compared with MPC56. However, the moisture content of the MPC85 cheese was higher than the MPC56 cheese. When lactose was added into the MPC85 cheese milk (section 5.3.3) the changes in moisture content of the cheese was not consistent with the changes in the total solid content.

The aged (Old) MPC samples might have better water binding properties than the new MPC samples, thus resulting in higher moisture content cheeses. Applying shear did not change solid content in the cheese milk nor the water binding properties of the MPC sample. Therefore, the moisture content of the MPC cheese was not affected by shear treatment.
The rearrangement of protein network during ripening (Noel, 1996) might explain the texture change from Day 1 to Day 7. The texture changes might also be due to the salt uptake and the further loss of moisture in the cheese (Van Den Berg, 1993). On the other hand, the decrease in hardness from Day 7 to Day 14 might be the result of the extensive proteolysis or deterioration of the cheese samples.

Shear treatment on the MPC solution had effects on the hardness of the resulting cast Feta cheese, but the effects were not significant for all the MPC samples. Brunner (1974) stated that increased adsorption of casein on the fat globule surface resulted in increasing weak points in the gel network. This phenomenon was suggested to be the cause of decreased curd tension on homogenized cheese. However, applying shear on the MPC85 solution without the presence of fat might increase the available micellar casein to join the gel network, thus increasing the curd strength.

Although the particle size of the 1-hour hydrated MPC solution had a negative correlation with the resulting cheese hardness values (sections 5.3.1 and 5.3.2), the correlation was not significant. This suggests that reducing the particle size of the hydrated MPC sample might not be the most important factor affecting cheese texture, especially for the low protein MPC samples. The increase in total solid content of the MPC85 cheese might have a greater effect on cheese hardness than the decrease in particle size of the hydrated MPC85 particles.

Salt in the brine that diffuses into cheese overtime decreases the moisture content of the cheese possibly slowing down proteolysis and retarding microbial growth in the cheese during storage (Abd El-Salam, 1987; Pierre et al., 1999). Low salt uptake by the
cheese resulted in high moisture retention and soft texture (Van Den Berg, 1993). In this study, the low salt concentration in the brine (2.5% vs. 7 to 10%) could explain the high moisture contents of the cheese samples. The salt concentration in the brine might not be enough to decrease proteolysis thus causing layer separation and liquefaction of some cheese samples after two weeks of storage.

According to Pierre et al. (1999) cheeses with low total solid content had large empty cavities forming a continuous void space. Observation on the MPC85 cheese samples (section 5.3.1) using the ESEM fits this description. The large empty spaces in the MPC85 cheese are where the liquid (water) forms a continuous phase, thus giving a much softer texture. The lumpy and compact structure in the MPC56 cheese gave a higher hardness value than the smooth gel structure did. The strong contrast in surface structure was demonstrated by the results of additional lactose in the MPC85 cheese milk.
CHAPTER 6

CONCLUSION

The particle characteristics of the MPC samples of various protein contents and from various sources were different. The low protein content MPC samples tended to have smaller particle sizes and the high protein content MPC samples tended to have larger particle sizes. However, the particle sizes of the MPC samples in the same protein content group were spread out in a wide range. The differences between dry and hydrated particle sizes of the same MPC sample gave a hint of particle changes occurring during hydration.

The dissociating agents caused different effects on each of the MPC samples. The reaction of the MPC sample to the dissociating agents was a guideline to determining the forces that hold the MPC particles together. Various degrees of hydrophobic interactions and disulfide linkages are involved in the particle shell formation to resist dissociation of the particles during hydration.

The particle sizes were also affected by treatments after hydration. Applying ultrasonication, heat, or shear to hydrated MPC particles resulted in dissociation of the particles. However, the degrees of dissociation were not the same. Combinations of two treatments, such as heat and shear, had synergistic effects. The wide variation of the particle characteristics
suggests that the particle properties are more closely related to processing parameters than to protein content of the MPC samples. However, the proposed hypothesis that higher protein content provides more interactions and results in a harder to dissociate particle shell is supported by the results of investigating the MPC samples of different protein contents from the same manufacturer.

Most of the MPC samples needed addition of CaCl₂ in order to form rennet-induced gels. The rennet gel strength increased as the rennet and calcium concentrations increased. Calcium concentration tended to have greater effects on gel strength than rennet. Treatments such as ultrasonication, heating, and shear of the MPC solution that affected the hydrated MPC particle size affected the rennet gel strength as well. The negative correlation between hydrated MPC particle size and rennet gel strength suggests the dissociated particles provide more casein micelles available for building the gel network, therefore improving the gel strength.

The age of the MPC sample had some impact on particle properties and the resulting cast Feta cheese texture. The old MPC samples had a larger particle size compared with their newer counterparts. The hardness of the cheese made from the new MPC samples tended to be higher than those made from their older counterparts. The hydration time differences in MPC solution did not result in significant differences in the resulting cheese hardness. Applying shear to MPC solution did not improve cheese hardness either. The proposed hypothesis that the dissociated MPC particles release more casein micelles was supported by the results of the rennet gel experiment; but was not supported by the results of the cast Feta cheese experiment.

