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A Study of the Effects of Proteolytic Adjunct Culture on the Physical and Functional Properties of Low-Fat Mozzarella Cheese

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A STUDY OF THE EFFECTS OF PROTEOLYTIC ADJUNCT CULTURE ON THE PHYSICAL AND FUNCTIONAL PROPERTIES OF LOW-FAT MOZZARELLA CHEESE

by

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ABSTRACT

A Study of the Effects of Proteolytic Adjunct Culture on the Physical and Functional Properties of Low-Fat Mozzarella Cheese

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As fat is removed from Mozzarella cheese, the resulting increase in protein content causes the cheese to become tough, thus decreasing the desired physical characteristics of meltability and stretch. Low-fat (6% fat) Mozzarella cheese was manufactured with the addition of several levels of a *Lactococcus lactis* adjunct culture that was proteinase positive and lactose deficient in an attempt to improve these physical properties. During cheese manufacture, milk was acidified to pH 6.0, then inoculated with *Lactobacillus helveticus* and *Streptococcus thermophilus*. Experimental vats were also inoculated with either 0.25, 0.50, or 1.0% of the adjunct culture. Cheeses made with the adjunct culture had increased melt properties at d 1. During the first 14 d of storage, cheeses manufactured with 0.50% and 1.0% adjunct culture melted more readily than the control; by 28 d, the meltability of all cheeses was similar. Breakdown of cheese body was more rapid in the experimental cheeses and was particularly apparent during shredding. The increase in softness was presumed to be the result of increased proteolysis in the cheeses. There were no significant differences in melt viscosity between control and experimental cheeses. Storage time, however, was significant, and between d 14 and d 28, melt viscosity decreased for all

cheeses. Protein hydrolysis was measured using SDS-PAGE, but no differences were observed in the disappearance of intact caseins.

In the second part of this study, part-skim (18% fat) Mozzarella cheese was manufactured from milk standardized to a casein-to-fat ratio of 1.2 and inoculated with *L. helveticus* strain and *S. thermophilus* strain. Low-fat (6% fat) Mozzarella cheese was manufactured from milk with a casein-to-fat ratio of 4.2 and inoculated with the same starter culture with (or without) addition of the proteinase positive, lactose deficient adjunct culture. The cheese was molded into 1.5-lb blocks and stored at 4°C. Meltability and melt viscosity of the cheese were measured during 28 d storage. Disappearance of α_{s1}-casein and β-casein was measured using free solution capillary electrophoresis, which separated intact proteins and large peptides. Micellar electrokinetic capillary chromatography was used to study the appearance of small peptides (<30 kDa) during storage. After 28 d storage, there were significant decreases in the amount of intact α_{s1}-casein remaining after 28 d, but no measurable change in β-casein in either the part-skim or low-fat cheeses. In part-skim cheese, 71% α_{s1}-casein remained, but in the low-fat cheeses only 20% intact α_{s1}-casein remained after 28 d. If adjunct culture was used in low-fat cheese, then only 14% α_{s1}-casein was found after 28 d. A similar increase in proteolysis in the low-fat cheeses was observed based on the amount of small peptides produced. Part of these differences may be a function of increased moisture content of the low-fat cheese, 61% vs 51% in part-skim cheese. During storage, part-skim Mozzarella showed a typical increase in melt with a corresponding decrease in melt viscosity. Melt increased from 10.6 cm at d 1 to 16.9 cm at d 28; melt viscosity at 80°C decreased from 1.0×10^6 cP at d 1 to 2.1×10^5 cP at d 28. There was less change in melt in the low-fat cheese during storage, 8.9 cm at d 1 and 10.9 cm at d 28. Melt viscosity decreased from 4.8×10^5 cP at d 1 to 1.9×10^5 cP at d 28. It appears that adding the adjunct culture increased initial meltability of the low-fat

cheese by accelerating proteolysis during the first 14 d but caused an increase in viscosity and decrease in melt after 14 d of refrigerated storage.

(66 pages)

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LIST OF ABBREVIATIONS

Lac^d = lactose-deficient

Prt^+ = proteinase-positive

Prt^d = proteinase-deficient

AV_{60} = apparent viscosity at 60°C

MNFS = moisture in nonfat substance

FSCE = free solution capillary electrophoresis

SDS-PAGE = sodium dodecyl sulfate-polyacrylamide gel electrophoresis

HPLC = high performance liquid chromatography

MECC = micellar electrokinetic capillary chromatography

UHT = ultra-high temperature

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

Recent awareness by consumers to decrease the amount of fat in their diets (27) has caused the demand for lower fat dairy products, including cheese. The increased popularity of pizza in recent years has been largely responsible for the increased consumption of Mozzarella cheese, which has shown a steady increase in production over the past twelve years (23). This popularity has prompted research to develop an acceptable low-fat Mozzarella cheese with appropriate functionality for pizza.

Low-fat Mozzarella does not develop acceptable melting qualities as quickly as does part-skim Mozzarella. It must be aged longer (6 wk compared with 2 wk for part-skim) to develop similar melting properties (36). Increased melting during aging has been related to the disappearance of α_{s1}-casein, which is a function of proteolysis occurring in the cheese. Increasing the melt while reducing the storage time for low-fat Mozzarella cheese would be highly desirable, particularly for the pizza industry, where young Mozzarella cheese is typically used (3).

We hypothesized that if we increase the rate of proteolysis, by using a more proteolytic culture system, acceptable melting characteristics should be achieved with less aging time. The objective was to determine if using proteolytic adjunct culture improves the melting properties of low-fat Mozzarella cheese by increasing the rate of proteolysis in the cheese, thus shortening the storage time required to obtain low-fat Mozzarella cheese with desirable melting properties.

RESEARCH OBJECTIVE

The objective of this study is to determine if using a proteolytic adjunct culture will improve the melting properties of low-fat Mozzarella cheese by increasing the rate of proteolysis in the cheese, thus shortening the storage time required to obtain cheese with desirable melting characteristics.

PRELIMINARY RESULTS

We began by obtaining cultures from a commercial culture company. The cultures were described as highly proteolytic, lactose-negative mesophiles. We wanted a culture that would contribute to proteolysis without utilizing lactose, thereby keeping the manufacturing time of the control cheese consistent with the proteolytic adjunct-treated cheeses. During the first trial the adjunct culture was added at increasing concentrations to the vats of milk with every other parameter held constant. During this first trial it was observed that the manufacture time of the adjunct-treated cheese was on average 1 h faster than the manufacture time of the control (Table 1). Therefore, it was suspected that the adjunct culture was utilizing lactose, contributing to the formation of lactic acid and subsequently driving down the pH of the curd at a faster rate than the control. As a result, the moisture content of the cheeses from the first trial was variable, ranging up to a 4.5% difference in moisture between the control and the adjunct-treated cheeses (Table 1). Therefore, our tests for measuring melt and viscosity could not be completely related to the degree of proteolysis in the adjunct-treated cheeses. To confirm the characteristics of the adjunct culture, purified culture was grown in nonfat UHT milk and monitored for acid development; an API ZYM (Analytab Products Inc.) assay was also conducted. The results showed that the cultures were proteolytic and had the ability to ferment lactose. However,

TABLE 1. Manufacturing time (renneting to stretching), pH at stretching, and moisture content for initial trials of low-fat Mozzarella cheese made with different levels of proteinase-positive adjunct culture.

	Time (min)		pH		Moisture (%)	
Adjunct level	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM
0	217	18.3	5.32	0.015	55.3	0.18
0.25	154	3.5	5.34	0.007	58.1	0.63
0.50	135	6.9	5.34	0.012	59.6	1.18
1.00	134	11.9	5.32	0.006	59.3	0.43

results from the acid development in nonfat milk showed their ability to produce acid was far less than that of the starter cultures (Figure 1). To compensate for the decreased manufacturing time of the adjunct-treated cheeses, the original inoculation percent of the primary starter culture was adjusted accordingly to keep the manufacture time of the control cheese consistent with the adjunct-treated cheeses. Several trials of cheese making were conducted until the proper ratio of primary starter culture to adjunct culture was achieved to give consistent manufacturing times, thereby reducing the variability of the moisture content. This ratio was then documented and used for subsequent trials in our research.

An unexplained observation made during our preliminary trials was the difference in the appearance of the whey. Cheese vats treated with the adjunct cultures produced whey that had a white, more cloudy appearance, while the whey from the control vats maintained a yellow, more transparent appearance. One theory on this observation is that the adjunct culture may be utilizing the riboflavin in the milk, which contributes to the yellow color, therefore causing the color change in the whey.

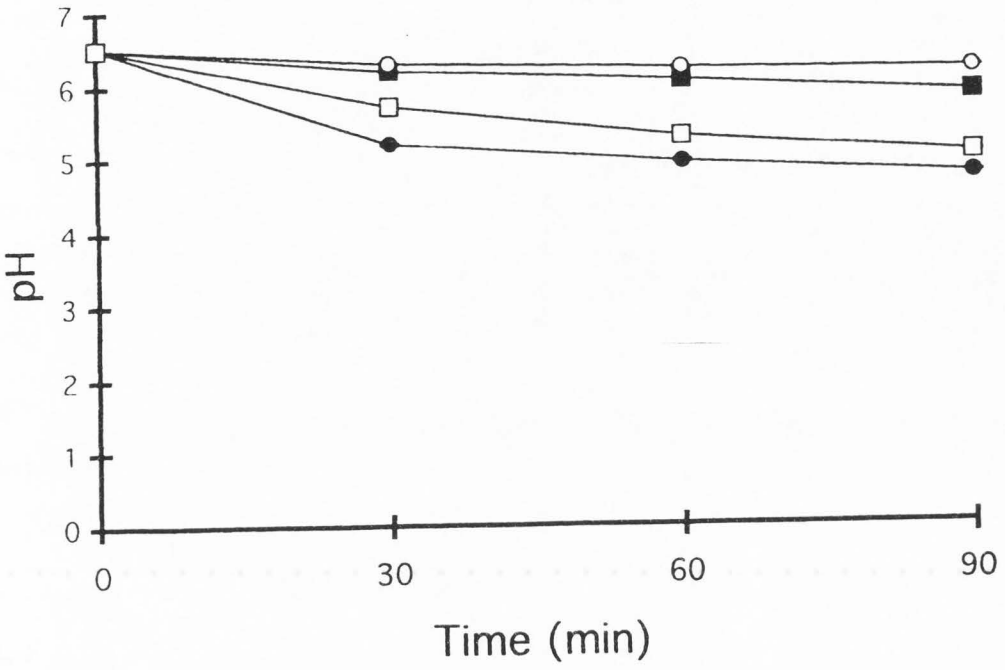

Figure 1. Mean pH measurements (±SEM, error bars obstructed by symbols) at 34°C of *S. thermophilus* (solid circle),*L. helveticus* (solid square), proteolytic adjunct culture (open circle),and equal parts of *S. thermophilus* and *L. helveticus* combined (open square) grown over time in UHT nonfat milk.

LITERATURE REVIEW

Low-Fat Mozzarella

Researchers have observed that a reduction in fat content of 33% in Cheddar-type cheeses produced an acceptable cheese, but when the fat content was reduced by 50% or more, the cheese had little flavor and poor physical properties (28). Textural characteristics and melting properties are significantly affected by a lowered-fat content in Mozzarella cheese (35, 37). Toughness increases as the fat is removed from Mozzarella cheese, which may be the result of an increase in protein concentration as well as differences in the distribution of moisture within the protein matrix (26). A loss in the elasticity (stretch) of

cheese is experienced as the ratio of fat to solids-not-fat increases (22). Reduced moisture levels in low-fat Mozzarella also contribute to a tough rubbery texture and poor melting and stretching characteristics (25). Desirable properties of Mozzarella cheese are medium firmness, sufficient melt, adequate stretchability, and ease of shredding (1), which vary with the age of the cheese, pH, moisture content, salt content, and type of starter culture (26). In low-fat, high-moisture Mozzarella cheese with only 41% of the fat content of full-fat Mozzarella there is a lack of fat droplets to provide the physical and textural characteristics of full-fat Mozzarella. The small and compact peptides formed from extensive proteolysis may assume the role of the fat droplets by acting as a filler (20). Also, as a result of casein proteolysis, the protein matrix of the cheese becomes less dense, allowing fat globules to coalesce, enhancing the flow of the melted fat.

When comparing reduced-fat (10% fat) Mozzarella with part-skim (20% fat), Mozzarella, Tunick et al. (36) observed that if the reduced-fat Mozzarella were aged for an increased period of time to allow continued degradation of α_{s1}-casein, it resulted in textural properties comparable to part-skim Mozzarella. Tunick et al. (37) showed that low-fat (9% fat), high-moisture Mozzarella cheese acquired the physical characteristics comparable to part-skim Mozzarella cheese only after 6 wk of refrigerated storage.

Stretch and melt are two of the properties that are the most characteristic of Mozzarella cheese quality (1). It is typically observed that during storage, melt and stretch are inversely related: melt increases during the first 14 d and stretch decreases (30,32). Tunick and Sheih (38) concluded that meltability in reduced-fat Mozzarella is dependent on moisture and refrigerated storage. Controlling moisture content by coagulating the milk at a lower pH can improve the quality of low-fat Mozzarella cheese. Chen et al. (6) demonstrated that by adding the milk coagulant at a lower pH (pH 6.05) the moisture content of the cheese was higher and resulted in cheese with improved melt and stretch characteristics. Therefore, by

controlling these variables a reduced-fat Mozzarella with texture and melt characteristics similar to full-fat Mozzarella may be achieved.

One component affecting stretch in Mozzarella cheese that has been suggested by investigators is the time allowed for acid development. Slower acid development results in a slower conversion of dicalcium para-casein to monocalcium para-casein causing the cheese to have less stretch (16).

A better understanding of the biochemical reactions taking place during cheese maturation is necessary to develop solutions to the problems affecting low-fat cheeses (34).

