Mozzarella Cheese: Impact of Milling pH on Chemical Composition and Proteolysis[1]

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ABSTRACT

The objective of this study was to determine the impact of milling pH on initial chemical composition and proteolytic changes in Mozzarella cheese during refrigerated storage. A new pilot-scale Mozzarella cheese-making method without brine salting was developed to produce cheese with homogeneous chemical composition within and among vats. Three milling pH (5.10, 5.25, and 5.40) were used to make three vats of cheese in 1 d. Cheese making was replicated on 3 d, on which the order of cheese making for each pH was selected so that effects of day and order of cheese making were blocks in a 3 × 3 Latin square design.

Milling pH affected cheese pH and titratable acidity. However, the initial chemical composition (i.e., moisture, fat, and protein) and amounts of nitrogen soluble in 12% TCA and in pH 4.6 acetate buffer were unaffected by differences in milling pH. During 50 d of refrigerated storage, differences in cheese pH among treatments were unchanged, the amount of nitrogen soluble in TCA and in acetate buffer increased, the amount of residual intact α_s-casein decreased, and the amount of intact β-casein remained constant. Proteolysis during refrigerated storage was unaffected by differences in milling pH.
(**Key words**: Mozzarella cheese, milling pH, composition, proteolysis)

Abbreviation key: **TA** = titratable acidity.

INTRODUCTION

Brine salting of Mozzarella, after the cheese is stretched in hot water, produces non-homogeneous chemical composition. Differences in chemical composition, especially gradients of salt concentration within each block of cheese, can affect chemical changes during ripening (14). Differences in block size and shape cause different patterns of non-homogeneity (10). Variations in chemical composition within blocks and resulting differences in proteolysis at different locations within blocks make sampling and interpretation of results difficult (4, 14) when Mozzarella cheese is brine salted. In addition, other problems are associated with brine salting. Brine can serve as a source of microbial contamination of cheese, and brine disposal can be an environmental problem. Efforts have been made to improve salting methods for Mozzarella cheese (8, 21, 24), but commercial Mozzarella cheese manufacturing still includes brine salting. A cheese-making method without brine salting that will produce Mozzarella cheese with homogeneous composition is needed. A "no-brine" salting method of making Mozzarella cheese was developed in the first phase of the present study.

After development of a "no-brine" cheese-making method, the new method was used for cheese manufacture in an experiment to determine whether variation in milling pH influences cheese composition or proteolysis. When dry salted cheese is made, curd is milled to ensure efficient whey drainage and more

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uniform salt distribution (27). The pH at milling is the most important determinant of final cheese pH in dry salted cheeses; in turn, the cheese pH indicates the extent of acid production throughout the cheese-making process (20). According to a survey (22), the pH of commercial Mozzarella cheeses ranged from 4.7 to 5.5. Mineral content (e.g., calcium content) of commercial Mozzarella cheese may also be variable because mineral retention in cheese depends on cheese pH (12). Calcium content or the ratio of calcium to protein in cheese may influence cheese texture (13). According to Kosikowski (17), the optimal pH for stretching of Mozzarella cheese (made with starter culture) is between 5.1 and 5.3.

Final cheese pH affects cheese quality, and researchers (20) have attempted to specify the optimal pH range for each cheese type. Cheese pH is also important because of its influence on proteolytic changes, which are considered to be the most important biochemical event during the ripening of many cheese varieties (9). Although the proteolysis in Mozzarella is considered to be lower than in Cheddar or Gouda (6), proteolysis in Mozzarella was evident (7).

The influence of differences in milling pH (and resulting cheese pH) on proteolysis in Mozzarella cheese made with starter culture has not been investigated. Therefore, this study was conducted to determine the impact of differences in milling pH on initial chemical composition and proteolytic changes in Mozzarella cheese during refrigerated storage.

MATERIALS AND METHODS

Preparation of Starter Culture

Direct-to-vat frozen starter cultures, Thermococcus C120® (*Streptococcus salivarius* ssp. *thermophilus*) and Thermorod R160® (*Lactobacillus delbrueckii* ssp. *bulgaricus*), were used (Rhône-Poulenc, Madison, WI). The streptococci and lactobacilli cultures were thawed and refrozen separately. Four cans of each culture (360 ml from the same production lot) were thawed by immersion in water at 25°C, opened, and mixed in a sterile Erlenmeyer flask. Portions (40 ml) were transferred into sterile vials (50-ml capacity; Capital Vial Corp., Fultonville, NY), quickly frozen in liquid nitrogen, and stored in a freezer (model U1786DOE; Revco Scientific, Ashville, NC) at –70°C. The concentration of cells in the streptococci and lactobacilli cultures was approximately 1×10^9 cells/ml.

Cheese-Making Method

A new pilot-scale cheese-making method without brine salting was developed to produce Mozzarella cheese with homogeneous chemical composition. Raw skim milk and raw cream were obtained from the Cornell University dairy plant and mixed to a final concentration of 2.25% fat. The standardized milk was pasteurized (model EOS-75 HTST pasteurizer; Cherry Burrell, Chicago, IL) at 72°C for 16 s, cooled to 4°C, divided into three equal 170-kg portions, and stored overnight at 4°C.