The hardness of the cheeses increased dramatically from Day 1 to Day 7 and stayed about the same or, in some cases, decreased from Day 7 to Day 14. The pH’s of the MPC cheeses did not change consistently; some decreased, some stayed the same, and some increased
during storage. The high pH of the MPC85 cheeses suggests that there might not be enough lactose in the MPC sample. Addition of lactose into the MPC85 cheese milk decreased the pH and improved the hardness of the resulting cheese. The negative correlation between pH and cheese texture suggests that pH of the cheese had a large impact on the cheese texture. The moisture content had a negative correlation with hardness of the cheese as well. The surface structure observed by the ESEM provided some explanation of the differences in the cheese texture.

Overall, the particle properties of the MPC samples might not be the major cause of the differences in the resulting cheese characteristics. Other factors, such as the age of the MPC sample, available lactose and calcium content in the cheese milk, etc. could have impacts on the cheese texture as well. The pH of most of the MPC cheese samples did not meet the standard of Feta cheese. Addition of lactose gave a significant reduction of the pH during storage and improved the texture of the cheese. Moreover, the salt concentration in the brine might be a factor for bad keeping quality of the MPC cheese samples that caused the layer separation of the cheese samples after 14-days of storage. Therefore, more work is needed to develop a good quality cheese from high protein content MPCs.
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<td>64.24</td>
<td>*65.87</td>
<td>42.48</td>
<td>*50.22</td>
<td>78.82</td>
<td>87.27</td>
</tr>
<tr>
<td>2-ME15</td>
<td>32.92</td>
<td>28.66</td>
<td>20.43</td>
<td>52.14</td>
<td>46.52</td>
<td>54.90</td>
<td>59.64</td>
</tr>
<tr>
<td>Average</td>
<td>47.05</td>
<td>47.62</td>
<td>31.99</td>
<td>43.52</td>
<td>42.65</td>
<td>62.84</td>
<td>64.69</td>
</tr>
</tbody>
</table>

* - obscuration was below 5% at the time of measurement

**Table A.2:** Average D(v, 0.5) of all MPC samples at 15 minute of treatment
<table>
<thead>
<tr>
<th>MPC50</th>
<th>US</th>
<th>Urea</th>
<th>SDS</th>
<th>2ME</th>
<th>D(v, 0.5)*</th>
<th>Obscuration%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q</td>
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<td>-8.51</td>
<td>-20.88</td>
<td>94.50</td>
<td>23.92</td>
</tr>
<tr>
<td>U</td>
<td>-23.79</td>
<td>-0.87</td>
<td>12.17</td>
<td>-4.31</td>
<td>63.20</td>
<td>27.17</td>
</tr>
<tr>
<td>R</td>
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<td>-2.51</td>
<td>76.48</td>
<td>7.97</td>
<td>47.38</td>
<td>27.81</td>
</tr>
<tr>
<td>S</td>
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<td>-26.45</td>
<td>-17.43</td>
<td>-41.33</td>
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<td>35.71</td>
</tr>
<tr>
<td>T</td>
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<td>-35.71</td>
<td>83.84</td>
<td>-96.38</td>
<td>19.64</td>
<td>19.57</td>
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<td>-30.99</td>
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<td>15.11</td>
<td>47.72</td>
<td>40.97</td>
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<table>
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<tr>
<th>MPC60-70</th>
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<th>Urea</th>
<th>SDS</th>
<th>2ME</th>
<th>D(v, 0.5)</th>
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</thead>
<tbody>
<tr>
<td>L</td>
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<td>-8.82</td>
<td>-26.81</td>
<td>99.20</td>
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<td>-6.62</td>
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<td>16.13</td>
<td>-9.29</td>
<td>57.74</td>
</tr>
<tr>
<td>O</td>
<td>-44.35</td>
<td>-26.90</td>
<td>51.63</td>
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<td>50.43</td>
</tr>
<tr>
<td>P</td>
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<td>-31.29</td>
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<td>-16.25</td>
<td>5.67</td>
<td>-34.76</td>
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<tr>
<td>SD</td>
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<td>9.57</td>
<td>30.91</td>
<td>36.31</td>
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<table>
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<th>SDS</th>
<th>2ME</th>
<th>D(v, 0.5)</th>
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</thead>
<tbody>
<tr>
<td>I</td>
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<tr>
<td>H</td>
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<td>75.41</td>
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<tr>
<td>J</td>
<td>-97.85</td>
<td>-30.56</td>
<td>19.30</td>
<td>-82.55</td>
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<tr>
<td>Average</td>
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<td>22.58</td>
<td>-44.02</td>
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<tr>
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<table>
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<th>2ME</th>
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<tbody>
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<td>G</td>
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<td>3.36</td>
<td>-12.66</td>
<td>85.93</td>
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<tr>
<td>F</td>
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<td>7.76</td>
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<td>-9.32</td>
<td>-0.03</td>
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<td>76.69</td>
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<td>D</td>
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<td>20.10</td>
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</tr>
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<td>E</td>
<td>-47.10</td>
<td>-10.69</td>
<td>16.14</td>
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<td>C</td>
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<td>7.96</td>
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</tbody>
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* - D(v, 0.5) of untreated hydrated particles

