A Study of Factors Controlling Physical Properties of Mozzarella Cheese and the Manufacture of a Reduced Fat Mozzarella Cheese

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A STUDY OF FACTORS CONTROLLING PHYSICAL PROPERTIES OF MOZZARELLA CHEESE AND THE MANUFACTURE OF A REDUCED FAT MOZZARELLA CHEESE

by

Richard Kevin Merrill

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

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Richard K. Merrill
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Variables affecting the physical properties of Mozzarella cheese were investigated. The effects of various milk-clotting enzymes were examined. The type of milk coagulating enzyme used played a significant role in determining physical properties of direct acid Mozzarella cheese. Cook color was not affected by enzyme type, but melt and stretch were significantly affected.

Proteolytic nature of starter cultures was reviewed and recommendations were given. Cheese made with proteinase-deficient strains had more stretch after holding for 14 and 28 d than cheese made with non-deficient strains. Cheese made with pairs or single strains of \( L. \) helveticus had the same melt, more stretch, and less cook color than cheeses made with paired strains of \( Lactobacillus \) delbrueckii ssp. bulgaricus and \( Streptococcus \) salivarius ssp. thermophilus.

Frozen storage, thawing, and shredding of Mozzarella cheese were described and suggestions given for optimizing shelf life. Shredding, freeze temperature, thaw temperature, and time of storage had no effect on cook color. Frozen, shredded cheese stretched more and melted less than non-shredded frozen cheese.

Reduced fat, high moisture Mozzarella cheese was made and found acceptable.
when compared to low moisture part-skim Mozzarella cheese. Reduced fat cheeses decreased in stretch and increased in melt throughout storage. Differences in stretch, melt, and cook color were not significant from one casein-to-fat ratio to another.

Reduced fat, high moisture Mozzarella cheese was made with partial or total replacement of \textit{L. helveticus} with \textit{L. casei} \textit{ssp. casei} and was found to compare well with low moisture part-skim Mozzarella cheese. Cheese made with \textit{L. casei} \textit{ssp. casei} cultures, paired with either \textit{S. salivarius \textit{ssp. thermophilus}} and \textit{L. helveticus} or just \textit{S. salivarius \textit{ssp. thermophilus}}, had the least stretch and the greatest melt.

(114 pages)
CHAPTER 1
MOZZARELLA CHEESE: A REVIEW

INTRODUCTION

"Like Italian art, architecture, music, and literature, Italian cheese is a product of ancient culture" with a history covering at least the past 2500 yr (36, 86). Cheese adorned the tables of the Caesars, served as rations for Roman armies, and today is part of most traditional Italian dishes (86). While Italy may not be ranked among the world's leading dairy producing countries, at least relative to its size, its cheese industry is of the highest order (36). Originating centuries ago in southern Italy were two well-known soft cheeses, Mozzarella and Ricotta. Few in the world appreciate the significance of the "pasta-filata" principle of cheese-making. In this system, the unique texture and grain of these cheeses are achieved through stretching the curd in hot water during manufacture. Provolone, Caccicavallo, and several other cheeses also owe their characteristic texture and body to this mechanical manifestation. Of all these less well known cheeses, the soft non-aged Mozzarella is the foundation for all pasta-filata-type cheeses (53). Production of Italian cheese varieties (Mozzarella, Provolone, Ricotta, Romano, and Parmesan) in the United States has been steadily rising over the past 30 yr. In 1991, over 2.3 billion pounds were produced, an increase over 1989 production levels of about 121 million pounds. Mozzarella cheese accounted for 78% of the total Italian cheese production in 1991, bringing total Mozzarella production to over 1.8 billion pounds (personal communication, Dan Buckner, USDA).

The increasing popularity of Mozzarella cheese parallels that of the well known Italian specialty, pizza (36, 53). Today's pizza market is slowly maturing. Competition is becoming much more intense with pizza wars and stiff competition in the delivery and carry-out business (33). However, the fame of Mozzarella cheese has also spread into
Western and Eastern Europe, Asia, and South America (2, 63). The continued growth and popularity of Italian cheese and pizza will depend on continued consumer acceptance (2). The Italian soft-cheese industry continues to grow at a rapid rate. To keep it healthy, new ideas and innovations are required. Many cheese manufacturers are becoming receptive to technological advances, and a wonderful opportunity exists for progress (63).

MOZZARELLA CHEESE QUALITY

Mozzarella cheese supplied to the distributor or processor must conform to standards of identity and user specifications—i.e., percent moisture, percent FDB, etc. The cheese must be free from extraneous matter and defects (98). Attributes of cheese quality include meeting customer requirements in terms of functionality of the cheese i.e., melting properties, texture, color, shredability, etc. (98). Since customers have different and often subjective expectations for Mozzarella cheese quality and functionality, cheese manufacturers must aim to meet these quality expectations (98). The Mozzarella cheese producer also directly affects the pizza business. The largest single item purchased by the pizza industry is Mozzarella cheese (69, 83). Acceptability of pizza is measured by the amount of cheese topping, the way cheese melts, and the stretch or stringiness of the melted cheese (36, 40). Pilcher et al. (83) found the most common quality concerns of pizza restaurants for cheese to be poor shredding, excessive free oil, “soupy” melt, and blistering. Various factors in processing, distribution, and handling of Mozzarella cheese can affect these desired properties. Some of these factors include: a) milk quality: percent milkfat, casein quality, whey protein content, enzymes present and their activity, mineral content; b) type of pasteurization process; c) bacterial starter cultures; d) activity and specificity of milk coagulant; e) degree of acidity; f) time and temperature of cooking; g) CaCl₂ levels; h) aging; i) packaging; j) storage temperature; k) handling at restaurant level; l) thawing, refrigeration, and temperature controls; m) type of oven,
temperature of cooking (2).

**Microbiological Considerations**

The microbial quality of cheese is largely dependent upon the microbial load of cheese milk as well as storage, processing, and handling of milk prior to cheese making. Among the microbiological quality problems in Mozzarella and pizza cheese are late gassing or blowing of the wrapping and soft and pasty body. The association of heterofermentative lactic acid bacteria with the "blowing" of the wrapping and "open" texture in Cheddar cheese has been reported. Laleye et al. (54) studied the problem of gas formations and open texture in Canadian Cheddar and oak cheeses. Their reports indicated that high numbers of lactobacilli, especially heterofermentative and citrate fermenting organisms, were associated with defective cheese. They did not detect any correlation between levels of coliforms, staphylococci, yeast, molds, and clostridia and formation of late gas during maturation process. The association of lactobacilli with a soft-body defect in commercial Mozzarella cheese was studied by Hull et al. in 1984 (44). Slicing and melt-down characteristics are detrimentally affected by this soft body defect. Microbial analysis showed $10^4$ to $10^6$ CFU of non-starter *Lactobacillus casei* per gram of Mozzarella cheese. These strains of *Lactobacillus casei* were able to survive pasteurization and were able to grow to 6°C in the presence of 5% NaCl. As a result, these bacteria are capable of growing in Mozzarella cheese at conditions that suppress growth of starter bacteria.

**MANUFACTURING**

A typical manufacturing process for low moisture, part-skim Mozzarella cheese in the United States involves standardization and pasteurization of milk followed by starter addition and rennet coagulation. After cooking of the curd and draining of the whey, the cheese curd is mechanically stretched, molded, and brined. Times, temperatures, and
desired acidity levels at each step vary depending on manufacturing conditions, customer requirements, and variation in the milk supply (36, 52, 98).

**Direct Acidification**

Addition of acid to milk prior to coagulation was suggested in early work on manufacture of cheese from pasteurized milk and has been traditionally practiced in making Ricotta cheese (84). Mozzarella cheese curd made from chemically acidified milk has been shown to possess excellent stretching properties at pH 5.6, whereas toughness and excessive fat losses are observed at pH 5.4 (10). This is in contrast to results reported by Kosikowski (52) who reported that curd generally would not stretch above pH 5.4 and that, although it would stretch below pH 5.2, the cheese became tough, and fat losses were excessive. Breene et al. (11) found that by decreasing the fat content of the cheese milk by adding skim milk, or by low pressure homogenization of the whole milk before standardization, fat losses could be reduced in directly acidified Mozzarella cheese. Demott (22) reported that yield of Mozzarella cheese made by the culture method from fluid milk and varying amounts of reconstituted nonfat dry milk had recoveries of fat, protein, and total solids greater than that made from fluid milk by direct acidification. However, product yield from reconstituted nonfat dry milk and cream by direct acidification was higher than that when the product was made from part fluid milk and part reconstituted nonfat dry milk by the culture method. Quarne et al. (84) noted that the type of acid has no affect on the handling properties of the curd during stretching and molding operations. On the other hand, the type of acid used to acidify milk before coagulation with rennet in direct acidification procedures for cheese manufacture has significant effects on the composition and characteristics of cheese as reported by Shehata et al. (91) and Quarne et al. (84). Phosphoric acid showed increased moisture loss and enhanced calcium retention (84, 91). Fat recovery in Mozzarella cheese is not affected by the type of acidulant used (84); however, recovery of solids-not-fat was greater in cheese
made with phosphoric acid than in cheese made with hydrochloric or lactic acids (84, 91). The increase in solids-not-fat recovery may have been due, in part, to increased retention of calcium in the curd, resulting from precipitation of calcium phosphate with the milk protein (91).

**Ultrafiltration**

Ultrafiltration (UF) technology has been used in cheese to increase cheese yield (92). Mozzarella cheese made from UF concentrated milk has poor stretching and melting properties (18), which seems to be more pronounced as the concentration of milk increases. Whey protein appears to cause this problem. Whey proteins precipitate on the casein network during heating, making it difficult or impossible for individual casein strands to move relative to each other (88). The high water-binding capacity of whey protein reduces the free water in cheese, thereby reducing the flow properties of the cheese (67). Fernandez and Kosikowski (30) reported that Mozzarella cheese made at an optimum volume concentration ratio (VCR) of 1.75:1 produced cheese with good melting properties, increased output per vat, and higher yield efficiency based on total solids. No difference in cheese moisture was evident between control and retentate cheese, even at retentate levels of 1.75:1 VCR, which may be due to the lower than normal cooking times (30 min) and curd being cut into larger cubes (30).

One of the most critical defects associated with Mozzarella cheese is fat leakage appearing on the surface of pizza during cooking. It has been reported that generally more fat leakage is found with cheese manufactured from UF milk during cooking, even when the cheese is made from milk concentrated less than two-fold (28, 67).

**Mozzarella Cheese from Nonfat Dry Milk**

Many cheese varieties can be manufactured satisfactorily from recombined milk using low-heat skim milk powder and anhydrous milk fat. In general, acceptable quality
cheese can be made from recombined milk using the same principles and standards as apply to the production of cheese from fresh milk (38). Fat and casein losses during cheese manufacture are no greater when recombined milk is used than for fresh milk, provided that a stable emulsion is achieved by efficient homogenization of the basic ingredients. Flanagan et al. (32) described the manufacture of Mozzarella cheese using low-heat skim milk powder and fresh cream as the fat source. They reported normal melt and stringiness characteristics in the Mozzarella but an absence of flavor development. Demott (22) reported that fat, protein, and total solids were greater when Mozzarella cheese made from reconstituted skim milk powder was made by the culture technique rather than by direct acidification.

Low Fat Mozzarella Cheese

Concern about diets high in fat has stimulated research into fat replacement in foods (15, 93, 97). Public health officials have stated the need for light dairy products: they want them to contain less fat, less cholesterol, and less saturated fatty acids (93). Sears and Spurgeon (89) used hydrocolloids to produce low fat process cheeses from natural Cheddar or blue cheeses. These spreads contained 3.8% to 15.7% fat, 8.9% to 15.5% protein, and 51.5% to 65.7% moisture. Much of the recent work in this area has involved Cheddar cheese. Attempts have been made to produce low fat hard and semi-hard cheeses containing 4%-27% fat, compared to regular Cheddar cheese (93, 97). However, little success has been realized in cheese flavor using the traditional manufacturing procedures (15, 93, 97). Tunick et al. (97) examined low fat, low moisture Mozzarella cheese and found it too hard and not meltable enough to be considered comparable to full-fat Mozzarella cheese. However, low fat, high moisture Mozzarella cheese (<30% FDB) after 6 wk of refrigerated storage does have textural characteristics similar to those of fresh high fat, low moisture Mozzarella cheese.
EFFECTS OF MILK-COAGULATING ENZYMES

Variation in the proteolytic characteristics of milk-clotting enzymes is well documented (13). Milk-clotting enzymes manifest a variety of proteolytic characteristics specific to each enzyme (14, 95). Quarne et al. (84) studied proteolysis and sensory characteristics in direct acid Mozzarella cheese made with several milk-clotting enzymes. They found that proteolysis, as measured by soluble nitrogen levels at pH 4.4, is greatest in cheese made with fungal coagulant and least in cheese made with bovine pepsin and that fungal rennet accelerates proteolysis and body development in pizza cheese. Also, cheese made with fungal rennet is least curdy, whereas bovine pepsin cheese is most curdy. Emmons et al. (27) found that Cheddar cheese made with bovine pepsin is more elastic than cheese made with calf chymosin and that elasticity decreases as proteolysis increases. Mickelsen and Fish (66) found that proteolysis of whole casein, α-casein (α-CN), and β-casein (βCN) is greater with fungal coagulants than with calf chymosin or bovine pepsin. Chymosin and bovine pepsin degrade casein at about the same rate and level, but porcine pepsin degrades β-CN more quickly than either calf chymosin or bovine pepsin (34). Commercial fungal coagulants are less active on α-CN than is chymosin. Preferential proteolysis of one casein over another by different milk-clotting enzymes may modify physical properties of cheese. Fungal coagulants, like M. miehei protease, are less proteolytic than calf chymosin towards α-CN (35). Creamer and Olson (20) showed that chymosin cleavage of α-CN weakens the protein network as cheese ripens, causing a rapid decrease in firmness during the early stages of Cheddar cheese ripening. The differences in casein degradation during clotting with different enzymes may affect the aggregation of casein micelles. Holmes et al. (43) showed that less than 6% of milk-clotting enzyme—whether porcine pepsin, rennet Mucor pucillus protease, or M. miehei protease—remains in Cheddar cheese curd. Mathesson (65) found
that residual chymosin is not active in Mozzarella cheese. owing to the high cooking
temperature. Creamer (19) said that the curd-heating procedure of Mozzarella
manufacture denatures chymosin. Some chymosin activity survives in Mozzarella
cheese, but it is limited compared with rennet activity of other cheese types (36). Eino et
al. (25) showed, using electron microscopy, that structural characteristics in the protein
framework formed during milk clotting are specific to the milk-clotting enzyme used.
Curds made with bovine pepsin and porcine pepsin are similar in structure; curd made
with rennet has a more compact and organized structure. Micelles interact differently
during clotting, depending on which milk-clotting enzyme is used, thus altering the
physical properties of the cheese.