Proteolysis in Cheese

Proteolysis of caseins may start with the formation of the curd and continue throughout refrigerated storage of the cheese, including non-ripened cheeses like low-fat, high-moisture Mozzarella (37). In most bacteria-ripened cheeses the caseins are the first to be hydrolyzed. In particular, the α-caseins are hydrolyzed, while there is little reduction in the β- and para-κ-caseins (28). Plasmin, which naturally occurs in milk, and chymosin, which is added to the milk during cheesemaking, may both contribute to the formation of peptides during proteolysis (20). Fragments of caseins formed by the action of chymosin and plasmin provide the necessary metabolic substrates for the proteases and peptidases produced by the starter culture (15). There is widespread acceptance that the enzymes in milk, added chymosin, and starter and non-starter bacteria all play a part in the flavor development and maturation of cheese (28). During cheese ripening, proteolysis may be defined as two separate phases. In the first phase immature cheese curd will typically lose its tough, rubbery texture within the first 7 to 14 d after manufacture. This indicates that casein proteolysis is occurring since the casein network is the primary component of the cheese microstructure. Creamer and Olsen (8) suggested that the casein network experiences a significant loss in stability when chymosin hydrolyzes only 20% of the α_{s1}-

casein to form the α_{s1}-I peptide. This peptide proves to be present in all types of cheese during early maturation (18). The second phase of ripening tends to extend into a period of months during which time the remainder of the α_{s1}- caseins and other caseins are hydrolyzed. Textural changes are more gradual during the second phase (8).

There is a significant relationship between casein proteolysis and textural characteristics in cheese (14). DeJong (9) attributed the softening of cheese over time to the hydrolysis of proteins in the cheese. Such proteolysis has been shown to affect the functional properties of Mozzarella cheese when it is heated (31, 32). Farkye et al. (13) reported an average decrease of 26.4% after 14 d of manufacture for the α_{s1}-casein band using urea-PAGE to assess proteolysis. Creamer (7) followed the proteolysis of Mozzarella, Cheddar, and Gouda cheese using gel electrophoresis and found that after 12 wk of storage, α_{s1}-casein in Mozzarella was greater than in Cheddar or Gouda cheeses. Lower levels of proteolysis in the Mozzarella were also indicated by the decrease in the amount of nonprotein and noncasein nitrogen (7). Creamer (7) attributed a greater amount of intact casein in Mozzarella to the lack of bacterial proteolytic ability resulting from the higher cook temperature used in the manufacture of Mozzarella. He also suggested a relationship between the presence of intact caseins and large peptides and the stretching properties of Mozzarella. Over a 6-wk storage period, low-fat, high-moisture Mozzarella will experience substantial proteolysis, with 40-50% of the α_{s1}-casein being broken down (19, 21, 37). The breakdown of α_{s1}-casein in reduced fat (< 10% fat), high-moisture Mozzarella cheese has been related to textural characteristics in the cheese, thereby, making the proteolysis of the caseins an important factor in the research and development of the textural properties of lower-fat Mozzarella (21, 36).

When MNFS in the cheese is high, proteolysis in the cheese is intensified, and the meltability of the Mozzarella cheese increases (38). Characterization of the components of

the cheese matrix in molecular terms will be necessary to bring about a better understanding of the mechanisms involved in casein breakdown and its influence on cheese texture (20).

Based on these observations, it should be feasible to improve the melting properties of low-fat Mozzarella cheese by using a more proteolytic culture system. Therefore, instead of having to age the low-fat Mozzarella for six or more weeks, a cheese with desired physical properties and functionality for use on pizza could be obtained in the same time that part-skim Mozzarella is aged (i.e., 1 to 2 wk). An increase in meltability at the time of manufacture proves to be a desirable characteristic when the cheese is manufactured for use on pizza. The storage time is greatly reduced, allowing the cheese to be used faster, similar to its full-fat counterpart (3).

During cheese ripening the predominate pathway leading to the breakdown of the caseins will involve rennet and plasmin, but only to a limited extent according to Visser (39). The proteinase/peptidase system of the starter as well as nonstarter bacteria are primarily responsible for the continued degradation of the polypeptides to small peptides and amino acids (39). The initial degradation of α_{s1}-casein seems to be directly affected by rennet, but the other casein fractions tend to be more resistant to this initial breakdown, particularly in the case of chymosin (18).

Use of Proteolytic Cultures in Cheese

Studies have shown that accelerated ripening accompanied by improved cheese body and texture in Cheddar Cheese resulted from the addition of thermophilic cultures used in conjunction with mesophilic starter cultures (29). Oberg et al. (32) demonstrated that differences in the proteolytic ability of the cultures used to manufacture Mozzarella cheese influenced the physical parameters of melt and stretch. Therefore, these physical parameters and their relationship to proteolytic activity have been the subject of much research (30). Cheese made with proteinase-positive (Prt^+) cultures had better melting characteristics than

cheese made with proteinase-deficient (Prt^d) cultures. After 14 d of storage the Prt^+ cheeses flowed 1.3 times further than the Prt^d cheeses (32). DiPalma et al. (12) manufactured Swiss cheese using strains of *Lactobacillus helveticus* varying in proteolytic ability and demonstrated that cheese made with the more proteolytic strains was soft and crumbly, but cheese made with the less proteolytic strains was firm and had a more elastic body. Casein hydrolysis was also accelerated when extracellular microbial proteinase was added to cheese (2). Investigation of the proteolytic enzyme systems in cheese starter cultures has been a major focus since the relationship between proteolytic ability of the cultures and body and texture in the cheese has been demonstrated (32). Characterization of the proteinases and peptidases in cheese starter and adjunct cultures is important because of the role these enzymes have in cheese flavor, texture, and body. Since these enzymes are diverse, different cultures may yield very different cheeses, making it difficult for manufacturers to produce consistent, good quality Mozzarella (28). In a study conducted by Oberg et al. (32) using thermolactic cultures, it was observed that differences in stretch properties were not as apparent based on the type of cultures used compared to the differences in melt properties. Some cheeses made using Prt^d cultures exhibited the same stretch properties as those made using Prt^+ cultures. Other Prt^+ cheeses had greater stretch (i.e., higher apparent viscosity) after 14 d of storage.

Measuring Proteolysis in Cheese

Analytical techniques including gas chromatography, mass spectrophotometry, HPLC, SDS-PAGE, and column chromatography have been depended on in the past for monitoring chemical changes in Cheddar during ripening (24). Such techniques as gel electrophoresis, gel filtration, ion exchange, and reversed-phase chromatography have been used to study degradation of α_{s1} and β-caseins by chymosin (17). Farkye et al. (13) used

urea-PAGE to illustrate the breakdown of α_{s1} and β-caseins in Mozzarella cheese by measuring the decreased intensity of the bands over 14 d of storage. DiMatteo et al. (11) were able to show about a 55.4% degradation of α_{s1}-casein to α_{s1}-I and α_{s1}-II caseins during Mozzarella storage using polyacrylamide gel electrophoresis. Disadvantages of the traditional protein separation techniques include long running times, difficulties with reproducibility in staining and gel properties, along with challenges in quantification (10).

A more recent technique now being employed in protein separation and analysis is free solution capillary electrophoresis (FSCE), which was first accomplished by Jorgenson and Lukacs (10). This technique is gaining widespread popularity as a tool in food analysis (10). The principle behind protein separation for FSCE involves endoosmotic flow, which is faster than electromigration. A high voltage is applied to the positive pole of the capillary, and the proteins are separated (10). The ability to directly quantify the eluted peaks in FSCE is a major advantage of this technique over SDS acrylamide gel electrophoresis (10), which is commonly performed for protein analysis (5). FSCE also has an advantage over HPLC since it has a high resolution potential, utilizes only a small amount of sample and buffer volumes, and the capillary tubes are inexpensive and easy to replace (33). Capillary electrophoresis is also a rapid technique with an average run time of only 20 min (4).

Kristiansen et al. (17) performed FSCE on caseins and casein hydrolysates prepared by the action of chymosin on αs and β purified caseins in solution. Results of their investigation indicated a good separation between the individual caseins. Peaks for κ-CN, β-CN, α_{s2}-CN, and α_{s1}-casein were identified. The κ-CN produced a double peak containing a narrow peak followed by a broader peak with one or two shoulders, while a tall, slender peak was caused by β-CN. Kristiansen et al. (17) concluded that this method was highly suitable for monitoring the formation of easily soluble peptides often lost in

traditional gel electrophoresis and that the method may be applied to identifying and measuring the peptides in cheeses.

REFERENCES

1 Alvarez, R. J. 1986. Expectations of Italian cheese in the pizza industry. Pages 130-131 *in* Proceedings 23rd Annual Marschall Invitational Italian Cheese Seminar., Marschall Products-Miles Laboratories Inc., Madison, WI.

2 Ardo, Y., and H. E. Petterson. 1988. Accelerated cheese ripening with heat treated cells of *Lactobacillus helveticus* and a commercial proteolytic enzyme. J. Dairy Res. 55:239-245.

3 Breene, W. M., W. V. Price, and C.A. Ernstrom. 1964. Manufacture of pizza cheese without starter. J. Dairy Sci. 47:1173-1180.

4 Cancalon, P. 1995. Capillary electrophoresis: a useful technique for food analysis. Food Tech. 49:52-58.

5 Cancalon, P., J. S. Elam, and L. M. Beidler. 1976. SDS gel electrophoresis of rapidly transported proteins in garfish olfactory nerve. J. Neurochem. 27:687-693.

6 Chen, C.M., M.E. Johnson, and J.J. Jaeggi. 1995. Manufacturing lower fat Mozzarella cheese by adding milk coagulant at different pH values. Pages 53-55 *in* Annual Report. Center for Dairy Research, University of Wisconsin, Madison.

7 Creamer, L. K. 1976. Casein proteolysis in Mozzarella-type cheese. N.Z. J. Dairy Sci. Technol. 11:130-131.

8 Creamer, L. K., and N. F. Olsen. 1982. Rheological evaluation of maturing Cheddar cheese. J. Food Sci. 47: 631-636.

9 DeJong, L. 1977. Protein breakdown in soft cheese and its relation to consistency. I. Proteolysis and consistency of "Noordhollanse Meshanger" cheese. Neth. Milk Dairy J. 30:242-253.

10 Deyle, Z., and R. Struzinsky. 1991. Capillary zone electrophoresis: its applicability and potential in biochemical analysis. J. Chromatogr. 569:63-122.

11 DiMatteo, M., G. Chiovitti, and F. Addeo. 1982. Variation in the composition of Mozzarella during storage. Sci. Tec. Latt. Cas. 33:197-213.

12 DiPalma, V. L., E. G. Hammond, and B. A. Glatz. 1987. Effect of lactobacilli on the properties of Swiss cheese. J. Dairy Sci. 70:733-737.

13 Farkye, N. Y., L. J. Kiely, R. D. Allshouse, and P. S. Kindstedt. 1991. Proteolysis in Mozzarella cheese during refrigerated storage. J. Dairy Sci. 74:1433-1438.

14 Fox, P. F. 1989. Proteolysis during cheese manufacture and ripening. J. Dairy Sci. 72:1379-1400.

15 Kok, J. 1990. Genetics of the proteolytic system of lactic-acid bacteria. FEMS Microbiol. Revs. 87:15-42.

16 Kosikowski, F.V. 1977. Cheese and Fermented Milk Foods. Edward Brothers, Ann Arbor, MI.

17 Kristiansen, K.R., J. Otte, M. Zakora, and K.B. Qvist. 1994. Capillary electrophoresis used to monitor the enzymatic hydrolysis of caseins and the fractionation of hydrolysis products. Milchwissenschaft. 49:683-688.

18 Lawrence, R. C., L. K. Creamer, and J Gillis. 1987. Texture development during cheese ripening. J. Dairy Sci. 70:1748-1760.

19 Malin, E. L., J. M. Banks, M. H. Tunick, A. J. R. Law, J. Leaver, and V. H. Holsinger. 1993. Texture enhancement of low-fat Mozzarella cheese by refrigerated storage. Int. Dairy J. 3:548.

20 Malin, E. L., and E. M. Brown. 1995. Influence of casein peptide conformations on the textural properties of cheeses. Pages 303-310 *in* Chemistry of Structure-Function Relationships in Cheese. E. L. Malin and M. H. Tunick, ed. Plenum Press, New York, NY.

21 Malin, E. L., M. H.Tunick, P. W. Smith, J. J. Sheih, B. C. Sullivan, and V. H. Holsinger. 1994. Effect of homogenization on the properties of low-fat Mozzarella cheese. Pages 487-493 *in* Cheese Yield and Factors Affecting Its Control. Int. Dairy Fed., Brussels.

22 Masi, P., and F. Addeo. 1986. An examination of some mechanical properties of a group of Italian cheese and their relation to structure and condition of manufacture. J. Food Eng. 5:217-229.

23 McMahon, D. J., C. J. Oberg, and W. McManus. 1993. Functionality of Mozzarella cheese. Aust. J. Dairy Technol. 48:99-104.

24 McSweeny, P. L. H., and P. F. Fox. Cheese: Methods of chemical analysis, Chapter 9 *in* Cheese: Chemistry, Physics and Microbiology, Vol. 1, P. F. Fox, ed. Chapman and Hall, London, 1993.

25 Mistry, V.V., and D.L. Anderson. 1993. Composition and microsctructure of commercial full-fat and low-fat cheeses. Food Struc. 12:259-266.

26 Merrill, R. K., C. J. Oberg, and D. J. McMahon. 1994. A method for manufacturing reduced fat Mozzarella cheese. J. Dairy Sci. 77:1783-1789.

27 National Academy of Sciences. 1989. Recommended Dietary Allowances. 10th ed. Natl. Acad. Press, Washington, DC.

28 Nauth, R. K., and D. Ruffie. 1995. Microbiology and biochemistry of reduced-fat cheese. Pages 345-357 *in* Chemistry of Structure-Function Relationships in Cheese. E.L. Malin and M. H. Tunick, ed., Plenum Press, New York, NY.

29 Oberg, C. J., and J. R. Broadbent. 1993. Thermophilic starter cultures: another set of problems. J. Dairy Sci. 76:2392-2406.

30 Oberg, C. J., R. K. Merrill, R. J. Brown, and G. H. Richardson. 1992. Effects of freezing, thawing, and shredding on low moisture, part-skim Mozzarella cheese. J. Dairy Sci. 75:1161-1166.

31 Oberg, C. J., R. K. Merrill, L. V. Moyes, R. J. Brown, and G. H. Richardson. 1991. Effects of *Lactobacillus helveticus* culture on the physical properties of Mozzarella cheese. J. Dairy Sci. 74:4101-4107.

