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INFLUENCE OF SALT, CALCIUM, MOISTURE, AND pH ON THE

STRUCTURE AND FUNCTIONALITY OF NONFAT DIRECTLY

ACIDIFIED MOZZARELLA CHEESE

by

Brian M. Paulson

A thesis submitted in partial fulfillment
of the requirements for the degree

of

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in

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Approved:

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ABSTRACT

Influence of Sodium Chloride, Calcium, Moisture, and pH on the Structure and

Functionality of Nonfat Directly Acidified Mozzarella Cheese

by

Brian M. Paulson, Master of Science

Utah State University, 2004

Major Professor: Dr. Donald J. McMahon
Department: Nutrition and Food Science

Experiment A explored the influence of sodium on direct acid, nonfat Mozzarella

cheese. Cheeses with differing salt levels were obtained by varying dry salt applications

(none, 0.5%, and 1.0% NaCl w/w) and hot brine stretching (0%, 5%, and 10% NaCl

wt/v). Salt application and salt content influenced cheese moisture, meltability,

expressible serum, micro- and ultra-structure, and color. Moisture was highest when

cheese was salted before stretching ($P = 0.03$). Melt was lowest in cheeses that were

unsalted ($P = 0.05$). Cheeses stretched in salt brine had < 1% of the amount of

expressible serum found in unsalted cheese ($P < 0.0001$). Unsalted cheeses had a more

open structure with pockets of serum distributed throughout the protein matrix giving it

an opaque, white appearance. Salted cheeses had a more homogeneous protein matrix

lacking light scattering surfaces, resulting in a translucent cheese. Neither salt

concentration nor method of salting affected the calcium content of the cheeses ($P >$

0.05).

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Experiment B explored the influence of calcium, moisture, and pH on cheese structure and functionality. Cheeses were manufactured using combinations of citric and acetic acids. Addition of EDTA to the whey during cooking, $CaCl_2$ fortification, and extended drain times were used to produce eight cheeses in a 2^3 factorial design with target pH levels of 5.8 and 5.3, 70% and 66% moisture, and 0.6% and 0.3% calcium levels. EDTA was unsuccessful in removing calcium from pH 5.8 cheese. Adding $CaCl_2$ successfully increased the calcium level of pH 5.3 cheese. Cheese with 0.3% calcium had greatest melt, decreased hardness and increased adhesiveness. Cheese with 0.6% calcium had decreased melt and adhesiveness, and increased hardness. When calcium content was held at 0.6% there was no significant difference in melting even when pH was varied from pH 5.8 to pH 5.3. The microstructure of the 0.6% calcium cheeses had an increase in protein folds and serum pockets. Low calcium cheeses had a very homogeneous structure.

Directly acidified nonfat Mozzarella cheese manufactured with 1.0% dry salt and hot water stretching produced the best cheese. This cheese contained 0.4% salt, 0.4% calcium, no expressible serum, and good meltability.

(80 pages)

ACKNOWLEDGMENTS

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CHAPTER 1

GENERAL INTRODUCTION

Fat plays a critical role in the structure and functionality of Mozzarella cheese, providing many of the characteristic properties consumers expect of cheese on a pizza. When fat is removed from the cheese matrix, as in reduced fat and nonfat mozzarella cheese, several undesirable characteristics develop including poor melt, a tough rubbery texture, and a greenish, translucent appearance which leads to decreased acceptability by the consumer.

Proper melt and shred fusion on a pizza is one of the most critical functional properties of Mozzarella cheese. In modern pizza ovens, reduced and nonfat cheeses perform very poorly as the cheeses fail to melt fully and cheese shreds fail to fuse together into a single molten mass.

Much work has been done to improve the functionality, mainly melt properties, of reduced fat cheeses through moisture retention. When cheese melts, the fat phase helps lubricate the proteins within the cheese allowing them to flow. Additionally, the fluid phase of the molten fat remains in the cheese while water can be readily lost through evaporation while the pizza cooks. In a cheese with little to no fat to remain fluid in the cheese, moisture loss can result in skin formation, greatly reducing melt performance. Thus, increased moisture cannot solve all of the problems that accompany the removal of fat from the cheese matrix.

A thorough study of the impact various components of Mozzarella cheese, including moisture, pH, salt, and calcium, has on the structure, functionality and appearance of the cheese matrix is needed. It is hoped that with a better understanding of

how these components interact within the cheese matrix, the quality and performance of Mozzarella cheese can be improved when fat is removed to meet consumer demands for reduced fat and nonfat products.

LITERATURE REVIEW

Introduction

The popularity of pizza in the diet has led to a dramatic increase in production and consumption of Mozzarella and other Italian style cheeses. Consumers are now more aware of the need to reduce daily fat intakes (US Dept. of Agriculture, 1995), which has led to a demand for pizza cheeses with lower fat contents to satisfy the health-conscious consumer's needs. Unfortunately, the removal of fat in Mozzarella cheese can lead to a decrease in cheese functionality, specifically melt and color (Konstance and Holsinger, 1992; Rudan et al., 1999), resulting in a less desirable product for the consumer. Therefore, a better understanding of the factors influencing the functionality of Mozzarella cheese when fat is removed is needed.

Mozzarella Structure

Modifying its protein structure creates the characteristic stretch, meltability, and stringiness of Mozzarella cheese. Mozzarella is a stretched, or pasta filata, style cheese where cheese curds are melted in a hot brine solution then kneaded and stretched to a smooth consistency before being cooled and packaged. When the molten cheese is stretched, the casein molecules within the cheese protein matrix unfold and re-align in long fibrous strands in the cheese. During this process, fat and moisture form channels which help keep the protein strands separate and allow the cheese to have good melt and

stretch characteristics (McMahon et al., 1993). This structure is also important to cheese appearance as will be discussed later.

Mozzarella Functionality

Mozzarella cheese is a complex system involving the interactions of many constituents that determine cheese functionality. These constituents include protein, fat, and moisture contents as well as sodium and calcium concentrations (Quarne et al., 1968: Keller et al., 1974; Cervantes et al., 1983), and pH. The most important functional properties for Mozzarella cheese are proper melt and cheese color (Kindstedt et al., 1991). Much of the current knowledge regarding cheese functionality has been obtained with research on part-skim Mozzarella cheeses. Very little is known about the interactions that determine cheese functionality in reduced fat and nonfat Mozzarella cheeses.

The ability of the casein molecules that make up the protein matrix of Mozzarella cheese to flow past each other when heated determines how well the cheese will melt. Barz and Cremer (1993) reported that when heat is applied to cheese, the protein fibers absorb the heat, begin to denature, unravel and deform. When enough heat has been absorbed, the proteins reach a point where they can flow aided by free moisture and liquid fat. In traditional cultured Mozzarella cheese, melt increases with age due to proteolysis of caseins within the matrix over time leading to a decrease in protein-to-protein interactions (Perry et al., 1997).

Fat plays a critical role in the functionality of Mozzarella cheese. When Mozzarella is heated and stretched during manufacture, the curd proteins melt and form long bundles of fibers separated by channels of fat and serum (Oberg et al., 1993). These

fat-serum channels reduce the cross linking of the protein fibers and reduce the overall protein-to-protein interactions within the cheese body. During cooking, the fat melts and provides a fluid phase to assist cheese flow and melt (Barz and Cremer, 1993). When fat is removed from Mozzarella cheese, these protein fibers compact and the increased protein interaction that occurs decreases cheese melt and flow (Oberg et al., 1993).

Fat globules and fat channels also play an important role in the visual appearance of Mozzarella cheese. Light scattering surfaces within the cheese matrix created by the fat channels and globules give the cheese a white appearance. When fat is removed, surface area for light scattering is reduced and the cheese appears more translucent and has a greenish tint (Merrill et al., 1994). As a result, colorants such as titanium dioxide are often added to nonfat Mozzarella cheese to make the cheese appear white. Dave et al. (2001) reported that the color and opacity of nonfat Mozzarella cheese is dependent on the chemical composition of the cheese, primarily the fat content.

Influence of Moisture on Mozzarella Functionality

Increased moisture has been reported to improve Mozzarella cheese meltability in low and reduced-fat Mozzarella cheeses (Barz and Cremer, 1993; Merrill et al., 1994) so several techniques have been developed to increase the final moisture content of Mozzarella cheese. Merrill et al. (1994) found that increasing the milk pasteurization temperature could increase the retained moisture in low-fat Mozzarella cheese and improve the functionality. If the combined fat and moisture content of Mozzarella cheese exceeds 70%, it has been claimed that cheese melt can be greatly improved with out the need for aging (Barz and Cremer, 1993). Another way to increase moisture content and improve the functionality of low fat Mozzarella cheese is to use

exopolysaccharide-producing cultures (Perry et al., 1997). Again, by increasing the moisture content of the cheese, protein-to-protein interactions are decreased and cheese-melt increases.

The moisture within the cheese matrix must be in the correct state in order to improve the functionality of the cheese. Freshly manufactured Mozzarella cheese usually has expressible serum due to free moisture existing in pockets within the cheese matrix. The amount of expressible serum decreases as cheese ages and is absorbed into the into the protein matrix of the cheese due to changes in the protein matrix (McMahon et al., 1999). The melt and functionality of the cheese is improved as the expressible serum is decreased (Guo and Kindstedt, 1995; Kindstedt, 1995). Thus, water must be incorporated into the protein matrix of the cheese in order for it to effectively decrease protein-to-protein interactions and improve functionality. Conversely, for water to exist as free expressible moisture, the protein bundles must become more dense and compact, such increased level of protein-protein interactions results in a cheese that is harder to melt.

Influence of Salt on Mozzarella Functionality

There are conflicting reports concerning the influence of salt on full fat or part-skim Mozzarella cheese. Sodium from added salt can exchange with calcium within the cheese matrix and disrupt phosphate-calcium-phosphate bridging leading to a weakened matrix. However, researchers report that high salt concentration leads to a more rigid cheese and suggests an increase in protein interactions (Olson, 1982; Kindstedt, 1991; Pastorino et al., 2003a). Kindstedt (1991) also reported that salting increases the water retention of Mozzarella cheese. This could have applications in increasing the moisture

of low and nonfat cheeses (Guo and Kindstedt, 1995; Guo and Kindstedt, 1996) and

modifying the protein interactions within the matrix of the cheese.

**Influence of Calcium and pH on Mozzarella
Functionality**

Much of the research on calcium content of cheese and it's impact on cheese

functionality concerns the influence of pH on the solubilization and removal of colloidal

calcium phosphate from the casein micelle as milk pH is reduced to 5.6 and lower (Visser

et al., 1986; Kiely et al., 1992; Lucey and Fox, 1993; Merrill et al., 1994). When milk for

cheese making is pre-acidified as is done in directly acidified cheese, the removal of

calcium may be enhanced by the more efficient diffusion of soluble calcium-phosphate

from the casein micelles before coagulation. Once coagulation takes place, and the

protein matrix density increases, permeability is reduced and soluble calcium is trapped

within the curd particle (Guinee et al., 2002). Thus, in directly acidified cheeses, similar

functional properties can be found at higher cheese pH (5.6) compared to cultured

cheeses manufactured with much a lower final pH (5.3) (Breene et al., 1964).

The type of acid used to pre-acidify milk has been found to influence the

demineralization of the casein micelle. Keller et al. (1974) showed that calcium-

chelating acids, such as citric acid, increase the meltability of directly acidified cheese

and increase moisture retention in the finished cheese when compared to non-chelating

acids at a similar pH. This suggests that the calcium content of the cheese is more

influential on cheese functionality and protein interactions than pH at typical

cheesemaking levels. This is supported by the work of Pastorino et al. (2003c) with acid

injection in cheddar cheese. As pH was reduced through acid injections, calcium

phosphate in the cheese matrix became soluble and protein interactions decreased leading to decreased hardness and increased flow. Once the pH was reduced below 5.0 and approached 4.8 (the isoelectric point of the caseins), protein interactions increased leading to an increase in cheese hardness and decreased melt. In another supporting study, Guo and Kindstedt (1996) also found that Mozzarella cheese manufactured with a low draw pH and low final pH had improved melt due to an increased solubility of calcium-phosphate at lower pH and an overall reduction in calcium in the finished cheese.

Finally, Pastorino et al. (2003b) found that increasing the calcium content of the cheese changed protein interaction within the cheese matrix and thus impacted the functional and structural characteristics of the cheese.

Application to the Industry

A greater understanding of the influence of salt, calcium, moisture, and pH on the protein matrix of mozzarella cheese can lead to Mozzarella manufacturing process modifications and refinements will improve the functionality, performance, and consumer acceptance of reduced fat and nonfat Mozzarella cheese.

HYPOTHESIS AND OBJECTIVES

It is hypothesized that protein interactions within the cheese matrix is the major factor that determines the meltability, structure, and functionality of Mozzarella cheese. It is recognized that salt, calcium, moisture, and pH influence the functionality of Mozzarella cheese and it is suspected that they do so via influence of protein interactions. The objectives of this research are to study the influence of these components on nonfat

Mozzarella cheese made by direct acidification. This will eliminate the influence of fat, and reduce the influence of proteolytic breakdown caused by starter cultures on cheese functionality. The use of direct acidification will give better control of the chemical composition of the cheese since culture activity for pH of the curd does not have to be considered. This research will be divided into two experiments.

Experiment A: Determine the influence of salt composition on the total composition, functionality, and structure of nonfat Mozzarella cheese.

Experiment B: Determine the influence of pH, calcium content, and moisture on the functionality, texture, and structure of nonfat Mozzarella cheese.

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CHAPTER 2

INFLUENCE OF SODIUM CHLORIDE ON APPEARANCE, FUNCTIONALITY, AND PROTEIN ARRANGEMENTS IN NONFAT MOZZARELLA CHEESE[1]

ABSTRACT

Nonfat Mozzarella cheese curd was manufactured in 227-kg batches on three separate d using direct acidification. Cheeses with differing NaCl concentrations were obtained by dividing curd into separate lots that received various applications of dry NaCl (0, 0.5, and 1.0% NaCl, wt/wt) and hot brine (0, 5, and 10% NaCl, wt/vol) stretching treatments. The NaCl, Ca, ash, fat, moisture, and protein contents as well as cheese meltability and expressible serum of each cheese were determined. In addition, observations were made on cheese color and functionality over 24 d of storage at 4°C. Transmission and scanning electron micrographs of unsalted and salted cheeses were evaluated to determine the differences in the protein matrix. The type of NaCl application and the NaCl content of the cheeses influenced the cheese moisture, meltability, expressible serum, microstructure, and ultra structure. The moisture content was highest in cheeses in which the curd was salted before stretching. The melt was the lowest in cheeses that were unsalted. Cheeses that were stretched in either 5 or 10% brine had <1% of the amount of expressible serum observed in unsalted cheese. Unsalted cheeses had a more open structure than did salted cheeses. Pockets of free serum were distributed throughout the protein matrix of the unsalted cheese, thus producing light-scattering

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surfaces and making the cheese opaque. In contrast, the salted cheeses had a more homogeneous protein matrix that lacked light scattering surfaces, resulting in a translucent cheese. Neither NaCl concentration nor method of salting affected the Ca content of the cheeses.