**STARTER CULTURES**

While some Mozzarella cheese is made in the U.S. using direct acidification
methods, most of the Mozzarella cheese produced is manufactured using starter cultures.
These starter cultures, along with rennet, and cheese milk make-up a dynamic system.
The cultures of greatest importance are Streptococcus salivarius ssp. thermophilus,
Lactobacillus delbrueckii ssp. bulgaricus, and Lactobacillus helveticus. Some cheese
makers are now including lactococcus species to obtain greater control over the early
cheese making process.

The more proteolytic *L. delbrueckii* ssp. *bulgaricus* stimulates *S. salivarius* ssp.
*thermophilus* by producing certain peptides and amino acids from milk caseins (42, 45,
61, 82, 87, 96). The symbiotic relationship between these two cultures has been utilized
for many years for production of Mozzarella cheese and yogurt. The growth of
*L. delbrueckii* ssp. *bulgaricus* is stimulated by formic acid and carbon dioxide produced
by *S. salivarius* ssp. *thermophilus* (8, 24, 87, 96).

Proteolytic enzyme systems encompass an important area of thermophilic starter
Investigators have shown a relationship between culture proteolysis and body and texture properties in cheese (23, 100). Because of the critical role proteinases and peptidases play in cheese flavor and body development, it is important to characterize properties found in the thermophilic starter cultures used to manufacture cheese. Different starter cultures produce a variety of peptides and amino acids in fermented products, due to the diversity of proteolytic systems found in thermophilic lactobacilli (71). Ayres et al. (6) found wide variation in the concentrations of tyrosine released by different strains. Oberg et al. (76) screened 33 commercial strains of *L. delbrueckii* ssp. *bulgaricus* and recorded a six-fold difference in total proteolysis with the o-phthalaldehyde test. When compared to other genera screened, these strains appear to exhibit a much wider range of proteolysis. The o-phthalaldehyde absorbance readings ranged from .20 to 1.29 among cultures incubated in 10% NDM for 12 h. The wide range of proteolytic activity noted among strains of *L. delbrueckii* ssp. *bulgaricus* was not observed with other thermophilic genera and species. Amino acid analysis was utilized to characterize proteolysis in milk during the growth of *L. delbrueckii* ssp. *bulgaricus* (76). Results indicated that each strain produces a distinct pattern of amino acid concentrations. Differences among amino acid profiles reflect variable proteinase, peptidase, and transport activities among the strains.

Proteolysis of *S. salivarius* ssp. *thermophilus* strains is minimal, particularly when compared with lactobacilli (41). Their overall proteolysis has been compared to that of proteinase-deficient strains of *Lactococcus lactis* (95).

Because of culture ratios and such proteolytic diversity, significant variability may be found in the final products. Because cultures vary so greatly in their proteolytic activity, Mozzarella manufacturers find it difficult to produce consistently uniform, high quality products. Because *L. delbrueckii* ssp. *bulgaricus* is often used in the manufacture of Mozzarella cheese, variation in proteolytic systems among strains of this organism has
implications on the physical properties of Mozzarella cheese.

**LACTIC CULTURE EFFECTS ON CHEESE PROPERTIES**

It remains difficult to correlate enzyme activity with organoleptic and textural changes in cheese products, but some investigators have been successful. Alvarez (2), in compiling a list of desirable Mozzarella cheese attributes, suggested that many of these features are influenced by starter bacteria.

One example involves culture activity as it relates to the rate of acid production and final pH of cheese products. Moisture content is related to the time for acid production to occur. Increased set and cook temperatures drive moisture out, making the temperature favorable for acid production by thermophiles. Studies have shown that optimum growth temperature seems to have no influence on growth of *L. delbrueckii* ssp. *bulgaricus* and *S. salivarius* ssp. *thermophilus* (64, 85). Rather, optimum temperatures for acid production range from 2 to 8°C above optimum growth temperature (64, 85, 87). Investigators have found that *S. salivarius* ssp. *thermophilus* strains reached greater numbers than *L. delbrueckii* ssp. *bulgaricus* at 37, 42, and 45°C, leading to rod:coccus ratios of 1:2.2, 1:8, and 1:2.4, respectively (85, 100).

Creamer (19) found that less casein degradation occurred in Mozzarella cheese than in Gouda or Cheddar and suggested that stretching properties of Mozzarella cheese may be related to the presence of intact casein and large peptides. He correlated higher concentrations of intact casein in Mozzarella with a decrease in bacterial proteolytic activity as a consequence of higher cook temperatures.

Oberg et al. (75) used single cultures of *L. delbrueckii* ssp. *bulgaricus* that varied in overall proteolytic ability to manufacture Mozzarella cheese. Single rod cultures were not recommended because the pH decrease is too slow. Less proteolytic strains of *L. helveticus* contribute to a firm, elastic body in Swiss cheese, whereas more proteolytic
strains produced a soft, short crumbly body (23).

Cheese browning during cooking is also affected by the type of starter culture used. Galactose-fermenting strains of *L. helveticus* and *S. salivarius* ssp. *thermophilus* reduced browning compared with cheese made with non-galactose fermenters. (79) Studies also showed that addition of galactose-fermenting *S. salivarius* ssp. *thermophilus* cultures to Cheddar cheese vats resulted in decreased browning. Law and Wigmore (55) noted that control of the relative rate of change in texture properties may be possible if a favorable peptidase-to-protease activity ratio in cheese can be established. Creamer and Olson (20) suggested endopeptidases that cleave α-CN, a key component of cheese, may be responsible for changes in body and texture properties of cheese. Di Palma et al. (23) found reduced proteolysis to be correlated with a more firm, elastic Swiss cheese body.

**Culture Adjuncts**

Recently *L. helveticus* cultures have become more popular for applications in the dairy industry, due to their different peptidolytic and proteolytic profiles. The ability of *L. helveticus* to utilize the galactose moiety of lactose has also increased the popularity of this culture. These metabolic differences may help to reduce the extent to which browning occurs in Mozzarella cheese. Mozzarella cheese made with galactose-positive cultures of *S. salivarius* ssp. *thermophilus* and *L. helveticus* showed the least browning after cooking (46). Hutkins et al. (45) suggested that a reduction in cook color occurs when galactose fermenting strains of *S. salivarius* ssp. *thermophilus* are used in conjunction with *L. helveticus*. A large number of Swiss and Mozzarella cheese manufacturers have replaced *L. delbrueckii* ssp. *bulgaricus* with *L. helveticus* in their coccus:rod mixture. Manufacturers suggest that excessive cheese softening can be prevented by using *L. helveticus* in Mozzarella cheese. Cholette and McKellar (17) have suggested that peptidases of *L. helveticus* are important in cheese ripening. Swiss cheese producers report that fewer body defects and a sweeter, nuttier flavor are obtained when
L. delbrueckii ssp. bulgaricus is replaced with L. helveticus. Hutkins et al. (45) examined whether galactose-fermenting strains of S. salivarius ssp. thermophilus could reduce browning and found that, unless the lactobacilli were also galactose-positive, cook color was not affected. These results indicate that L. helveticus strains with high galactose fermentation activity may be better when reduced cook color is desired.

Consumer demand for reduced fat, low fat, and nonfat dairy products has not diminished. Meltability and stretch are the most important characteristics associated with Mozzarella cheese. Decreasing or removing fat from cheese often results in textural and flavor changes (12). Low fat cheese tends to be hard and exhibits poor melt and stretch properties (12, 27, 28, 30, 32). Stringent consumer expectations have led to a surge of investigations into factors that will contribute to development of acceptable lower fat dairy products.

Changes in Mozzarella cheese texture are highly attributed to proteolysis by lactic starter bacteria and milk coagulants (73, 74, 75, 84). The proteolytic capability of bacterial starter cultures can alter the physical properties of Mozzarella cheese, especially stretch and melt (70, 75). Changes in proteolytic characteristics of thermolactic starter cultures, particularly L. delbrueckii ssp. bulgaricus, modify stretch, melt, and cook color of Mozzarella cheese (26, 41, 75). A variety of proteolytic, peptidolytic, and esterolytic enzymes have been found and partially characterized in the lactobacilli (60).

Recently lactobacilli have gained much attention due to their strong peptidolytic, esterolytic, and lipolytic activities and the importance these enzymes have in accelerated-ripened cheese and enzyme-modified cheese (60). Lactobacillus casei ssp. casei has been shown to produce strong proteolytic and peptidolytic activities (4, 5, 60). Lee and Lee (60) reported a higher number and greater activities of peptidases and esterases in L. casei ssp. casei species when compared with other starter strains. Hull et al. (44) reported that L. casei ssp. casei species were associated with a soft-body defect in Mozzarella cheese.
In Cheddar cheese *L. casei* ssp. *casei* increased the rate of softening but did not increase soluble nitrogen; it increased the development of flavor but later caused an acid flavor and "short" body (57, 58, 59). A soft or pasty-body was observed in Mozzarella cheese where \( >10^4 \) *L. casei* ssp. *casei* cells per gram were found (44).

The use of *L. casei* ssp. *casei* species in Cheddar cheese and its effect on body and texture are well documented. Thus, complete or partial substitution of *L. delbrueckii* ssp. *bulgaricus* with *L. casei* ssp. *casei* species may enhance the physical properties of low fat Mozzarella cheese.

**BRINING OF MOZZARELLA CHEESE**

Salting is an important phase of the cheesemaking process and provides three important functions: 1) controls the growth of starter and non-starter bacteria, 2) facilitates whey drainage and formation of the rind, and 3) imparts its characteristic taste and enhances the flavor of cheese (99).

The five most critical factors to watch in the brining process are: 1) salt concentration, 2) brine pH, 3) mineral concentration in the brine, 4) brine temperature, and 5) microbial contamination.

With increasing time in the brine, cheese absorbs increasingly more salt; however, the rate of salt absorption will decrease with time due to the reduced salt differential between the cheese moisture and the brine (99). Salt absorption is linearly related to the surface-to-volume ratio of the cheese. With this in mind it is important that not only the size of the curd be consistent but also that the concentration of the brine be consistent. Guinee and Fox (39) reported that the quantity of salt absorbed by the cheese is proportional to the square root of brining time. Lawrence and Gilles (56) suggest that curd is more soluble at higher pH values and will result in higher salt retention by the curd. The mineral concentration of the brine, especially calcium, is the primary factor
leading to most failures with new brines. The calcium content of the brine must be similar to that of the cheese placed in the brine. In a newly prepared brine, calcium will be leached from the surface of the cheese and casein solubilized, thereby yielding a cheese with a soft rind (37). Breene et al. (9) found that curd tempered to 90°C absorbed less salt than curd, at lower or higher temperatures. This was due to a layer of exuded fat on the surface of the curd which restricted salt absorption. At lower temperatures there was not as much free fat at the surface, and at higher temperatures the fat was liquid and dispersed in the brine. Geurts et al. (37) reported that increasing brine temperatures resulted in increased salt absorption in the cheese, but that the temperature of the cheese going into the brine should be as close to the brine temperature as possible. Salt absorption is increased as moisture of the curd increases. With increased moisture in the cheese, the pore sizes in the protein matrix are larger, thus reducing the frictional effect of inward-diffusing salt molecules (37).

The major microbial contamination in brine involves salt-tolerant yeasts. However, listeria and other pathogens may also be a problem in brine tanks in Italian cheese plants. These organisms may not grow in the brine, but they tolerate the brine conditions until the cheese is pulled from the brine and growth conditions on the surface of the cheese become more favorable for the growth of the organism (99). To control microbial contamination in the brine, the brine should be routinely filtered to remove particulate matter, and the sodium chloride level should be properly maintained. If yeasts or bacteria are a potential problem, the brine could be vat-pasteurized to eliminate potential pathogens (99).

Immersion in a cold brine is traditionally the method of choice for salting Mozzarella cheese. Nilson and LaClair (68) salted Mozzarella cheese with salt tablets added directly to cheese, using a pressure gun, but the process impaired cheese meltability and stretching. Olson (77) incorporated salt into Mozzarella cheese by high
pressure injection. Ferris (31) stated that standard cold brining is costly, and Olson (78) observed that it was difficult to attain uniform salt concentration in Mozzarella cheese because salt uptake depends on time, temperature, and concentration of the brine. Nilson and LaClair (69) in a national survey of Mozzarella cheese observed a wide range of from 1.0 to 4.5% in salt. Fernandez and Kosikowski (29), using hot brine stretching and molding, showed more uniform salt distribution but slightly lower salt concentration than brine salted controls. They concluded that more uniform salt distribution was due to more efficient salt penetration. Kinstedt et al. (51) found that surface samples had significantly higher NaCl and apparent viscosity and lower moisture, Ca, and free oil than center samples, and that these locational differences in melting characteristics may be caused in part by exchange of Na with casein-bound Ca at the cheese surface.

To successfully brine Mozzarella cheese the following points should be considered: 1) produce an even uniformly sized milled curd particle, 2) consider the surface to mass ratio when selecting milled particle sizes in order to improve salt diffusion into the curd matrix for maximum salt retention, 3) apply salt uniformly to the curd, 4) equilibration within the cheese does not occur, so the importance of uniform salting cannot be too strongly stressed (99).

**FROZEN STORAGE**

Increases in Mozzarella cheese production over the last 20 years and instability of key physical properties, such as stretch and melt, have led producers to freeze Mozzarella cheese just after manufacture (2, 69, 83). Mozzarella cheese is often shredded prior to freezing to decrease freezing time and to facilitate use when thawed. Few have studied the effects of freezing, frozen storage, and thawing on body and texture of Mozzarella cheese. Cervantes et al. (16) studied the effect of freezing, thawing, and salt concentration on the texture of low moisture, part-skim Mozzarella cheese that had been
frozen at -15°C and thawed at 5.6°C. Texture, as assessed by compression and beam bending, was not significantly affected by freezing or thawing. Cheese softened when frozen storage time and time after thawing were increased. Dahlstrom (21) studied the effects of commercial freezing conditions on 2.25-kg loaves of Mozzarella cheese. Freezing by forced air required 5 to 131 h to reduce the cheese temperature through the freezing zone (from -1.1 to -6.7°C). Immediately after thawing, Mozzarella cheese had poor cohesiveness and melt, along with fat leakage and free surface moisture. Normal Mozzarella characteristics returned when cheese had been refrigerated for 1 to 3 wk following thawing.

Shanon (90) found that Cheddar cheese stored at -29, -18, 7, and 21°C for either 30 or 90 d was different in body and texture; frozen samples had a mealy texture. Alichanidis et al. (1) froze drained Teleme curd at -40°C for 12 h and then stored it at -20°C. Curd was thawed at 5°C for 24 h, made into finished cheese, and stored for 4 mo. Proteolysis increased, even though frozen cheese contained fewer starter bacteria than unfrozen curd. Taste panels rated the texture of cheese that had been frozen as being inferior to that of unfrozen.