32 Oberg, C. J., A. Wang, L. V. Moyes, R. J. Brown, and G. H. Richardson. 1991. Effects of proteolytic activity of thermolactic cultures on the physical properties of Mozzarella cheese. J. Dairy Sci. 74:389-397.

33 Otte, J., L. Midtgaurd, and K.B. Qvist. 1995. Analysis of caseinmacropeptide(s) by free solution capillary electrophoresis. Milchwissenschaft 50: 75-79.

34 Strickland, M., B. C. Weimer, and J. R Broadbent,. 1996. Capillary electrophoresis of cheddar cheese. J. Chromatogr. A, 731:305-313.

35 Tuckey, S. L. 1974. The phenomena of curd stringiness and matting. Paper No. 13 *in* Proceedings 11th Annual Marschall Invitational Italian Cheese Seminar Marschall Division, Miles Lab., Madison, WI.

36 Tunick, M. H., K. L. Mackey, J. J. Shieh, P. W. Smith, P. Cooke, and E. L. Malin. 1993. Rheology and microstructure of low-fat Mozzarella cheese. Int. Dairy J. 3:649-662.

37 Tunick, M. H., K. M. Mackey, P. W. Smith, and V. H. Holsinger. 1991. Effects of composition and storage on the texture of Mozzarella cheese. Neth. Milk Dairy J. 45:117-125.

38 Tunick, M. H., and J. J. Sheih. 1995. Rheology of reduced-fat Mozzarella. Pages 7-19 *in* Chemistry of Structure-Function Relationships in Cheese. E. L. Malin and M. H. Tunick, ed., Plenum Press, New York, NY.

39 Visser, S. 1981. Proteolytic enzymes and their action on milk proteins. Neth. Milk Dairy J. 35:65-88.

CHAPTER 2

EFFECTS OF PROTEOLYTIC ADJUNCT CULTURE ON THE PHYSICAL PROPERTIES OF LOW-FAT MOZZARELLA CHEESE

ABSTRACT

As fat is removed from Mozzarella cheese, the resulting increase in protein concentration causes the cheese to become tough, thus decreasing the desired physical characteristics of meltability and stretch. Low-fat (6% fat) Mozzarella cheese was manufactured with the addition of several levels of a *Lactococcus lactis* adjunct culture that was proteinase positive and lactose deficient in an attempt to improve these physical properties. During cheese manufacture, milk was acidified to pH 6.0, then inoculated with *Lactobacillus helveticus* and *Streptococcus thermophilus*. Experimental vats were also inoculated with either 0.25, 0.50, or 1.0% of the adjunct culture. Cheeses made with the adjunct culture had increased melt properties at d 1. During the first 14 d of storage, cheese manufactured with 0.50% and 1.0% adjunct culture melted more readily than the control; by 28 d, the meltability of all cheeses was similar. Breakdown of cheese body was more rapid in the experimental cheeses and was particularly apparent during shredding. This increase in softness was presumed to be the result of increased proteolysis in the cheeses. There were no significant differences in melt viscosity between control and experimental cheeses. Storage time, however, was significant and between d 14 and d 28, melt viscosity decreased for all cheeses. Protein hydrolysis was measured using SDS-PAGE, but no differences were observed in disappearance of intact caseins.

INTRODUCTION

Recent interest by consumers to reduce the amount of fat in their diets (12) has created a demand for lower fat dairy products, including cheese. Concurrently, an increased popularity of pizza has caused a steady rise in consumption and production of Mozzarella cheese (10). These factors have prompted researchers to develop a low-fat Mozzarella cheese with appropriate functionality for use on pizza.

However, textural characteristics and melting properties of cheese are affected by lowered fat content (17, 19). Toughness increases as fat is removed from Mozzarella cheese because protein concentration increases and changes occur in distribution of moisture within the protein matrix (11). Desirable properties of Mozzarella cheese are medium firmness, sufficient melt, adequate stretchability, and ease of shredding (1). These properties vary with age, pH, moisture, and salt content of the cheese, as well as the type of starter culture (11). Tunick et al. (19) showed that reduced-fat (9% fat) Mozzarella cheese acquired the physical characteristics comparable to part-skim Mozzarella cheese only after 6 wk of refrigerated storage.

DeJong (7) attributed the softening of cheese over time to hydrolysis of proteins in the cheese. Such proteolysis has been shown to affect the functional properties of Mozzarella cheese when it is heated (14, 15). Creamer (6) studied proteolysis of Mozzarella, Cheddar, and Gouda cheese and observed that after 12 wk of storage, α_{s1}-CN in Mozzarella was greater than in Cheddar or Gouda cheeses. Farkye et al. (9) reported an average decrease of 26% of intact α_{s1}-CN in Mozzarella cheese after 14 d of storage using urea-PAGE. It has also been shown (3) that casein hydrolysis can be accelerated by adding extracellular microbial proteinases to cheese.

Tunick et al. (18) attributed improved physical properties of reduced-fat (10% fat) Mozzarella cheese after 6 wk storage to degradation of α_{s1}-CN. Oberg et al. (15) showed

that differences in the proteolytic ability of cultures used to manufacture Mozzarella cheese influenced cheese melt and stretch. Cheese made with proteinase-positive (Prt^+) cultures had better melting characteristics than cheese made with proteinase-deficient (Prt^d) cultures. After 14 d of storage the Prt^+ cheeses flowed 1.3 times further than the Prt^d cheeses (15). It is typically observed that during storage, melt and stretch are inversely related: melt increases during the first 14 d and stretch decreases (14). Differences in stretch (i.e., apparent viscosity) properties were not so dependent on whether Prt^+ or Prt^d cultures were used.

Based on these observations, it should be feasible to improve the melting properties of low-fat Mozzarella by using a more proteolytic culture system. Instead of having to age low-fat Mozzarella for six or more weeks, a cheese with desired properties could be obtained in the same time that part-skim Mozzarella is aged (i.e., 1 to 2 weeks). The objective of this study was to increase the proteolytic activity in low-fat Mozzarella cheese by adding a Prt^+, lactose-deficient (Lac^d) adjunct culture. We then determined whether this improved the initial physical properties of low-fat Mozzarella cheese so that it does not have to be aged 6 wk before it is ready for use on a pizza.

MATERIALS AND METHODS

Milk and Cultures

Skim milk and cream, pasteurized at 80°C for 29 s, then cooled to 4°C, were obtained from the Richardson Dairy Products Laboratory (Utah State University, Logan). Milk was standardized to 0.6% fat by blending pasteurized cream and skim milk in the desired proportions. Starter cultures were direct-vat set, lyophilized cultures consisting of *Streptococcus thermophilus* TA061 and *Lactobacillus helveticus* LH100 (Rhodia, Marschall Products, Madison, WI). Cultures were weighed individually into sterile containers and stored at -20°C prior to use. Direct-vat set adjunct culture consisting of Prt^+

Lac^d strains of *Lactococcus lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris* (CR213 ; Chr. Hansen Laboratories, Milwaukee, WI) was frozen and stored at -70°C. Adjunct culture was weighed into sterile containers and added directly to cheese vats 55 min after starter culture was added.

To standardize manufacturing time from rennet addition to stretching the curd, starter culture/adjunct culture combinations were adjusted to provide equivalent acidification rates during cheese manufacture. Inoculations levels selected were 0.015% (approximately 0.9 activity units) of *L. helveticus* LH100 and 0.015% (approximately 0.6 activity units) of *S. thermophilus* TAO61 for the control vat, while the vats that were to receive 0.25, 0.50, or 1.0% adjunct culture were inoculated with 0.009%, 0.007%, or 0.005% of *L. helveticus* LH100 and 0.009%, 0.007% or 0.005% of *S. thermophilus* TAO61, respectively.

Cheese Manufacturing Procedure

Ten-kilogram samples of standardized milk were placed into each of four stainless steel vats (35 x 22 x 22 cm). Milk (4°C) was pre-acidified to pH 6.0 with acetic acid diluted 1 to 10 with distilled water. Vats were placed in a water bath and heated to 34°C. Starter culture was added and the milk ripened for 55 min at 34°C, then the adjunct was added if required, and the milk was clotted with 0.75 ml of double strength rennet (Chymax; Pfizer Dairy Products Division, Pfizer Inc., Milwaukee, WI) diluted in 10 ml of water. Curd was cut (25 min after set) using 1.9-cm knives. After healing for 15 min, curd was gently stirred for 30 s every 15 min. The temperature of the whey and curd was increased to 38°C over 10 min, and then one-half of the whey (5 kg) was drained from each vat. Curd was left in the remaining whey (with occasional stirring) at 38°C until the curd reached pH 5.3, then the remainder of the whey was drained. The curd remained in the vat at room temperature until the pH reached 5.2, then 10 g of salt was added. The curd was hand stretched in a hot brine solution (5% NaCl) at 82°C for 3 min until smooth and elastic, placed into stainless

steel molds (9 x 9 x 9 cm), then cooled in an ice bath for 1 h. Cheese blocks were removed from the molds, individually vacuum-sealed and stored at 4°C. On d 1, 7, 14, and 28, samples were removed for analysis with the remaining cheese vacuum-sealed and stored at 4°C.

Proximate Analysis

One day after manufacture, cheese was analyzed for fat by the Babcock method (16), proteins by the Kjeldahl method (2), and moisture by vacuum oven at 100°C for 16 to 18 h (16). Cheese samples (2.0 g) were placed in aluminum weigh pans and held under vacuum at 100°C for 16 to 18 h, allowed to cool to room temperature in a desiccator, and reweighed to determine moisture loss.

Gel Electrophoresis

Twenty-milligram samples of cheese from each sample block were prepared on d 1, 7, 14, and 28 by addition of 1 ml of Tris buffer (10 m*M* Tris, and 1 m*M* EDTA, pH 8.0), 350 μl of SDS, and 50 μl of 2-mercaptoethanol. Samples were then placed in boiling water for 5 min, mixed by vortexing for 2 to 3 s, and boiled for an additional 5 min. This process was repeated until cheese samples were completely in solution. One-dimensional SDS-PAGE was performed using the PhastSystem™ (Pharmacia LKB Biotechnology, Piscataway, NJ) on PhastGel™ (homogeneous 20) gels. Bromophenol blue (3 μl) was added to the sample as a tracking dye before electrophoresis. Skim milk, centrifuged at 2000 rpm for 10 min to remove remaining fat, served as a control. Gels were stained with Coomassie Blue (PhastGel™ Blue R, Pharmacia LKB Biotechnology AB Uppsala, Sweden), destained, dried, and scanned into a computer using PhastImage™ software (Pharmacia LKB Biotechnology).

Viscosity Test

A Brookfield DV II + helipath viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA) equipped with a T-bar spindle (T-F with a 9.1-mm crossbar) was submerged into a 25 x 150-mm test tube containing 15 g of melted cheese (tempered for 10 min at 60°C) held in a jacketed water bath (60°C). The viscometer speed was set at 1.5 rpm, and when the peak measurement was achieved, the helipath was set in motion. As the helipath raised the T-bar spindle out of the test tube, an IBM-compatible computer equipped with DV Gather + version 1.0 (Brookfield Engineering Laboratories, Inc.) was set to read the apparent viscosity (AV_{60}) every 5 s for 10 min. Apparent viscosity of the cheese was determined by taking the average value of AV_{60} between 30 s and 90 s while the spindle was still completely submerged in the cheese.

Melt Test

Fifteen-gram samples of cheese were placed into 24-cm long glass tubes and packed to 4 cm in height. Tubes were stoppered at both ends, horizontally positioned on a stainless steel rack, and held at 4°C for 30 min. Samples were then placed in a convection oven for 1 h at 135°C. Following a period of cooling to room temperature, the distance the melted cheese traveled was measured from the end of the stopper to the tip of the melted cheese.

Statistical Analysis

The experiment was designed as a split-plot, randomized block with repeated measures using three replications. Analysis of variance was calculated using Minitab 7.2 (Minitab Inc., State College, PA) for the dependent variables of melt and viscosity as measured on d 1, 7, 14, and 28. Analysis of variance for melt and viscosity was also calculated for individual day and treatment. The means were compared using least significance difference, and significance was declared at $P \leq 0.05$.

RESULTS

Cheese Composition

Moisture contents of the cheeses manufactured using the adjunct culture were slightly higher than were moisture contents of the control cheeses as shown in Table 2. This occurred even though manufacturing times (set to stretch) for the cheeses were consistent with industry practices, ranging from 2.3 to 2.5 h. Larger differences in moisture content were observed in preliminary trials in which starter culture inocula were not adjusted when the adjunct culture was used and manufacturing times were ≤ 2 h. Total protein of the cheeses made with adjunct culture was slightly lower than the control cheese but consistent with the increased moisture.

Cheese Melting

There was no overall significant difference between treatments for cheese melt as illustrated in Table 3. Also, because the cheese was sampled in a way that required a repeated measures design to be used, no statistical difference between storage times was observed.

TABLE 2. Composition and manufacturing time (renneting to stretching) for low-fat Mozzarella cheese made with different levels of proteinase-positive adjunct culture.

	Moisture		Fat		Protein		Time	
Adjunct level	$\overline{X}$	SEM	$\overline{X}$	SEM	$\overline{X}$	SEM	$\overline{X}$	SEM
	(%)						(min)	
0	59.6	0.5	7.0	0.3	25.2	0.3	150	10
0.25	60.8	1.4	6.4	0.1	24.1	0.8	138	9
0.50	62.8	0.6	6.2	0.2	22.8	0.7	138	9
1.00	61.7	1.3	6.2	0.1	23.4	1.4	144	10

TABLE 3. Analysis of variance for melt and viscosity in low-fat Mozzarella cheese made with different percentages of Prt^+ Lac^d adjunct culture.

Source of variation	df	Melt		Viscosity	
		MS	F	MS $(x10)^{12}$	F
Replicate (R)	2	9.69 ^{NS}	.893	4.91 *	8.88
Treatment (T)	3	11.50 ^{NS}	1.06	0.254 ^{NS}	0.46
T x R (error A)	6	10.85	5.93	0.553	0.67
Day (D)	3	34.06 ^{NS}	2.27	150.53 ***	40.7
R x D (error B)	6	15.00	8.20	3.70	4.51
T x D	9	2.14 ^{NS}	1.17	2.02 ^{NS}	2.46
R x T x D (error C)	18	1.83	5.98	0.82	2.89
Corrected total	47				

^{NS} $P > 0.05$.