INTRODUCTION

When fat is removed from Mozzarella cheese, several undesirable characteristics develop during cooking, including poor melt, a tough and rubbery texture, translucent color, and rapid skin formation (Masi and Addeo, 1986; Lelivere and Lawrence, 1988; Konstance and Holsinger, 1992; Mistry and Anderson, 1993; Oberg and McMahon, 1996). These changes occur because fat globules normally acts as a filler between the protein fibers that are formed during hot stretching of the cheese curd (Paquet and Kaleb, 1988; McMahon et al., 1993, Oberg et al., 1993), thus, reducing the interactions among proteins within the protein matrix. Consequently, nonfat cheese, because of the increased protein concentration and increased interactions between proteins within the cheese matrix, would require more energy to melt when heated than full fat cheese would. It has been claimed (Barz and Cremer, 1993) that melt can be greatly improved when the combined fat and moisture content of Mozzarella cheese is $\geq 70\%$. Applied to nonfat cheese, this principle suggests that the optimum melting of nonfat cheese could be obtained when the moisture content is increased because of the decrease in interactions between proteins as the protein concentration within the cheese is decreased.

When the fat content of a cheese is reduced, the moisture is usually increased to compensate for the lower fat content (Merrill et al., 1994). When considered on a fat-free basis, however, the moisture content often does not increase sufficiently (Fife et al.,

1996), and the ratio of moisture to protein remains comparable with that of the full fat

cheese. Increased moisture content can help soften the cheese to avoid a rubbery texture;

however, at high moisture contents, the cheese may become so soft that it cannot be

shredded or sliced (Perry et al., 1997). Another pitfall is that, if the moisture content is

increased without consideration of other parameters, such as the water-holding capacity

of the proteins, continued syneresis can occur after packaging, resulting in a whey

expulsion in the package (Merrill et al., 1996). Thus, manufacturing protocols have to be

chosen carefully so that the moisture content can be elevated, but the moisture in the

cheese must be stabilized and held within the protein matrix.

When Mozzarella cheese is heated, the fat melts and becomes fluid, which helps

the molten cheese to flow. Concomitantly, the protein matrix absorbs energy, which

affects the interactions that are maintaining the protein structure (Myers, 1990).

Interactions under entropic control (e.g., hydrophobic interactions) become stronger (up

to 60 to 80°C) while those under enthalpic control (i.e., electrostatic and van der Waals'

interactions, and hydrogen bonds) become weaker. Because of these opposing

temperature dependencies, many proteins unfold in the temperature range of 60 to 80°C

(the range to which Mozzarella cheese is typically heated to bring about melting). The

same temperature dependence would also exist for interactions among protein molecules.

Therefore, as the cheese is heated, the enthalpically controlled interactions between

proteins are disrupted, allowing the cheese to deform and flow as the proteins move past

one another.

At temperatures achieved during the initial stages of cooking a pizza, fat is stable

and remains in the cheese or on the cheese surface as moisture is lost to evaporation. Fat

protects the protein fibers from dehydration during cooking by adsorbing heat energy

(Barz and Cremer, 1993) and retarding moisture evaporation. When low or nonfat cheese

is used on a pizza that is cooked in a forced-air convection oven, the cheese rapidly chars

unless oil or water is placed on the surface of the moisture (personal observation) because

the surface moisture is cooked away (Paquet and Kalab, 1988). Because there is little or

no fat to protect the proteins once the moisture is cooked away, the proteins dry and form

a skin on the surface of the cheese. As the pizza continues to cook, the dehydrated

proteins rapidly char. Consequently, nonfat cheese that has a high moisture content and

can retain its moisture will have better melt characteristics.

It has been reported (Cervantes et al., 1983; Ramkumar et al., 1997) that NaCl

alters the water-binding properties of casein within the cheese matrix and, thus,

influences the physical properties of the cheese. Guo and Kindstedt (1995, 1996) showed

that unsalted blocks of part-skim Mozzarella cheese had higher levels of expressible

serum than did brined part-skim Mozzarella cheese, which suggests that salting of the

cheese increases the water-holding capacity of the cheese matrix by increasing the

hydration of the casein molecules that make up the cheese matrix. At the same time, the

amount of soluble proteins (e.g., β-CN) also increases (Guo and Kindstedt, 1996).

The objective of this study was to determine whether variation in the amount of

NaCl, applied using either dry-salting, by stretching the curd in hot brine or a

combination of both methods, could increase the moisture retention and alter the structure

of the cheese matrix. Such changes could lead to improved melt and functionality of

nonfat Mozzarella cheese. Directly acidified nonfat Mozzarella cheese curd at pH 5.4 was

used as a model to reduce the secondary proteolytic changes to the cheese matrix during

storage and to minimize other confounding effects from the starter cultures. The curd

manufacture was based on the methods of Breene et al. (1964b); hot brine stretching

(Fernandez and Kosikowski, 1986) was used to evenly distribute the NaCl and to

eliminate brining of the cheese after stretching the curd.

MATERIALS AND METHODS

Cheese Manufacture

Skim milk (227 kg) fortified with 1.0% NDM was pasteurized at 80°C for 29 s

(Konstance and Holsinger, 1992) and then cooled to 4°C overnight. The milk was placed

in an open, rectangular vat and acidified to pH 5.40 ± 0.03 using glacial acetic acid

diluted 1:10 with distilled water. The milk was heated to 35°C, and 16 ml of single

strength calf rennet (Rhône-Poulenc, Madison WI) was added. After 15 min, the curd was

cut with 1.9-cm knives and allowed to heal for 15 min. The curd was then stirred at 35°C

for 45 min, and the whey was drained. The curd was divided into three equal portions,

which were dry-salted with 0, 0.5, or 1.0% (wt/wt) NaCl in a random order. The NaCl

was sprinkled over the curd, and the curd stirred to distribute the salt evenly. The curd

was then allowed to stand for 5 min (at 35°C) and occasionally stirred to prevent matting.

Each portion of curd was then subdivided into three equal lots, which were stretched in

either hot (82°C) water, 5% (wt/wt) brine, or 10% (wt/wt) brine in a random order. The

cheeses were stretched by hand until smooth and assessed for ease of stretching. The

cheeses were placed in stainless steel molds (9 x 9 x 9 cm), cooled in ice water for 1 h,

and then vacuum-packaged and stored at 4°C.

Cheese Analysis

The cheese was shredded in a hand-held electric shredder (Presto Professional SaladShooter, National Presto Industries, Inc., Eau Claire, WI) prior to analyses. The cheese moisture was determined on d 1 and 24 using a vacuum oven (AOAC, 1990). Expressible serum was measured using the centrifugation method of Guo and Kindstedt (1995); sample size was 125 g. Protein was determined using the Kjeldahl method (AOAC, 1990). Fat was determined using the modified Babcock test (Richardson, 1985). Melt was determined at d 1, 8, 16, and 24 using the horizontal melt tube method and a cooking temperature of 115°C (Olson and Price, 1958). Calcium was determined using inductively coupled plasma atomic emission spectroscopy (US EPA, 1992). Ash was measured using the AOAC dry ash method for cheese (AOAC, 1990).

Total NaCl content was measured on d 1 using a chloride analyzer (model 926; Corning Scientific, Medfield, MA). The cheese was homogenized with a blender using distilled deionized H2O and a dilution factor of 1:20. The slurry was filtered through #1 filter paper, and the filtrate was used for NaCl analysis according to the specifications given by the manufacturer. All analyses were conducted in duplicate.

Electron Microscopy

Samples for transmission and scanning electron microscopy were collected from 7-d-old cheeses that had the lowest and the highest NaCl contents (cheeses A1 and C3 in Table 1). The cheeses were cut into slices (1 mm x 1mm x 5 mm) and fixed in a 2% glutaraldehyde solution overnight. Samples for scanning electron microscopy were prepared according to the methods of McManus et al. (1993). Samples for transmission electron microscopy were cut again into cubes (1 mm x 1mm x 1 mm) and placed in 1%

OsO4 in 0.2 M cacodylate buffer for 1 h, dehydrated in a graded ethanol series to 100%

ethanol, infiltrated with Spurr's epoxy overnight, transferred to Beem. capsules filled

with Spurr's epoxy, and heated to 70°C for 24 h. Thin sections, 70-nm, were cut on an

Ultracut ultramicrotome (Leica Inc., Deerfield, IL), transferred to 300-hex mesh grids,

and counterstained with uranyl acetate and lead citrate. Sections were examined on a

Zeiss 902 electron microscope (Carl Zeiss, Inc., Thornwood, NY) at an accelerating

voltage of 80 kV. All chemicals and grids were obtained from Electron Microscopy

Sciences (Fort Washington, PA).

Image Analysis

High magnification (140,000x) transmission electron micrographs were scanned

using a Macintosh computer and a 24-bit color flatbed scanner. A 512 x 512-pixel image,

representing a 180-nm square, was cropped from the scanned micrographs. The images

were processed according to Cooke et al. (1995) using NIH Image 1.60 software

(National Institutes of Health, Washington, DC). A 6 x 512-pixel grid with spaces

representing 15.9 nm was pasted to the top of the processed image. Spacing between

electron-dense regions (i.e., protein aggregates) in the micrographs were calculated using

the fast Fourier transform function of the image analysis software.

Statistical Analysis

Cheese was made in triplicate using different batches of milk. Data were analyzed

using a split-plot design with dry-salting treatments as the whole plot and hot brine

stretching as the subplots. All ANOVA for dependent variables were calculated

separately using SAS (SAS User's Guide, 1991). When significant ($P \leq 0.05$), the

differences between means were analyzed using least significant difference.

RESULTS

Cheese Physical Properties

The curd of cheeses that was stretched in water became sticky when placed in the hot water and adhered readily to the rubber gloves worn during hand-stretching. After the curd was worked slightly, the adhesiveness lessened, and the cheese stretched well. All cheeses that were stretched in 5 or 10% hot brine solutions showed good elasticity during stretching without adhering to the rubber gloves but did exhibit a less elastic texture.

The color and appearance of the cheese were also affected by the medium in which the cheeses were stretched. All cheeses were opaque while being stretched. After the cheeses were cooled to room temperature (ca. 22°C), the cheeses stretched in hot water were white and opaque, although their opacity decreased with increased dry-salt treatments. When cooled to 4°C, these cheeses became translucent, as is typical for nonfat cheeses. In contrast, the cheeses that had been stretched in brine lost opaqueness more quickly and were translucent by the time they were cooled to room temperature (ca. 22°C). During the melt test, when the cheeses were heated to ca. 90°C, all cheeses became opaque and white. Then, after subsequent cooling, all of the cheeses became translucent again.

Cheeses with low NaCl concentrations shredded better than did cheeses with high NaCl concentrations throughout the 24 d of storage and analysis, regardless of moisture content. At approximately 0.8% NaCl content, cheese shredding became difficult, and cheese would adhere to the knives and crumble in the shredder.

Cheese Composition

The composition of the cheese is shown in Table 1. Fat contents for all cheeses were ≤ 0.30%. When the curd was stretched in hot water, only about half of the NaCl that was applied to the curd was retained in the cheese. The addition of 1% (wt/wt) of NaCl to the curd produced a cheese with a total NaCl content of 0.68%; the unsalted cheese had a baseline level of 0.14% NaCl. The remainder of the NaCl was lost in the water used for stretching the cheese. As NaCl content of the stretching water was increased, the final NaCl content of the cheese also increased. Stretching of the unsalted curd in 5% brine yielded a cheese with an NaCl content of 0.85%; stretching of the curd that had received 1.0% NaCl prior to stretching in 5% brine produced a cheese with 1.47% NaCl. This result is similar to that obtained by Barbano et al. (1994) who added 2.2% NaCl to curd and then stretched the curd in a 6% brine solution to produce a cheese with 1.76% NaCl.

Cheese moisture contents were in the range of 62.1 ± 1.4 and were influenced by dry-salting of the curd. The addition of 1% NaCl to the cheese curd before stretching increased the cheese moisture by 2%; stretching in brine did not effect the cheese moisture. Consequently, cheeses that were not dry-salted before stretching had the lowest total moisture contents, regardless of the stretching conditions. They also had lower ratios of moisture to protein content. The mean Ca content for the directly acidified cheeses was 0.37%, which was considerably lower than the 0.7% Ca typically found in Mozzarella cheese (USDA, 1976; Barbano et al., 1994; Guo and Kindstedt, 1995). Although the dry-salted cheeses tended to have lower Ca contents than cheese that was not dry salted (Table 1), these differences were not significant ($P = 0.30$). The effect of brine concentration at stretching on Ca content was also not significant ($P = 0.17$)

Table 1. Mean (± SEM) for d 1 moisture, protein, ash, fat, salt and Ca concentration of direct-acid nonfat Mozzarella cheese manufactured with varying levels of application of dry salt to the curd, and stretched in hot water or brine solutions.

	Salt treatment		Moisture		Protein		Ash		Fat		Salt		Ca	
No.	Dry[1]	Brine[2]							(%)					
			X	SEM	X	SEM	X	SEM	X	SEM	X	SEM	X	SEM
A1	0.0	0	61.2	0.29	32.3	0.46	1.94	0.04	0.22	0.03	0.14	0.02	0.42	0.03
A2	0.5	0	63.2	0.28	29.8	0.46	1.83	0.10	0.27	0.02	0.40	0.07	0.39	0.04
A3	1.0	0	63.0	0.20	30.3	0.31	2.05	0.04	0.28	0.03	0.68	0.07	0.37	0.09
B1	0.0	5	60.7	0.29	31.8	0.51	2.32	0.21	0.23	0.03	0.85	0.13	0.39	0.03
B2	0.5	5	61.9	0.60	29.0	0.79	2.43	0.17	0.25	0.03	1.01	0.12	0.35	0.01
B3	1.0	5	63.4	0.31	30.1	0.54	2.86	0.17	0.28	0.02	1.47	0.14	0.36	0.01
C1	0.0	10	60.9	0.50	31.3	0.46	2.88	0.28	0.30	0.03	1.36	0.19	0.37	0.05
C2	0.5	10	62.1	0.47	29.9	0.79	3.00	0.21	0.27	0.04	1.72	0.13	0.38	0.03
C3	1.0	10	62.3	0.36	29.9	0.58	3.51	0.27	0.28	0.02	2.18	0.14	0.32	0.05

[1]Percentage of salt added to curd before stretching.
[2]Salt concentration added to water used for stretching the curd.

Expressed Serum

Expressible serum was affected ($P < 0.0001$) by the hot brine stretching treatment.