**PHYSICAL PROPERTIES OF MOZZARELLA CHEESE**

Physical properties of Mozzarella cheese, particularly free oil and water formation, stretch, melt, and cook color, determine Mozzarella cheese quality. Precise definitions for these attributes and standard objective methods for their assessment do not exist (48). In general terms, meltability refers to the capacity of cheese particles to form a continuous melt, stretchability is the ability of the melted cheese to form fibrous strands that elongate without breaking under tension, and elasticity, or "strength of the stretch," is the ability of the fibrous strands to resist permanent elongation when stretched (48). Few studies concern physical properties of Mozzarella cheese, possibly because objective
methods to measure these properties are lacking. The most simple and straightforward method of measuring the functionality of Mozzarella cheese is to subjectively evaluate the performance of melted cheese on a pizza under commercial conditions. This type of subjective measurement may be useful for industrial quality control programs, but it is not useful from the standpoint of research unless combined with objective measurements.

Melt

Nilson and LaClair (69) measured melt by placing a 6.2-cm\(^3\) discs of cheese 5-mm thick on filter paper, heating, and comparing the change in area. Fernandez and Kosikowski (30) used the same method to study meltability of Mozzarella cheese made with ultrafiltered whole milk retentates and also measured changes with an Instron Universal Testing Machine. A major limitation of these methods is in obtaining measurements that are representative of the sample (i.e., the meltability as measured from a single disc of cheese, will depend largely upon the position in the cheese from which the sample was taken). Oberg et al. (72, 75) made modifications to the Olson and Price (80) tube flow method to measure meltability of low moisture part-skim Mozzarella cheese. In this test 15 g of shredded cheese are packed to a 4 cm depth into the end of a 30 x 250 mm Pyrex glass tube. The sealed tube is tempered at 4°C for 30 min and then placed horizontally in a conventional oven at 110°C for 60 min. The tube is removed from the oven and the flow distance is measured. Park and Rosenau (81) found that the Arnott test more accurately measured melt in Mozzarella cheese than the Schreiber test. In the Arnott test (3) a cylinder of cheese approximately 17 x 17 mm is placed on a glass tray and heated for 15 min at 100°C.

Stretch

Helical viscometry is used by the food industry for various applications in measuring the rheological properties of very viscous foods. Kinstedt and Rippe (48, 49)
found that a helical viscometer most accurately measures the apparent viscosity of Mozzarella cheese. In this procedure, a rotating t-bar spindle is raised through a column of melted cheese at 60°C, and the resistance exerted on the spindle is measured. Cheeses that form tough fibrous strands that accumulate around the rotating spindle exert greater resistance than those that form gelatinous soft strands or no strands. Oberg et al. (75), after making modifications to the method of Kinstedt et al. (48, 49), used helical viscometry to measure stretchability of Mozzarella cheese.

**Free Oil**

The separation of fat from melted cheese resulting in the formation of fat pools is termed free oil formation. Due to consumer concerns over fat in the diet, this defect associated with Mozzarella cheese has become of concern to Mozzarella cheese manufacturers and the pizza industry.

Oiling off may be measured by melting disks of cheese of specified dimensions on filter paper under defined conditions of time and temperature. Free oil formation is then measured as the area of the oil ring that absorbs into the filter paper.

Kinstedt and Rippe (50) developed a method for determination of free oil formation in melted Mozzarella cheese using standard Babcock equipment. In this test 18 g of cheese are weighed into Paley-Babcock bottles. Bottles are immersed in boiling water for 4 min to melt cheese. Distilled water (20 ml at 57.5°C) is added and the bottles are centrifuged hot (57.5°C) for 10 min. A portion of 1:1 distilled water and methanol is added to a final volume in the upper region of the calibrated neck and centrifuged for 2 min. Bottles are then hand rocked for 10 s and centrifuged for 2 min; this is then repeated a second time. Finally, bottles are tempered in 57.5°C water for 5 min, and the fat column is then measured with glymol. Eighteen replicates of low moisture and low moisture part-skim Mozzarella gave coefficients of variation of 3.3 and 6.2%, respectively. Kinstedt et al. (51) found that lower free oil is associated with lower
moisture and higher NaCl concentrations found at the surface of Mozzarella cheese. Also, they found that higher free oil found in low moisture than low moisture, part skim cheeses was undoubtedly related to the higher fat content in the former. Low NaCl concentrations in the cheese center combined with the high fat content of low moisture cheeses apparently led to an accelerated destabilization of fat during aging, resulting in a higher percentage of total cheese fat becoming free oil. The inverse relationship between free oil formation and NaCl concentration that was observed suggests that NaCl enhances the emulsification process of fat within the cheese matrix. Lelievre et al. (62) found that as homogenization pressure increased, free oil formation decreased. Kinstedt and Kiely (47) found that increased cheese age and fat-in-dry basis enhance the formation of free oil while increased salt content aids in elimination of free oil. In Mozzarella cheese, sodium exchange with casein-bound calcium could lead to enhanced emulsification of the resulting curd leading to decreased free oil formation.

**Cook Color**

Cook color (browning) increases with time as lactose is further broken down to its glucose and galactose moieties. Glucose is readily metabolized by starter and non-starter bacteria; however, the galactose moiety is released into the cheese matrix where it is free to react with α- or ε-amino groups and undergo Maillard browning reactions when cooked. Johnson and Olson (46), using a Hunterlab colorimeter, found a positive correlation between galactose concentration and brown color intensity in Mozzarella cheese during cooking. Bley et al. (8) also used a Hunterlab colorimeter to develop a method to predict the tendency of cheese to turn brown when heated. Oberg et al. (75) developed a method using a reflectance colorimeter to measure the tendency of Mozzarella cheese to brown during cooking. In this test 15 g of grated cheese are placed in a test tube and immersed in a boiling water bath for 1 h. Color differences are measured using a Minolta Chroma Meter. The bottom of the test tube is clamped to the
measurement head, and eight readings are taken with the tube being rotated approximately 45° between each reading. The mean b* values indicating color change differences from yellow to blue were used to compare cook colors. As mentioned previously the use of *L. helveticus* alone or in conjunction with galactose-positive *S. salivarius* ssp. *thermophilus* will significantly reduce the tendency for Mozzarella cheese to brown during cooking.

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CHAPTER 2
EFFECTS OF MILK-CLOTTING ENZYMES ON PHYSICAL PROPERTIES OF MOZZARELLA CHEESE

ABSTRACT

Direct acid Mozzarella cheese was made in 6-L vats using calf chymosin, bovine pepsin, porcine pepsin, or *Mucor miehei* protease. Four cheeses were made with each enzyme. Stretch, melt, cook color (reflectance colorimeter), moisture, and pH were measured at 1, 7, 14, and 28 d. Correlation coefficients among these parameters were calculated, and the effects of choice of enzyme and storage time at 4°C on these parameters were evaluated by analysis of variance. Cook color was not affected by enzyme type and changed only slightly during storage. Melt was affected by choice of enzyme and increased significantly with time. During the 28-d ripening period, the melt of cheese made with calf chymosin increased the most. The smallest increase in melt was in cheese made with porcine pepsin. Stretch was significantly affected by enzyme and by storage time. Stretch decreased rapidly in all cheeses between d 1 and 7 and stabilized during the next 21 d. Cheese made with porcine pepsin had the greatest stretch and cheese made with calf chymosin had the least stretch, between d 7 and 28. Melt increased and stretch decreased during storage of all cheeses. The type of milk-clotting enzyme used played a significant role in determining physical properties of direct acid Mozzarella cheese.

INTRODUCTION

A steady increase in Mozzarella cheese production over the past 10 yr and stringent expectations by the industry (1) concerning physical properties of Mozzarella cheese

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1 Coauthored by C. J. Oberg, R. K. Merrill, R. J. Brown, and G. H. Richardson. Richard K. Merrill was the major contributor. Permission to reprint this chapter granted by the *Journal of Dairy Science.*
mean that all production parameters must be considered to improve quality. The proteolytic capability of bacterial starter cultures can alter the physical properties of Mozzarella cheese, especially stretch and melt (21). Proteolytic action of milk clotting enzymes may also produce desirable properties in Mozzarella cheese.

Milk clotting enzymes manifest a variety of proteolytic characteristics specific to each enzyme (3, 26). Quarne et al. (23) studied proteolysis and sensory characteristics in direct acid Mozzarella cheese made with several milk clotting enzymes. They found that proteolysis, as measured by soluble nitrogen levels at pH 4.4, is greatest in cheese made with fungal rennet and least in cheese made with bovine pepsin and that fungal rennets accelerate proteolysis and body development in pizza cheese. They did not determine how enzymes affect stretch, melt, or cook color. Emmons et al. (8) found that Cheddar cheese made with bovine pepsin is more elastic than cheese made with calf chymosin and that elasticity decreases as proteolysis increases.

Specificities of the milk clotting enzymes toward the caseins also suggest that these enzymes may influence the physical properties of Mozzarella cheese. Mickelsen and Fish (20) observed that proteolysis on whole casein, \( \alpha \)-casein (\( \alpha \)-CN), and \( \beta \)-casein (\( \beta \)-CN) is greater with fungal rennets than with calf chymosin or bovine pepsin. Chymosin and bovine pepsin degrade casein at about the same rate and level, but porcine pepsin degrades \( \beta \)-CN more quickly than either calf chymosin or bovine pepsin (10). Commercial fungal rennets are less active on \( \alpha \)-CN than chymosin. The differences in casein degradation during clotting with different enzymes may affect the aggregation of casein micelles. Agglomeration characteristics of micelles modified by chymosin are different than those of micelles treated with rennet substitutes, implicating the extent and specificity of \( \kappa \)-casein (\( \kappa \)-CN) hydrolysis (11) in prescribing the mechanism of curd formation. Electron micrographs by Eino et al. (7) show that the structures of curds made with bovine pepsin or porcine pepsin are similar; curd made with calf chymosin is more compact.
MATERIALS AND METHODS

Milk-Coagulating Enzymes

Rennet units (RU) (9) were used to compare milk-clotting enzyme activities on a Formagraph instrument (3, 18, 19) using low-heat NDM reconstituted in .01 M CaCl₂ and equilibrated at 5°C for 20 h as substrate. Calf rennet (New Zealand CO-OP-Renco, Eltham, New Zealand, 185 RU/ml), bovine pepsin (Chris Hansen's Lab., Inc., Milwaukee, WI; 113 RU/ml), Mucor miehei protease (Marschall-New Marzyme, Marshall Products, Madison, WI, 118 RU/ml), and porcine pepsin (Marshall Products, Madison, WI, 124 RU/ml) were purchased. The appropriate volumes of individual enzymes were added for a final concentration of 280 RU/6 L of milk.

Direct Acid Mozzarella Cheese Manufacture

Direct acid Mozzarella cheese was made using the method of Breene et al. (2). Six liters of raw commingled milk from the Utah State Dairy Products Laboratory was standardized to a casein:fat ratio of 1.2:1, followed by pasteurization at 63°C for 30 min. Milk was cooled to 5°C then acidified to pH 5.6 with lactic acid. Acidified milk was warmed to 37°C and set for 30 min with the appropriate volume of enzyme solution diluted in 30 ml of cold tap water. Curd was cut cooked at 37°C for 80 min, and then the curd container was placed in a water bath to raise the temperature to 49°C in 5 min. Whey was drained, and curd patties were milled, mixed, and molded in 82°C water for approximately 5 min until the curd ball was smooth and elastic. Molded curd balls were placed in ice water to firm the curd and then soaked in 25% salt brine for 8 h at 22°C. The brine solution was reused for all of the cheese trials; additional salt added before each trial. Cheese was stored at 4°C.
Curd Analysis

Cheese was analyzed for moisture using the CEM Microwave Model AVC 80 (CEM Corp., Matthews, NC). Curd pH was measured with a Beckman Model 60 pH Meter (Beckman Instruments Inc., Fullerton, CA) and an Orion 8163 Ross% combination pH electrode (Orion, Inc., Cambridge, MA). Moisture and pH were measured at 1, 7, 14, and 28 d with cheese equilibrated to 22°C.

Stretch Test

Stretch was determined at 1, 7, 14, and 28 d using a modified version of the helical viscometer method of Kindstedt et al. (15). Fifteen grams of shredded cheese were tamped into a 25 mm x 150 mm test tube and set in a 60°C water bath for 10 min. A Brookfield LVT (Brookfield Engineering Laboratories, Inc., Stoughton, MA) helipath viscometer was fitted with a t-bar spindle (TF with a 1.075 cm crossbar). The t-bar spindle was gradually submerged in the tempered cheese until it reached the bottom by turning on the helipath stand. The helipath stand was then turned off and the viscometer was adjusted to a speed of 1.5 rpm. When a full-scale reading was attained, the helipath stand was turned on to raise the rotating spindle. Ten readings, one per minute, were taken while the helipath raised the viscometer spindle out of the tube. Relative peak areas were recorded for each 10-min measurement period. Readings greater than 100 were adjusted to 100 when relative peak areas were calculated.

Browning Test

Test tubes of grated cheese used in the stretch test were put into a boiling water bath for 60 min. Color differences were measured with a Minolta Chroma Meter CR 221B (Minolta Corp., Ramsey, NJ) (24). The selector switch was set to Illuminant C (6774K) and L*a*b* was the mode used for measuring chromaticity. The bottom of the test tube was in contact with the measuring head of the reflectance colorimeter. Eight readings
were taken from each tube, and the tube was rotated 45° between readings. The $b^*$ values, indicating color change differences from yellow to blue, were used to evaluate differences in cook color. Cheese samples were analyzed at 1, 7, 14, and 28 d.

**Melt Test**

A modification of the method developed by Olson and Price (22) was used to measure melt. Fifteen grams of shredded cheese were put in one end of a Pyrex glass tube (30 mm ¥ 250 mm). The end containing the cheese was sealed with a number 7 solid rubber stopper and the other end was closed with a stopper with a hole to allow gas to escape. The tubes were tempered at 4°C for 30 min while positioned vertically with shredded cheese at the bottom, then held horizontally at 110°C for 60 min. After cooling to room temperature, cheese flow was measured.

**Statistical Analysis**

Analysis of variance was conducted separately for the dependent variables pH, moisture, cook color, melt, and stretch (14). Four independent replicates were run with milk prepared separately for each replicate. Therefore, variation between milk was included in the replicate variable. Each enzyme was used in each replicate, so variation between milks was not confounded with the variable enzyme. Measurements of dependent variables were made at 1, 7, 14, and 28 d. Expected mean squares for the analysis of variance model are shown in Table 1 (25). Correlations, means, and analyses of variance were calculated using JMP™ software from SAS® (14).
TABLE 1. Expected mean squares used to calculate F ratios in the analyses of variance for pH, moisture, color, melt, and stretch.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Expected mean squares</th>
<th>F Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicate</td>
<td>MSR = $s^2_{abr} + 4 s^2_{ag} + 16 s^2_r$</td>
<td>MSR/MSAR</td>
</tr>
<tr>
<td>Enzyme</td>
<td>MSA = $s^2_{abr} + 4 s^2_{ar} + 16 \frac{S a^2}{3}$</td>
<td>MSA/MSAR</td>
</tr>
<tr>
<td>Error A</td>
<td>MSAR = $s^2_{abr} + 4 s^2_{ar}$</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>MSB = $s^2_{abr} + 16 \frac{S b^2}{3}$</td>
<td>MSB/MSABR</td>
</tr>
<tr>
<td>Enzyme ¥ Time</td>
<td>MSAB = $s^2_{abr} + 4 \frac{S (ab)^2}{9}$</td>
<td>MSAB/MSABR</td>
</tr>
<tr>
<td>Error B</td>
<td>MSABR = $s^2_{abr}$</td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

Cheese Manufacture

Each of the milk-coagulating enzymes produced direct acid Mozzarella cheese with normal characteristics. Moisture and pH were measured each time that cheese samples were tested for stretch, melt, and color. There was not a significant correlation ($P > .05$) between stretch, melt, or color and either pH or moisture with any of the enzymes. Analyses of variance showed that neither pH nor moisture content of the cheese was affected by choice of enzyme or storage time (Table 2).