* $P \leq 0.05$.

*** $P \leq 0.001$.

When melting behavior at individual days was compared, there was some significant differences throughout the 28 d storage period (Table 4). Cheese made with 0.50% adjunct culture had the highest melt score, but by 28 d, the difference between the control and experimental cheeses was smaller although it was still statistically significant. The advantage of using the Prt^+ Lac^d adjunct culture was that cheese made with adjunct culture had melting properties after 7 d of storage that were similar to the melt properties of the control after 14 d storage.

Cheese Viscosity

Addition of adjunct culture had only a minor influence on viscosity of melted cheese, and was not significantly different (Tables 3 and 5). Storage time significantly affected AV_{60} (Table 3) although all the cheeses at d 1, 7, and 14 had high viscosity. A significant decrease in AV_{60} occurred between d 14 and d 28 (Table 5). Such decreases in AV_{60} of melted cheese during storage have been reported for part-skim Mozzarella (14, 15) and reduced fat Mozzarella (11). For part-skim Mozzarella this decrease in apparent viscosity usually occurs between d 1 and d 14 after manufacture.

TABLE 4. Meltability of low-fat Mozzarella cheese made with different levels of Prt+ adjunct culture during 28 d storage at 4°C.

Adjunct level	Melt			
	d 1	d 7	d 14	d 28
(%)	---------- (cm) ----------			
0	10.2^{aA}	11.0^{aB}	12.8^{aC}	11.3^{bB}
0.25	10.9^{bA}	12.6^{bB}	12.7^{aB}	10.5^{aA}
0.50	11.5^{cA}	13.8^{cB}	14.8^{cC}	11.1^{bA}
1.00	11.5^{cA}	12.8^{bB}	13.9^{bC}	11.8^{cA}

$^{a, b, c}$Means within column with different superscripts differ ($P \leq 0.05$)
LSD: d1 = 0.396, d7 = 0.837, d14 = 0.325, d28 = 0.319

$^{A, B, C}$ Means within row with different superscripts differ ($P \leq 0.05$)
LSD: 0 = 0.518, 0.25 = 0.633, 0.50 = 0.510, 1.0 = 0.618

Cheese Shredding

When cheese was shredded by hand in preparation for the melt test it was observed that during storage all of the cheeses became softer and more difficult to shred. By 28 d the control cheese was still shreddable, whereas, by 14 d the adjunct cheeses were sticky and difficult to shred. The softest cheese was that made with 0.5% adjunct and also had the highest moisture content. The cheese made with 0.25% adjunct was also too soft for shredding by 14 d even though the moisture content was only 1.2% greater than the control. Curiously, in other experiments we have conducted (data not shown), low-fat Mozzarella with up to 63% moisture has been made, and this stickiness problem was not observed.

Proteolysis

Gels from SDS-PAGE analysis of the protein breakdown in the cheeses between 1 d and 28 d of refrigerated storage showed the disappearance of the α_{s1}-CN band in the

TABLE 5. Apparent viscosity of low-fat Mozzarella cheese made with different levels of Prt+ adjunct culture during 28 d storage at 4°C.

Adjunct Level	Apparent Viscosity			
	d 1	d 7	d 14	d 28
(%)	----------(cp x 10^6)----------			
0	7.1^{aA}	6.2^{aB}	6.9^{aC}	1.5^{bD}
0.25	6.6^{aA}	7.4^{bB}	6.0^{bC}	1.2^{aD}
0.50	6.5^{aA}	7.5^{bB}	6.0^{bA}	1.4^{bC}
1.00	6.4^{aA}	7.1^{bB}	6.1^{bA}	2.6^{cC}

$^{a, b, c}$Means within column with different superscripts differ ($P \leq 0.05$)
LSD: d1 = 8.42, d7 = 4.88, d14 = 4.53, d28 = 2.09

$^{A, B, C}$ Means within row with different superscripts differ ($P \leq 0.05$)
LSD: 0 = 4.23, 0.25 = 2.68, 0.50 = 7.64, 1.0 = 6.02

control cheese (Figure 2) as well as in the cheese made with 0.50% adjunct culture (Figure 3). The appearance of a band between the α_{s1}-CN and β-CN bands was clearly visible and became more prominent over the 28 d storage period. This band was assumed to be α_{s1}-I CN, which would be consistent with the results of Tunick et al. (18) showing that α_{s1}-CN in fresh Mozzarella cheese is broken down into α_{s1}-I CN and other peptides during storage. However, no differences in proteolysis were observed in the SDS-PAGE gels between experimental and control cheeses at 28 d of storage (Figure 4).

DISCUSSION

Manufacturing Procedure

The melt performance of the low-fat cheeses, especially the experimental cheeses, was of similar magnitude to part-skim Mozzarella cheeses we have made in previous experiments (11) even though the low-fat cheeses contained only 6 to 7% fat. This was

attributed to maintaining a high moisture content in the fat-free component (64 to 67%) of the low-fat cheeses. Part-skim Mozzarella cheese containing 20% fat would have moisture in the fat-free component of 60 to 65% if it contained 48 to 52% moisture. Tunick et al. (19) have shown that improved functional properties of reduced-fat (10% fat) Mozzarella cheese can be obtained if the cheese is made with an increased moisture content. Their reduced-fat cheese with higher moisture content (55 to 59%) melted better than reduced-fat cheese with lower moisture levels and had similar melt properties to part-skim Mozzarella with comparable moisture. Also, adding the milk coagulant at a lower pH resulted in cheese

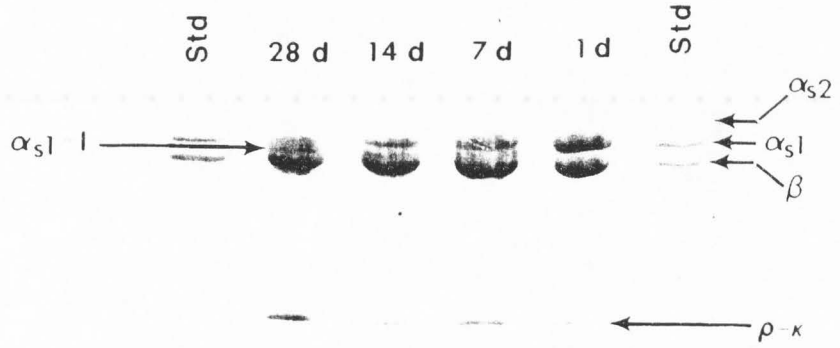

Figure 2. SDS-PAGE electropherogram of control low-fat Mozzarella cheese illustrating caseins present at 1, 7, 14, and 28 d. The gel was run from top (-) to bottom (+). Skim milk was used as a standard.

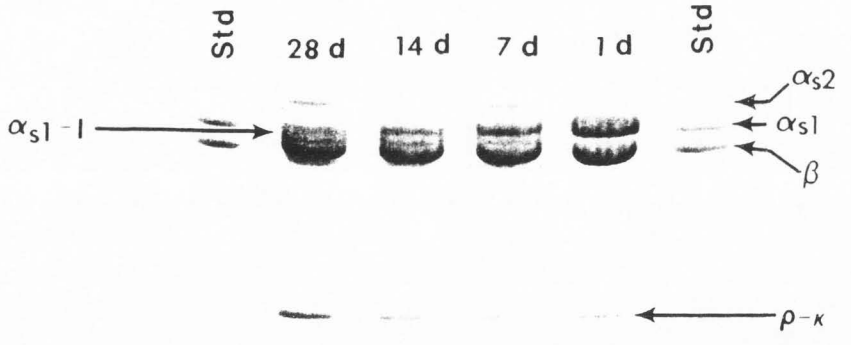

Figure 3. SDS-PAGE electropherogram of low-fat Mozzarella cheese made with 0.50% inoculum of proteinase-positive adjunct culture illustrating the caseins present at 1, 7, 14, and 28 d. The gel was run from top (-) to bottom (+). Skim milk was used as a standard.

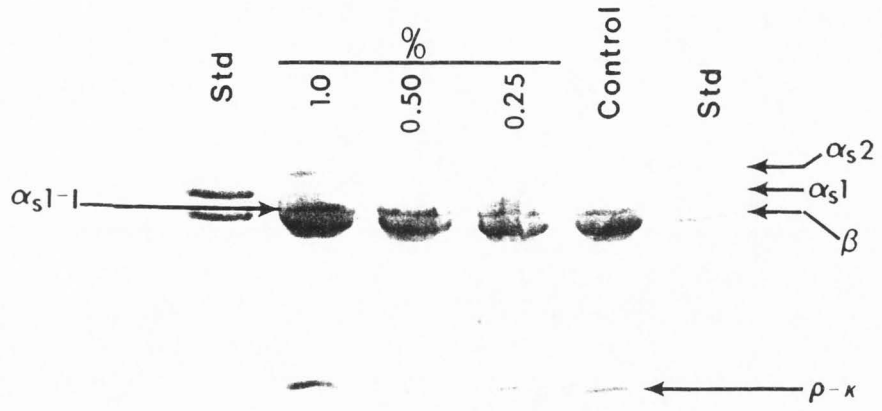

Figure 4. SDS-PAGE electropherogram of low-fat Mozzarella cheese made with 0.25, 0.50, and 1.0% inoculum of adjunct culture or with starter culture only (control) at 28 d. The gel was run from top (-) to bottom (+). Skim milk was used as a standard.

with a higher moisture content, which was attributed to increased meltability and improved functionality (5).Using a low cook temperature is necessary to make higher moisture cheeses, and this would also facilitate survival of rennet, starter, and adjunct cultures in the cheese curd, which should enhance breakdown of α_{s1}-CN, thereby improving melt properties (18).

Even after adjusting the levels of starter and adjunct cultures there were still some slight differences in manufacturing times (Table 3). The largest difference was between the control cheese with a manufacturing time of 2 h 30 min and the cheese made with 0.50% adjunct, which reached a pH of 5.2 about 15 min sooner. There was a 3% difference in moisture content between these cheeses. This was probably not a function of manufacturing time because the cheese made with 0.25% adjunct also had a shorter manufacturing time but had only a moisture content of 60.8% (Table 3). In preliminary trials where starter culture inoculation was kept consistent, manufacturing time was reduced

by 90 min when 1.0% adjunct culture was added (from 4 h to 2.5 h), which resulted in a 4.5% increase in cheese moisture content. Thus, it is unlikely that the 3% increase in moisture obtained in this study was a result of differences in manufacturing time. A difference of 15 min would be expected to change moisture content by less than 1%.

Cultures

Initially the adjunct culture was obtained because it reportedly did not utilize lactose, (i.e., classified as lactose negative) and was not expected to influence acid development during cheese manufacture. However, it became apparent that acid production during cheese manufacture was increased when starter cultures were supplemented with the adjunct culture. When grown in nonfat UHT milk, the adjunct cultures produced far less acid than the starter cultures grown under the same conditions (data shown in preliminary results). Consequently, we classified them as Lac^d rather than lactose negative and adjusted starter culture inocula accordingly so as to obtain comparable manufacturing times.

The influence of these Prt^+ Lac^d adjunct cultures on starter culture performance may have been a consequence of either their acid production or their proteolytic ability. The adjunct cultures may have utilized a small amount of lactose (or galactose) for its growth thus increasing the overall rate of acid production during cheese making. An alternative explanation may be that because the adjunct cultures were more proteolytic, they provided additional peptides and amino acids to stimulate growth of the starter (13), thereby increasing the rate of acid production during cheese manufacture.

Proteolysis

Differences in melting and shredability between the control and adjunct cheeses indicate that adding the Prt^+ Lac^d adjunct culture had changed the physical characteristics of the low-fat Mozzarella cheese. Such changes could not be explained by differences in moisture

content of the cheeses. Increased meltability of Mozzarella cheese during aging has been associated with breakdown of caseins (specifically α_{s1}-CN) (9, 19) and so has softening of cheese body (7, 8). Although no quantitative differences in the SDS-PAGE gel patterns were observed (Figure 4) this may not mean that the protein breakdown patterns were identical. There was too much overlap between the α_{s1}-CN and α_{s1}-I CN bands of the SDS-PAGE gels to differentiate between them using densitometry. Methods other than SDS-PAGE for detecting protein breakdown may be more successful in visualizing differences in proteolysis patterns.

Melt

The increased melting displayed by the adjunct-treated cheeses directly after manufacture is a desirable characteristic because it would reduce the storage time required for low-fat Mozzarella cheese before it was ready for use on pizza (4, 19). Cheeses made with the Prt^+ Lac^d adjunct culture were observed as softer than the control cheese from 1 d, and this softness continued to increase throughout the storage period. Softness of body was particularly apparent when the cheeses were shredded for use on pizza.

CONCLUSIONS

We modified the Utah State University reduced-fat Mozzarella manufacturing procedure to produce a low-fat (6% fat) Mozzarella cheese containing a Prt^+ Lac^d adjunct culture. The moisture content of these cheeses ranged from 61 to 63%, and the protein content ranged from 23 to 24%. During the first 2 wk of storage, use of a 0.50% adjunct culture increased the meltability of low-fat Mozzarella cheese. This may make it possible to reduce the storage time necessary to achieve melt properties suitable for use on pizza. However, during storage these cheeses became very soft, sticky, and difficult to shred, much faster

than cheese made without adjunct culture. As storage time was increased past 14 d, differences in melt were less apparent. These changes in melt properties could not be related to disappearance of intact α_{s1}-CN. Optimizing the adjunct culture inoculum level or selecting other cultures with enhanced proteolytic properties may allow melting to be improved while not inducing excessive body softening. More sensitive measures of protein breakdown may also provide elucidation of what changes occur in cheese proteins during storage.

REFERENCES

1 Alvarez, R. J. 1986. Expectations of Italian cheese in the pizza industry. Page 130 *in* Proceedings 23rd Annual Marschall Invitational Italian Cheese Seminar, Marschall Products-Miles Laboratories Inc., Madison, WI.

2 Association of Analytical Chemists. 1990. Official Methods of Analysis. 15th ed. AOAC, Arlington, VA.

3 Ardo, Y., and H. E. Petterson. 1988. Accelerated cheese ripening with heat treated cells of *Lactobacillus helveticus* and a commercial proteolytic enzyme. J. Dairy Res. 55:239-245.