Cheeses stretched in 5 and 10% hot brine solutions had no expressible serum on d 1 or

subsequently. These cheeses had salt contents ranging from 0.85% to 2.18%. Ramkumar

et al. (1997) reported a similar occurrence in directly acidified curd; no expressible serum

was obtained when the milk was coagulated at pH 5.4 or 5.1. Cheeses stretched in hot

water had expressible serum, the quantity of which decreased as concentrations of dry-

salt treatment and age increased (Figure 1). By d 24, some of the serum could still be

expressed from these cheeses. These cheeses also suffered from syneresis when held at

room temperature (ca. 22°C) and had some whey in the packages during storage at 4°C.

Unsalted cheese had the highest level of expressible serum even though it had the lowest

moisture content. Those cheeses that contained expressible serum also exhibited

syneresis when the cheese was heated, as was evident from the free serum that separated

from the molten cheese during the melt test.
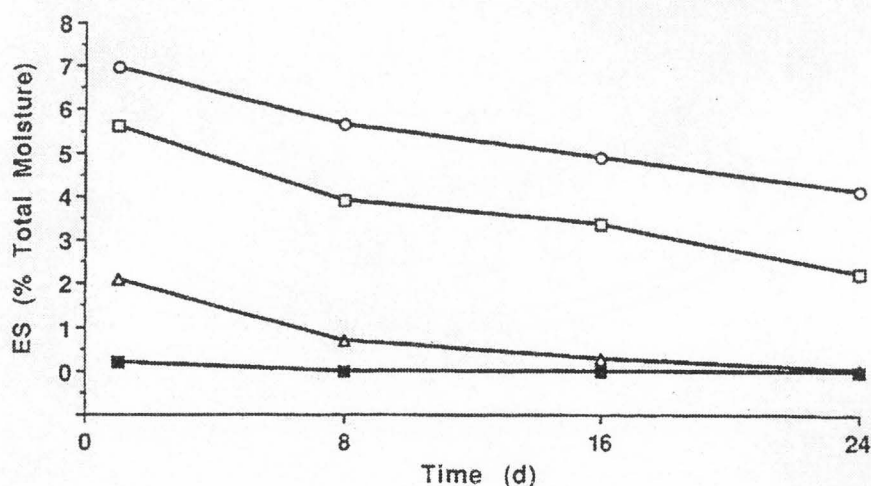
Figure 1. Decline in expressed serum (ES; weight of expressed serum/total cheese moisture) over 24 d storage at 4°C for cheeses A1 [O], A2 [□], A3 [◇], and B1[■] from Table 1

Cheese Melt

Salting of the cheese influenced melt: unsalted cheese had 1-cm lower melt on d 1 than did all other cheeses ($P = 0.04$). Cheeses that had received any of the salt treatments (and had NaCl contents of 0.4 to 2.2%) all had similar melt and did not change significantly during storage. Similarly, no change occurred in the functional properties of directly acidified part-skim Mozzarella cheese during storage (Oberg et al., 1992). Meltability of the unsalted cheeses increased by 0.5 cm during 24 d of storage, and no significant differences were observed between cheese melt for any of the cheeses by the end of the experiment (Figure 2).

Cheese Microstructure and Ultrastructure

The addition of NaCl to cheese had an impact on both the microstructure and ultrastructure of nonfat Mozzarella cheese. When the microstructure of the cheeses was examined at low magnification, differences were observed between salted and unsalted Mozzarella cheeses (Figure 3). When the fractured surface of the unsalted cheeses was examined using the scanning electron microscope, many fissures and folds (1 to 25 mm in size) were found to be distributed throughout the protein matrix (Figure 3A). These structures were pockets of whey that remained trapped within the cheese curd after stretching. The larger folds were similar to the fat-serum channels formed during hand-stretching of higher fat Mozzarella cheese (Merrill et al., 1996), although these folds were smaller and less numerous in the nonfat cheese. In contrast, the salted cheeses had a more homogeneous microstructure that lacked areas of entrapped whey (Figure 3B).

This difference among cheeses was also observed within the cheese matrix at higher magnification using transmission electron microscopy (Figure 4). Areas of lower
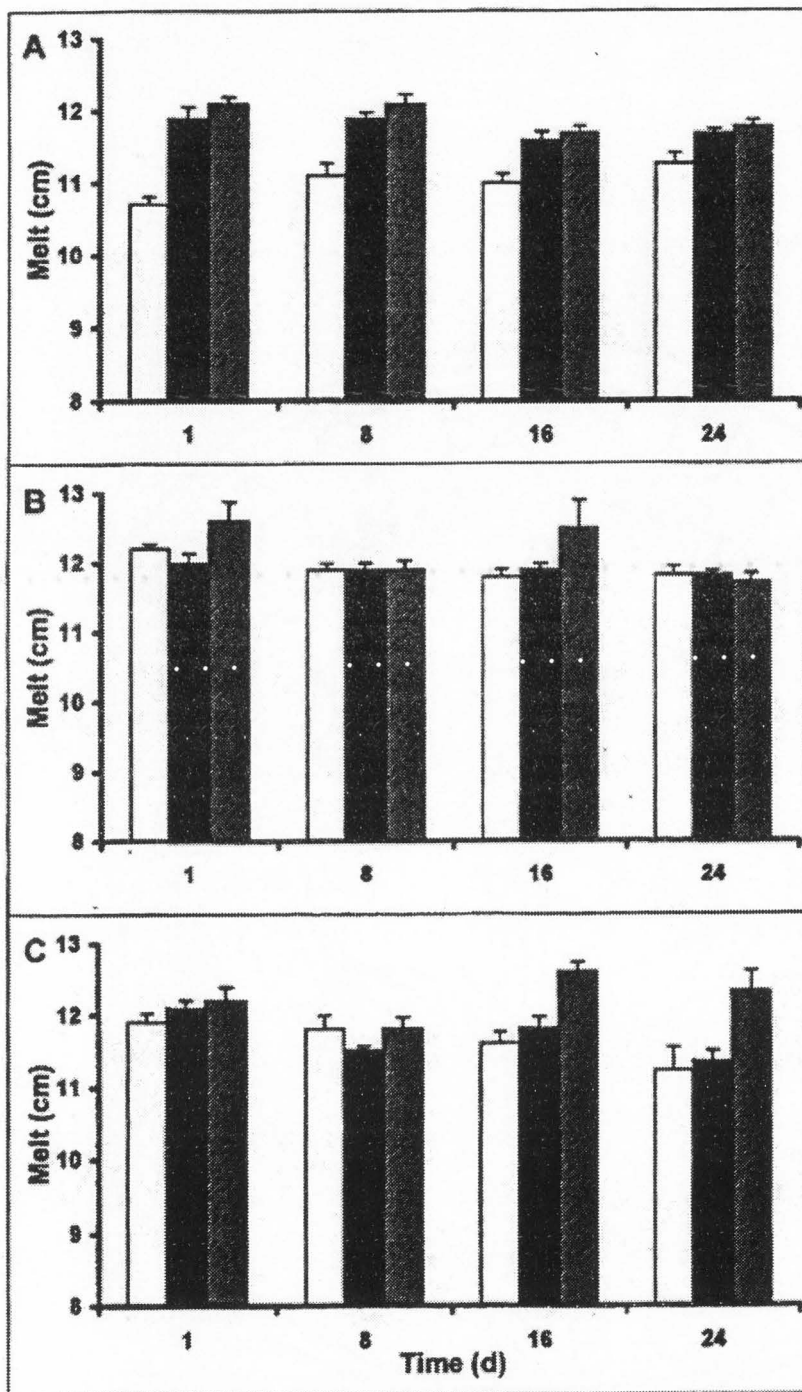
Figure 2. Mean (±SEM) melt measurements for Mozzarella cheeses manufactured using hot water stretching (A), 5% hot brine stretching (B), and 10% hot brine stretching (C). Within each stretching treatment were cheeses with 0.0% dry (white bars) 0.5% dry (black bars), and 1.0% dry (gray bars) salt applications. Error bars represent standard errors of the mean.
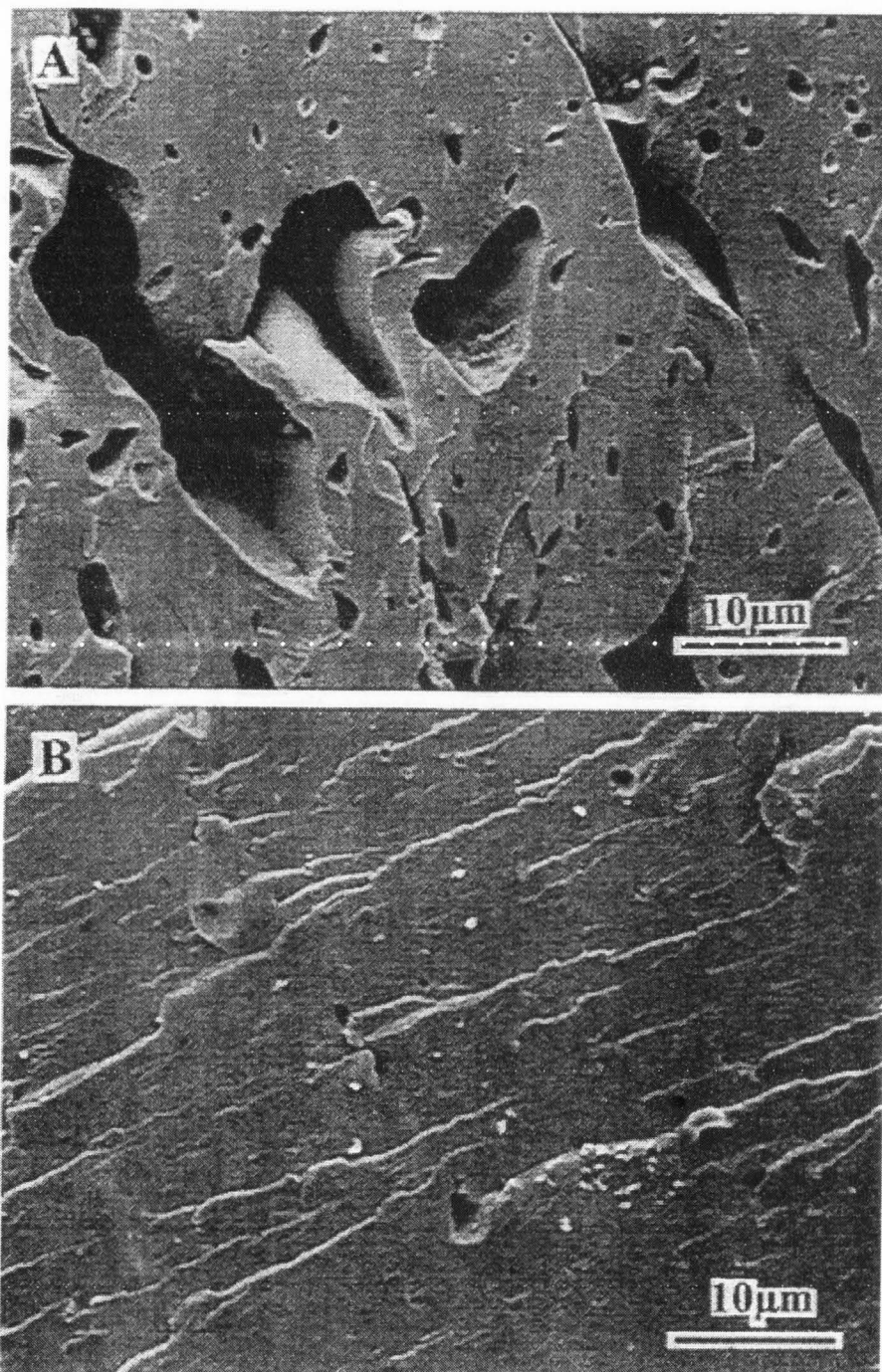
Figure 3. Scanning electron micrographs of d 7 nonfat Mozzarella cheese. Unsalted cheese (A) was opaque in appearance and had measurable expressible serum. Salted cheese (B) was translucent in appearance and had no expressible serum.

electron density (20 to 200 nm in size) were present in the unsalted cheese (Figure 4A). Such regions were fewer and smaller in the salted cheese, giving them a more homogeneous protein matrix (Figure 4B).

At high magnification (140,000x), differences in the ultrastructure were also observed between the unsalted and salted cheeses (Figure 5). The unsalted cheese had larger and more distinct protein aggregates, and the micrographs of those cheeses had a grainy appearance (Figure 5A). In contrast, the salted cheese had a more evenly dispersed protein arrangement (Figure 5B), and large protein clusters were less obvious.

Image Analysis

Processed binary images of the high magnification micrographs used for the Fourier transform calculations are shown in Figure 6. The black regions represent protein aggregates, and the white regions represent the spacing between aggregates. Unsalted cheese had larger protein aggregates and greater space between aggregates (Figure 6A). In contrast, salted cheeses had smaller protein aggregates and smaller spacing between them (Figure 6B). The spacing between bars on the grid along the top of these two images represents 15.9 nm. The average distance between protein aggregates in both the salted and unsalted cheeses were calculated using Fourier transforms of the binary images (Figure 6, C and D), which were generated from Figure 6, A and B, respectively. The cluster of pixels around the origin in Figure 6, C and D, represent the inverse spacing of the electron-dense regions calculated from the processed images. The lines along the equator represent inverse spacings of 1/15.9 nm. The average spacing of the electron-dense regions in the micrographs was calculated to be 3.5 nm in the unsalted cheese and 2.4 nm in the salted cheese.