Cook Color

Cook color was not affected by choice of enzyme, but changed during storage ($P =$
Color decreased over time; the most rapid decrease was between d 7 and 14. Little cook color (browning) was seen in any of the direct acid Mozzarella cheese, because $b^*$ values less than 5 cannot be visually distinguished from the natural white color of unheated cheese. All cheeses appeared white throughout the 28-d storage period.

**Melt**

Melt was affected by choice of enzyme ($P = .0154$), by storage time ($P < .0001$) and (because of the difficulty of measurement) by replicate (Table 2). Melt of all cheeses increased over time; the sharpest increase was between d 7 and 14. Cheese made with porcine pepsin had the least melt over time (Figure 1). Melt of cheese made with bovine pepsin and *M. miehei* protease increased by similar amounts during the storage period. Cheese made with calf chymosin had the largest increase during the first 14 d of storage and had the highest melt at d 7 through 28.

**Stretch**

Stretch was significantly affected by the enzyme used ($P = .0385$), as shown by analysis of variance (Table 2). Because of the difficulty of running this test, replicate was also significant. Stretch also decreased significantly ($P < .0001$) during storage, particularly during the first 7 d (Figure 2). Stretch was greatest at d 7 in cheese made with porcine pepsin, followed by cheese made with bovine pepsin, *M. miehei* protease, and calf chymosin. Cheese made with calf chymosin had the least stretch from d 7 through 28, whereas cheese made with either porcine pepsin or bovine pepsin had the most stretch over this period. Stretch and melt were inversely correlated (Figure 3). This relationship has been observed previously (21). The milk-clotting enzymes that produced the most stretch over time also produced the least amount of melt, whereas those that produced the least stretch showed the greatest melt.
TABLE 2. Analyses of variance for pH, moisture, color, melt, and stretch.

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Figure 1. Melt measurements of Mozzarella cheese made by direct acidification using different coagulants. Bars represent standard errors (n = 4).
Figure 2. Stretch measurements (relative units) of Mozzarella cheese made by direct acidification using different coagulants. Bars represent standard errors (n = 4).
Figure 3. Correlation between stretch and melt in Mozzarella cheese made by direct acidification. Bars represent standard errors (n = 16). The line is an exponential fit of the points.
DISCUSSION

Variation in the proteolytic characteristics of milk-clotting enzymes is well documented (3). Different milk-clotting enzymes vary in their proteolytic effects on the physical properties of Mozzarella cheese.

Though direct acid Mozzarella cheese appears white, the test used here was sensitive enough to detect cook color changes after storage. Cultured Mozzarella cheese develops much darker cooked color during storage (21). A high concentration of sugar is available for browning in direct acid Mozzarella, but amino acid levels (also required for browning) are lower than in cultured cheese.

Holmes et al. (13) showed that less than 6% of milk-clotting enzyme, whether porcine pepsin, rennet, *Mucor pusillus* protease, or *M. miehei* protease, remains in Cheddar cheese curd after pressing. Fox (11) reported that approximately 6% of rennet and less porcine pepsin remains in Cheddar cheese curd. Mathesson (17) found that residual rennet is not active in Mozzarella cheese owing to the high cooking temperature. Creamer (5) said that the curd-heating procedure of Mozzarella manufacture denatures rennet. Some rennet activity survives in Mozzarella cheese, but it is limited compared with rennet activity of other cheese types (12).

Stretch and melt of direct acid Mozzarella cheeses vary with choice of enzyme. Because Mozzarella cheese contains little enzyme beyond cooking and stretching, differences in enzymic proteolysis must occur prior to stretching. This cannot be seen in cheese made with bacterial cultures because starter proteolysis masks the effects of milk-clotting enzymes. Quarne et al. (23) saw differences in soluble nitrogen at pH 4.4 and in formol nitrogen among cheeses made with fungal rennet, pepsin, and veal rennet.

Variations in stretch and melt among direct acid Mozzarella cheeses made with different enzymes may be affected by enzyme specificities toward individual caseins. Fox (10) found that proteolysis products of sodium caseinate treated with rennet or
bovine pepsin produce similar products, which differ from those prepared with porcine pepsin. Fungal milk clotting enzymes cause more proteolysis than calf chymosin or bovine pepsin in whole casein, α-CN, and β-CN (20). Proteolysis is greatest in pizza cheese made with fungal rennet and least in cheese made with bovine pepsin (23). Also, cheese made with fungal rennet is least curdy, whereas bovine pepsin cheese is most curdy. Extent and specificity of proteolysis of different milk-clotting enzymes on individual caseins appears to affect the way that cheese stretches and melts.

Casein degradation between clotting enzyme addition and stretching might affect micelle aggregation, causing differences in melt and stretch. Differences in aggregation characteristics of micelles treated with rennet substitutes and those treated with calf chymosin (16) may reflect differences in extent of proteolysis of κ-CN and probably other caseins. Eino et al. (7) showed using electron microscopy, that structural characteristics in the protein framework formed during milk-clotting are specific to the milk-clotting enzyme used. Curds made with bovine pepsin and porcine pepsin are similar in structure; curd made with rennet has a more compact and organized structure. Micelles interact differently during clotting, depending on which milk-clotting enzyme is used, thus altering the physical properties of the cheese.

Preferential proteolysis of one casein over another by different milk-clotting enzymes may modify physical properties of cheese. Fungal rennets, like M. miehei protease, are less proteolytic than calf chymosin toward α-CN (11). Creamer and Olson (6) showed that chymosin cleavage of α-CN weakens the protein network as cheese ripens, causing a rapid decrease in firmness during the early stages of Cheddar cheese ripening. Mozzarella cheese made with calf chymosin in this study had the largest reduction in stretch and the largest increase in melt, which was consistent with greater proteolysis of α-CN with calf chymosin than with the other milk clotting enzymes. Cheese made with porcine pepsin had the most stretch and least increase in melt, indicating there was less proteolysis with
Porcine pepsin. Porcine pepsin degrades β-CN more quickly than does calf chymosin or bovine pepsin when measured using sodium caseinate in .1 M sodium phosphate buffer (10). Porcine pepsin preferentially degrades β-CN over α-CN, causing less weakening of the protein network. As the protein network of cheese breaks, the cheese melts better and stretches less.

CONCLUSIONS

Milk-clotting enzymes affect the physical properties of direct acid Mozzarella cheese. These effects may be obscured by starter culture proteolysis, so they have not been well observed before. Proteolysis, even that which occurs prior to stretching of Mozzarella cheese, affects stretch and melt. This may be caused by modifications of micelle interactions specific to the various milk-clotting enzymes.

REFERENCES


CHAPTER 3
EFFECTS OF LACTOBACILLUS HELVETICUS CULTURE ON PHYSICAL PROPERTIES OF MOZZARELLA CHEESE¹

ABSTRACT

Six-liter vats of Mozzarella cheese were made using either single strains of *Lactobacillus helveticus* or paired strains of *L. helveticus* and *Streptococcus salivarius* ssp. *thermophilus*. Lactobacillus strains were either strongly or weakly proteolytic as established by the o-phthaldialdehyde test. Three cheeses were made with each culture type and stored at 4°C. Stretch, melt, color, moisture, and pH values were determined at 1, 7, 14, and 28 d. All cheeses lost stretch rapidly from d 1 to 7 and slowly declined between d 7 and 28. Melt increased rapidly for all cheeses from d 1 to 7 and then remained constant. Differences in stretch and melt from one culture type to another were not significant. Cheese made with proteinase-deficient strains had more stretch after holding for 14 and 28 d than cheese made with nondeficient strains. Time of storage significantly affected both stretch and melt over 28 d. Cheeses made from all four culture types decreased in cook color over 28 d storage. There were no significant strain differences in cook color, but the culture by time interaction was significant. Cheese made with pairs or single strains of *L. helveticus* had the same melt, more stretch, and less cook color than chees made with paired strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *S. salivarius* ssp. *thermophilus* studied previously.

INTRODUCTION

Mozzarella cheese production in the US has risen 10% annually for 10 yr (28). A recent nationwide survey indicated that only 92% of Mozzarella cheese in the US is

¹Coauthored by C. J. Oberg, R. K. Merrill, L. V. Moyes, R. J. Brown, and G. H. Richardson. Richard K. Merrill was the major contributor. Permission to reprint this chapter granted by the Journal of Dairy Science.
acceptable (19). In pizza restaurants in Vermont in 1991, more than 50% of the Mozzarella cheese had poor shredding properties, blistering, soupy melt, or other defects (25). These observations and stringent expectations by the industry (1) have led to an examination of factors that contribute to development of physical properties crucial to Mozzarella cheese quality and functionality.

Changes in the physical properties of cheese, particularly in body and texture, have been monitored for many years using a variety of techniques and instruments. Recent use of the helical viscometer and the application of reflectance colorimetry now make it possible to examine the role starter cultures play in development of these properties (17, 21).

Changes in proteolytic characteristics of thermolactic starter cultures, particularly *Lactobacillus delbrueckii* ssp. *bulgaricus*, modify stretch, melt, and cook color of Mozzarella cheese (11, 13, 21, 22). A variety of proteolytic and peptidolytic enzymes has been found and partially characterized in the lactobacilli (16, 20, 27). Activity differences of these enzymes among bacterial strains could contribute to differences in physical properties of Mozzarella cheese. *Lactobacillus helveticus* has higher peptidase activity relative to protease activity than *L. delbrueckii* ssp. *bulgaricus* (12). Law and Wigmore (18) found that the peptidase to protease activity ratio in cheese affects the rates of change in textural properties. Creamer and Olson (8) attributed changes in cheese body and texture to the endopeptidases that cleave a-casein, a key structural component of cheese. Creamer (7) found higher concentrations of a-casein in Mozzarella cheese than in Gouda or Cheddar. Peptidase stability appears to be an important factor for cheese ripening (5). Di Palma et al. (10) showed this in Swiss cheese in which a firmer, more elastic body resulted from less proteolysis. Thus, replacing *L. delbrueckii* ssp. *bulgaricus* with *L. helveticus* in Mozzarella cheese cultures could change cheese properties.

Because *L. helveticus* is able to use galactose, it can reduce levels of residual
galactose in Mozzarella cheese, thus reducing cook color and possibly blistering. Johnson and Olson (15) found that Mozzarella cheese made with galactose-positive (Gal+) cultures of *Streptococcus salivarius* ssp. *thermophilus* and *L. helveticus* has less browning during cooking. They also found that browning increased with levels of residual galactose.

The objective of this study was to investigate the effects of single proteinase-positive (Prt+) and proteinase-deficient (Prt-) strains of *L. helveticus* and pairs consisting of one strain each of *S. salivarius* ssp. *thermophilus* and Prt+ and Prt- strains of *L. helveticus* and the effects of storage time on physical properties of Mozzarella cheese.

**MATERIALS AND METHODS**

**Cultures**

Cultures of *L. helveticus* and *S. salivarius* ssp. *thermophilus* were obtained from the Department of Microbiology culture bank at Weber State University. Cultures were propagated in either 10% NDM, MRS, or basal media (29). Activity tests based on the method of Okigbo et al. (23), in which the incubation temperature increased from 30°C to 40°C prior to cheese making, were run to select strains with adequate make times. Strains of *L. helveticus* were selected according to their proteolytic activity as measured by the o-phthaldialdehyde test (6). Previous research indicates that culture proteolysis affects physical properties of Mozzarella cheese (21).

**Bulk Starter Preparation**

Bulk starter cultures were prepared in 10% Miles Marschall CR (Rhone-Polenc, Madison, WI) starter with internal pH control. One percent of yeast extract was added to the starter media. Starter media was reconstituted, heated to 90°C, and held at that temperature for 40 min. Media was rapidly cooled to 40°C and inoculated with 1% of culture grown in 10% NDM. The bulk starter medium was then incubated for 7 h at 40°C (9).
Mozzarella Manufacturing Procedure

Mozzarella cheese was made with single strains of *L. helveticus* that were either strongly or weakly proteolytic as determined by the *o*-phthalaldehyde test (6). Mozzarella cheese was also made from paired cultures that contained a 1:1 ratio of single, common, strain of *S. salivarius* ssp. *thermophilus* coupled with a strain of *L. helveticus* that was either strongly or weakly proteolytic. Six-liter vats of Mozzarella cheese were made from raw milk from Utah State Dairy Products Laboratory that had been standardized to a casein:fat ratio of 1.2:1 and pasteurized at 63°C for 30 min. The milk was cooled to 32°C and put into stainless steel cheese vats, and 2% starter was added. A water bath was used to control milk temperature in the vats. Inoculated cheese milk was ripened for 1 h at 32°C. Single-strength calf rennet (New Zealand CO-OP-Renco, Eltham, NZ), 1.5 ml diluted 1:20 in cold water, was added, and the milk was set for 30 min. The curd was then heated to 41°C over 30 min with periodic stirring. Whey was drained when whey pH reached 5.9 (measured at 41°C). Curd patties were cheddared until pH decreased to 5.2 and were milled, mixed, and molded in fresh 82°C water until curd balls were smooth and elastic (approximately 5 min for each sample). Molded curd balls were transferred to ice water to firm the curd and then placed in a saturated NaCl brine for 8 h at 22°C. The brine solution was reused for all the cheese trials; additional salt was added before each trial. Each cheese sample was held at 4°C until tested.

Curd Analysis

Cheese was analyzed for moisture using the CEM Microwave Model AVC 80 (CEM Corp., Mattews, NC). Curd pH readings were taken with a Beckman Model 60 pH Meter (Beckman Instruments Inc., Fullerton, CA). Moisture and pH measurements were taken at 1, 7, 14, and 28 d.
Stretch Test

Cheese samples were analyzed at 1, 7, 14, and 28 d for stretch. The helical viscometer method of Kindstedt et al. (17) was used with modifications. Fifteen grams of shredded cheese were tamped into a 25 × 150-mm test tube and placed in a 60°C water bath for 10 min. A Brookfield LVT (Brookfield Engineering Laboratories, Inc., Stoughton, MA) helipath viscometer T-bar spindle (TF with a 1.075 cm crossbar) was gradually submerged into the tempered cheese sample until it reached the bottom by turning on the helipath stand. The helipath stand was turned off, and the viscometer was then set on a speed of 1.5 rpm. When a full-scale reading was reached, the helipath stand was turned on to raise the rotating spindle. Ten readings were taken in the 10 min that it took for the helipath to raise the viscometer out of the tube. Stretch is defined as the relative peak area recorded for a 10-min measurement period. Readings greater than 100 were adjusted to 100 when relative peak areas were calculated.