4 Breene, W. M., W. V. Price, and C. A. Ernstrom. 1964. Manufacture of pizza cheese without starter. J. Dairy Sci. 47:1173-1180.

5 Chen, C.M., M.E. Johnson, and J.J. Jaeggi. 1995. Manufacturing lower fat Mozzarella cheese by adding milk coagulant at different pH values. Pages 53-55 in Annual Report, Center for Dairy Research, Univ. of Wisconsin, Madison, WI.

6 Creamer, L. K. 1976. Casein proteolysis in Mozzarella-type cheese. N.Z. J. Dairy Sci. Technol. 11:130-131.

7 DeJong, L. 1977. Protein breakdown in soft cheese and its relation to consistency. I. Proteolysis and consistency of "Noordhollanse Meshanger" cheese. Neth. Milk Dairy J. 30:242-253.

8 DiPalma, V. L., E. G. Hammond, and B. A. Glatz. 1987. Effect of lactobacilli on the properties of Swiss cheese. J. Dairy Sci. 70:733-737.

9 Farkye, N. Y., L. J. Kiely, R. D. Allshouse, and P. S. Kindstedt. 1991. Proteolysis in Mozzarella cheese during refrigerated storage. J. Dairy Sci. 74:1433-1438.

10 McMahon, D. J., C. J. Oberg, and W. McManus. 1993. Functionality of Mozzarella cheese. Aust. J. Dairy Technol. 48:99-104.

11 Merrill, R. K., C. J. Oberg, and D. J. McMahon. 1994. A method for manufacturing reduced fat Mozzarella cheese. J. Dairy Sci. 77:1783-1789.

12 National Academy of Sciences. 1989. Recommended Dietary Allowances. 10th ed. Natl. Acad. Press, Washington, DC.

13 Oberg, C. J., and J. R. Broadbent. 1993. Thermophilic starter cultures: another set of problems. J. Dairy Sci. 76:2392-2406.

14 Oberg, C. J., R. K. Merrill, L. V. Moyes, R. J. Brown, and G. H. Richardson. 1991. Effects of *Lactobacillus helveticus* culture on the physical properties of Mozzarella cheese. J. Dairy Sci. 74:4101-4107.

15 Oberg, C. J., A. Wang, L. V. Moyes, R. J. Brown, and G. H. Richardson. 1991. Effects of proteolytic activity of thermolactic cultures on the physical properties of Mozzarella cheese. J. Dairy Sci. 74:389-397.

16 Richardson, G. H., ed. 1985. Standard Methods for the Examination of Dairy Products. 15th ed. Am. Pub. Health Assoc., Washington DC.

17 Tuckey, S. L. 1974. The phenomena of curd stringiness and matting. Paper 13 *in* Proceedings 11th Annual Marschall Invitational Italian Cheese Seminar Marschall Division, Miles Lab., Madison, WI.

18 Tunick, M. H., K. L. Mackey, J. J. Shieh, P. W. Smith, P. Cooke, and E. L. Malin. 1993. Rheology and microstructure of low-fat Mozzarella cheese. Int. Dairy J. 3:649-662.

19 Tunick, M. H., K. M. Mackey, P. W. Smith, and V. H. Holsinger. 1991. Effects of composition and storage on the texture of Mozzarella cheese. Neth. Milk Dairy J. 45:117-125.

CHAPTER 3

MONITORING PROTEOLYSIS IN PART-SKIM AND LOW-FAT MOZZARELLA CHEESE BY CAPILLARY ELECTROPHORESIS

ABSTRACT

Part-skim (18% fat) Mozzarella cheese was manufactured from milk standardized to a casein-to-fat ratio of 1.2 and inoculated with *Lactobacillus helveticus* and *Streptococcus thermophilus* starter cultures. Low-fat (6% fat) Mozzarella cheese was manufactured from milk with a casein-to-fat ratio of 4.2 and inoculated with the same starter cultures with (or without) addition of a proteinase-positive, lactose-deficient adjunct culture. The cheese was molded into 1.5-lb blocks and stored at 4°C. Meltability and melt viscosity of the cheese were measured during 28 d of storage. Disappearance of α_{s1}-CN and β-CN was measured using free solution capillary electrophoresis. Micellar electrokinetic capillary chromatography was used to study the appearance of small peptides (<30 kDa) during storage. There were significant decreases in the amount of intact α_{s1}-CN remaining after 28 d, but no measurable change in β-CN in either the part-skim or low-fat cheeses. In the part-skim cheese, 71% of the α_{s1}-CN remained, while in the low-fat control cheeses only 20% of the α_{s1}-CN remained after 28 d. Use of the adjunct culture in the low-fat cheese resulted in only 14% of the α_{s1}-CN remaining after 28 d. Micellar electrokinetic capillary chromatography showed a similar increase in proteolysis in the low-fat cheeses based on the increased concentration of small peptides produced. Increased moisture content of the low-fat cheese (61%) compared to the part-skim cheese (51%) may account for some of the differences observed. During storage, part-skim Mozzarella showed a typical increase in melt with a corresponding decrease in melt viscosity. Melt distance increased from 10.6 cm at d 1 to 16.9 cm at d 28, while melt viscosity (at 80°C) decreased from 1.0×10^6 cP to 2.1

x 10^5 cP. There was less change measured in the low-fat cheese during storage, with melt distance increasing from 8.9 cm at d 1 to 10.9 cm at d 28, and melt viscosity decreasing from 4.8 x 10^5 cP to 1.9 x 10^5 cP. Adding the adjunct culture increased initial meltability of the low-fat cheese by accelerating proteolysis during the first 14 d, but caused an increase in melt viscosity and decrease in melt after 14 d.

INTRODUCTION

There is a significant relationship between casein proteolysis and textural characteristics in cheese (9). Breakdown of α_{s1}-CN in reduced fat (< 10% fat), high-moisture Mozzarella cheese has been related to changes in the textural characteristics of the cheese, thereby making casein proteolysis an important factor in the development of acceptable textural properties of lower fat Mozzarella (14, 25). Proteolysis of caseins starts during curd formation, then continues through refrigerated storage of the cheese, including non-ripened cheeses such as low-fat, high-moisture Mozzarella (26). Creamer (4) followed the proteolysis of Mozzarella, Cheddar, and Gouda cheeses using gel electrophoresis and found after 12 wk of storage a greater amount of α_{s1}-CN present in the Mozzarella than in either Cheddar or Gouda cheese.

Changes in stretch and melt of Mozzarella cheese have also been associated with proteolysis during aging (20, 21). Oberg et al. (21) demonstrated that changes in the proteolytic ability of cultures used in the manufacture of Mozzarella cheese influences melt and stretch characteristics. When MNFS in the cheese is high, proteolysis in the cheese is intensified, and the meltability of Mozzarella cheese is increased (28). DiPalma et al. (6) manufactured Swiss cheese using strains of *Lactobacillus helveticus* varying in proteolytic ability and demonstrated that cheese made with more proteolytic strains was soft and crumbly, but cheese made with less proteolytic strains was firm with a more elastic body.

Textural characteristics, as well as melt properties, are significantly affected by decreasing the fat content in Mozzarella cheese (24, 26). Medium firmness, sufficient melt, adequate stretchability, and ease of shredding are some of the most valuable properties of Mozzarella cheese (1). These properties are contingent on the age of the cheese, curd pH, moisture content, salt content, and type of starter culture used (18). An increase in toughness can be observed as fat is removed from Mozzarella cheese; this is a result of increased protein concentration in the cheese as well as differences in moisture distribution within the cheese matrix (18). Tunick et al. (26) showed that reduced-fat (<10% fat), high-moisture Mozzarella was not comparable in physical characteristics to part-skim Mozzarella cheese until after 6 wk of refrigerated storage. Over a 6-wk storage period, low-fat, high-moisture Mozzarella experiences substantial proteolysis, with 40-50% of the α_{s1}-CN being broken down (12, 26). A better understanding of the biochemical reactions during cheese maturation is necessary in the development of solutions to problems associated with low-fat cheese (23).

In most bacteria-ripened cheeses the first casein to be hydrolyzed is α_{s1}-CN with little initial reduction in β-CN and para-κ-CN (19). Plasmin, which naturally occurs in milk, and chymosin, which is added to the milk during cheese making, both contribute to the formation of peptides during proteolysis (13). Chymosin- and plasmin-hydrolyzed caseins provide substrates for starter culture proteases and peptidases, which produce peptides and amino acids necessary for bacterial metabolism (11). There is widespread acceptance that the indigenous milk enzymes, rennet, and starter and non-starter bacteria all play a part in the flavor development and maturation of cheese (19).

Characterization of cheese matrix components in molecular terms is necessary to better understand the mechanisms involved in casein breakdown and its influence on cheese texture (13). Analytical techniques including gas chromatography, mass

spectrophotometry, HPLC, PAGE, and column chromatography have been used in the past for monitoring chemical changes in cheese during ripening (17). Disadvantages of these traditional protein separation techniques include long running times, difficulties with reproducibility in staining and gel properties, along with challenges in quantification (6). A technique now being employed in protein separation and analysis is free solution capillary electrophoresis (FSCE). This technique is gaining widespread popularity as a tool in food analysis. The principle behind protein separation for FSCE involves endoosmotic flow, which is faster than electromigration. A high voltage is applied to the positive pole of the capillary, and the proteins are separated (5). The ability to directly quantify the eluted peaks in FSCE is a major advantage of this technique over SDS acrylamide gel electrophoresis, which has typically been used to monitor proteolysis in Mozzarella cheese (7). Capillary electrophoresis is also a more rapid technique with an average run time of only 20 min (3).

The objective of this study was to determine if capillary electrophoresis could be used as a more sensitive analytical technique than conventional gel electrophoresis to monitor proteolysis and its relation to changes in the functional properties of part-skim Mozzarella and low-fat Mozzarella cheese during maturation.

MATERIALS AND METHODS

Milk and Cultures

Low-fat Mozzarella. Skim milk and cream obtained from the G.H. Richardson Utah State University Dairy Products Laboratory were pasteurized at 80°C for 29 s then cooled to 4°C. Milk was standardized to 0.6% fat by blending pasteurized cream and skim milk in the desired proportions. Starter cultures were direct-vat-set, lyophilized cultures consisting of *Streptococcus thermophilus* TA061 and *Lactobacillus helveticus* LH100 (Rhodia, Marschall Products, Madison, WI). Cultures were weighed individually into sterile containers and stored at -20°C prior to inoculation. Direct-vat-set adjunct cultures

consisting of proteinase positive (Prt^+) lactose deficient (Lac^d) strains of *Lactococcus lactis* ssp *lactis* and *Lactococcus lactis* ssp *cremoris* blend CR213 (Chris Hansen Laboratories, Milwaukee, WI) were frozen and stored at -70°C. Adjunct culture was weighed into sterile containers and added directly to cheese vats 55 min after starter culture had been added.

Part-skim Mozzarella. Raw milk standardized to a casein/fat ratio of 1.2 was vat pasteurized at 63°C for 30 min then cooled to 34°C. The same starter cultures were used for the manufacture of low-fat Mozzarella as described above.

Mozzarella Manufacturing Procedure

Low-fat Mozzarella. Ten-kilogram samples of standardized milk were placed into each of two stainless steel vats (35 × 22 × 22 cm). Milk (4°C) was pre-acidified to pH 6.0 with acetic acid diluted 1 to 10 in distilled water. Vats were then placed in a water bath and heated to 34°C. One vat was inoculated with 1.5 g each of *L. helveticus* LH100 and *S. thermophilus* TAO61, and the remaining vat was inoculated with 0.7 g each of the two starter cultures. Inoculated milk was ripened for 55 min at 34°C and then 50 g of adjunct culture (CR213) was added to the vat inoculated with the lower level of starter culture. Both vats were set with 0.75 mL of US double-strength recombinant rennet (Chymax, Pfizer Dairy Products Division, Pfizer Inc., Milwaukee, WI) diluted in 10 mL of water. The curd was cut 25 min after rennet addition using 1.9-cm knives. After healing for 15 min the curd was gently stirred for 30 s every 15 min until it reached the drain pH. The temperature of the curd was increased to 38°C over 10 min; then, one-half of the whey (5 kg) was drained from each vat. Curd was left in the remaining whey and held at 38°C until the curd reached a pH of 5.3. The remainder of the whey was then drained. Drained curd was left to stand in the vat at room temperature until the pH reached 5.2 and was dry salted with 10 g of NaCl (0.1% w/w). Salted curd was hand stretched in a hot brine solution (5% NaCl) at 82°C for 3 min until smooth and elastic, molded into stainless steel boxes (9 × 9 ×

9 cm), and placed in an ice bath for 1 h. Cheese blocks were removed from the molds and individually vacuum sealed. On d 1, 3, 7, 14, 21, and 28, samples were removed from the same block of cheese for analysis with the remaining cheese vacuum sealed and stored at 4°C.

Part-skim Mozzarella. Ninety-one kilograms of standardized milk was placed into a 270-L capacity stainless steel rectangular vat and pasteurized at 63°C for 30 min. Milk was cooled to 34°C and pre-acidified to pH 6.0 with dilute acetic acid, then inoculated with starter culture. Starter was allowed to ripen for 45 min before adding 6.8 ml of rennet diluted in 90 ml distilled water. Curd was cut 20 min after set with 1.9-cm knives and allowed to heal for 15 min. Healed curd was heated to 40°C with gentle agitation. Whey was drained at pH 5.9, and the curd was cheddared until it reached pH 5.2-5.3. The curd was milled and dry salted (0.1% w/w), then hand stretched in a hot brine solution (5% NaCl) at 82°C for 3 min until smooth and elastic. Stretched curd was molded into stainless steel boxes (9 × 9 × 9 cm), and placed in an ice bath for 1 h. Cheese blocks were removed from the molds, individually vacuum sealed, and stored at 4°C. Cheeses were analyzed on d 1, 3, 7, 14, 21, and 28.

Chemical Analysis of Cheese

One day after manufacture, cheese was analyzed for moisture, protein, fat, and salt. Moisture was determined with a vacuum oven (Model 5331, National Appliance Co. Chicago, IL). Shredded cheese samples (2.0 g) were placed in aluminum weigh pans and held at 100°C overnight (16-18 h), allowed to cool to room temperature in a desiccator, and reweighed to determine moisture loss (22). The Kjeldahl procedure was used to determine protein content (2). Fat content was determined by the Babcock method (22). Salt content was measured using chloride analysis (Model 926 Chloride Analyzer; Corning Glass Works, Medfield, MA 02052).