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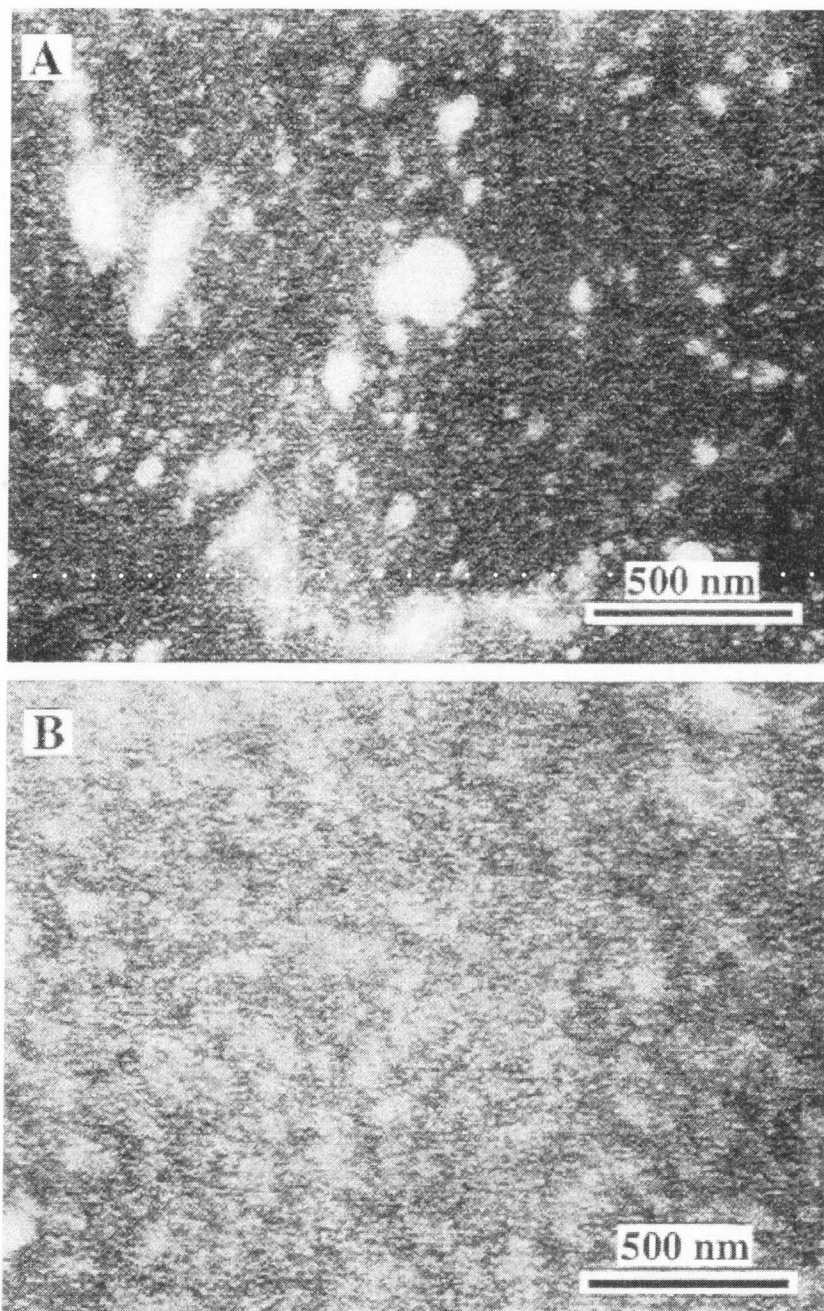
Figure 4. Transmission electron micrographs of d 7 nonfat Mozzarella cheese showing pockets in unsalted cheese (A) and homogeneous structure of salted cheese (B).
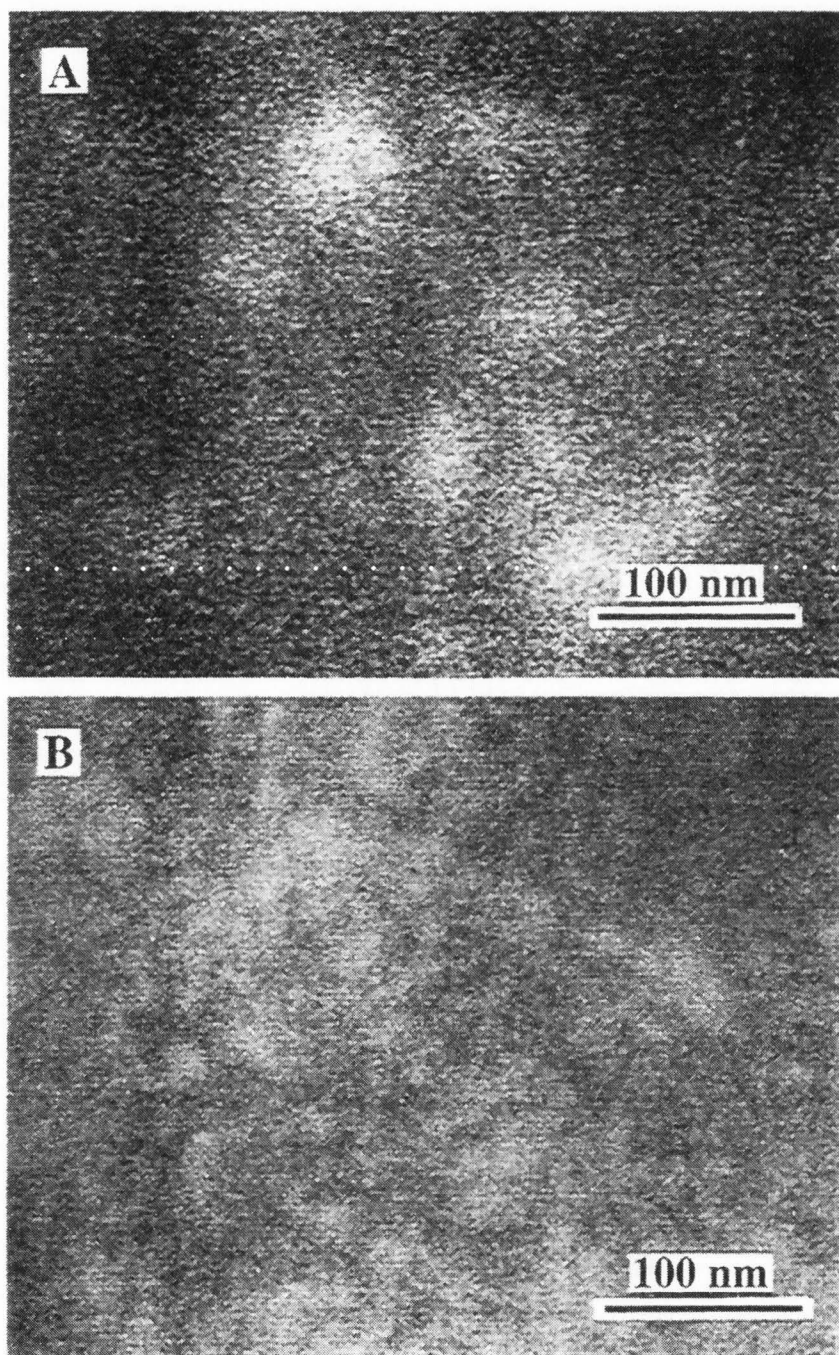
Figure 5. Transmission electron micrographs, at identical magnification, of d 7 nonfat Mozzarella cheese at high magnification, showing distinct protein aggregates in unsalted cheese (A) and a more diffuse appearance of proteins in salted cheese (B).
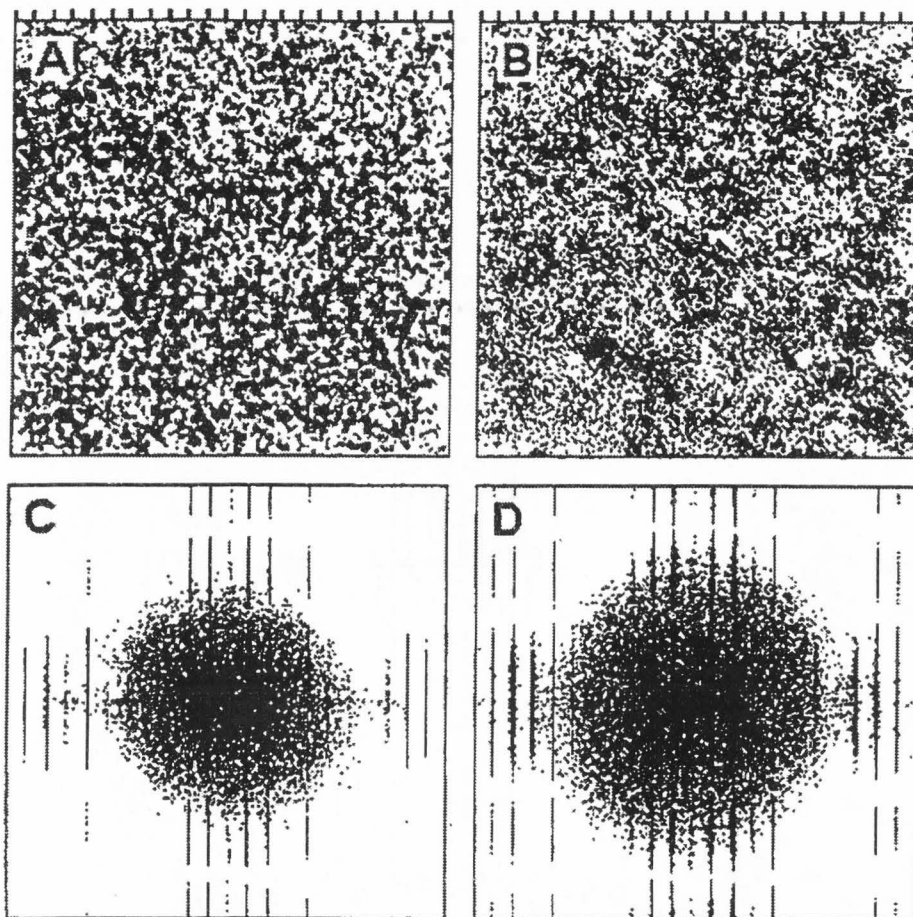
Figure 6. Binary images (from Figure 5) of the unsalted cheese matrix (A) and the salted cheese matrix (B) and the respective Fourier transforms for unsalted cheese (C) and salted cheese (D) that were used to calculate the average spacing between the protein aggregates. Spacing of lines in grating (A and B) are 15.9 nm. Spacing of lines on equator (C and D) represents 1/15.9 nm.

The actual distance between protein aggregates would be an order of magnitude greater than that calculated for the micrographs because of the manner in which the micrographs were obtained. For transmission electron microscopy, an ultra-thin section of plastic-embedded cheese is the object viewed in the microscope. Although these sections are only 70 nm thick, this is significant on a molecular basis, so the conversion from a three-dimensional section to a two-dimensional micrograph must be considered when measuring the objects, and, more importantly, the distances between objects must be considered when those distances are much less than the thickness of the section.

Thus, the number of electron-dense regions shown in Figure 6, A and B, represent not only the number of protein aggregates distributed in a 184 x 184-nm square but rather the total number of protein aggregates distributed in a 184 x 184 x 70-nm block (Figure 7). Consequently, the average spacing between aggregates in the cheeses would be of the order of 20 to 30 nm.

The same dimensional compression must also be considered when the size of the protein aggregates is calculated. However, the actual size of the protein aggregates may be slightly smaller than that observed in the micrographs because of the way objects are viewed in the transmission electron micrograph. As the electron beam passes through the sample section, electrons scatter when they interact with electron-dense regions (proteins that have been stained with Os, Ur, and Pb) of the section. Then, as the electrons that passed through the section without being scattered are collected, a gray-scale image is formed that represents the proportion of electrons passing through the sample. Consequently, the shadows formed by two electron-dense particles that may be separated by up to 70 nm can appear to be linked together or as a single larger shadow (Figure 7).

Micrograph
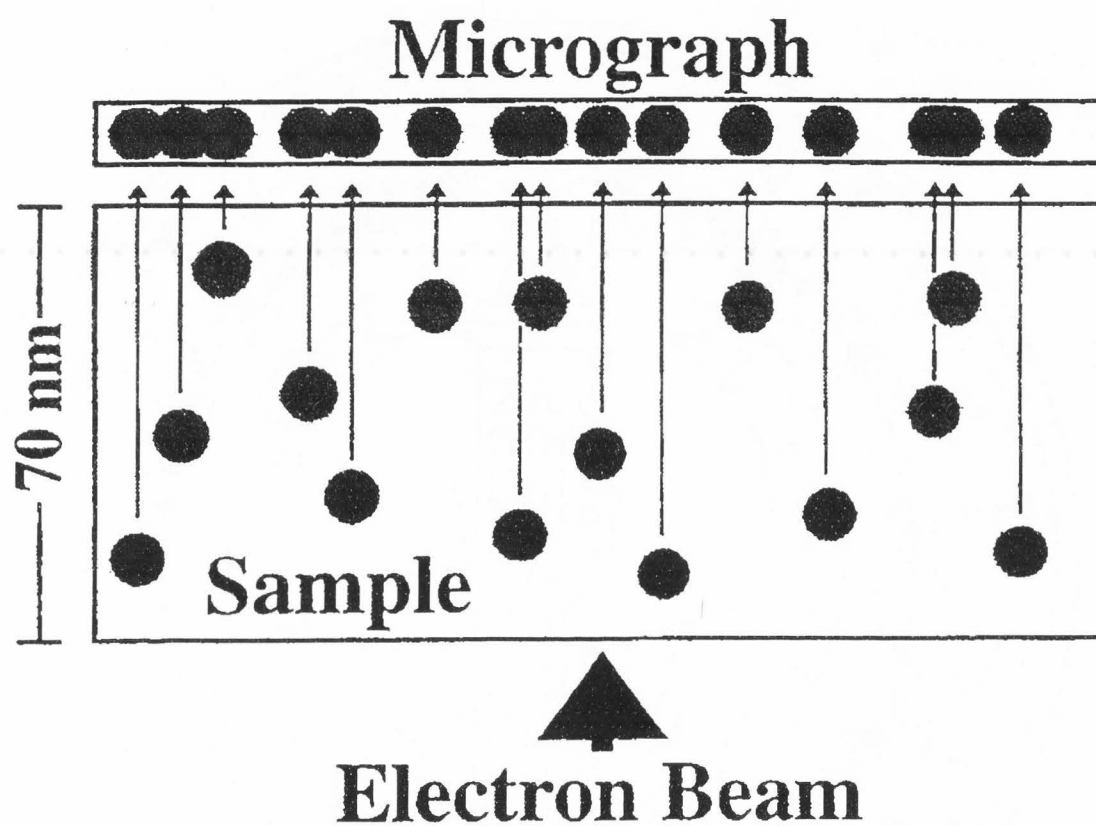
Figure 7. Schematic diagram of the dimensional compression that occurs with transmission electron microscopy.

DISCUSSION

Differences in curd behavior during manufacture, cheese appearance, moisture content, and meltability can all be related to how NaCl affected the organization of the proteins in the cheese matrix.

Cheese Manufacture

During the preliminary trials, we determined that two modifications to the Breene et al. (1964a) method were necessary when direct acidification was used to manufacture a nonfat cheese at pH 5.4. Breene et al. (1964a, 1964b) found that curd from milk acidified to pH 5.6 had better characteristics than curd formed at pH 5.3 to 5.4. However, we wanted to have the curd at a lower pH so that our model would be close to actual Mozzarella cheese-making practices. Skim milk only produced a weak curd when it was renneted and was too weak to cut satisfactorily. The addition of 1% NDM before acidification increased the milk protein concentration sufficiently to produce a firm curd that did not fragment when cut. Also, heat treatment of the curd and whey to 49°C after cutting caused the nonfat curd to melt and stick to the vat, so this heating step was eliminated, and the set temperature of 35°C was maintained throughout curd manufacture.

The tackiness of directly acidified cheese curd during stretching (especially curd with pH <5.6) has been reported by other researchers (Larson et al., 1967; Keller et al., 1973), although no explanation has been given for such tackiness. This problem did not occur when the curd was stretched in hot brine. A possible explanation is the tendency for para-casein in the curd to associate with hydrophobic materials (such as rubber gloves)

rather than with the solvent water. The addition of NaCl to the stretching water apparently increased the protein hydration in the nonfat curd, and, by implication, reduced the surface hydrophobicity of the proteins in the curd. Whether an exchange of Na^+ for Ca^{++} occurred was not determined. The cheeses stretched in brine tended to have lower Ca contents (Table 1), but additional experiments would be required to show a statistically significant difference.