Melt Test

A modification of the method developed by Olson and Price (24) was used to measure meltability. Fifteen grams of shredded cheese were put in one end of a Pyrex glass tube (30 mm × 250 mm). The end containing the cheese was closed with a number 7 solid rubber stopper, and the opposite end was plugged with a stopper of the same size with a hole for gas escape. The tubes were tempered at 4°C for 30 min while positioned vertically with shredded cheese at the bottom and then were held horizontally at 110°C for 60 min. Melt is defined as the distances that cheese flows as measured after cooling the samples to room temperature.

Cook Color Test

Test tubes of grated cheese used in the stretch test were placed in a boiling water bath for 60 min. Color differences were measured with a Minolta Chroma Meter CR-
221B (Minolta Corp., Ramsey, NJ) (26) set at Illuminant C (6774K) with \( L^* a^* b^* \) as the chromaticity measuring mode. The bottom of the test tube was clamped in contact with the measuring head. Eight readings were taken from each tube, and the tube was rotated 45° between readings. The \( b^* \) values, indicating color change differences from yellow to blue, were used to evaluate differences in cook color. Cheese samples were analyzed for cook color at 1, 7, 14, and 28 d.

**Statistical Analysis**

Analyses of variance were run separately for the dependent variables pH, moisture, cook color, melt, and stretch. There were three independent replicates of each culture type. Each dependent variable was measured at 1, 7, 14, and 28 d. Correlations, means, and analyses of variance were calculated using JMP™ software from SAS® (14).

**RESULTS**

**Cheese Manufacture**

All starters examined in this study produced Mozzarella cheese with normal physical characteristics. Moisture and pH were measured each time cheese samples were tested for stretch, melt, and color. There were no significant correlations between stretch, melt, or color and either pH or moisture with any of the cultures. Analyses of variance showed that moisture content of the cheese was affected by storage time, but pH had no effect (Table 3). Pairs of cultures took approximately 2 h 50 min from set to milling at pH 5.2; single strains took 3 h 15 min.

**Stretch**

Cheese made with *L. helveticus* cultures, either single strains or paired with the *S. salivarius* ssp. *thermophilus* strain, showed a significant decrease \((P < .0001)\) in stretch over time (Table 3). Cheese made with the pair that included the Prt+ strain of
*L. helveticus* retained the greatest stretch from d 14 through 28 (Figure 4). Cheese made with single strains of *L. helveticus* had less stretch after d 14. Analysis of variance showed no significant differences in stretch among the cultures. Stretch was inversely related to melt ($r = -.997$) (Figure 5).

**Melt**

Melt of all cheeses increased rapidly through the first 7 d of storage (Figure 6). After d 7, there was little change in melt, except in cheese made with the Prt⁻ single strain of *L. helveticus*, in which melt decreased from d 14 through 28. Analysis of variance showed no difference in melt among culture types, but melt was significantly affected by storage time ($P = .001$) (Table 3).

**TABLE 3. Analyses of variance for pH, moisture, color, melt, and stretch.**

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**Cook Color**

The cook color of three of the four cheeses decreased over time (Figure 7), but cheese made with the pair that included the Prt⁻ strain of *L. helveticus* increased in cook
color over time as is typically seen in Mozzarella cheese (21). Cheese made with the Prt- single strain of *L. helveticus* had the least cook color from d 1 through 28. Time significantly affected cook color (*P* = .035), as did the time versus culture interaction (*P* = .003) (Table 3). Cook color increased with cheese pH (*r* = .461). As storage progressed beyond 14 d, the effects of Prt+ and Prt- strains of *L. helveticus* became significant.
Figure 4. Stretch measurements (± SEM) of Mozzarella cheese made with either single strains of proteinase-positive (Prt+) or proteinase-deficient (Prt-) Lactobacillus helveticus or mixed single strain pairs of Prt+ or Prt- L. helveticus and Streptococcus salivarius ssp. thermophilus (n = 3). Solid bar indicates Prt+ L. helveticus; dark striped bar, Prt- L. helveticus; open bar, Prt+ L. helveticus and S. salivarius ssp. thermophilus; light striped bar, Prt- L. helveticus and S. salivarius ssp. thermophilus.
Figure 5. Correlation between overall stretch (±SEM) and overall melt (±SEM) (n = 12).
Figure 6. Melt measurements (± SEM) of Mozzarella cheese made with either single strains of proteinase-positive (Prt+) or proteinase-deficient (Prt-) Lactobacillus helveticus or mixed single strain pairs of Prt+ or Prt- L. helveticus and Streptococcus salivarius ssp. thermophilus (n = 3). Solid bar indicates Prt+ L. helveticus; dark striped bar, Prt- L. helveticus; open bar, Prt+ L. helveticus and S. salivarius ssp. thermophilus; light striped bar, Prt- L. helveticus and S. salivarius ssp. thermophilus.
Figure 7. Color measurements (± SEM) of Mozzarella cheese made with either single strains of proteinase-positive (Prt⁺) or proteinase-deficient (Prt⁻) Lactobacillus helveticus or mixed single strain pairs of Prt⁺ or Prt⁻ L. helveticus and Streptococcus salivarius ssp. thermophilus (n = 3). Solid bar indicates Prt⁺ L. helveticus; dark striped bar, Prt⁻ L. helveticus; open bar, Prt⁺ L. helveticus and S. salivarius ssp. thermophilus; light striped bar, Prt⁻ L. helveticus and S. salivarius ssp. thermophilus.
DISCUSSION AND CONCLUSIONS

Oberg et al. (21) showed that paired cultures of \textit{L. delbrueckii} ssp. \textit{bulgaricus} and \textit{S. salivarius} ssp. \textit{thermophilus} have an average make time of 3 h 30 min; make times with single strains of \textit{L. delbrueckii} ssp. \textit{bulgaricus} are approximately 5 h 30 min. In this study, pairs of \textit{L. helveticus} and \textit{S. salivarius} ssp. \textit{thermophilus} took only 2 h 50 min from set to milling at pH 5.2 and single strains of \textit{L. helveticus} took 3 h 15 min. Unlike \textit{L. delbrueckii} ssp. \textit{bulgaricus}, \textit{L. helveticus} is able to ferment the galactose moiety of lactose. Consequently, \textit{L. helveticus} produces more acid from the same amount of lactose. Replacing \textit{L. delbrueckii} ssp. \textit{bulgaricus} Mozzarella starter strains with \textit{L. helveticus} strains would be expected to decrease make times.

During the first 7 d of storage, all cheese in this study increased in melt and decreased in stretch (Figures 4 and 6). These two measurements were strongly correlated (Figure 5), each being affected differently by proteolysis during the 1st wk of storage. There were no significant differences in melt or stretch between single strains and pairs or between Prt+ and Prt- strains. Oberg et al. (21) found significant differences in melt and stretch between paired cultures of \textit{L. delbrueckii} ssp. \textit{bulgaricus} and \textit{S. salivarius} ssp. \textit{thermophilus} and single strains of \textit{L. delbrueckii} ssp. \textit{bulgaricus}. Activity was improved, and physical properties were maintained in Mozzarella cheese when \textit{L. helveticus} was paired with \textit{S. salivarius} ssp. \textit{thermophilus} instead of \textit{L. delbrueckii} ssp. \textit{bulgaricus}. Lactobacillus also appeared superior to \textit{L. delbrueckii} ssp. \textit{bulgaricus} when single strains were used to make Mozzarella cheese.

Strains of \textit{L. helveticus} exhibit greater peptidase activity than \textit{L. delbrueckii} ssp. \textit{bulgaricus} (11, 12, 13). Ardo and Pettersson (3) studied accelerated cheese ripening and found that heat-treated cells of \textit{L. helveticus} do not accelerate breakdown of casein, but do accelerate breakdown of peptides, thus increasing levels of amino acid nitrogen. Ardo et al. (2) studied the production of a low fat, semi-hard cheese and found that addition of
heat-treated *L. helveticus* enhances peptidolytic activity even during the first few weeks, which they attributed to an aminopeptidase from the cells. Bartels et al. (4) found strains of *L. helveticus* to be more proteolytic and peptidolytic than *L. delbrueckii* ssp. *bulgaricus*. They also noted that *L. helveticus* degrades medium-sized peptides. This higher level of proteolysis, coupled with the reducing sugar available in Mozzarella cheese, suggests an increase in cook color for cheese made with *L. helveticus*, especially with Prt+ strains of *L. helveticus*. The decrease in color with single strains must be due to fermentation of residual lactose to produce galactose. Color increases with time with the paired strains (proteolysis) when galactose is available, so increase in color with paired strains indicates lack of *L. helveticus* to metabolize galactose. The decrease in cook color over time in this study fits the facts: *L. helveticus* cultures are Gal+, but *L. delbrueckii* ssp. *bulgaricus* cultures do not ferment galactose. The Prt+ versus Prt- difference in cook color became apparent in cheese made with the single strains of *L. helveticus* after 28 d storage and in the paired cultures after 14 d of storage (Figure 7). There was less browning in the cheeses made in this study than in cheese made with *L. delbrueckii* ssp. *bulgaricus* cultures.

*Lactobacillus delbrueckii* ssp. *bulgaricus* can be replaced with *L. helveticus* in Mozzarella cheese cultures. In this study, stretch increased, melt was maintained, cook color decreased, and make times decreased. In addition, *L. helveticus* appeared superior to *L. delbrueckii* ssp. *bulgaricus* when single strains were used to make Mozzarella cheese. A strain of *L. helveticus* that is Prt+ and Gal+ would be best for reducing cook color. Total elimination of browning requires optimizing cultures and processing parameters.
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CHAPTER 4
EFFECTS OF FREEZING, THAWING, AND SHREDDING ON LOW MOISTURE, PART-SKIM MOZZARELLA CHEESE

ABSTRACT

Commercially manufactured low moisture, part-skim Mozzarella cheese (2.25 kg loaves) was either shredded or cut into 5-×10-×7-cm blocks. Shredded cheese and blocks were either frozen at -196°C and stored at -70°C or frozen and stored at -20°C. Cheese was then thawed at either 4.4, 12.8, or 25°C. Samples were analyzed for stretch by helical viscometry, for melt by the modified tube test, and for cook color by reflectance colorimetry just prior to freezing and at 7, 14, 21, and 42 d of storage. Mozzarella cheese stored at 4.4°C was used as the control. Shredding, freeze temperature, thaw temperature, and time of storage had no effect on cook color. Frozen cheese showed greater stretch than unfrozen cheese. Shredded cheese stretched more after frozen storage than unshredded cheese. Frozen shredded cheese melted less than frozen unshredded cheese. Cheese frozen at -20°C melted more after frozen storage than cheese frozen at -196°C. Cheese stored at 4.4°C melted more than frozen cheese. Thawing temperature by itself had no effect on either stretch or melt. Stretch was greatest in Mozzarella cheese frozen at -196°C, shredded, and held for at least 21 d. Melt was greatest in cheese in blocks, frozen at -20°C, and stored for only a short time.

INTRODUCTION

Rapid increase in Mozzarella production in the US and instability of key physical properties, such as stretch and melt, have led producers to freeze Mozzarella just after manufacture (2, 13, 19). Mozzarella cheese is often shredded prior to freezing to

1 Coauthored by C. J. Oberg, R. K. Merrill, R. J. Brown, and G. H. Richardson. Richard K. Merrill was the major contributor. Permission to reprint this chapter granted by the Journal of Dairy Science.
decrease freezing time and to facilitate use when thawed. Mozzarella curd is sometimes frozen for storage and then heat processed into finished product (10). Oberg et al. (14, 15, 16) showed that changes in bacterial starter cultures and milk-coagulating enzymes can modify physical properties in Mozzarella cheese, particularly stretch, melt, and cook color. Stretch decreased and melt increased during storage at 4.4°C (16). Conditions of freezing, frozen storage, and thawing may modify physical properties of Mozzarella cheese.

Few have studied the effects of freezing, frozen storage, and thawing on body and texture of Mozzarella cheese. Cervantes et al. (3) studied the effect of freezing, thawing, and salt concentration on the texture of low moisture, part-skim Mozzarella cheese that had been frozen at -15°C and thawed at 5.6°C. Frozen storage never exceeded 1 wk. Texture, as assessed by compression and beam bending, was not significantly affected by freezing or thawing. Cheese softened when frozen storage time and storage time after thawing were increased. Dahlstrom (4) studied effects of commercial freezing conditions on 2.25-kg loaves of Mozzarella cheese. Freezing by forced air required 5 to 131 h to reduce the cheese temperature through the freezing zone (from -1.1°C to -6.7°C). Immediately after thawing, Mozzarella cheese had poor cohesiveness and melt, along with fat leakage and free surface moisture. Normal Mozzarella characteristics returned when cheese had been refrigerated for 1 to 3 wk following thawing. A 14- to 21-d tempering period after thawing was suggested.

Others have addressed how freezing affects the physical properties of cheeses other than Mozzarella. Shannon (24) found Cheddar cheese stored at -29, -18, 7, and 21°C for either 30 or 90 d was different in body and texture; frozen samples had a mealy texture. Alichanidis et al. (1) froze drained Teleme curd at -40°C for 12 h and then stored it at -20°C. Curd was thawed at 5°C for 24 h, made into finished cheese, and stored for 4 mo. Proteolysis increased, even though frozen cheese contained fewer starter bacteria than
unfrozen curd. Taste panels rated the texture of cheese that had been frozen as being inferior to that of unfrozen cheese. Freezing, storing for 7 wk at -18°C, then thawing did not adversely affect the texture of Quarg cheese (5). Risoi et al. (23) found that cottage cheese frozen and stored at -17.8 or -23.3°C for 9 mo was less acceptable than fresh cottage cheese. The texture and flavor of cottage cheese decreased progressively as frozen storage time increased (12).

It is difficult to assess extent of physical changes caused by freezing cheese. Fennema (6) reviewed the research on cheese freezing and reported conflicting results. Tressler et al. (25) suggested that, to prevent deterioration of body and texture, cheese should not be frozen. Luck (11) suggested that Gouda and Cheddar cheese are not suitable for freezing, but that young Camembert, Brick, and cream cheese can be frozen. Fennema (6) said that high moisture cheeses, such as Mozzarella, which have short storage lives, are potential candidates for freezing.

This study assessed the effects of freezing temperature, storage time and temperature, shredding, and thaw time and temperature on stretch, melt and cook color of low moisture, part-skim Mozzarella cheese.