Capillary Electrophoresis

Capillary electrophoresis was performed on a P/ACE 2100 automated capillary electrophoresis system (Beckman Instruments, Fullerton, CA, USA equipped with System Gold software, version 7.11) by the method of Strickland et al. (23). Differences in peak areas were measured by calculating the average percent area for each peak for the three trials. Final peak area percentages of remaining caseins (Figure 8) were calculated on the initial peak area at d 1.

Free solution capillary electrophoresis. Free solution capillary electrophoresis was performed using a run buffer containing 4*M* urea, 100 *mM* phosphate at pH 7.2 to separate intact caseins and large peptides. Twenty-milligram samples of cheese were added to 400 μl of 8*M* urea in a 1.5-ml microfuge tube and mixed by vortexing for 3 to 5 min until the cheese was completely dissolved. After centrifugation at 12,000 x *g* for 10 min, the solution under the floating lipid layer was recovered with a plastic pipette tip and filtered through a 0.2-μm low protein binding filter (Gelman Science, Ann Arbor, MI, USA) to remove suspended lipids. The filtrate was diluted 2-fold with water, which resulted in a urea concentration similar to the run buffer. This diluted sample was centrifuged at 12,000 x *g* for 10 min before injection. All samples were assayed soon after preparation to avoid artifacts due to modification of the proteins by the urea solution.

Micellar electrokinetic capillary chromatography. Micellar electrokinetic capillary chromatography (MECC) was performed with a run buffer containing 40 m*M* SDS in 100 m*M* Na borate, pH 8.5, to separate small molecules and peptides. Twenty grams of cheese and 180 ml of distilled water were blended and centrifuged at 10,000 x *g* for 30 min. After centrifuging, fat from the samples was skimmed off the surface, and the samples were filtered through Whatman GF/A and No. 5 filter paper. The supernatant was centrifuged through 30K centricons (Amicon, Beverly MA, USA), and the filtrate was diluted 4-fold in

50 *mM* Na borate buffer and centrifuged at 12,000 x g for 10 min before injection into the capillary electrophoresis equipment.

Melt Viscosity Test

A Brookfield DV II + helipath viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA) equipped with a T-bar spindle (T-F with a 1.075 cm crossbar) was used to measure melt viscosity. Fifteen-gram samples of shredded cheese were placed into a 25 × 150-mm test tube and tempered for 10 min at 80° C. The T-bar spindle was submerged into the tube of melted cheese, held in a water-jacketed sample holder at 80°C. Viscometer speed was set at 0.3 revolutions per min. When the peak measurement was achieved, the helipath was set in motion and viscosity data were collected every 5 s for 10 min using an IBM-compatible computer equipped with DV Gather + version 1.0 (Brookfield Engineering Laboratories, Inc.). Melt viscosity of the cheese was determined by taking the mean value of viscosity for readings between 30 s and 90 s while the T-bar spindle was still completely submerged in the melted cheese.

Melt Test

Fifteen-gram samples of shredded cheese were placed into 24-cm long glass tubes and lightly packed in one end of the tube to 4 cm in height. Tubes were stoppered at both ends, horizontally positioned on a stainless steel rack, held at 4°C for 30 min, and then placed in a convection oven for 1 h at 110° C. Tubes were allowed to cool on the stainless steel rack until reaching room temperature. The distance the melted cheese traveled in the tube was measured from the end of the stopper to the rounded tip of the melted cheese.

Statistical Analysis

The experimental design was a split-plot randomized block using repeated measures with three replications. Part-skim Mozzarella cheese samples were used only for

observational comparison against the low-fat Mozzarella cheese samples and were not included in the statistical analysis for comparison to the low-fat cheese samples. Analysis of variance for the dependent variables of melt and melt viscosity of the low-fat Mozzarella samples was evaluated separately. Analysis of variance was calculated using Minitab 7.2 (Minitab Inc., State College, PA).

RESULTS

Cheese Composition

Composition of the part -skim and low-fat Mozzarella cheese is given in Table 6. The part-skim Mozzarella cheese was within the moisture and fat ranges required for low-moisture, part-skim Mozzarella cheese although its salt content was slightly low. The low-fat cheeses met the requirement of <6% fat necessary for the low-fat food category. The manufacturing procedure used for the low-fat cheese resulted in moisture content being increased when considered on a total component basis (61% compared to 51% for the part-skim cheese) or on a fat-free basis (65% compared to 63% for the part-skim cheese).

Cheese Shredding

As cheese was shredded by hand in preparation for the melt test, it was observed that as storage time increased, the low-fat adjunct-treated cheeses became softer and more difficult to shred. The texture of the adjunct-treated, low-fat cheeses were pasty and more sticky than the low-fat control and part-skim Mozzarella cheese. By d 28, the low-fat control, and part skim cheeses were still easily shreddable, while by d 14 the low-fat cheese containing the Prt^+ adjunct culture was very sticky with the cheese shreds clumping together during shredding.

TABLE 6. Mean (±SEM) percentage of moisture, fat, protein and salt in part skim Mozzarella cheese and in low-fat Mozzarella cheese made with and without Prt^+ Lac^d adjunct culture.

	Moisture		Fat		Protein		Salt	
	(%)							
Cheese	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM	$\bar{X}$	SEM
Part skim	51.3	1.79	18.5	0.59	21.7	1.13	0.8	0.06
Low-fat (control)	61.0	0.35	5.7	0.17	23.1	0.15	1.2	0.01
Low-fat (adjunct)	61.5	0.22	5.2	0.17	23.0	0.12	1.2	0.02

Melt

Observations based on Figure 5 indicate a steady increase in the melt of the part-skim Mozzarella until d 14. After d 14 a slight decrease in melt was observed. Overall, the melt increased from 10.6 cm on the day after manufacture, reached a peak of 18.6 cm at d 14, and then slightly decreased to 16.9 cm by d 28.

While there was no overall significant effect of adjunct culture addition on the melt properties of the low-fat cheeses, a significant interaction between treatment and storage time was observed (Table 7). Improved melting properties ($P \leq 0.05$) in the low-fat cheese made with the adjunct culture can be seen on d 3 after manufacture (Figure 5). At d 3, the low-fat control cheese melted to 9.0 cm and the low-fat Prt^+ adjunct cheese to 10.6 cm. However, by d 14, the melt of the adjunct-treated cheese decreased ($P \leq 0.05$) to less than the control. Melting properties of the cheese made with the Prt^+ adjunct culture at 7 d were comparable to the melt properties of the control low-fat cheese after 14 d storage.

Melt Viscosity

Observation of the melt viscosity of the part-skim cheese showed a declining trend over the 28-d storage period (Figure 6), displaying the inverse relationship with the melt

distance seen in previous studies (18, 20, 21). Similarly with meltability, there was no overall significant effect of adjunct culture addition on melt viscosity of the low-fat cheese. There was a significant effect from storage time (Table 7). Just as the melt properties of the low-fat cheese with the adjunct culture declined at d 14 ($P \leq 0.05$), the viscosity of the Prt^+ adjunct-treated cheeses showed an increase on d 14 (Figure 6), illustrating the inverse relationship between these two properties. Viscosity of the adjunct-treated, low-fat cheese went from 3.6×10^5 cP at d 1 to 5.0×10^5 cP at d 14, then dropped back to 3.5×10^5 cP at d 28.

TABLE 7. Analysis of variance for melt and viscosity in low-fat Mozzarella cheese made with and without the addition of 0.50% Prt^+ Lac^d adjunct culture.

Source of variation	df	MS	
		Melt	Viscosity ($\times 10^{-10}$)
Replicate (R)	2	7.57	4.86
Treatment (T)	1	6.36	5.66
Error a	2	5.15	2.61
Day (D)	5	2.95	4.18*
T x D	5	10.61**	8.26**
Error b	20	1.49	1.10
Subsamples	36	0.56	0.252
Total	71		

*$P \leq 0.05$.
**$P \leq 0.01$.

In contrast, the low-fat control cheese continued to decrease in viscosity over the 28 d of storage. It started at 4.8×10^5 cP at d 1 and decreased ($P \leq 0.01$) to 1.9×10^5 cP by d 28. Although the change in melt properties was not as dramatic in the low-fat cheeses as in the part-skim cheese, the inverse relationship between melt viscosity and melt distance can still be observed in these cheeses (compare Figures 5 and 6). The decreasing trend in melt viscosity of cheese during storage corresponds to our previous observations for part-skim (15, 16), reduced fat (12), and low-fat (8) Mozzarella cheeses.

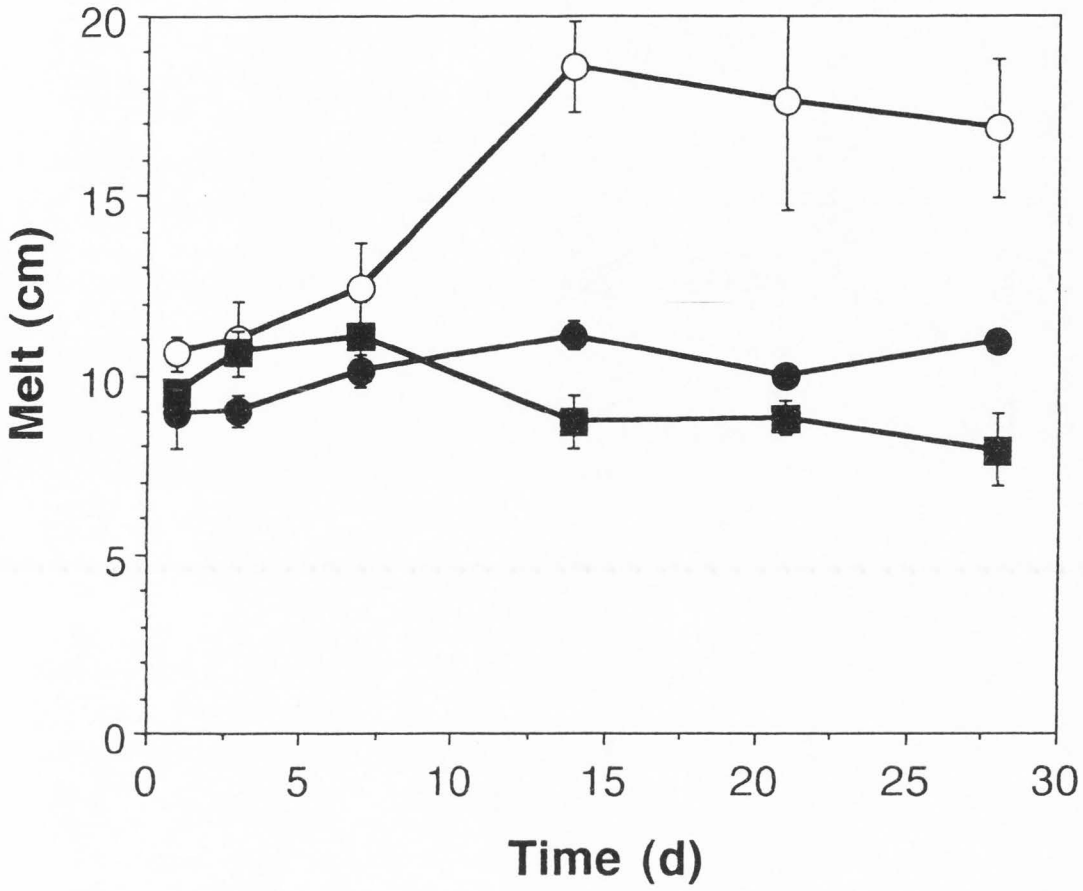

Figure 5. Mean (±SEM) melt measurements (centimeters) after heating for 60 min at 110°C of part-skim Mozzarella cheese(open circle) and low-fat Mozzarella cheese made with(solid square) and without(solid circle) the addition of a 0.50% inoculum of Prt^+ Lac^d adjunct culture.

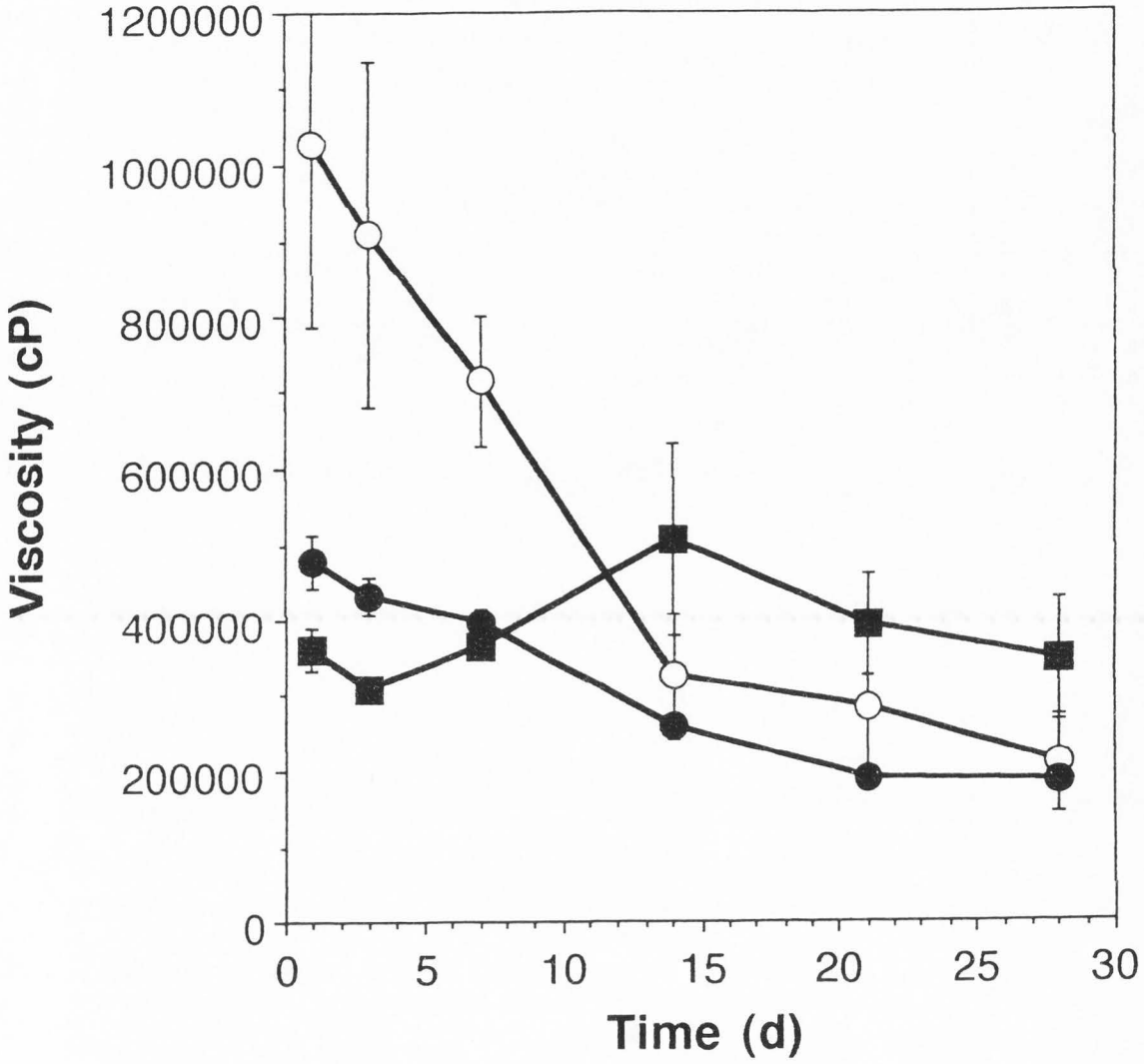

Figure 6. Mean(±SEM) viscosity measurements (centipoise) at 80°C of part-skim Mozzarella cheese(open circle) and low-fat Mozzarella cheese made with(solid square) and without(solid circle) the addition of a 0.50% inoculum of Prt^{+} Lac^{d} adjunct culture.