Protein Interactions

Before the cheese curd is salted, the proteins in the cheese at pH 5.4 can be considered still to be undergoing aggregation. This drive toward aggregation (initiated by the renneting of the milk) occurs because the free energy of the cheese curd can be lowered as the hydrophobic regions of the proteins are shielded from water as the proteins aggregate. Such hydrophobic interactions increase in strength as the temperature increases (Myers, 1990), and any functional characteristics of the curd that are based upon hydrophobic interactions become more apparent when the curd is heated during the stretching process.

The addition of NaCl to the nonfat cheese apparently increased the interactions between proteins and the surrounding water, thus reducing the hydrophobic interactions between protein molecules. Consequently, less aggregation of the protein into protein dense groupings was observed. Although the actual distance between protein aggregates is somewhat uncertain because of dimensional compression in the micrograph images, the differences observed among the cheeses provides an indication of the change in the ultrastructure caused by salting. In the salted cheeses, the spacing between the aggregates decreased 31% compared to that in the unsalted cheese. Such increased hydration of

proteins, as shown at the ultrastructural level, demonstrates why cheese curd in the pH range of 5.6 to 5.2 is soluble in warm NaCl solutions (Kosikowski and Mistry, 1997).

As the protein hydration increases, voluminosity of the cheese matrix also increases, causing a migration of water from the large and small voids into the protein matrix. The increased water-holding capacity of the proteins with increased NaCl concentration is further demonstrated when the ratios of moisture to protein of the cheeses are compared. The unsalted cheese contained only 1.9 g of water/g of protein, and, if the quantity of expressible serum was used as a first approximation of the amount of water in the serum pockets, then the hydration of the cheese matrix in the unsalted cheese would be even lower (<1.8 g of water/g of protein). In contrast, the salted cheese contained 2.1 g of water/g of protein, which, although greater than the unsalted cheese, is still considerably less than the hydration of native casein micelles, which is 3.7 g of water/g of protein (McMahon and Brown, 1984).

When milk is renneted, a series of changes in how the proteins interact with each other is initiated. The subsequent coagulation and syneresis that occur after renneting reflects the sum of all thermodynamic forces acting on the casein proteins, including nonspecific hydrophobic and electrostatic interactions as well as specific interactions (such as Ca bridging) between the renneted para-casein micelles (Kosikowski and Mistry, 1997). Hydrophobic interactions are important in the destabilization and aggregation of the casein micelles by rennet and continue to be important in the syneresis of the resultant coagulum (Renault et al., 1997). This syneresis, or whey expulsion, is enhanced 1) by heating the curd, which increases the strength of hydrophobic interactions and, therefore, increases interactions among proteins so that more water is excluded from the cheese

curd, and 2) by lowering curd pH, which reduces the net charge of the caseins as they approach their isoelectric point so that the proteins become less soluble. By the time the cheese curd is ready to be pressed (or stretched in the case of Mozzarella cheese), the protein matrix usually has reached a point at which little moisture can be removed from the curd without mechanical force, and cheese curd (which is essentially hydrated paracasein) contains less than half of the hydration of native casein micelles (Creamer, 1985). Then, when NaCl is added, the ionic environment around the proteins changes such that the various components of the cheese curd system may be redistributed to obtain an equilibrium state of minimum energy (Ramkumar et al., 1997). For the directly acidified nonfat cheese curd, salt addition results in an increase in protein hydration.

Cheese Composition

The higher moisture of the nonfat cheeses that were dry-salted before stretching suggests a behavior for curd made by direct acidification that is different from that typically observed. It is generally accepted that the cheese moisture content is inversely proportional to the NaCl content (Guinee and Fox, 1993) because of the increase in whey release that takes place when Cheddar cheese curd (Sutherland, 1974) or Mozzarella cheese curd (Barbano et al., 1994) is salted. One of the differences between the directly acidified cheese curd and curd made using starter cultures is that there is no internal pH gradient within directly acidified curd. The drop in the pH of the cheese curd induces more syneresis than that occurring if milk has previously been brought to the same pH (Walstra et al., 1985). So, instead of a continual acidification that affect the curd proteins, the protein conformations within the directly acidified curd are set by the initial acidification of the milk before the curd is cut. The subsequent whey drainage from the

curd above that pH resulting from renneting and cutting the curd can only be induced by mechanical agitation or heating.

Another difference is that the directly acidified cheese had a much lower Ca content because of the acidification of milk to pH 5.4 before renneting. At this pH, all of the inorganic phosphate and 75% of the Ca have been solubilized from the casein micelles (Van Hooydonk et al., 1986). Consequently, when the whey is drained from the directly acidified curd, loss of Ca and PO_4 from the curd is greater. The resultant cheese contained only 0.37% Ca (i.e., 1.2 g of Ca/g of protein). In comparison, Mozzarella and Cheddar cheeses made using starter cultures typically contain 2.7 and 2.9 g of Ca/g of protein, respectively (USDA, 1976). Directly acidified curd would, therefore, be more susceptible to the peptizing action of NaCl, which has been shown to cause cheese curd to absorb water if the Ca concentration of the NaCl solution is low (ca. 0 to 0.5%) (Geurts et al., 1972). Such peptizing action causes the cheese curd to absorb water so that the casein matrix swells to form a hydrated gel.

In a study (Ovanova et al., 1971) of syneresis of milk gels, syneresis was inhibited when NaCl was added to whey surrounding the curd, which was attributed to an increase in hydrophilic capacity of the proteins because of their interaction with Na+, which resulted in an increase in water-holding capacity of the proteins. Creamer (Creamer, 1985) also observed that the addition of NaCl increased the water retention of renneted milk. He suggested this increase was a result of a displacement of Ca from the protein matrix, leading to an increase in the number of ionic groups in the matrix and a consequent increase in volume of the matrix. Robertson et al. (1975) observed that NaCl addition to whey before draining the curd resulted in an increase in the moisture content

of the drained curd. Other researchers (Grufferty and Fox, 1985; Walstra et al., 1985; Pearce and MacKinlay, 1989), however, have reported less influence of salt on curd syneresis when NaCl is added to the milk prior to rennet coagulation.

Normally, when the curd is salted, some of the salt dissolves on the curd surface, which induces a counterflow of water from the curd onto the surface (because of osmotic pressure) and produces a concentrated brine layer on the surface. This expulsion of whey from the curd is usually further enhanced by a localized contraction of proteins on the curd surface as they are salted-out. Evidently, for the directly acidified cheese curd, there must be an induced expansion of the protein network sufficient to counteract these osmotic forces so that this release of whey does not occur. The pore width of the protein matrix in nonfat curd has been observed (Guinee and Fox, 1993) to be smaller than for a cheese curd with higher fat content (ca. 20% fat), which reduces the diffusion coefficient by 30% and would also affect how the curd reacts to salt addition. Apparently, whether salting of the cheese curd promotes or inhibits syneresis is determined by the physico-chemical environment of the proteins that constitute the gel matrix of the curd particles, including pH (and pH gradient within the curd particles), Ca (and PO_4) concentrations, moisture content, and temperature. As stated by Guinee and Fox (1993), although a considerable amount of information is available on the significance of NaCl in cheese, many gaps in the knowledge remain. Whatever the reason, in our experiments with directly acidified curd, the addition of NaCl to the curd resulted in a salting-in of the proteins rather than the salting-out observed (Guinee and Fox, 1993) for proteins at milled-curd surfaces during Cheddar cheese manufacture.

Expressible Serum

The water in the large serum pockets, observed in unsalted cheese, would correspond to the water that can be removed by centrifugation as expressible serum. These pockets, formed by the entrapment of water between the protein strands during stretching, must be interconnected to some extent or the serum would not be able to diffuse as readily from the cheese. The pockets can be considered analogous to an open cell foam with water contained in open cells within the relatively solid protein matrix. The smaller pockets (observed as areas of lower electron density) appear as closed cells and probably do not contribute to expressible serum. The rate of decline of expressible serum during storage of those nonfat cheeses that had any expressible serum was much slower than that typically observed for cultured part-skim Mozzarella cheese. In Mozzarella cheese made with starter culture, expressed serum decreases to 0% within 10 to 20 d (Guo and Kindstedt, 1995), although unbrined Mozzarella continues to express serum longer (Guo and Kindstedt, 1996). In the directly acidified low NaCl, nonfat Mozzarella cheeses, the expressed serum only decreased by 40% after 24 d. This result suggests that, without added Na+, there is a delay in the changes occurring within the protein matrix that increases the water-holding capacity of the matrix.

Cheese Appearance

The change in opacity of the cheeses as a result of salting can be attributed to changes in the protein matrix. In unsalted curd, the proteins are more aggregated, and the protein matrix has numerous pockets of free serum throughout. The edges of these pockets would provide a surface at which light can be scattered, giving the cheese an opaque appearance. In addition, the aggregation of the protein may also contribute

somewhat to light scattering, although the small size of the aggregates compared with the size of the serum pockets suggests that most of the light scattering occurs at the serum-matrix interface. Salting the cheese results in the absorption of free serum into the matrix, giving a homogeneous matrix with few discontinuities or surfaces to cause light scattering. Thus, the salted cheese becomes translucent.

The cheeses stretched in brine became translucent by the time the cheese was cooled to room temperature, which suggests a change in the protein structure upon addition of NaCl. This result would be expected as the cheese curd and brine are intimately mixed during stretching. In contrast, cheeses that were stretched in hot water had lower NaCl contents, and, consequently, absorption of serum into the matrix would take longer. In this case, not until the cheeses were cooled to 4°C did they lose their opaqueness. This temperature dependence of opaqueness is a strong indication that hydrophobic interactions are involved.

The effect of temperature on the association and disassociation of caseins in milk is well known (McMahon and Brown, 1984) and has also been used to explain changes in protein content of expressed serum of Mozzarella cheese (Guo and Kindstedt, 1995). At pH 5.4 (the pH of the cheeses in this study), the dissociation of casein from micelles has also been shown to be much greater at 4°C than at 20°C (Du, 1994). The importance of the hydrophobic interactions was also evident in the cycling from translucency to opaqueness and back to translucency that occurred as the cold cheese was heated during stretching (or during the melt test) and subsequently cooled. Whether serum pockets were reformed during heating or whether the light scattering was solely a function of change in

protein aggregation size was not determined as samples for electron microscopy were
only prepared from cheese on d 1.

Cheese Melt

The lower meltability of unsalted cheese can be explained in terms of the energy
required to disrupt the matrix network and to allow the proteins to flow. Proteins that are
more highly aggregated would require more thermal energy to disrupt the aggregates and
disassociate the proteins. In contrast, salted cheeses with smaller protein aggregates and a
more hydrated protein matrix had better melt. The slight increase in the melt of the
unsalted cheeses during storage corresponds with the decrease in the expressible serum
over the same time and implies that the increased meltability observed with cheese of
greater moisture content (Barbano et al., 1994; Perry et al., 1997) is a function of the
moisture being held within the cheese matrix and not the total cheese moisture. As water
migrates into the protein matrix, the interactions of protein to water increase, and the
hydration sphere of the proteins increases to accommodate the extra water molecules.
Concomitantly, the volume of the protein matrix increases, resulting in the protein matrix
filling the spaces previously occupied by the serum pockets and voids.

CONCLUSIONS

The influence of salting on the color, expressible serum, and melting of nonfat
Mozzarella cheese can be explained by the changes in the microstructure and
ultrastructure of the cheese. Unsalted cheese had larger protein aggregates with free
serum existing in small and large pockets within the protein matrix, and salted cheese had
a more homogeneous structure with more hydrated proteins. There was no expressible

serum in the cheeses with a NaCl content $\geq 0.85\%$. These cheeses were also relatively translucent in appearance. Salted cheeses also had a slightly improved meltability; however, the meltability did not change during 24 d of storage. When all factors were considered, the nonfat cheese made from curd that had 1.0% NaCl that was added before stretching and was stretched subsequently in hot water produced the best cheese. This cheese had only 0.4% NaCl content but similar melt characteristics to cheeses manufactured with a higher NaCl content; this cheese was also more opaque at room temperature and shredded better.

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CHAPTER 3

INFLUENCE OF CALCIUM, PH, AND MOISTURE ON THE FUNCTIONALITY, TEXTURE, AND STRUCTURE OF NONFAT, DIRECTLY ACIDIFIED MOZZARELLA CHEESE

ABSTRACT

The influence of calcium, moisture, and pH on the structure and functionality of direct-acid, nonfat Mozzarella cheese was studied. Cheeses were manufactured on three separate days using combinations of citric and acetic acids. Addition of EDTA to the whey during cooking to chelate calcium, calcium chloride fortification, and extended drain times were used to produce eight cheeses in a 2^3 factorial design with target pH levels of 5.8 and 5.3, 70% and 66% moisture, and 0.6% and 0.3% calcium levels. Using EDTA was unsuccessful in removing calcium from the pH 5.8 cheese while adding calcium chloride was successful in increasing the calcium level of pH 5.3 cheese. Cheeses were analyzed for calcium, meltability, moisture, texture, and cheese microstructure after 2 wk storage. Calcium content of cheese had most influence on cheese melt and hardness with the greatest melt occurring in cheeses with the lower calcium level (0.3%). These low calcium cheeses were also softer and more adhesive than the cheeses containing 0.6% calcium. When calcium content was held constant at 0.6%, there was no significant difference in melting even when pH was varied from pH 5.8 to pH 5.3. The microstructure of cheese with 0.6% calcium had an increase in protein folds and serum pockets in comparison to the low calcium cheeses that had a very homogeneous structure.

INTRODUCTION

When fat is removed from Mozzarella cheese, several undesirable characteristics develop including poor melt and shred fusion when cooked on a pizza, short or decreased stretch, and an increase in cheese hardness (Konstance and Holsinger, 1992; Mistry and Anderson, 1993; Tunick et al., 1993). Unlike part-skim and full-fat Mozzarella cheese, in which fat occupies spaces between protein fibers that are created during the stretching process in Mozzarella cheese manufacture (Oberg et al., 1993), in nonfat cheese there is no physical hindrance to fusion of protein strands. Also, when cooked, the molten fat provides a liquid phase that aids the flow of molten cheese (Tunick et al., 1993; Tunick and Shieh, 1995).

One strategy to alleviate the effects of fat reduction on the functional properties of Mozzarella cheese is to increase cheese moisture content. Unfortunately, if too much moisture is left in the cheese matrix there can be an excessive amount of expressible serum. To accommodate additional water, the water holding capacity of the protein matrix needs to be increased. Lowering the calcium content of nonfat Mozzarella cheese by using direct acidification produces a more hydrated protein matrix and improved meltability (see Chapter 2).