**MATERIALS AND METHODS**

**Experimental Samples**

Commercially manufactured (Star Valley Cheese, Western Dairymen Cooperative Inc., Star Valley, WY) 2.25-kg loaves of low moisture, part-skim Mozzarella cheese were obtained from the same vat. One sample from each 2.25-kg loaf of low moisture, part-skim Mozzarella cheese was analyzed for moisture using the CEM Microwave Model AVC 80 (CEM Corp., Mattews, NC). Cheese pH was measured with a Beckman Model 60 pH Meter (Beckman Instruments Inc., Fullerton, CA). Fat content of the cheese was measured using the Babcock test (21). The cheese contained 47.8% moisture and 33.3 %
fat on a dry basis.

Freezing of Cheese

Freshly manufactured 2.25-kg loaves of low moisture, part-skim Mozzarella cheese from a single batch were obtained from a local commercial manufacturer. Loaves were either shredded (shred size 0.5- x 0.5- x 7-cm) using a food processor (Hamilton Beach Model 702AL, Washington, NC) or cut into 5 x 10 x 7 cm blocks. Shredded and cut cheese blocks were vacuum sealed in plastic cheese packaging bags. Each sample weighed approximately 250 g. Half of the shredded and unshredded samples was frozen at -196°C in a liquid nitrogen tank for 15 min and then stored at -70°C. The remaining samples were frozen and stored at -20°C in a conventional freezer. Nonreplicated controls of shredded and unshredded cheese were stored at 4.4°C.

Thawing of Cheese

Shredded and unshredded cheese samples were removed from frozen storage and thawed at either 4.4°C for 3 h, 12.8°C for 2 h, or 25°C for 45 min. After thawing, samples were held at 4.4°C and tested within 6 h. Three independent replicates were thawed at each temperature for each set of freezing and shredding conditions.

Physical Property Tests

Shredded and unshredded samples were analyzed for stretch by helical viscometry (9), melt by modified tube test (18), and cook color by reflectance colorimetry (22). Methods were modified as previously described (14). Relative peak areas for stretch were recorded for each 10-min measurement period. Readings greater than 100 were adjusted to 100 when relative peak areas were calculated. Samples were tested just prior to freezing and at 7, 14, 21, and 42 d of storage. Cost of Mozzarella cheese inventories prohibit storing cheese longer than 42 d.
Statistical Analysis

Analyses of variance were run separately for the dependent variables stretch, melt, and cook color. The three dependent variables [freezing temperature (-196 or -70°C), form (block or shredded), and thawing temperature (4.4, 12.8, or 25°C)] were treated as a completely randomized block split over time (7, 14, 21, and 42 d of storage). All interactions were included in the model, and all experiments were replicated three times. Statistical calculations were done using JMP™ software (7). Actual probability levels for all analysis of variance comparisons are shown in Table 4.
TABLE 4. Analyses of variance for stretch, melt, and color.

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RESULTS AND DISCUSSION

Stretch

Stretch was significantly affected by shredding and by storage time, but not by freezing temperature or by thawing temperature (Table 4). Frozen cheese showed more stretch over time than did refrigerated control cheese (Figure 8). Frozen shredded cheese showed more stretch than frozen unshredded cheese. The stretch of both forms of frozen cheese rapidly increased from d 7 to 14; after 14 d, the stretch of unshredded cheese slowly decreased, whereas the stretch of shredded cheese continued to increase to d 42. The longer the storage time, the greater the difference in stretch between shredded and unshredded frozen cheese. Freezing temperature, -196 versus -20°C, did not affect stretch. The stretch of refrigerated control cheese rapidly decreased from d 7 to 14 and then decreased slowly for the remainder of the testing period (Figure 8). Oberg et al. (16) also saw this rapid decrease in stretch of refrigerated cheese followed by a gradual decline.

Melt

Melt was significantly affected by shredding, freezing temperature, and storage time (Table 4). Frozen cheese melted less than refrigerated control cheese over the entire storage period (Figure 9). Shredded frozen cheese melted significantly less than unshredded frozen cheese. The interaction between shredding and freezing temperature was also significant (Table 4). The melt of shredded frozen cheese decreased from d 7 to 21 and then remained stable to d 42. There was less reduction in the melt of shredded cheese, both frozen and refrigerated, between d 7 and 21. Unshredded control cheese decreased rapidly in melt from d 7 to 21.

The temperature at which cheese was frozen also affected melt over time (Figure 10). After 14 d, cheese frozen at -20°C had more melt than cheese frozen at -196°C. The melt
of cheese frozen at -196°C decreased rapidly from d 7 to 21 and then remained constant. The decrease in melt at d 21 was less dramatic in cheese frozen at -20°C, but melt continued to decrease between d 21 and 42. Thaw temperature by itself did not affect melt, but the time by thaw temperature interaction was significant (Table 4).

**Stretch and Melt**

The inverse relationship between stretch and melt of Mozzarella cheese seen in other studies (8, 14, 15) also occurred in this study. Mozzarella cheese with the greatest stretch had the least melt. Shredded frozen cheese had more stretch than unshredded frozen cheese. The opposite was true for melt. Frozen cheese stretched more than unfrozen control cheese, but unfrozen control cheese melted more than frozen cheese.

Cohesiveness in curd protein structure is required for stretch but has the opposite effect on melt. Proteolysis has been correlated with changes in the stretch and melt properties of Mozzarella cheese (15, 16). Proteinase-positive cultures produced cheese with more melt than cheese made with proteinase-deficient cultures (16). Protein breakdown in cheese reduced cohesiveness and softened the body, thus increasing melt. Tempering of thawed Mozzarella cheese for 2 to 3 wk to improve melt probably allows proteolysis to break down cheese body (4), which was consistent with the greater melt of control cheese than of the frozen cheeses in this study.

**Cook Color**

Cook color was not affected by freezing temperature, storage time, shredding, or thawing temperature (Table 4). Cook color for unfrozen control cheese and frozen cheese was similar throughout the 42-d testing period.
Figure 8. Stretch measurements (relative peak area) for frozen shredded and non-shredded Mozzarella cheese and for non-shredded and shredded refrigerated Mozzarella cheese (frozen, n = 3; control, n = 1).
Figure 9. Melt measurements of shredded and non-shredded Mozzarella either stored frozen (pooled -20 and -70°C samples) of refrigerated (4.4°C) (frozen, n = 3; control, n = 1).
Figure 10. Melt measurements of Mozzarella cheese frozen at either -196°C and stored at -70°C or frozen and stored at -20°C (n = 3).
CONCLUSIONS

The temperature and speed with which Mozzarella cheese is frozen plays an important role in the melt properties of thawed cheese. Freezing temperature had a pronounced effect on melt in this study. Price (20) noted that the drop in temperature from -1.1°C to -6.7°C during freezing is critical to textural changes. Olson (17) suggested that, the more rapidly cheese passes through this freezing zone, the less damage is done to the cheese by ice crystal formation. Fennema (6) suggested that liquid nitrogen or liquid carbon dioxide be used to freeze cheese rapidly instead of using slower methods like forced-air freezing. In the present study, cheese frozen at -20°C melted more than cheese frozen at -196°C, which suggests that slower freezing (-20° vs. -196°C) results in large ice crystals and greater breakdown of cheese.

The form in which Mozzarella cheese is frozen (block or shredded) also affected the stretch and melt properties of the thawed cheese. The melt and stretch of shredded cheese differed from unshredded cheese. Fennema (6) suggested that cheese should be cut into pieces no larger than .11 kg (.25 lb) to facilitate freezing. Stretch was greatest in shredded frozen cheese, apparently because rapid freezing limited the size of ice crystals. The fact that the internal structure was not extensively altered allowed the cheese to retain its maximum cohesiveness, which was manifested as increased stretch and decreased melt. Frozen shredded cheese melted less and stretched more than frozen unshredded cheese. Therefore, both low temperature and shredding increase the rate of freezing, minimize damage to the cheese, and reduce the rate of melting.

Freezing, storing, and thawing significantly affect the stretch and melt of Mozzarella cheese but do not affect cook color. Stretch of frozen stored Mozzarella cheese was greatest when it was shredded, frozen as quickly as possible (-196°C in this study), and held for at least 21 d. For greatest melt, it should be left in block form, not frozen or frozen as slowly as possible (-20°C in this study), and stored for as short a time as
possible. Manufacturers must balance stretch and melt, because freezing and frozen storage affect stretch and melt oppositely.

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CHAPTER 5
EFFECTS OF HIGH MOISTURE ON PHYSICAL PROPERTIES OF REDUCED FAT MOZZARELLA CHEESE

ABSTRACT

Mozzarella cheese was made with a 50% reduction in fat and with increased moisture as compared to conventional low moisture part-skim Mozzarella cheese. Single strains of Lactobacillus helveticus and Streptococcus salivarius ssp. thermophilus were used to inoculate milk which had been standardized to a casein-to-fat ratio of either 1.2, 1.6, 2.0, or 2.4. Changes in manufacturing procedures were made to elevate moisture levels. Three cheeses were made at each of the four casein-to-fat ratios and stored at 4°C. Stretch, melt, and cook color were evaluated at 1, 7, 14, and 28 d. Cook color was not found to be significant by analysis of variance. All cheeses decreased in stretch from d 1 to d 28. Melt increased for all cheeses during storage. Differences in stretch, melt, and cook color were not significant from one casein-to-fat ratio to another. Cheese made with a casein-to-fat ratio of 2.4 retained more stretch over 28 d than cheese made with lower casein-to-fat ratios. Time of storage significantly affected both stretch and melt over 28 d. Mozzarella cheese with 50% less fat had stretch and melt characteristics that were similar to low moisture part-skim Mozzarella cheese.

INTRODUCTION

Production of Italian cheese varieties (Mozzarella, Provolone, Ricotta, Romano, and Parmesan) in the United States continued to rise in 1991, to over 2.3 billion pounds. Mozzarella cheese accounted for 78% of the total Italian cheese production. Reduced fat, low fat, and nonfat dairy products are becoming more prevalent in the dairy industry.

1Coauthored by R. K. Merrill, C. J. Oberg, and D. J. McMahon. Richard K. Merrill was the major contributor.
However, decreasing or removing fat from cheese results in physical and flavor changes which often lead to poorer quality products. Brown (2) found that in low fat cheese, texture was noticeably affected by decreasing the amount of milkfat, and the resulting cheese was very hard and tough. Manufacture of low fat Mozzarella cheese has been attempted, but typically less than 30% of the milkfat is removed. As more fat is removed from Mozzarella cheese, a very tough curd that has poor melt and stretch properties is usually produced, leading to loss of consumer acceptability (2, 7-9, 13).

Similarly modifications in the protein or fat level of Mozzarella cheese made with recombined milk also reduces stretch and melt (11). In Mozzarella cheese made with retentate supplemented milk, cohesiveness and hardness increase compared to conventional Mozzarella cheese (11). Creamer and Olson (5) attribute changes in cheese body and texture to the endopeptidases that cleave a-casein. Creamer (4) found higher concentrations of a5-casein in Mozzarella cheese than in Gouda or Cheddar. *L. helveticus* has a higher peptidase activity relative to protease activity than does *L. delbrueckii* ssp. *bulgaricus* (10). Law and Wigmore (16) found that the peptidase to protease activity ratio in cheese affects the rates of change in textural properties.

The physical properties of Mozzarella cheese vary depending on cheese age, pH, moisture and fat levels, salt content, and the starter cultures used (3, 13, 14, 18-20, 23). With increased moisture or increased fat on dry basis (FDB), Mozzarella cheese becomes softer and less shreddable (9, 17). In direct acidification of Mozzarella cheese, physical properties change according to the type of acid used and the pH (13). Increasing the moisture level of Mozzarella cheese and using of *L. helveticus* may significantly alter the physical properties and offer a possible solution to manufacturing lowfat Mozzarella cheese. This study assesses the effects of reduced milkfat and increased moisture levels on the stretch, melt, and cook color of Mozzarella cheese and reports the results of an investigation of processing parameters that can be varied to achieve a low fat Mozzarella
cheese with acceptable physical properties.

MATERIALS AND METHODS

Materials

Milk was obtained from the Utah State University Dairy Products Laboratory and pasteurized at 80°C for 29 s, then cooled overnight to 4°C. Milk was standardized to a casein-to-fat ratio (c/f) of 1.2 (control), 1.6, 2.0, and 2.4 (reduced fat). Single strength calf chymosin was diluted 3 ml into 50 ml cold water prior to use. Direct set lyophilized culture consisting of *L. helveticus* LH100 and *Streptococcus salivarius* ssp. *thermophilus* TA061 (Lacto-Labo, Rhone Poulenc, Dange Saint-Romain, France) were individually weighed into sterile test tubes and stored at 4°C until used.

Mozzarella Manufacturing Procedure

Seven liters of milk were placed into each of four stainless steel vats (21.6 x 21.6 x 21.6 cm). Three batches of milk (standardized to casein-to-fat ratios of 1.6, 2.0, 2.4) were then acidified to pH 6.0 with 80% lactic acid diluted 1:2 with distilled water. All four cheese vats were placed in a water bath and the milk warmed to 33.9°C. Each vat was inoculated with .75 grams of each culture and allowed to ripen 45 min at 33.9°C. Diluted rennet (3 ml) was added (Lacto-Labo, Rhone Poulenc, Dange Saint-Romain, France) to the milk and allowed to coagulate.

Ten minutes after rennet addition the three fat-reduced curds (c/f of 1.6, 2.0, 2.4) were cut using 1.905 cm knives, while the control sample (c/f of 1.2) was cut 50 min after adding rennet. Each vat with its cut curd was left undisturbed for 15 min, followed by 30 s of gentle agitation every 15 min. The curd was heated to 37.8°C over 10 min and held at that temperature until the whey titratable acidity reached .17; then the whey was drained.

Cheese curd was then cheddared, by manually rotating curd patties, every 20 min.
with curd blocks being piled two deep on the first turn. When the curd reached a
titratable acidity of .60, the curd was hand molded and stretched in fresh 82°C water until
the molten curd was smooth and elastic (approximately 2.5 min). Molded curd was
placed in an 8.9 × 8.9 × 8.9 cm stainless steel box and placed under ice to form a small
loaf. Blocks of cheese were then placed in a saturated NaCl brine for 4 h at 4°C. Each
cheese was individually vacuum packaged and stored at 4°C until tested.

**Chemical and Physical Analysis of Cheese**

Cheese was analyzed for moisture using the CEM Microwave Model AVC 80
(CEM Corp., Matthews, NC). Fat was determined using modified Babcock method.
Samples were analyzed for melt by modified tube test (21), and cook color, by reflectance
colorimetry (24). Melt and cook color methods were modified as previously described
(18). Samples were tested at 1, 7, 14, and 28 d of storage.