Table A.3: Percent change of D(v, 0.5) of all MPC samples after 5 minutes of dissociating treatments
Table A.4: Average $D(v, 0.5)$, of MPC A70 and MPC A85 at various treatments

<table>
<thead>
<tr>
<th>D(v, 0.5) - um</th>
<th>1H</th>
<th>US</th>
<th>H60</th>
<th>HO</th>
<th>HUS</th>
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</thead>
<tbody>
<tr>
<td>A70</td>
<td>50.32</td>
<td>20.91</td>
<td>26.77</td>
<td>12.94</td>
<td>0.37</td>
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<tr>
<td>A85</td>
<td>78.65</td>
<td>49.92</td>
<td>47.30</td>
<td>31.57</td>
<td>11.71</td>
</tr>
</tbody>
</table>
Figure A.1: Particle size distribution of MPC40 samples at 0 minutes of treatment

Figure A.2: Particle size distribution of MPC50 samples at 0 minutes of treatment
Figure A.3: Particle size distribution of Q (MPC50) after 0, 5, 10, and 15 minutes of ultrasonication treatment

Figure A.4: Particle size distribution of Q (MPC50) after 0, 5, 10, and 15 minutes of urea treatment
Figure A.5: Particle size distribution of Q (MPC50) after 0, 5, 10, and 15 minutes of SDS treatment

Figure A.6: Particle size distribution of Q (MPC50) after 0, 5, 10, and 15 minutes of beta-mercaptoethanol treatment
Figure A.7: Particle size distribution of MPC60-70 samples at 0 minutes of treatment

Figure A.8: Particle size distribution of L (MPC70) after 0, 5, 10, and 15 minutes of ultrasonication treatment
Figure A.9: Particle size distribution of L (MPC70) after 0, 5, 10, and 15 minutes of urea treatment

Figure A.10: Particle size distribution of L (MPC70) after 0, 5, 10, and 15 minutes of SDS treatment
Figure A.11: Particle size distribution of L (MPC70) after 0, 5, 10, and 15 minutes of beta-mercaptoethanol treatment.

Figure A.12: Particle size distribution of MPC80 samples at 0 minutes of treatment.
Figure A.13: Particle size distribution of K (MPC80) after 0, 5, 10, and 15 minutes of ultrasonication treatment

Figure A.14: Particle size distribution of K (MPC80) after 0, 5, 10, and 15 minutes of urea treatment
Figure A.15:  Particle size distribution of K (MPC80) after 0, 5, 10, and 15 minutes of SDS treatment

Figure A.16:  Particle size distribution of K (MPC80) after 0, 5, 10, and 15 minutes of 2-mercaptoethanol treatment
Figure A.17: Particle size distribution of MPC85 samples at 0 minute of treatment

Figure A.18: Particle size distribution of B (MPC85) after 0, 5, 10, and 15 minutes of ultrasonication
Figure A.19: Particle size distribution of B (MPC85) after 0, 5, 10, and 15 minutes of urea treatment

Figure A.20: Particle size distribution of B (MPC85) after 0, 5, 10, and 15 minutes of SDS treatment
Figure A.21: Particle size distribution of B (MPC85) after 0, 5, 10, and 15 minutes of 2-mercaptoethanol treatment

Figure A.22: Particle size distribution of MPC NZ56 sample
Figure A.23: Particle size distribution of MPC NZ70 sample

Figure A.24: Particle size distribution of MPC NZ85 sample
APPENDIX B

ADDITIONAL PARTICLE SIZE DISTRIBUTION CURVES FOR MPC56, 70, AND 85 SAMPLES FROM THE SAME MANUFACTURER
Figure B.1: Particle size distribution of the "New" MPC56 solution after 1, 3, and 5-hour hydration

Figure B.2: Particle size distribution of the "New" MPC70 solution after 1, 3, and 5-hour hydration
Figure B.3: Particle size distribution of the “New” MPC85 solution after 1, 3, and 5-hour hydration

Figure B.4: Particle size distribution of the “Old” MPC56 solution after 1, 3, and 5-hour hydration
Figure B.5: Particle size distribution of the "Old" MPC70 solution after 1, 3, and 5-hour hydration

Figure B.6: Particle size distribution of the "Old" MPC85 solution after 1, 3, and 5-hour hydration
Figure B.7: Particle size distribution of the MPC56 1-hour hydrated solution and cheese milk

Figure B.8: Particle size distribution of the MPC70 1-hour hydrated solution and cheese milk
Figure B.9: Particle size distribution of the MPC85 1-hour hydrated solution and cheese milk

Figure B.10: Particle size distribution of the MPC85 1-hour hydrated solution with additional 0%, 5%, and 12% lactose


De Castro, M. 1999. *Influence of Spray Dryer Air Outlet Temperature and Nozzle Orifice Diameter on Characteristics of High-Protein Milk Protein Concentrate (MPC)*. PhD. Dissertation. The Ohio State University.