There are several ways to lower calcium content of cheese including decreased pH at draining (Kindstedt, 1991), pre-acidification of milk, use of chelating agents and acids, and curd washing. Breene et al. (1964) observed that direct acid cheese made using calcium chelating acids, such as critic acid, had functional properties similar to cheeses with lower pH. Similar work on cheddar cheese has shown that injection of acid after cheese manufacture influenced the protein interactions within the cheese by

solubilizing calcium phosphate in the cheese matrix and improved the meltability of the cheese (Pastorino et al., 2003b). This work supports the model that the impact of charged ions, specifically calcium, on protein-to-protein interactions within the matrix plays a significant role in cheese functionality (see Chapter 2; Pastorino et al., 2003a; Joshi et al., 2003). When calcium is directly injected into the cheese matrix, differences in protein arrangements were observed (Pastorino, 2003a). This indicates that calcium is the key component in determining the structural arrangements of casein within the matrix of Mozzarella cheese.

Proper cheese melt is one of the primary functional concerns when the performance of Mozzarella cheese on a pizza is considered. To meet consumer expectations, Mozzarella cheese must melt and fuse properly without excessive burning or blistering. Reduced-fat, low fat, and nonfat cheeses typically have very poor melt due to the increased protein interactions in the cheese. When cheese is melted, the protein fibers unfold, lose their defined structure, and begin to flow past one another. As protein density increases and protein interactions increase within the cheese matrix, as is the case when fat is removed, more energy is required to disrupt the protein bonding within the matrix and allow the molten cheese to flow. Also of concern during cooking is excessive drying of the protein matrix. Moisture is readily lost due to evaporation while cooking whereas fat, which does not evaporate, can remain in its fluid state during cooking. This results in cheese shreds that dry out before melting and becoming part of the molten cheese. Thus, replacement of fat with water will not completely solve the functional issues and a better understanding of how the protein interactions within the cheese matrix affect cheese functionality is needed.

Little work has been done to evaluate the effects of calcium, pH and moisture on directly acidified nonfat Mozzarella cheese. Using directly acidified Mozzarella cheese as a model system, acid type and manufacturing conditions can be selected to generate cheeses with varying pH, moisture, and calcium levels so each component, and it's effect on the protein matrix of the cheese, can be studied independently. Further, by using direct acidification, proteolysis effects on the protein matrix due to culture activity is eliminated.

MATERIALS AND METHODS

Cheese Manufacture

Skim milk, fortified with 1.0% NDM, was pasteurized at 80°C for 29 s and cooled to 4°C overnight. The chilled milk, 10-kg per vat, was placed in eight open rectangular vats and acidified and fortified with calcium chloride using the milk treatments described in Table 2. Cheese vats were heated to 35°C in a jacketed water bath. Milk in each vat was set using 1.0 ml of single strength calf rennet (Rhodia, Inc., Madison WI). After 15 min, the curd was cut with 1.9-cm knives, allowed to heal for 15 min, then stirred constantly for the time listed in Table 2. To aid in reducing calcium content, 15 g EDTA was added to the whey during cheese making in two of the treatments (Table 2). After whey draining, some treatments also were dry stirred to remove additional moisture (Table 2). Curds were dry salted with 0.4 g NaCl and hand stretched in 82°C hot water containing 5% (w/w) NaCl. Molten cheeses were stretched until smooth, then placed in stainless steel molds (9 cm x 9 cm x 9 cm). The molded cheese was cooled in ice water for 1 h, then vacuum packed and stored at 4°C.

Cheese Composition

Cheese was shredded in a hand-held electric shredder (Professional Salad Shooter, National Presto Industries, Inc., Eau Claire, WI) prior to analysis. All analyses were run on 14-d old cheeses. Cheese moisture was determined in duplicate using a vacuum oven (AOAC, 1990). Protein was determined without subsampling using the Kjeldahl method (AOAC, 1990). Calcium was determined without subsampling using inductively coupled plasma atomic emission spectroscopy (US EPA, 1992).

Cheese Functionality

Cheese melt was determined in duplicate by a modified melt tube method using a hot oil bath at 90°C (McMahon et al., 1999). Overall melt was measured as distanced traveled by the molten cheese after heating for 16 minutes (maximum distance was 220 mm). Texture analysis; hardness, adhesiveness, gumminess, chewiness, and springiness of 14-d old cheese was performed in duplicate using the Texture Profile Analysis function on a Stevens Farnell texture analyzer (Model 25, Stevens Farnell, Dunmorow, UK) with sample preparation as described by Fife et al. (2002).

Electron Microscopy

Samples for transmission and scanning electron microscopy were collected from 14-d-old cheeses from two replicates. The cheeses were cut into slices (1 mm x 1 mm x 5 mm) and then fixed in a 2% glutaraldehyde solution overnight. Samples for scanning electron microscopy were prepared according to the methods of McManus et al. (1993). Samples for transmission electron microscopy were cut again into cubes (1 mm x 1 mm x 1 mm) and placed in 1% OsO_4 in 0.2 M cacodylate buffer for 1 h, dehydrated in a graded ethanol series to 100% ethanol, then infiltrated with Spurr's epoxy overnight, transferred

Table 2. Individual treatments applied per 10 kg of skim milk (fortified with 1.0% NDM).

Cheese	Milk Treatment	pH at set	Whey Treatment	Cut-to-Drain Time (Min)	Dry Stirring Time (Min)
HPH1	105 ml 1/10 acetic acid	5.8	None	50	0
HPH2	105 ml 1/10 acetic acid	5.8	None	50	40
HPH3	80 ml 1/10 acetic acid 2.5 g citric acid	5.8	15g EDTA*	50 min stir	0
HPH4	80 ml 1/10 acetic acid 2.5 g citric acid	5.8	15g EDTA*	50 min stir	40
LPH1	230 ml 1/10 acetic acid 14 g calcium chloride	5.3	None	40	0
LPH2	230 ml 1/10 acetic acid 14 g calcium chloride	5.3	None	40	30
LPH3	230 ml 1/10 acetic acid	5.3	None	40	0
LPH4	230 ml 1/10 acetic acid	5.3	None	40	30

* EDTA added 15 minutes before whey drain

to Beem® capsules filled with Spurr's epoxy, and heated to 70°C for 24 h. Thin sections, 70-nm thick, were cut on an Ultracut ultramicrotome (Leica, Inc., Deerfield, IL), transferred to 300-hex mesh grids, and then counterstained with uranyl acetate and lead citrate. Sections were examined on a Zeiss 902 electron microscope (Carl Zeiss, Inc., Thornwood, NY) at an accelerating voltage of 80 kV. All chemicals and grids were obtained from Electron Microscopy Sciences (Fort Washington, PA).

Statistical Analysis

Five replicates of cheese were made using different batches of milk. Means were calculated from duplicate analyses and analyzed by Statistica™ (Statsoft Inc., Tulsa, OK) using MANOVA function with one main effect of eight treatments. When significant ($P \leq 0.05$), differences between means were analyzed using least significant difference.

RESULTS

Cheese Composition

Cheese composition is shown in Table 3. Fortification of cheese milk with 14-g $CaCl_2$ in the low pH cheeses (LPH1 and LPH2) increased calcium content in the finished cheese to 0.6% and was the same as the pH 5.8 cheeses. Acidification of cheese milk to 5.3 without calcium fortification produced cheese with lower (0.3%) calcium content.

Addition of citric acid in the manufacture of direct acid cheese or the use of EDTA during cooking of high pH cheeses (HPH3 and HPH4) did not decrease calcium levels in the finished cheese as proposed. Consequently, statistical analysis could not be performed as a 2 x 2 x 2 factorial, but was instead analyzed as a completely randomized

Table 3. Means for moisture, protein, and calcium content of directly acidified nonfat Mozzarella cheese manufactured with treatments described in Table 2. Means with the same letter superscript within the same column were not significantly different.

Cheese	Acidification pH	Moisture	Protein	Calcium
		----------------------(%)----------------------		
HPH1	5.8	69.9^{bc}	23.6^{bc}	0.56^{b}
HPH2	5.8	65.7^{a}	27.4^{d}	0.63^{bc}
HPH3	5.8	70.3^{c}	23.0^{ab}	0.57^{b}
HPH4	5.8	66.0^{ab}	26.2^{cd}	0.59^{bc}
LPH1	5.3	70.3^{c}	24.2^{bc}	0.56^{b}
LPH2	5.3	65.9^{ab}	27.4^{d}	0.67^{c}
LPH3	5.3	73.1^{d}	20.4^{a}	0.30^{a}
LPH4	5.3	67.9^{b}	23.0^{ab}	0.34^{a}

design with one main effect of eight treatments. Cheese moisture increased as calcium was reduced from 0.6% to 0.3% in the LPH3 and LPH4 cheeses.

Cheese Meltability

The calcium content of directly acidified nonfat Mozzarella cheese had an influence on the meltability of the finished cheese (Figure 8). When the calcium was reduced to 0.3%, cheese-melt increased dramatically with molten cheese flowing the entire length of the melt tube (220 mm). When calcium in the cheese was increased to 0.6%, near the level found in commercially manufactured low moisture, part-skim Mozzarella cheese (USDA, 1980), the ability of the molten cheese to flow was decreased. Cheese melt was similar in all cheeses when the calcium content was 0.6% and did not depend on pH or moisture content in this system.
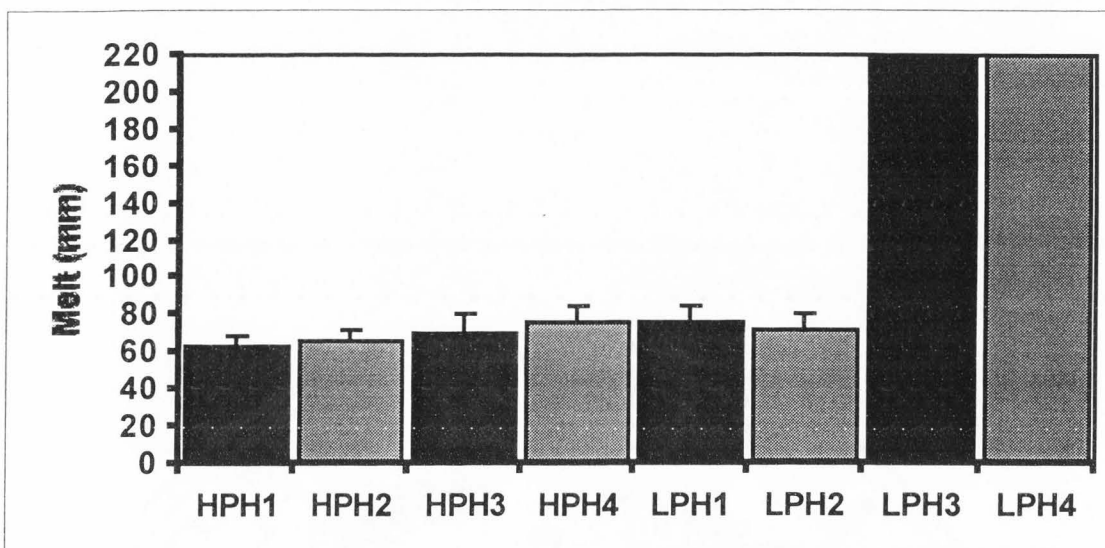
Figure 8. Mean cheese melt measurements of directly acidified nonfat Mozzarella. Matching colors received similar moisture treatments during manufacture. Cheeses HPH1, HPH2, HPH3, HPH4, LPH1, and LPH2 have similar calcium (0.6%). Cheeses LPH 3 and LPH4 have lower calcium (0.3%). Bars are SEM.

Cheese Texture

Texture of directly acidified nonfat Mozzarella cheese can be modified by adjusting calcium and moisture content (Figure 9). When calcium content was 0.6%, the protein matrix was firm. While at low calcium (0.3%) the cheese was softer. Total moisture also influences cheese hardness. A reduction of ca. 5% moisture in the cheese matrix led to an increase in cheese hardness. Together, reducing calcium and increasing moisture produced cheeses with very soft texture (see cheese LPH3 in Figure 9).

Calcium had a similar effect on adhesiveness of the cheese matrix. Cheeses with calcium levels of 0.3% had an increase in adhesive properties in comparison to cheeses with twice the calcium (0.6%). Moisture did not appear to influence the adhesiveness of the cheese matrix when the calcium was 0.6%. However, when calcium was reduced to 0.3%, an increase in moisture led to an increase in adhesiveness (Figure 10).
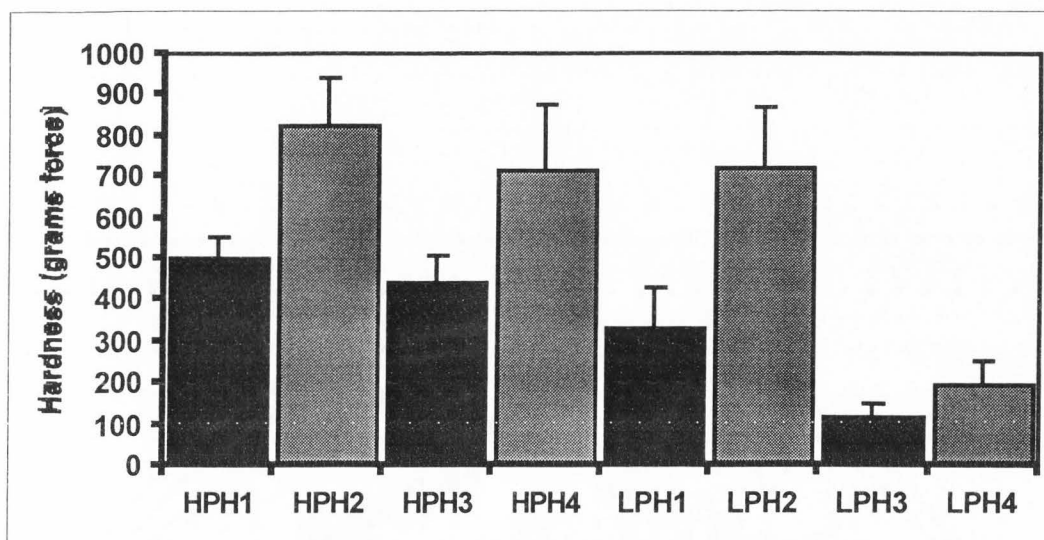
Figure 9. Mean cheese hardness measurements of directly acidified nonfat Mozzarella cheese. Matching colors received similar moisture treatments during manufacture. Cheeses HPH1 through LPH2 have similar calcium level (0.6%). Cheeses LPH3 and LPH4 have lower calcium (0.3%). Bars are SEM.

Cheese Microstructure

The overall calcium content of the finished cheese had an impact on the microstructure of directly acidified nonfat Mozzarella cheese. Differences in cheese microstructure were observed between cheeses with 0.6% calcium and 0.3% calcium (LPH3). When fractured surfaces were observed, cheeses with 0.6% calcium (HPH1, HPH3 and LPH1) had numerous protein folds and serum pockets while cheese with 0.3% calcium (LPH3) had a much more homogeneous structure with no visible folds or pockets (Figure 11). The heterogeneous structure of cheese containing 0.6% calcium was evident at both pH 5.8 (HPH1 and HPH2) and cheese at pH 5.4 (LPH1).