Proteolysis

The use of FSCE enabled the disappearance of both α_{s1}-CN and β-CN, as well the appearance of large peptides produced by proteolysis, to be observed during storage of Mozzarella cheese (Figure 7). It was observed that in the part-skim cheese there was a slight decrease in amount of intact α_{s1}-CN during the 28 d storage at 4°C (Figure 8). By d 28, 71% of the d 1 level of α_{s1}-CN remained intact in the part-skim cheese. This is similar to the 50% intact α_{s1}-CN remaining in part-skim Mozzarella cheese after 29 d storage reported by Kiely et al. (10). In contrast, both Fife et al. (8) and Farkye et al. (7) observed a larger decrease in intact α_{s1}-CN in part-skim Mozzarella cheese with only 25% remaining after 28 d or 14 d, respectively. The hydrolysis of α_{s1}-CN can also be followed by observing the appearance of such breakdown peptides as α_{s1}-I-CN, which is presumed to be part of Group 3 peaks observed in Figure 3. Tunick et al. (27) have reported that in part-skim and low-fat Mozzarella cheeses, α_{s1}-I-CN represents up to 24% of the total α- and β-caseins after 6 wk of storage. If a high cooking temperature (e.g., 46°C) was used during curd manufacture to inactivate residual rennet, then less proteolysis occurred and α_{s1}-I-CN accounted for only 6 to 14% of α- and β-caseins.

Observation of Figure 8 reveals minimal change detected in the amount of intact β-CN remaining in the cheese during the 28-d storage period. This is in agreement with Kiely et al. (10), who also observed no breakdown of β-CN during 29 d of storage. A 40% decrease in intact β-CN was observed by Farkye et al. (7); however, the cheese they studied was made using *Cryphonectria parasitica* rennet, which is known to be more proteolytic towards β-CN than chymosin. A more rapid disappearance of α_{s1}-CN was observed from the FSCE electropherograms of the low-fat cheeses (Figure 8). After 28 d of storage, 20% of the d 1 level of intact α_{s1}-CN was measured in the low-fat control samples. For the low-fat cheese made using the Prt^+ adjunct culture, the amount of intact

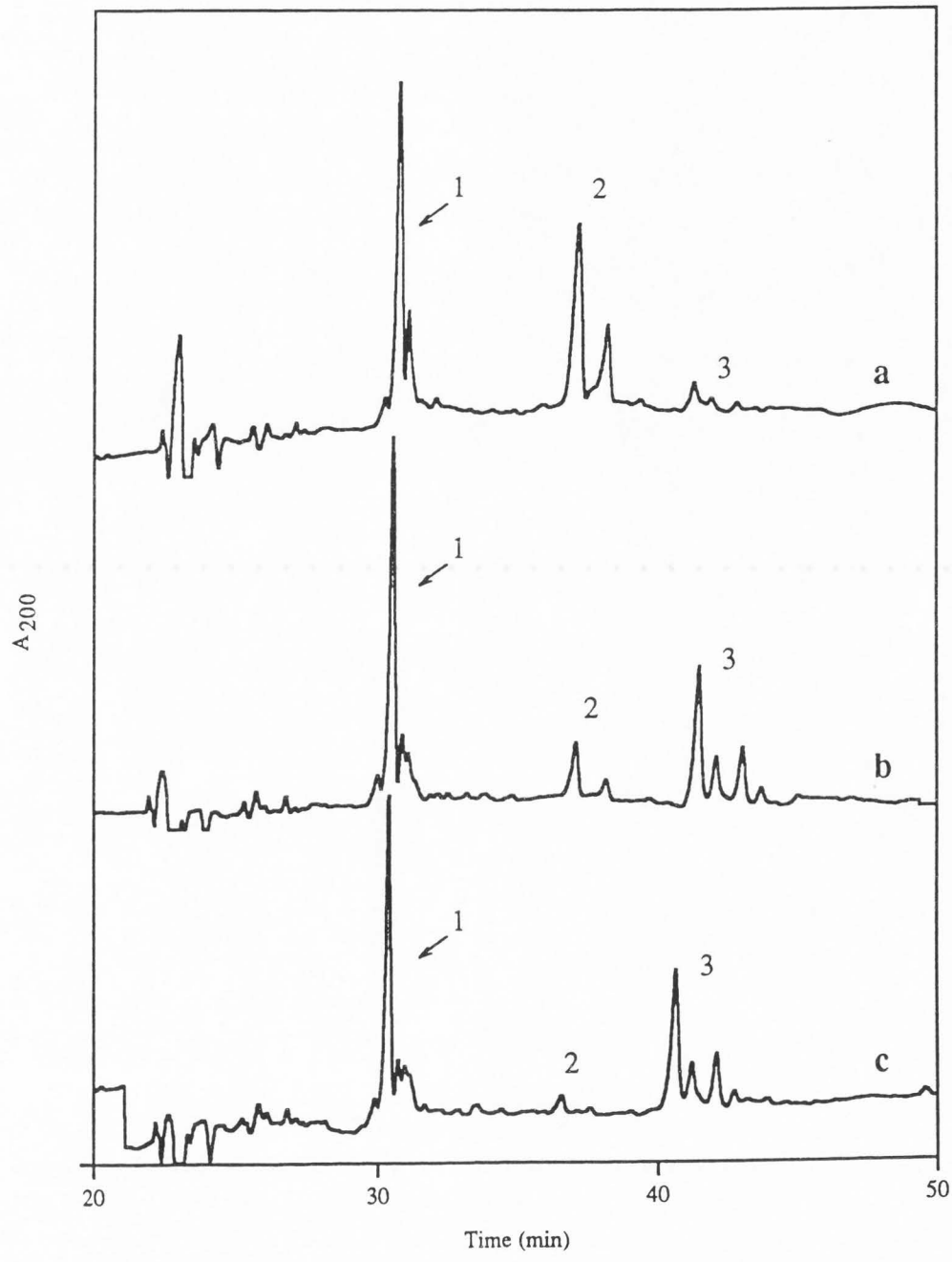

Figure 7. Free solution capillary electrophoresis of low-fat Mozzarella cheeses after storage at 4°C for 1 d (a), 14 d (b) and 28 d (c). Peaks identified with purified caseins include 1, β-CN ; 2 (two peaks), α_{s1}-CN ; 3 (four peaks), breakdown products. A_{200} = absorbance at 200 nm wavelength.

α_{s1}-CN casein remaining after 28 d was measured at only 14% of the amount present at d 1. The more extensive proteolysis that occurred in the low-fat cheese was also observed using the MECC method (Figure 9). At d 14 of storage, there was an increased formation of peptides in the low-fat cheeses compared to the part-skim cheese. Average total peak area units in the part-skim cheese MECC electropherogram was 699, while the total peak areas for the low-fat control and adjunct-treated cheese were 913 and 1018, respectively.

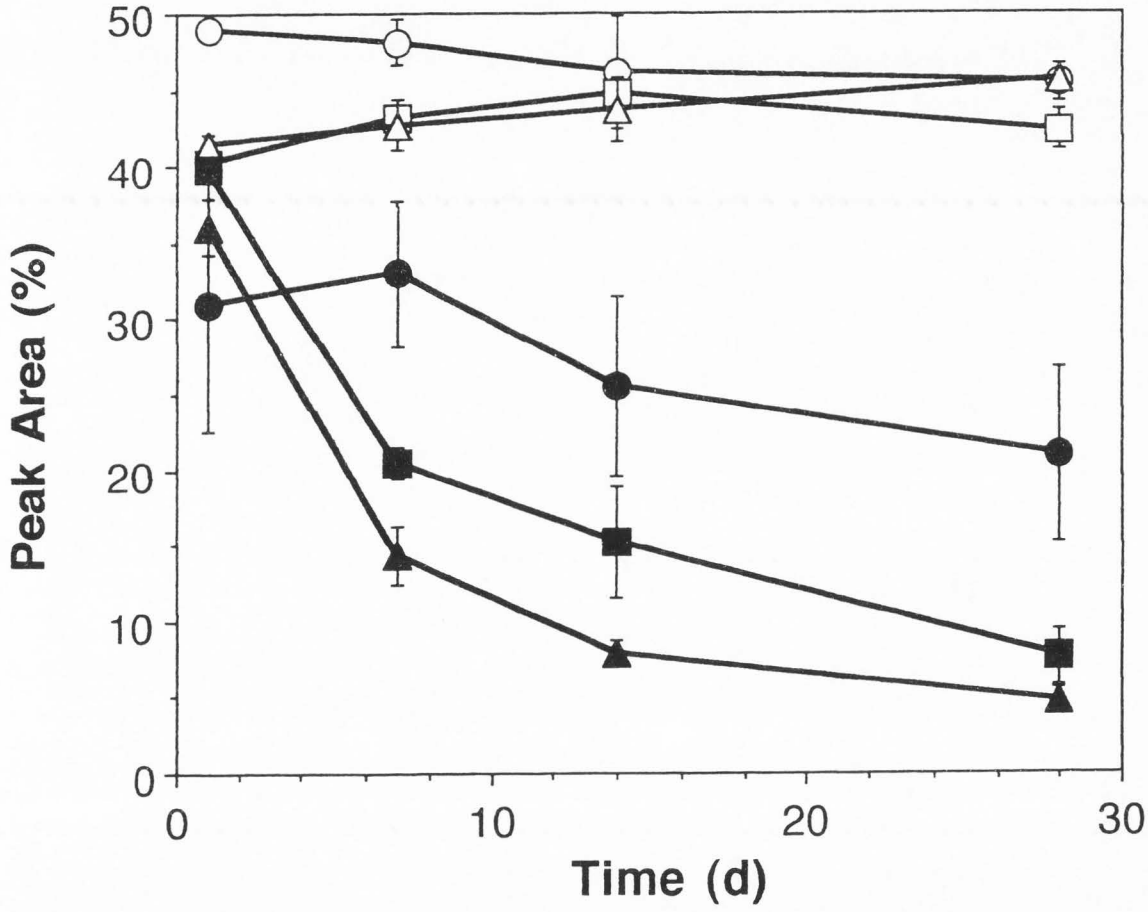

Figure 8. Mean percent peak areas (±SEM) of β-CN (open symbols) and α_{s1}-CN (solid symbols) measured by free solution capillary electrophoresis during 28 d storage at 4°C of part-skim Mozzarella (circles) and low-fat Mozzarella cheese made with (triangles) and without (squares) the addition of a 0.50% inoculum of Prt^+ Lac^d adjunct culture.