**Stretch Test**

Stretch was determined at 1, 7, 14, and 28 d using a modified version of the
helical viscometer method of Kindstedt et al. (15). Fifteen grams of shredded cheese
were tamped into a 25 mm × 150 mm test tube and tempered in a 60°C water bath for 10
min. A Brookfield DV II+ (Brookfield Engineering Laboratories, Inc., Stoughton, MA)
helipath viscometer was fitted with a T-bar spindle (TF with a 1.075-cm crossbar). The
T-bar spindle was gradually submerged in the tempered cheese until it reached the bottom
by turning on the helipath stand. The helipath stand was then turned off and the
viscometer adjusted to a speed of 1.5 rpm. When a full-scale reading was attained, the
helipath stand was turned on. By using an IBM compatible computer equipped with DV
Gather + version 1.0 (Brookfield Engineering Laboratories, Inc., Stoughton, MA), 120
readings, one per 5 s, were taken while the helipath raised the viscometer spindle out of
the tube. Relative peak areas were derived for each 10 min measurement period.
Readings greater than 100 were adjusted to 100 when relative peak areas were calculated.

**Statistical Analysis**

Analyses of variance were run separately for the dependent variables cook color, melt, and stretch. There were three independent replicates for each casein-to-fat ratio. Each dependent variable was measured at 1, 7, 14, and 28 d. Correlations, means, and analyses of variance were calculated using JMP™ software from SAS® (12).

**RESULTS**

**Cheese Manufacture**

Mozzarella cheese produced at each of the casein-to-fat ratios exhibited normal characteristics. Other processing methods were tried, such as removing the curd at a low titratable acidity, reducing the cook temperature to 35.5°C, and removing whey followed by addition of water, but did not improve the product. The following changes in manufacturing procedures of an elevated pasteurization temperature (80°C for 29 s), pre-acidification of milk to pH 6.0, larger cutting knives, reduced cooking temperature (37.8°C), periodic agitation during cooking instead of constant stirring, and less frequent turning during cheddaring, produced a reduced fat high moisture Mozzarella cheese with characteristics comparable to those of a part-skim low moisture Mozzarella cheese. Cheese with lower fat levels did, however, exhibit a greenish tint, because of reduced light scattering centers in the cheese. Moisture and fat were measured after 1 d of refrigerated storage. Cheese contained levels of moisture ranging from 50.47 to 53.72%, and FDB of 21.28 to 43.09% (Table 5).

**Stretch**

Reduced fat high moisture Mozzarella cheese made with various casein-to-fat ratios showed a significant decrease ($P < .010$) in stretch over time (Table 6). The casein-to-fat
was not significant \((P<.338)\) showing that reduced fat cheese had stretch comparable to the part-skim Mozzarella control cheese. Stretch decreased significantly throughout 28-d of storage for all four cheeses with the greatest change occurring over the first 7 d (Figure 11). Cheese made with a casein-to-fat ratio of 2.4 (lowest fat highest protein levels) retained the greatest stretch over the 28-d storage period. Stretch was greatest at d 7 in cheese made with a casein-to-fat ratio of 1.6, followed by cheese made with casein-to-fat ratios of 1.2, 2.0, and 2.4. Cheese made with a casein-to-fat ratio of 1.6 had the greatest stretch between d 7 and 28, whereas cheese made with a casein-to-fat ratio of 2.4 had the least.

<table>
<thead>
<tr>
<th>Casein-to-Fat Ratio</th>
<th>Fat (%)</th>
<th>Moisture (%)</th>
<th>Solids</th>
<th>FDB</th>
<th>MNFS</th>
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</thead>
<tbody>
<tr>
<td>1.2</td>
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<td>53.69</td>
<td>46.31</td>
<td>41.75</td>
<td>66.56</td>
</tr>
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<td>47.56</td>
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<td>61.69</td>
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<td>12.3</td>
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<td>2.4</td>
<td>10.3</td>
<td>53.01</td>
<td>46.99</td>
<td>21.99</td>
<td>59.12</td>
</tr>
</tbody>
</table>

Melt

Cheese made with a c/f ratio of 1.2 had the largest change in melt during the first 7-d and had the greatest melt from d 7 through 28 (Figure 12). Melt increased similarly throughout the storage period in cheese with a casein-to-fat ratio of 1.6, 2.0, and 2.4. Analysis of variance showed no significant difference in melt among the different casein-to-fat ratios \((P<.2365)\). Melt was significantly affected by time \((P<.0000)\) and time x casein-to-fat interaction \((P<.0158)\).
Cook Color

Cook color for all cheeses increased steadily during storage (Figure 13). The greatest increase in cook color occurred between d 1 and 14. Cheese made with a casein-to-fat ratio of 1.2 showed the least amount of cook color, while cheese with a casein-to-fat ratio of 2.0 had the greatest change in cook color during storage. Analysis of variance showed no difference between cheeses for the various casein-to-fat ratios ($P < .3616$) or over time ($P < .2796$).

TABLE 6. Analyses of variance for moisture, FDB, stretch, melt cook color.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Moisture</th>
<th>FDB</th>
<th>Stretch</th>
<th>Melt</th>
<th>Color</th>
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<tbody>
<tr>
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<td>F</td>
<td>P</td>
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<td>P</td>
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<td></td>
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</tr>
<tr>
<td>(Error)</td>
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<td></td>
</tr>
<tr>
<td>Time</td>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>Time × Casein:Fat</td>
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<tr>
<td>Replicate × Time</td>
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<td>[Casein:Fat] (Error)</td>
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<tr>
<td>Corrected Total</td>
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</table>
Figure 11. Stretch measurements (relative peak area) (±SEM) of reduced fat Mozzarella cheese made from milk with a casein-to-fat ratio of 1.2, solid bar; 1.6, dark striped bar; 2.0, open bar; and 2.4, light striped bar.
Figure 12. Melt measurements (relative peak area) (±SEM) of reduced fat Mozzarella cheese made from milk with a casein-to-fat ratio of 1.2, solid bar; 1.6, dark striped bar; 2.0, open bar; and 2.4, light striped bar.
Figure 13. Cook color measurements (relative peak area) (±SEM) of reduced fat Mozzarella cheese made from milk with a casein-to-fat ratio of 1.2, solid bar; 1.6, dark striped bar; 2.0, open bar; and 2.4, light striped bar.
Oberg et al. (19) found that culture activity was improved, and physical properties were maintained in Mozzarella cheese when *L. helveticus* instead of *Lactobacillus delbrueckii* ssp. *bulgaricus* was paired with *S. salivarius* ssp. *thermophilus*. Strains of *L. helveticus* also exhibit greater peptidase activity than strains of *L. delbrueckii* ssp. *bulgaricus* (10). Ardo and Pettersson (1) studied the production of a low fat, semi-hard cheese and found that addition of heat treated *L. helveticus* enhances the peptidolytic activity even during the first few weeks, which they attributed to an amino peptidase from the *L. helveticus* cells.

This higher level of peptidolysis, coupled with the reducing sugar available in Mozzarella cheese, suggests an increase in cook color for cheese made with *L. helveticus*. Mozzarella cheese made with single strains of *L. helveticus* actually shows a decrease in cook color, while cook color increases with time in cheese made with paired strains of proteinase positive (Prt+) *L. helveticus* and *S. salivarius* ssp. *thermophilus* (19). Change in cook color of reduced fat Mozzarella cheese made with *L. helveticus* supports the research of Oberg et al. (19).

Changes in the manufacturing procedure increased moisture levels and maintained the physical properties of reduced fat Mozzarella cheese. Elevated pasteurization temperatures denature whey proteins which are then trapped in the curd matrix and aid in water retention (22). Reduced cooking temperature, larger cutting knives, and periodic agitation create larger curd particles resulting in decreased syneresis and improved moisture retention in the curd during cooking (6). Less frequent turning during cheddaring keeps the curd on top cool; while acidity is being developed, whey expulsion is decreased thereby retaining moisture in the curd (6).

As increasingly more fat is removed from Mozzarella cheese, it becomes tougher and more difficult to melt. Once enough heat has been applied, the cheese does melt, but
cools rapidly and is no longer pliable. Mozzarella cheese made with elevated moisture and FDB levels becomes soft and difficult to shred (9, 17). High moisture Mozzarella made from reconstituted NDM also has poor body characteristics (9). Tunick et al. (25) reported that cheese made with low fat and low moisture was too hard and not meltable enough. In addition, the low fat, high moisture Mozzarella was comparable to low moisture part-skim Mozzarella cheese only after 6 wk of refrigerated storage.

In this study, Mozzarella cheese was manufactured with reduced fat, increased moisture levels, and L. helveticus instead of L. delbrueckii ssp. bulgaricus used in the starter culture. Throughout the 28-d storage period, all cheeses in this study decreased in stretch and increased in melt, with the majority of change occurring in the first 7 d. These two measurements were strongly correlated, each being affected differently by proteolysis during the first week of storage. The comparable performance between high moisture, low fat Mozzarella and low moisture, part-skim Mozzarella is apparent and shows acceptable reduced fat Mozzarella cheese is possible without the addition of fat substitutes.

REFERENCES


6 Danish Government Research Institute for Dairy Research


CHAPTER 6

EFFECTS OF LACTOBACILLUS CASEI SSP. CASEI ON PHYSICAL PROPERTIES OF HIGH MOISTURE, LOW FAT MOZZARELLA CHEESE

ABSTRACT

Reduced fat high moisture Mozzarella cheese was produced using either Streptococcus salivarius ssp. thermophilus or Lactobacillus helveticus, with either total or partial replacement of the L. helveticus with Lactobacillus casei ssp. casei. Stretch, melt, and cook color were determined at 1, 7, 14, and 28 d. All cheeses rapidly lost stretch and gained melt during the first seven days of storage. Differences from one culture type to another were not significant. Cheese made with L. helveticus and S. salivarius ssp. thermophilus showed the greatest stretch over time. Cheese made with a 2:1:1 ratio of S. salivarius ssp. thermophilus, L. helveticus and L. casei ssp. casei had the least stretch and greatest melt at day 28. Time of storage significantly affected stretch and melt throughout 28 d. Reduced fat cheese made with partial or total replacement of L. helveticus with L. casei ssp. casei had less stretch, more melt, and less cook color than reduced fat or low moisture part-skim Mozzarella made with S. salivarius ssp thermophilus and L. helveticus. Overall, the physical properties of reduced fat Mozzarella cheese made in this study compared favorably with low moisture, part-skim Mozzarella cheese.

INTRODUCTION

Consumer demand for reduced fat, lowfat, and nonfat dairy products has not diminished (4). Consumption of Mozzarella cheese, mainly as an ingredient on pizza, continues to rise (personal communication, Dan Buckner, USDA). Meltability and

1Coauthored by R. K. Merrill, C. J. Oberg, and D. J. McMahon. Richard K. Merrill was the major contributor.
stretch are the most important characteristics associated with Mozzarella cheese. Decreasing or removing fat from cheese often results in textural and flavor changes (3). Low fat cheese tends to be hard and to exhibit poor melt and stretch properties (3, 6, 7, 8, 9, 14). Stringent consumer expectations have led to a surge of investigations into factors that will contribute to development of acceptable lower fat dairy products.

Changes in Mozzarella cheese texture are highly attributed to proteolysis by lactic starter bacteria and milk coagulants (20, 21, 22, 25). Quarne et al. (25) found that different milk coagulating enzymes accelerate proteolysis and body development. Cheddar cheese made with bovine pepsin is more elastic than cheese made with calf chymosin and that elasticity decreases as proteolysis increases (6). Oberg et al. (21) found that milk-clotting enzymes affect the physical properties of direct acid Mozzarella cheese. The proteolytic capability of bacterial starter cultures can alter the physical properties of Mozzarella cheese, especially stretch and melt (20, 22). Changes in proteolytic characteristics of thermolactic starter cultures, particularly *Lactobacillus delbrueckii* ssp. *bulgaricus*, modify stretch, melt, and cook color of Mozzarella cheese (5, 11, 22, 23). A variety of proteolytic, peptidolytic, and esterolytic enzymes have been found and partially characterized in the lactobacilli (15, 19, 28).

Recently lactobacilli have gained much attention due to their strong peptidolytic, esterolytic, and lypolytic activities and the importance these enzymes have in accelerated-ripened cheese and enzyme-modified cheese (17). *Lactobacillus casei* ssp. *casei* has been shown to produce strong proteolytic and peptidolytic activities (1, 2, 17). Lee et al. (17) reported a higher number and greater activities of peptidases and esterases in *L. casei* species when compared with other starter strains. Hull et al. (12) reported that *L. casei* species were associated with a soft-body defect in Mozzarella cheese. In Cheddar cheese *L. casei* increased the rate of softening but did not increase soluble nitrogen; it increased the development of flavor but later caused an acid flavor and "short" body (29). A soft or
pasty-body was observed in Mozzarella cheese where $>10^4$ \textit{L. casei} cells per gram were found (12).

The use of \textit{L. casei} species in Cheddar cheese and its effect on body and texture are well documented. Thus, complete or partial substitution of \textit{L. delbrueckii} ssp. \textit{bulgaricus} with \textit{L. casei} species may enhance the physical properties of low fat Mozzarella cheese. This study examines the effects of storage time, reduced fat, elevated moisture levels, and \textit{L. casei} on the physical properties of Mozzarella cheese.

**MATERIALS AND METHODS**

**Materials**

Milk was obtained from Utah States Dairy Products Laboratory and pasteurized at 176°C for 29 sec and then cooled overnight to 4°C. Milk was standardized to a casein-to-fat ratio of 1.2 (control) and 2.4 (reduced fat). Single strength calf chymosin was diluted 3 ml into 50 ml cold water prior to use.

**Cultures**

Cultures of \textit{Lactobacillus delbrueckii} ssp. \textit{bulgaricus} LH100, \textit{Streptococcus salivarius} ssp. \textit{thermophilus} TA061, and LF200 (Rhone-Poulenc, Madison WI), and \textit{L. casei} ssp. \textit{casei} LC10 (Cultech Inc., Millville, UT) were selected based upon proteinase, peptidase, and lipase characterization for Mozzarella cheese manufacture. Bulk starter cultures were prepared individually in 7.4% Thermo-Lac (Cultech Inc., Millville, UT) culture medium with internal pH control. Two percent yeast extract was added to the starter medium for growth of \textit{L. casei} ssp. \textit{casei}. The medium was reconstituted using deionized water, heated to 90°C, and held at that temperature for 40 min. Medium was rapidly cooled to 40°C and inoculated with 1% of culture grown in 10% NDM. Medium containing 2% yeast extract was cooled to 30°C and inoculated with \textit{L. casei} ssp. \textit{casei}.
Mozzarella Manufacturing Procedure

Part-skim and reduced fat Mozzarella cheese was made with a 1:1 ratio of *L. delbrueckii* ssp. *bulgaricus* and *S. salivarius* ssp. *thermophilus*. Reduced fat Mozzarella cheese was also made using a 2:1:1 ratio of *L. delbrueckii* ssp. *bulgaricus* *S. salivarius* ssp. *thermophilus*, and *L. casei* ssp. *casei*, or a 1:1 ration of *S. salivarius* ssp. *thermophilus*, and *L. casei* ssp. *casei*.