Similar differences in protein matrix structure were also observed when thin sections of cheese were examined using transmission electron microscopy (Figure 12). The cheeses containing 0.6% calcium (HPH2, HPH4 and LPH2) had numerous serum pockets dispersed throughout a densely-stained protein network structure. While the
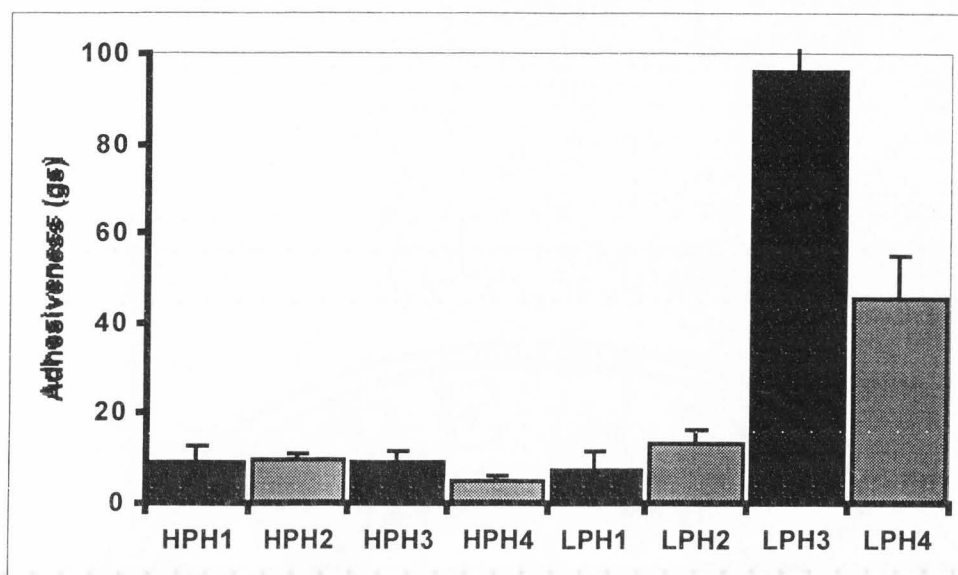
Figure 10. Mean cheese adhesiveness measurement of directly acidified nonfat Mozzarella cheese. Matching colors received similar moisture treatments during manufacture. Cheeses HPH1 through LPH2 have similar calcium level (0.6%). Cheeses LPH3 and LPH4 have lower calcium (0.3%). Bars are SEM.

cheese containing only 0.3% calcium (LPH4) exhibited a more open protein network structure with few serum pockets even though it had a higher moisture content (68.5%) than the other cheeses (65.7 to 66.0% moisture). When the electron dense areas of cheese were examined at higher magnification (Figure 13), the proteins in the higher calcium cheese were observed to be in a more aggregated state with larger spacing between the protein aggregates, than were the proteins in the low calcium cheeses.

At each of the pH-calcium treatment combinations there was cheese made at two moisture levels. Lower moisture contents were obtained by dry stirring the curd after the whey was drained (as described in Table 2). The only difference observed in micrographs of such pairs of cheese was that there was less fusion observed to occur between protein strands in the cheeses with the shorter stirring times (Figure 14A) than in cheese that was (Figure 14B). This implies that when the curd was salted and stretched

Figure 11. Scanning electron micrographs of pH-5.8 cheeses HPH1 (A) and HPH3 (B), and pH-5.4 cheeses LPH1 (C) and LPH4 (D), these cheeses contained 0.6% calcium (A, B and C) or 0.3% calcium (D). Bar = 1 μm.

Figure 12. Low magnification transmission electron micrographs of pH-5.8 cheeses HPH2 (A) and HPH4 (B) and pH-5.4 cheeses LPH2 (C) and LPH4 (D), these cheeses contained 0.6% calcium (A, B and C) or 0.3% calcium (D). Bar = 1 μm.

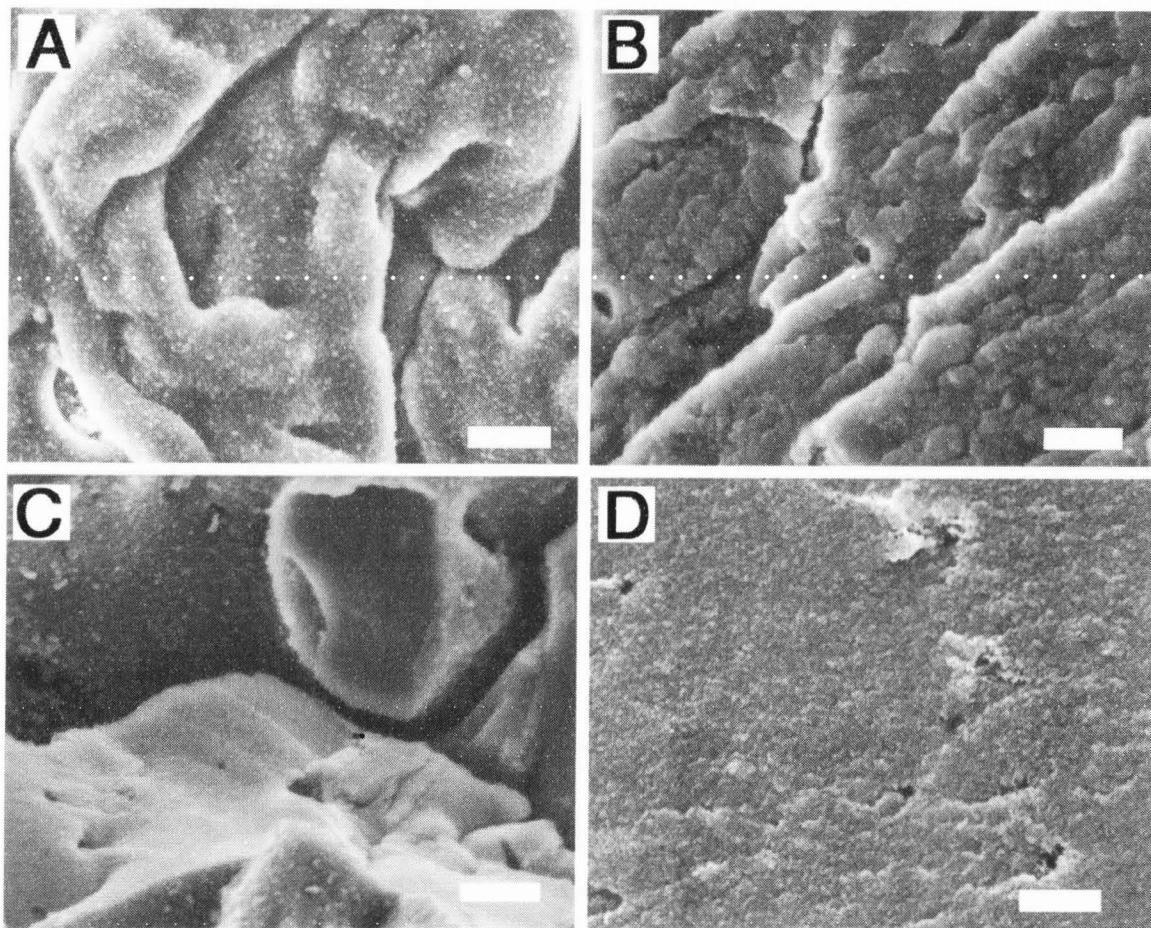**Figure 13.** High magnification transmission electron micrographs of pH-5.8 cheeses HPH1 (A) and HPH3 (B), and pH-5.4 cheeses LPH1 (C) and LPH3 (D), these cheeses contained 0.6% calcium (A, B and C) or 0.3% calcium (D). Bar = 100 nm.
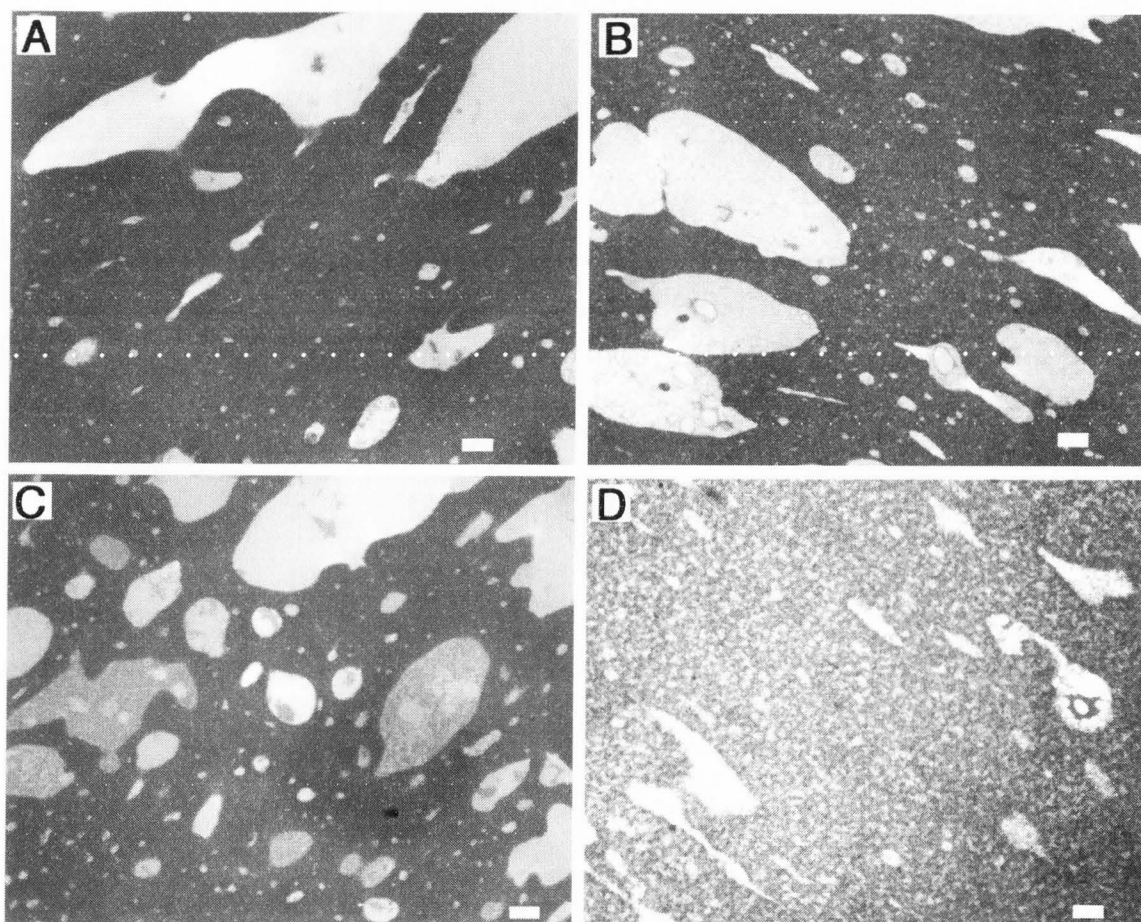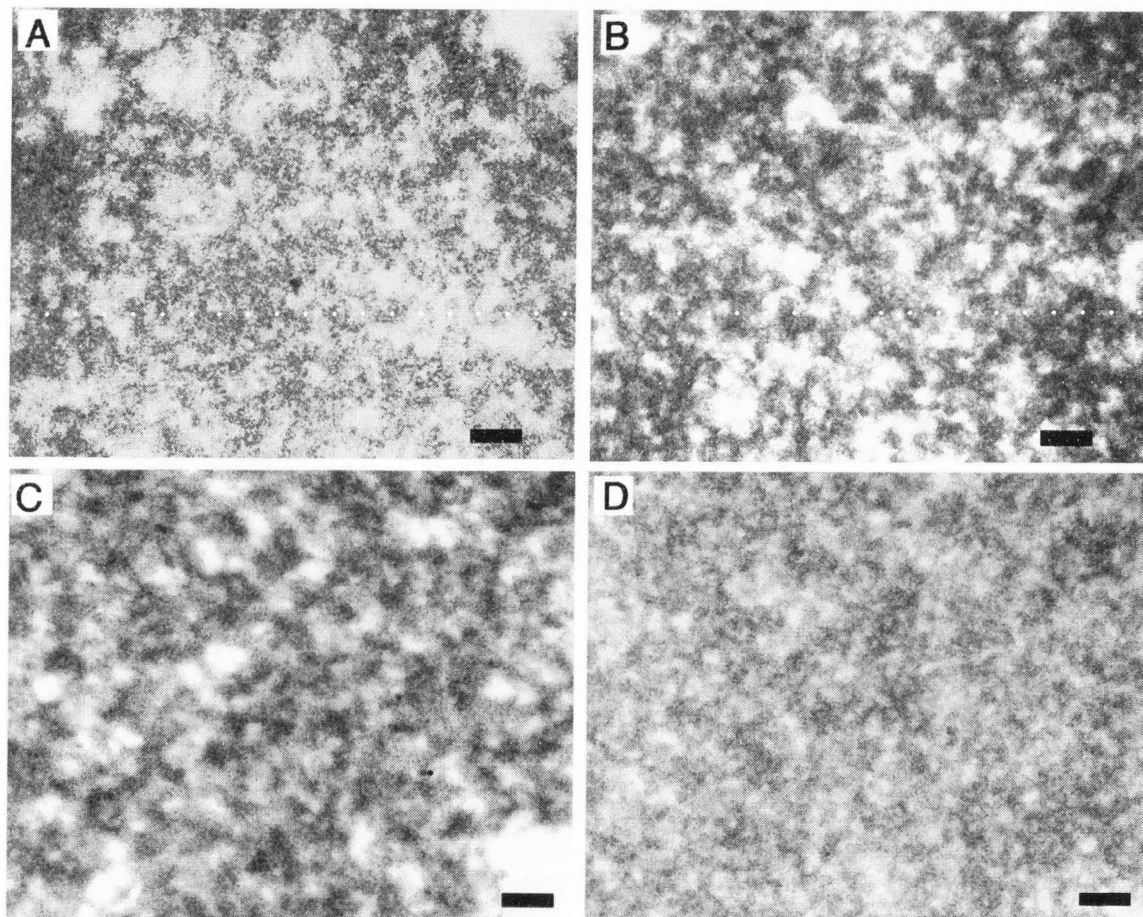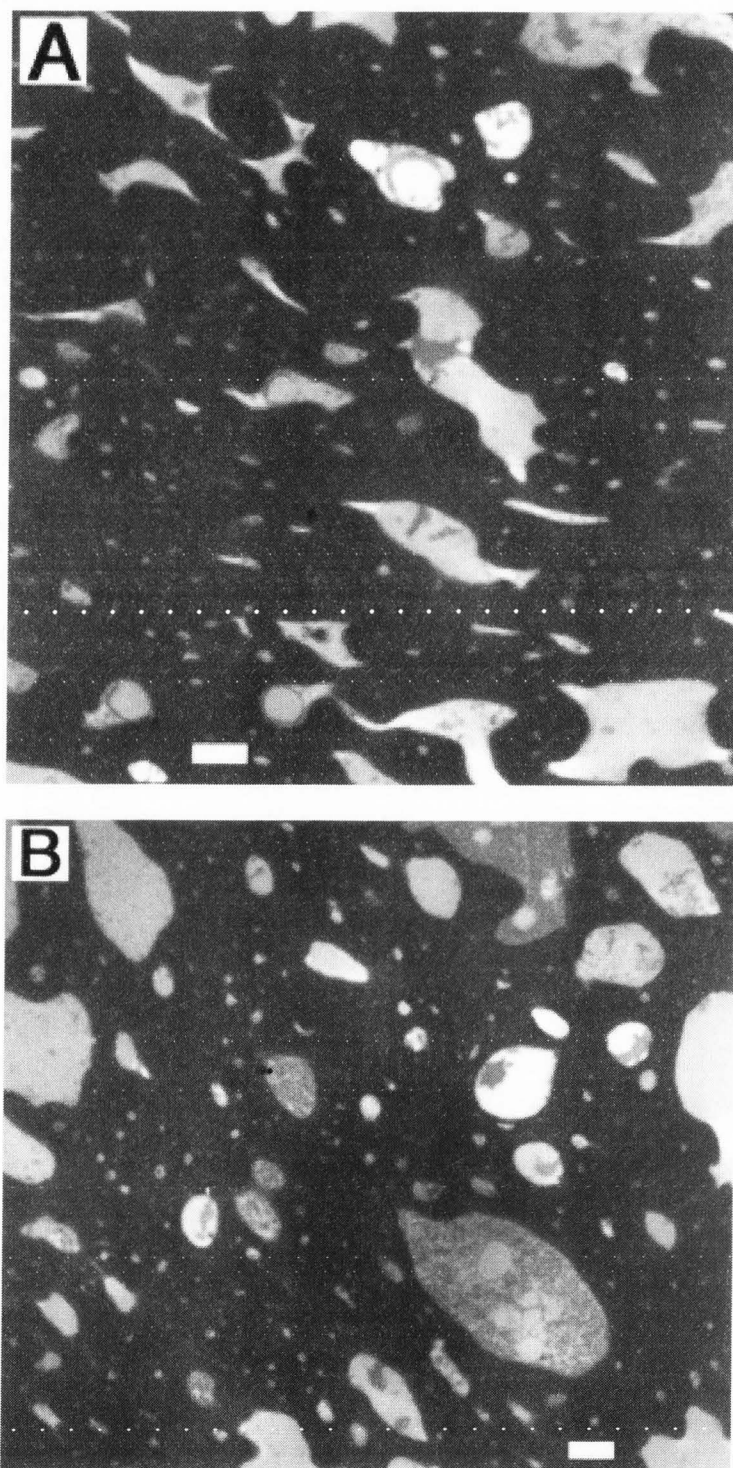
Figure 14. Transmission electron micrographs of pH-5.4 cheeses LPH1 (A) and LPH2 (B) that were made using a short and long dry-stirring time, respectively. Bar = 1 μm.