On the next day, the cold, standardized, pasteurized milk (ca. 170 kg per vat) was poured into a cheese vat (model 4MX; Kusel Equipment Co., Watertown, WI) and heated to 36°C. The starter culture mixture was added (.30 ml of lactobacilli and .30 ml of strepococci/kg of milk), and milk was ripened for 60 min at 36°C. At the end of ripening, single-strength calf rennet (Rhône-Poulenc) was added (.20 ml/kg of milk). Following a 30-min set, the milk coagulum was cut with a 1.2-cm wire knife and allowed to heal for 5 min. The curds were then stirred gently without heat for 10 min and then heated at 36 to 41°C over 15 min with continuous agitation. Temperature was maintained at 41°C, and agitation continued until the whey pH reached 6.40 ± .02; then whey was drained, and curd was piled in the center of the vat. Curd slabs were turned (cheddared) every 15 min until the curd reached the desired pH for milling (i.e., pH 5.40, 5.25, and 5.10).

The cheese curd (ca. 18 kg) was milled to 2 × 2 × 4 cm and salted at a total rate of 2% (wt/wt). Salt was divided and added in two equal applications and mixed for 1 min; 4 min of mellowing time were allowed after each application. After the second salting (1 min of mixing and 4 min of mellowing), the curd was mixed again for 1 min, and an additional 4 min were allowed for further mellowing. Curd temperature was maintained at 41°C during cheddaring, salting, and mellowing. The salting took about 15 min; during this time, acid

development by the culture continued. The yield of salted curd was about 15 kg per vat.

A pilot-scale, twin-screw Mozzarella mixer (model 640; Stainless Steel Fabricating, Columbus, WI) was used to stretch the curd. The mixer had separate thermostatic temperature control of the circulating salt brine in the mixer that contacted the curd directly and the water jacket that controlled cheese temperature in the portion of the mixer where the screws and curd were not submerged in the circulating brine. Salted curd (about 3 kg/min) was placed into the mixer and stretched in hot circulating brine. The temperatures for the steam heated circulating brine and the electrically heated jacket were both set at 57°C.

The circulating brine was prepared by dissolving 3 kg of salt in 27 kg of water (10% wt/wt) and heating the solution to 57°C in a steam-jacketed kettle. The heated brine was poured into the mixer about 3 min before curd was added. The temperature of circulating brine was maintained at 57°C in the mixer by the automatically controlled culinary steam injection system. Steam injection diluted the salt concentration in the circulating brine from 10% to about 8.7% during the initial temperature equilibration prior to curd addition. The salt content of the circulating brine decreased to about 7.5% by the end of stretching. The screw speed of the mixer was set at 50% of its full speed (about 12 rpm). Completion of the stretching took about 15 min.

Stretched cheese was extruded into stainless steel, cylindrical tubes (7.5 cm i.d. × 30 cm long). The first .5 kg of cheese from the mixer was removed and discarded. Tubes filled with 1.2 kg of cheese were placed in ice water. After the cheese had cooled for 60 min, the internal temperature reached 20°C. The cheese was removed from the tube, vacuum packaged (Multi Vac model 160; Koch, Kansas City, MO) in a barrier bag (model B150; Cryovac, Duncan, SC), and stored at 4°C. Six cylinders of cheese were made per vat. The third and fourth cylinders (sequence of extrusion) were used for chemical analyses.

Chemical Analyses

Milk, Whey, and Cheese. Changes in titratable acidity (TA) of milk and whey (25) and pH of milk, whey, and cheese were monitored during cheese making. A Xerolyt electrode (model HA405; Ingold Electrode, Wilmington, MA) and Accumet pH meter (model 915; Fisher Scientific, Springfield, NJ) were used for pH measurements. Temperature of whey and cheese during pH measurement was 38°C. The electrode was immersed in 3 *M* KCl storage solution at 38°C between pH measurements to improve stability. Reference solutions (Fisher Scientific) for pH 4 (SB107-500) and pH 7 (SB101-500) were tested at 38°C. The actual pH of the reference buffers were calculated for 38°C based on the recommended temperature coefficients provided by the manufacturer.

Fat content of milk (1), cream (1), whey (25), and cheese (25) were determined using Babcock tests. All nitrogen determinations were by the Kjeldahl method (1, 2). Percentages of nitrogen from the analyses of noncasein nitrogen (11) and total nitrogen (1) were multiplied by 6.38 to give milk protein equivalents. Total nitrogen and noncasein nitrogen measurements for milk were performed in triplicate. All other chemical analyses, except for cheese moisture, were performed in duplicate.