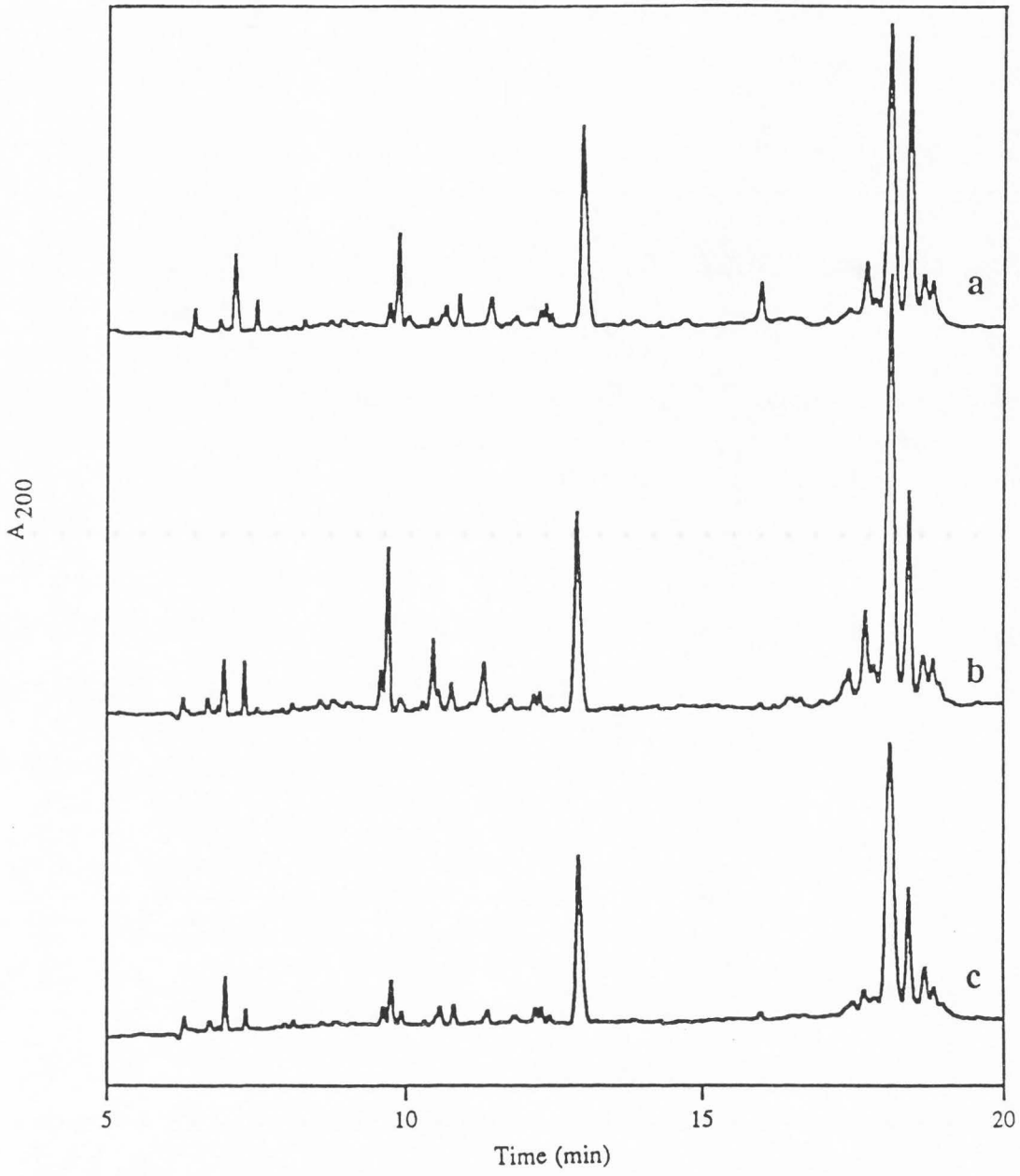

Figure 9. The 5- to 20-min region of micellar electrokinetic capillary chromatographs obtained from the aqueous fraction of low-fat Mozzarella cheese made without (a) and with (b) the addition of 0.50% inoculum of Prt^+ Lac^d adjunct culture and of part-skim Mozzarella cheese (c), after 14 d storage at 4C. A_{200} = absorbance at 200nm wavelength.

DISCUSSION AND CONCLUSIONS

Proteolysis

As an analytical tool, capillary electrophoresis (utilizing both FSCE and MECC) allowed the disappearance of intact caseins and the appearance of peptides to be quantitated during the storage of Mozzarella cheese. Previous experiments we conducted, using SDS-PAGE to measure proteolysis in low-fat cheese over time, had not been as successful because while using SDS-PAGE we had not been able to detect differences between cheeses made with or without Prt^+ adjunct culture, even though physical and functional differences between the cheeses were observed. There was some variability between the electropherograms of the low-fat Mozzarella cheeses. This may be the result of variance introduced during sample preparation. Very small samples of cheese (20 mg) were used for FSCE analysis (which is an advantage if only small amounts of the test substance are available), but better repeatability could be obtained if a larger cheese sample (such as a cross-section of the 1 kg cheese blocks) were used. The MECC method provided information on the number and volume of smaller peptides generated in the two low-fat cheeses that we could not quantitate with SDS-PAGE. When the Prt^+ adjunct culture was used for making low-fat Mozzarella cheese, more small peptides were produced than were produced in the control low-fat cheese.

Cheese Meltability

Increasing the rate of proteolysis in the low-fat cheese by using the Prt^+ adjunct culture allowed a cheese with better meltability to be obtained in a shorter time. However, the stickiness defect was evident by 14 d. From d 1 to d 7 after manufacture, the melting characteristics of the low-fat cheese with adjunct culture were comparable to that of the part-skim cheese during the same time period. However, while we observed a dramatic

increase in the part-skim meltability between d 7 and d 14, the low-fat cheese with adjunct showed a significant decrease ($P \leq 0.05$) in meltability between those same days (Figure 5). The control low-fat cheese followed a pattern similar to the part-skim cheese. As the amount of intact α_{s1}-CN decreased (Figure 7), the melt for both part-skim and low-fat Mozzarella increased (Figure 5).

The melt viscosity patterns (Figure 6) for both the low-fat control and part-skim cheeses showed a decreasing trend during the storage time. However, the low-fat adjunct cheese displayed a different pattern. There was an increase in viscosity at d 14 after manufacture, before a decreasing trend was seen. One possible explanation for this phenomenon may be the result of too much protein breakdown resulting in an increased adhesiveness of the proteins corresponding to the increase in viscosity and the stickiness defect seen at this point in storage time.

REFERENCES

1 Alvarez, R. J. 1986. Expectations of Italian cheese in the pizza industry. Page 130-138 in Proceedings of 23rd Annual Marschall Invitational Italian Cheese Seminar, Rhone-Poulenc, Madison, WI.

2 Association of Analytical Chemists. 1990. Official Methods of Analysis. 15th ed. AOAC, Arlington, VA.

3 Cancalon, P. 1995. Capillary electrophoresis: a useful technique for food analysis. Food Tech. 49:52-58.

4 Creamer, L. K. 1976. Casein proteolysis in Mozzarella-type cheese. N.Z. J. Dairy Sci. Technol. 11:130-131.

5 Deyle, Z., and R. Struzinsky. 1991. Capillary zone electrophoresis: its applicability and potential in biochemical analysis. J. Chromatogr. 569:63-122.

6 DiPalma, V. L., E. G. Hammond, and B.A. Glatz. 1987. Effect of lactobacilli on the properties of Swiss cheese. J. Dairy Sci. 70:733-737.

7 Farkye, N. Y., L. J. Kiely, R. D. Allshouse, and P. S. Kindstedt. 1991. Proteolysis in Mozzarella cheese during refrigerated storage. J. Dairy Sci. 74:1433-1438.

8 Fife, R. L., D. J. McMahon, and C. J. Oberg. 1996. Functionality of low-fat Mozzarella cheese. J. Dairy Sci. 79:1903-1910.

9 Fox, P. F. 1989. Proteolysis during cheese manufacture and ripening. J. Dairy Sci. 72:1379-1400.

10 Kiely, L. J., P. S. Kindstedt, G. M. Hendricks, J. E. Levis, J. J. Yun, and D. M. Barbano. 1993. Age related changes in the microstructure of Mozzarella cheese. Food Struct. 12:13-20.

11 Kok, J. 1990. Genetics of the proteolytic system of lactic-acid bacteria, FEMS Microbiol. Revs. 87:15-42.

12 Malin, E. L., J. M. Banks, M. H. Tunick, A. J. R. Law, J. Leaver, and V. H. Holsinger. 1993. Texture enhancement of low-fat Mozzarella cheese by refrigerated storage. Int. Dairy J. 3:548 (Abst.)

13 Malin, E. L., and E. M. Brown. 1995. Influence of casein peptide conformations on the textural properties of cheeses. Pages 303-310 *in* Chemistry of Structure-Function Relationships in Cheese. E. L. Malin and M. H. Tunick, ed. Plenum Press, New York, NY.

14 Malin, E. L., M. H.Tunick, P. W. Smith, J. J. Sheih, B. C. Sullivan, and V. H. Holsinger. 1994. Effect of homogenization on the properties of low-fat Mozzarella cheese. Page 487-493 *in* Cheese Yield and Factors Affecting Its Control, Publ. No. 9402, Int. Dairy Fed., Brussels.

15 Masi, P., and F. Addeo. 1986. An examination of some mechanical properties of a group of Italian cheese and their relation to structure and condition of manufacture. J. Food Eng. 5:217-229.

16 McMahon, D. J., C. J. Oberg, and W. McManus. 1993. Functionality of Mozzarella cheese. Aust. J. Dairy Technol. 48:99-104.

17 McSweeny, P. L. H., and P. F. Fox. 1993. Cheese: methods of chemical analysis. Pages 341-388 *in* Cheese: Chemistry, Physics and Microbiology, Vol. 1. P. F. Fox, ed., Chapman and Hall, London.

18 Merrill, R. K., C. J. Oberg, and D. J. McMahon. 1994. A method for manufacturing reduced fat Mozzarella cheese. J. Dairy Sci. 77:1783-1789.

19 Nauth, R. K., and D. Ruffie. 1995. Microbiology and biochemistry of reduced-fat cheese. Page 345-357 *in* Chemistry of Structure-Function Relationships in Cheese. E. L. Malin and M. H. Tunick, ed., Plenum Press, New York.

20 Oberg, C. J., R. K. Merrill, L. V. Moyes, R. J. Brown, and G. H. Richardson. 1991. Effects of *Lactobacillus helveticus* culture on the physical properties of Mozzarella cheese. J. Dairy Sci. 74:4101-4107.

21 Oberg, C. J., A. Wang, L. V. Moyes, R. J. Brown, and G. H. Richardson. 1991. Effects of proteolytic activity of thermolactic cultures on the physical properties of Mozzarella cheese. J. Dairy Sci. 74:389-397.

22 Richardson, G. H., ed. 1985. Page 351 *in* Standard Methods for the Examination of Dairy Products. 15th ed. Am. Pub. Health Assoc., Washington DC.

23 Strickland, M., B. C. Weimer, and J. R Broadbent,. 1996. Capillary electrophoresis of cheddar cheese. J. Chromatogr. A, 731:305-313.

24 Tuckey, S. L. 1974. The phenomena of curd stringiness and matting. Paper No. 13 in Proceedings 11th Annual Marschall Invitational Italian Cheese Seminar Marschall Division, Miles Lab., Madison, WI.

25 Tunick, M. H., K. L. Mackey, J. J. Sheih, P. W. Smith, P. Cooke, and E. L. Malin. 1993. Rheology and microstructure of low-fat Mozzarella cheese. Int. Dairy J. 3:649-662.

26 Tunick, M. H., K. M. Mackey, P. W. Smith, and V. H. Holsinger. 1991. Effects of composition and storage on the texture of Mozzarella cheese. Neth. Milk Dairy J. 45:117-125.

27 Tunick, M. H., E. L. Malin, P. W. Smith, J. J. Sheih, B. C. Sullivan, K. L. Mackey, and V. H. Holsinger. 1993. Proteolysis and rheology of low-fat and full fat Mozzarella cheeses prepared from homogenized milk. J. Dairy Sci. 76:3621-3628.

28 Tunick, M. H., and J. J. Sheih. 1995. Rheology of reduced-fat Mozzarella. Page 7-19 *in* Chemistry of Structure-Function Relationships in Cheese. E. L. Malin and M. H. Tunick, ed., Plenum Press, New York, NY.

CHAPTER 4

GENERAL SUMMARY

During the first stage of our research we produced a low-fat (6% fat) Mozzarella cheese containing a Prt^+ Lac^d adjunct culture (at 0.25, 0.50 and 1.0%) using a modified version of the Utah State University reduced-fat Mozzarella manufacturing procedure. The average moisture and protein contents of the cheeses were 62% and 23.5%, respectively. The meltability and melt viscosity of the cheeses were measured during 28 d storage at 4°C. The use of the Prt^+ Lac^d adjunct culture at the 0.5% concentration increased the meltability of the low-fat cheese by an average of 2.0 cm during the first 2 wk of storage. These results indicate the possibility of reducing the storage time of low-fat Mozzarella while maintaining the typical melt characteristics seen in regular part-skim Mozzarella. However, the adjunct-treated cheeses expressed a stickiness defect after 14 d of storage, making it difficult to shred, which was not apparent in the control cheese. A similar pattern in melt viscosity was seen between the control and adjunct-treated cheese; the 0.50% concentration showed the most variability. Samples of the cheese were analyzed using SDS-PAGE. Based on the gels, we were unable to determine a different pattern in protein breakdown between the control and the experimental samples. Although differences in protein breakdown could not be quantitated using SDS-PAGE, it was apparent that some difference in protein breakdown had occurred, simply by observing the difference in physical and functional characteristics between the control and experimental cheese. Initially, the adjunct culture was obtained for its classification as a highly proteolytic, lactose negative culture and was not expected to influence the acid development during cheese manufacture. However, in the prior trials it was observed that the cultures were contributing to acid development. Additional tests on the adjunct cultures were performed

to confirm our observations. These tests included an API ZYM test kit assay and acid development in nonfat UHT milk of the pure cultures. Both tests confirmed the ability of the cultures to ferment lactose. However, their ability was far less than that of the starter cultures grown under the same conditions. Therefore, we chose to classify the adjunct cultures as lactose deficient rather than lactose negative.

In the second stage of our research, part-skim and low-fat (6% fat) Mozzarella cheese was manufactured with or without the addition of the same Prt^+ Lac^d adjunct culture used in the previous trials, but a lower inoculation level of starter was used to compensate for the increased acid production contributed by the adjunct culture. Meltability and melt viscosity of the cheese were measured over 28 d of storage. Disappearance of α_{s1}-casein and β-casein was measured using FSCE. Micellar electrokinetic capillary chromatography was used to study the appearance of small peptides (<30kDa) during storage. Using the Prt^+ Lac^d adjunct cultures increased proteolysis and the meltability of the low-fat Mozzarella with a shorter storage time. From d 1 to d 7 after manufacture the melting characteristics of the low-fat cheese with adjunct culture were comparable to those of part-skim cheese during the same time period, but again, the stickiness defect was evident by d 14 after manufacture. Some correlation between the amount of intact α_{s1}-casein and the melt performance of both the part-skim and the low-fat Mozzarella could be seen from the electropherograms. As the amount of intact α_{s1}-casein decreased, the meltability of the cheese increased. However, the melt viscosity of the adjunct-treated low-fat cheese displayed a different pattern than did the low-fat control or the part-skim. The adjunct-treated cheese showed an increase in viscosity at d 14, and the low-fat control and the part-skim showed a decreasing trend throughout the 28 d of storage. One of the most interesting observations of our investigation was the opposite behavior in melt and melt viscosity displayed by the Prt^+ Lac^d adjunct-treated low-fat cheese and the low-fat control, although

ANOVA showed no significant effect on the melt and melt viscosity from the addition of these cultures. These differences observed in Figures 5 and 6 are a consequential observation. However, the cause of this change in behavior is unknown and would require further investigation. Overall, as an analytical tool, capillary electrophoresis allowed us to quantify the disappearance of intact caseins and the appearance of peptides during Mozzarella cheese storage.