immediately after whey drainage, curd shrinkage and whey expulsion had been interrupted and it could be expected that further syneresis might occur during storage of such cheese. Similar observations were made by Merrill et al. (1994) during their development of a procedure for manufacturing a reduced-fat mozzarella cheese. Increasing the moisture content of cheese requires a chemical intervention that results in an increase in the water holding capacity of the cheese matrix rather than a physical intervention such as shortening the manufacturing time. An example of such a chemical intervention would be to lower the calcium content of the cheese so that the proteins that comprise the cheese matrix become more hydrated as observed in the LPH3 and LPH4 cheeses. Both of these cheeses had moisture contents above that which was planned.

DISCUSSION

Calcium Interactions

Cheeses with similar calcium levels had similar microstructure and melt performance. At a calcium level of 0.6%, the fractured surface of the cheese showed protein fibers and numerous serum pockets. This indicates that the presence of calcium led to strong protein-to-protein interactions within the cheese matrix and, through syneresis, led to an exclusion of moisture from the cheese matrix.

Conversely, as calcium was decreased to 0.3% the protein matrix was observed to be more homogeneous with very few serum pockets even though moisture levels were typically 2 to 3% higher in the low calcium cheeses compared to their higher calcium counterparts. The reduction in calcium, and subsequent reduction in protein-to-protein interactions, allowed the moisture to diffuse into the protein matrix and increased protein hydration.

Since a large quantity of the moisture exists outside of the cheese matrix when the calcium is 0.6% in comparison to 0.3% cheese, it can be said that in any given volume of cheese, the protein density is higher and more compact in the 0.6% calcium cheeses than it is in the 0.3% calcium cheeses. This increased protein density led to a more rigid structure, increased hardness, and decreased melt.

A similar effect was reported by Pastorino et al. (2003a) when calcium chloride was injected directly into low moisture part-skim Mozzarella cheese. As calcium levels of the cheese were increased though a series of injections, the protein fibers in the cheese matrix contracted and water was excluded from the protein matrix. The compacted protein matrix led to decreased cheese melt and increased cheese hardness.

Calcium ions interact with casein molecules within the protein matrix The positively charged calcium ions associate with negatively charged regions of the caseins and neutralize charge repulsion; leading to increased protein interactions between casein molecules. The divalent nature of the calcium ion also contributes to bridging between proteins and a stronger, more cross-linked, protein matrix (Pastorino et al. 2003a). Monovalent ions, such as sodium, have a dissimilar effect. Previous research has shown that increasing the sodium content of directly acidified nonfat Mozzarella cheese leads to increased protein hydration and fewer protein-to-protein interactions (Chapter 2). This suggests that protein cross-linking within the cheese matrix is more important than charge neutralization when considering protein interactions and their effects on cheese functionality.

Furthermore, in agreement with Pastorino et al. (2003b), at cheese pH >5.0 the effect of pH on cheese is related to its influence on residual calcium content in the

cheese. Typically a higher pH cheese has higher calcium content than a lower pH cheese as demonstrated in the comparison of cheeses HPH1 and HPH2 that contained 0.6% calcium to that of LPH3 and LPH 4 that contained only 0.3% calcium. These are the calcium contents in cheese that result from direct acidification of milk to pH 5.8 and pH 5.3 using acetic acid. To obtain cheese (LPH1 and LPH2) from milk acidified to pH 5.3 that had a higher than normal calcium content it was necessary to fortify the milk with calcium before renneting (see Table 2). Such pH-5.3 cheese had similar structural and functional characteristics to the pH-5.8 cheeses. Thus, if calcium content is maintained at the same level, differences in pH would not be expected to influence cheese performance over the pH range of 5.0 to 5.8.

It had been expected that by acidifying milk to pH 5.8 with a combination of acetic acid and citric acid, and by adding EDTA as a calcium-chelator into the whey that cheeses would be obtained (HPH3 and HPH4) with a level of calcium comparable to the pH 5.3 cheeses. However, this was not the case and these cheeses had calcium contents of 0.6% and had similar structural and functional characteristics as HPH1 and HPH2. A possible explanation for this was reported by Pastorino et al. (2003c) who injected sodium citrate into cheddar cheese but did not observe any solubilization of protein-bound calcium. Instead, it was found that the citrate treatment increased the level of soluble phosphate in the cheese suggesting that the interaction of phosphate with the protein matrix plays a critical role along with calcium interactions.

The addition of EDTA to the whey to chelate calcium did not reduce total calcium content of the curd as was expected and did not alter the cheese structure or functionality. Syneresis occurs while curds are cooked and stirred in the whey and moisture and

solubilized minerals leave the curd and enter the whey. Evidently, there was inadequate transfer of EDTA into the curd particles to solubilize calcium so that it be transferred out of the curd as whey was expressed. A better means to chelate calcium in the curd might be to add chelating agents, such as EDTA, to the hot water/brine solution during cooking and stretching of the curd.

Moisture will migrate within the cheese matrix seeking the lowest free energy state. In cheeses with a high calcium content and densely compacted protein bundles, moisture exists in free serum pockets within the cheese matrix because the system favors protein-to-protein interactions. As calcium is decreased in the cheese matrix, protein-protein interactions within the cheese matrix are decreased and protein-water interactions are increased. Thus, it becomes more thermodynamically favorable for the water to diffuse into the protein matrix and the overall protein matrix becomes more hydrated.

Cheese Functionality

The differences in protein structure between cheeses with 0.6% calcium and cheeses with 0.3% calcium explains the differences in melt, hardness, and adhesiveness of the cheese. An increase in protein density and cross linkages through the interactions of the calcium ions would lead to an increased structural rigidity of the cheese matrix and overall increased cheese hardness. And indeed, in this study, cheeses with higher calcium were harder than cheeses with lower calcium.

Similarly, this structural rigidity explains the decreased melt performance of the higher calcium cheeses. As protein-to-protein interactions within the cheese matrix increase, more energy is required to disrupt the bonds within the cheese matrix and allow the proteins to flow past one another. In addition, a larger portion of the moisture exists

in pockets of free serum that once exposed as the protein matrix collapses during melt, can evaporate quickly in a pizza oven.

In a pizza oven supplying constant heat over a set period of time, cheeses with increased protein-to-protein interactions can be expected to take longer to melt as energy is absorbed to melt the cheese. If too much moisture is lost from the cheese surface before sufficient heat is absorbed to melt the cheese and begin to flow, melt can be reduced. In the low calcium cheeses, with highly hydrated protein matrices and fewer protein interactions, the bonds between proteins are much weaker and require less energy to break. As a result, melt will be more rapid and presumably take place before skin formation can prevents full melt.

Adhesiveness of the proteins in the 0.3% calcium cheeses was increased in comparison to cheeses with 0.6% calcium. Again, this can be explained by the protein structure of the cheese matrix. Higher calcium cheeses had more moisture present in serum pockets and when cut, this moisture is free to coat the surface of the cheese and prevent direct contact between the protein fibers and the external surfaces. A similar effect was found in previous research when hand stretching directly acidified nonfat Mozzarella cheese with high salt contents and highly hydrated protein matrices (Chapter 2). In this experiment, cheeses with hydrated matrices adhered readily to the researchers gloves and were very tacky in feel. It is expected that the expanded protein matrix in these cheeses can allow more contact with charged regions as the matrix is loosely associated internally and has a greater degree of flexibility. As moisture increased in the low calcium cheeses, adhesiveness increased indicating a progressive weakening of the matrix with increased water content. In the higher calcium cheeses, with compact

bundles of proteins, the charged regions of the protein matrix are tightly associated with each other and less are available for external interactions.

CONCLUSIONS

Reducing the calcium content to 0.3% in directly acidified Mozzarella cheese led to increased cheese melt, a softer body, and a homogeneous microstructure throughout the cheese. These changes occurred independently of pH (pH 5.3 compared to pH 5.8) or the moisture content (approximately 66 to 70%) of the nonfat cheese. When calcium content of the cheese was retained at 0.6%, the cheeses had similar functional performance. This confirms observations that at pH > 5.0, it is calcium content that controls cheese functionality, and that the influence of cheese pH is related to its effect on calcium solubilization (and loss into the whey) during cheesemaking. Such differences in cheese functionality can be explained by differences in the microstructure of the cheese matrix. As calcium content was increased, the protein bundles become larger and denser with a corresponding increased in serum pockets as water is excluded from the protein network matrix. Reducing the calcium increases the hydration of the protein matrix and weakening protein interactions leads to softening of the cheese and improved flow as the cheese is heated and melts.

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CHAPTER 4

GENERAL SUMMARY

Protein interactions within the Mozzarella cheese matrix play a significant role in the functionality of the cheese. These protein interactions provide the structural characteristic of the cheese and by changing how the proteins interact; the functionality of the cheese can be modified. This is important in nonfat Mozzarella cheeses where the only main components of the cheese matrix are protein and water.

The mineral content, specifically sodium and calcium, of the cheese is an important determining factor when considering cheese functionality. Sodium, a positively charged monovalent ion, promotes hydration and swelling of the cheese matrix by associating with negatively charged regions of the protein matrix and reducing charge repulsion. This allows moisture to be absorbed into the cheese matrix as protein solubility increases and reduces overall protein interactions in the cheese matrix. This swollen and hydrated matrix changes the appearance of the cheese. A highly hydrated cheese matrix loses many of the serum pockets and becomes a homogeneous mass. This homogenous mass lacks light scattering surfaces and becomes translucent and loses the characteristic white color of Mozzarella cheese. The hydrated matrix also loses much of its structural rigidity and the cheese body becomes soft. Lastly, the hydrated matrix, with the decreased protein interactions is easier to melt due to a decrease in protein bonding in the cheese.

In contrast to the effect sodium has on the cheese matrix, calcium, a positively charged divalent ion, has a dissimilar effect on the cheese when the pH is above 5.0. Calcium also associates with the negatively charged regions in the protein cheese matrix

similarly to sodium ions, however, calcium promotes cross linkages within the cheese matrix and leads to a stronger protein matrix. As proteins associate together in the cheese matrix, the protein bundles become more dense and exclude water from the protein mass. This leads to an increase in free serum within the cheese matrix. The increased density of the protein bundles gives the cheese matrix a harder texture. The increased protein density, caused by an overall increase in protein interactions within the cheese, requires more energy to disrupt and melt. Thus the melt performance is decreased when calcium is increased. Lastly, the presence of serum pockets in the cheese matrix provides light scattering surfaces and cheese have a white appearance typical of Mozzarella cheese.

A highly functional nonfat Mozzarella cheese can be manufactured using the parameters described for cheese B1 in the first experiment. This cheese contains approximately 0.4% sodium chloride obtained by dry salting the curd with 1.0% salt and stretching in hot water. This cheese also contains a low calcium content (0.4%) that improved cheese melt in comparison to higher calcium cheeses. This cheese also has low expressible serum and increased protein hydration in comparison to higher calcium and lower sodium cheeses. Finally, this cheese has sufficient hardness to perform well in mechanical shredding operations.

APPENDIX

Table 4. ANOV of dependent variables for cheese composition and functionality for cheese making trials conducted in Chapter 3, involving 8 treatments and 5 replications.

Dependent Variable	Mean Square	MSE	$F_{7,32}$	P
Moisture	37.	2.4	15.86	< 0.0001
Protein	10.	1.7	5.66	0.0026
Calcium	0.0928	0.0037	24.94	< 0.0001
Melt	24,158	236.7	102.05	< 0.0001
Hardness	391,081	51,553.4	7.59	< 0.0001
Chewiness	6,146,078	897,548.5	6.85	< 0.0001
Adhesiveness	5,737	1,251.7	4.58	0.0012
Springiness	0.	0.1	1.71	0.1410
Gumminess	193,847	49,017.5	3.95	0.0032