Seven liters of milk were placed into each of four stainless steel vats (21.6 × 21.6 × 21.6 cm). The batches of milk (standardized to a casein-to-fat ratio of 1.2 or 2.4) were then acidified to pH 6.0 with lactic acid. All of the cheese vats were then placed in a water bath and the milk was warmed to 33.9°C (93°F). Each vat was inoculated with 2% of each culture and allowed to ripen 45 min at 33.9°C (93°F). Diluted rennet (Rhone-Poulenc, Madison WI) was added to the milk and allowed to coagulate.

After 10 min the fat reduced curd was cut using 3/4 inch knives; the control sample (casein-to-fat of 1.2) was cut 50 min after adding rennet. After cutting, each vat was left undisturbed for 15 min followed by gentle agitation every 5 min for 30 s. The curd was heated to 37.8°C (100°F). When whey titratable acidity read 0.17, the whey was drained.

The cheese curd was then cheddared with rotation every 20 min, and blocks were piled two deep on the first turn. The curd at a titratable acidity of .60 was then hand molded and stretched in fresh 82°C (179.6°F) water until the molten curd was smooth and elastic (approximately 2.5 min). Molded curd was placed in 3.5 × 3.5 × 3.5 inch stainless steel boxes and placed under ice to form the curd. Blocks of cheese were then placed in a saturated NaCl brine for 4 h at 4°C. Each cheese was individually vacuum packaged and stored at 4°C until tested.

Physical Property Analysis

Cheese was analyzed for moisture using the CEM Microwave Model AVC 80 (CEM Corp., Matthews, NC). Fat was determined using modified Babcock method (26).
Samples were analyzed for melt by modified tube test (24), stretch by helical viscometry (16), and cook color by reflectance colorimetry (27). Methods were modified as previously described (21). Samples were tested at 1, 7, 14, and 28 d of storage.

**Statistical Analysis**

Analyses of variance were run separately for the dependent variables cook color, melt, and stretch. There were three independent replicates of each culture type. Each dependent variable was measured at 1, 7, 14, and 28 d. Correlations, means, and analyses of variance (Table 7) were calculated using JMP™ software from SAS® (13).

**TABLE 7. Analyses of variance for moisture, FDB, stretch, melt cook color.**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Moisture</th>
<th>FDB</th>
<th>Stretch</th>
<th>Melt</th>
<th>Color</th>
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<td>df</td>
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</table>

**RESULTS**

**Cheese Manufacture**

Each of the starter cultures used in this study produced reduced fat high moisture Mozzarella cheese with characteristics comparable to those of low moisture, part-skim
Mozzarella cheese. Cheese containing lower fat did, however, contain a greenish tint because of reduced light scattering centers in the cheese. Moisture and fat were measured after 1 d of refrigerated storage. Cheese contained levels of moisture ranging from 53.69 to 52.12%, and FDB of 41.75 to 20.66% (Table 8).

**Table 8.** Average percentages of fat, moisture, solids, FDB, and moisture nonfat solids (MNFS) for Mozzarella cheese made at different c/f ratios.

<table>
<thead>
<tr>
<th>Casein-to-Fat Ratio</th>
<th>Fat (%)</th>
<th>Moisture (%)</th>
<th>Solids</th>
<th>FDB</th>
<th>MNFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2</td>
<td>19.3</td>
<td>53.69</td>
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<td>52.44</td>
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<td>58.16</td>
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</table>

**Stretch**

Reduced fat Mozzarella cheese made with and without *L. casei* ssp. *casei* showed a significant (*P* < .01) decrease in stretch over time (Table 7). The time x culture interaction was not significant (*P* = .09). Stretch decreased significantly throughout the 28-d storage period with the greatest change occurring over the first 7 d (Figure 14). Reduced fat cheese made with strains of *L. helveticus* and *S. salivarius* ssp. *thermophilus* showed the greatest stretch over time. Mozzarella cheese made with *L. casei* ssp. *casei* cultures, paired with either *S. salivarius* ssp. *thermophilus* and *L. helveticus* or just *S. salivarius* ssp. *thermophilus*, had the least stretch over the 28-d storage period.

**Melt**

Cheese made with a casein-to-fat ratio of 1.2 (control) had the largest change during the first 7 d and had the greatest melt at d 7 through 14 (Figure 15). Cheese made with a
2:1:1 ratio of *S. salivarius* ssp. *thermophilus*, *L. helveticus* and *L. casei* ssp. *casei* had the greatest melt at d 28. Melt increased similarly throughout the storage period in reduced fat cheese. Analysis of variance showed no significant change in melt among cultures used; however, melt was significantly affected by time (*P* < .01) and time x culture interaction (*P* = .02).

**Cook Color**

Cook color for the control cheese increased steadily during storage, as is typical for Mozzarella cheese (Figure 16). Cook color for reduced fat cheeses made with *S. salivarius* ssp. *thermophilus*, *L. helveticus*, and either partial or total replacement of the rod portion with *L. casei* ssp. *casei* increased through d 7, then decreased during the remainder of the storage period. Cheese made with a 1:1 ratio of *S. salivarius* ssp. *thermophilus* and *L. casei* ssp. *casei* showed the least amount of cook color, while control cheese made with a 1:1 ratio of *S. salivarius* ssp. *thermophilus* and *L. helveticus* had the greatest color change during storage. Analysis of variance showed replicate to be significant (*P* = .02).
Figure 14. Stretch measurements (relative peak area) (±SEM) of reduced fat Mozzarella cheese made from milk with either a casein-to-fat ratio (c/f) of 1.2 (low moisture part-skim) or 2.4 (high moisture reduced fat), with combinations of *Streptococcus salivarius* ssp. *thermophilus* (ST), *Lactobacillus helveticus* (LH), and *Lactobacillus casei* ssp. *casei* (LC). Solid bar indicates c/f of 1.2 using ST and LH (1:1 ratio); dark striped bar, c/f of 2.4 using ST and LH (1:1 ratio); open bar, c/f of 2.4 using ST, LH, and LC (2:1:1 ratio); light striped bar, c/f of 2.4 using ST and LC (1:1 ratio).
Figure 15. Melt measurements (±SEM) of reduced fat Mozzarella cheese made from milk with either a casein-to-fat ratio (c/f) of 1.2 (low moisture part-skim) or 2.4 (high moisture reduced fat), with combinations of *Streptococcus salivarius* ssp.* thermophilus* (ST), *Lactobacillus helveticus* (LH), and *Lactobacillus casei* ssp. *casei* (LC). Solid bar indicates c/f of 1.2 using ST and LH (1:1 ratio); dark striped bar, c/f of 2.4 using ST and LH (1:1 ratio); open bar, c/f of 2.4 using ST, LH, and LC (2:1:1 ratio); light striped bar, c/f of 2.4 using ST and LC (1:1 ratio).
Figure 16. Cook color measurements (±SEM) of reduced fat Mozzarella cheese made from milk with either a casein-to-fat ratio (c/f) of 1.2 (low moisture part-skim) or 2.4 (high moisture reduced fat), with combinations of *Streptococcus salivarius* ssp. *thermophilus* (ST), *Lactobacillus helveticus* (LH), and *Lactobacillus casei* ssp. *casei* (LC). Solid bar indicates c/f of 1.2 using ST and LH (1:1 ratio); dark striped bar, c/f of 2.4 using ST and LH (1:1 ratio); open bar, c/f of 2.4 using ST, LH, and LC (2:1:1 ratio); light striped bar, c/f of 2.4 using ST and LC (1:1 ratio).
DISCUSSION AND CONCLUSIONS

Changes in Mozzarella cheese texture and physical properties correlate highly with
the extent to which proteolysis takes place by lactic acid bacteria and milk coagulants
(20, 21, 22, 25). Quarne et al. (25) found that proteolysis and body development were
accelerated in Mozzarella cheese depending upon which milk coagulant was used. Milk-
clotting enzymes affect the physical properties of Mozzarella cheese (21). The
proteolytic capabilities of bacterial starter cultures can also alter the physical properties of
Mozzarella cheese, especially stretch and melt. Oberg et al. (20) found that activity was
improved, and physical properties were maintained in Mozzarella cheese when
*L. helveticus* was paired with *S. salivarius* ssp. *thermophilus* instead of *Lactobacillus
delbrueckii* ssp. *bulgaricus*. Strains of *L. helveticus* exhibit greater peptidase activity than
*L. delbrueckii* ssp. *bulgaricus* (10).

Recently a variety of proteolytic, peptidolytic, and esterolytic enzymes have been
purified and partially characterized from lactobacilli, and much attention is being given to
these cultures and their enzymes for use in reduced and low fat cheeses (17).
*Lactobacillus casei* ssp. *casei* has been shown to possess enhanced proteolytic and
peptidolytic activities over other lactobacilli species used in manufacture of cheese (1, 2,
17, 30). Changes in the manufacturing procedures (18) and partial or total replacement of
*L. helveticus* with *L. casei* ssp. *casei* maintained the physical properties of reduced fat
Mozzarella cheese. Throughout the 28 d all cheese increased in melt and decreased in
stretch (Figures 14 and 15). There were no significant differences in stretch and melt
between cheese made with *S. salivarius* ssp. *thermophilus* and *L. helveticus* and cheese
made with partial or total substitution with *L. casei* ssp. *casei*.

Lee et al. (17) reported a higher number and greater activities of peptidases and
esterases in *L. casei* species when compared to other starter strains. Hull et al. (12)
reported that a soft body defect in Mozzarella cheese was caused by *L. casei* species
when found at $10^4$ cells per gram or higher. Tittsler et al. (29) found that in Cheddar cheese the rate of body softening and flavor development could be enhanced through addition of \textit{L. casei} species.

In this study, cheese was manufactured with reduced fat, increased moisture levels, and \textit{S. salivarius} ssp. \textit{thermophilus}, \textit{L. helveticus} and with either partial or total replacement of \textit{L. helveticus} with \textit{L. casei} ssp. \textit{casei}. Throughout the 28-d storage period, all cheese in this study decreased in stretch and increased in melt, with the majority of change occurring in the first 7 d. Cheese made with a 2:1:1 ratio of \textit{S. salivarius} ssp. \textit{thermophilus}, \textit{L. helveticus} and \textit{L. casei} ssp. \textit{casei} had the least stretch and the greatest melt at d 28, suggesting a possible synergistic relationship between \textit{L. helveticus} and \textit{L. casei} ssp. \textit{casei} and resulting in greater breakdown of the cheese body. The enhanced proteolysis causes stretch to decrease and melt to increase more rapidly than cheese made with only \textit{S. salivarius} ssp. \textit{thermophilus} and \textit{L. helveticus}. All reduced fat cheeses compared favorably to the low moisture, part-skim control cheese, suggesting that \textit{L. casei} ssp. \textit{casei} may be used to improve the textural characteristics of reduced fat Mozzarella cheese.

**REFERENCES**


CHAPTER 7
GENERAL SUMMARY

Some desirable physical properties of Mozzarella cheese deteriorate with time. Factors that cause this deterioration are not well defined. This research has looked at some of the most likely factors that affect the maintenance of Mozzarella cheese's physical properties.

Variation in stretch and melt among direct acid Mozzarella cheeses made with different enzyme coagulants may be affected by enzyme specificities toward individual caseins. Mozzarella cheese made with calf chymosin had the largest reduction in stretch and the largest increase in melt. Cheese made with porcine pepsin had the most stretch and the least melt, indicating there was less proteolysis with porcine pepsin than with calf chymosin.

Changes in proteolytic characteristics of thermolactic starter cultures modify stretch, melt, and cook color. When *Lactobacillus delbrueckii* ssp. *bulgaricus* was replaced with *Lactobacillus helveticus*, stretch increased, melt was maintained, cook color decreased, and make-time decreased. Strains of *L. helveticus* that are Prt- and Gal+ are best for reducing cook color and prolonging the physical properties of Mozzarella cheese.

The temperature and speed with which mozzarella cheese is frozen play an important role in the melt properties of thawed cheese. Frozen shredded cheese melted less and stretched more than non-shredded cheese. The fact that the internal structure was not extensively damaged allowed the cheese to retain its maximum cohesiveness, as was manifest by increased stretch and decreased melt. Both low temperature and shredding increase the rate of freezing, minimize damage to the cheese, and reduce the rate of melting. Stretch of frozen stored Mozzarella cheese was greatest when it was shredded, frozen as quickly as possible, and held for at least 21 d. For greatest melt, it
should be left in block form, not frozen or frozen as slowly as possible, and stored as short a time as possible.

As more fat is removed from Mozzarella cheese, its melting properties become nonexistent. If sufficient heat is applied, it does melt but cools rapidly to a hardened mass. In reduced fat Mozzarella cheese, Mozzarella cheese made with *L. helveticus* stretch decreased, melt increased, and cook color decreased. Physical properties of reduced fat Mozzarella cheese were comparable with low moisture, part-skim Mozzarella cheese. Acceptable reduced fat Mozzarella cheese with elevated moisture levels can be produced without addition of fat substitutes.

Enhanced proteolysis and peptidolysis of *Lactobacillus casei* ssp. *casei* can improve the physical properties of reduced fat Mozzarella cheese. Cheese manufactured with reduced fat, increased moisture levels, and *S. salivarius* ssp. *thermophilus*, *L. helveticus* and either partial or total replacement of *L. helveticus* with *L. casei* ssp. *casei* decreased in stretch and increased in melt. Cheese made with a 2:1:1 ratio of *S. salivarius* ssp. *thermophilus*, *L. helveticus*, and *L. casei* ssp. *casei* had the least stretch and the greatest melt, suggesting a possible synergistic relationship between *L. helveticus* and *L. casei* ssp. *casei*, resulting in greater breakdown of the cheese body.

For greatest stretching properties, Mozzarella cheese should be manufactured using porcine pepsin, and proteinase-deficient starter cultures; it should be shredded, frozen as quickly as possible, and held for at least 21 d.

For optimal melting characteristics, cheese should be made using calf chymosin, and proteinase-positive cultures; it should be left in block form, not frozen or frozen as slowly as possible, and stored for as short a time as possible.

To optimize cook color, Mozzarella cheese should be made with proteinase-deficient, galactose-positive strains of *L. helveticus* of *L. casei*. If total elimination of browning is desired, Mozzarella cheese should be manufactured by direct acidification.
Mozzarella cheese manufactured for the pizza industry is usually frozen and stored until its final use on a pizza; thus a high quality product is provided consistently. Consumers of Mozzarella cheese obtained in the supermarket often are purchasing cheese that may be up to 35-d old, during which time the physical properties have deteriorated and undesirable flavors have developed. In order to provide the highest quality product to the supermarket consumer, Mozzarella cheese should be sold shredded from the freezer case rather than the refrigerated dairy shelf, thereby preventing physical property deterioration and providing the highest quality product to consumers.
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APPENDIXES
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Research Consultant, Auro Tech. Inc. Menomonee Falls, Wisconsin. Coordinated development of a thermophilic starter culture media for use June 1989 in manufacture of yogurt and Italian cheeses. Directed research involving identification and characterization of starter strains. Selected strains were observed in a model milk system for growth performance and products of casein degradation. Results were analyzed and recommendations communicated directly to Auro Tech's CEO.

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