Cheese samples were ground in a blender (model 31BL92; Waring, New Hartford, CT) to obtain a particle size of about 2 to 3 mm. Ground samples were packed in a 50-ml plastic snap-lid vial, without headspace, to minimize moisture loss from cheese during storage at 4°C (up to 2 d prior to analyses). Salt content in cheese was determined by the Volhard procedure (25). Cheese moisture was determined gravimetrically, in quadruplicate, by drying 2 g of cheese at 100°C in a forced-air oven (model OV-490A-2; Blue M, Blue Island, IL) for 24 h (25). Calcium concentration in cheese was determined by complexometric titration (15).

The TA was determined (1) for cheese at 3 d of age: 10 g of cheese and 95 ml of 60°C distilled water were blended (Waring) for 30 s and filtered (Number 1; Whatman International Ltd., Maidstone, England). The filtrate (25 ml) was titrated with .1*N* NaOH, and the acid content of the cheese was calculated as a percentage of lactic acid. Cheese pH were determined at 3, 8, 15, 21, 29, and 50 d of refrigerated storage. Before pH measurements, the samples and buffers were tempered to 20°C.

Soluble Nitrogen. To measure proteolysis, contents of nitrogen soluble in pH 4.6 acetate buffer and in 12% TCA were determined after 3, 8, 15, 21, 29, and 50 d of storage at 4°C (3). Nitrogen values were converted to protein equivalents using the 6.38 factor. Values for pH 4.6- and 12% TCA-soluble nitrogen were expressed as percentages of total nitrogen contents of cheese.

Electrophoresis. Analysis of cheese by SDS-PAGE (28) was used to monitor the proteolysis of α_s- and β-caseins during refrigerated storage. Instead of a gradient of 10 to 20%, a constant acrylamide concentration of 15% was used for the running gel. Ground cheese (1 ± .05 g) and electrophoresis sample buffer (10 ml, 10 m*M* Tris·HCl, pH 6.8, 1% SDS, 20% glycerol, .02% bromophenol blue tracking dye) were added to a 20-ml tissue homogenizer (model 3431-k10; Thomas, Swedesboro, NJ), and the mixture was homogenized. The cheese homogenate (.1 ml) was transferred into a 2-ml sample vial, and .9 ml of electrophoresis sample buffer containing .8% (wt/vol) dithiothreitol was added. All vials were capped, inverted to mix, placed in boiling water for 5 min (to enhance binding of the SDS by the proteins), cooled to room temperature (22°C), and stored at −20°C.

Samples (3, 15, 29, and 50 d) of cheeses manufactured on the same day at three different milling pH were run together on the same gel. Prior to electrophoresis, frozen samples were warmed to room temperature and placed in boiling water for 5 min. Samples were cooled to room temperature, and 8 μl of each cheese sample were loaded onto the gel.

The amounts of α_s- and β-caseins were determined by scanning gels using a video densitometer (model 620; The 1-D Analyst; BioRad Laboratories, Rockville Center, NY) equipped with a filter for 600 nm for the fluorescent cool white light source. Gels were stained with Coomassie blue R-250. Sample loading, staining, and destaining procedures were adjusted to yield a consistent total absorbance (sum of all bands) among samples and across gels. Amounts of residual caseins were estimated as the area for zone 1 (α_{s1}- and α_{s2}-caseins) or zone 2 (β-casein) as a percentage of the total area of all major bands for each sample.

Experimental Design and Statistical Analysis

Three vats of cheese made using milling pH 5.10, 5.25, and 5.40 were made in 1 d, and the cheese making was replicated on 3 different d. On each day, the order of cheese making for the three milling pH was changed so that the effects of day and order of cheese making were blocks in a 3 × 3 Latin square design. The data for initial chemical composition were analyzed using PROC ANOVA of SAS (SAS Institute Inc., Cary, NC).

For the proteolytic changes during refrigerated storage, a split-plot design was used in which the whole-plot factor (milling pH) was replicated in a 3 × 3 Latin square design in which the effect of day and order were blocks. The factors, degree of freedom, and the statistical model are shown in Table 1; PROC GLM

TABLE 1. Statistical model used for data analyses.

Factors	df	Analyzed as
Whole-plot factor		
Milling pH	2	Classification
Day of cheese making	2	Block
Order of cheese making	2	Block
Error	2	
Subplot factor		
Age	1	Quantitative
Age × age	1	Quantitative
Interaction of milling pH × age	2	Classification × quantitative
Interaction of milling pH × (age × age)	2	Classification × quantitative
Error	39[1]	

[1]The degrees of freedom of the error term for the subplot factor error for electrophoresis results were 21 instead of 39 because only four times of aging were used instead of six.

of SAS (SAS Institute Inc., Cary, NC) was used. The level of significance was $P < .05$ throughout the paper.

RESULTS

Composition of Milk, Whey, and Cheese

The average fat, protein, and casein contents of milks used for cheese making were 2.25, 3.26, and 2.49%, respectively. Casein as a percentage of total nitrogen was 76.2%. The average fat content of whey was .20%.

The rates of pH and TA change during cheese making were the same for all treatments (data not shown). As expected, cheese pH and TA were different ($P < .05$) depending on milling pH (Table 2). However, cheese moisture, fat, protein, salt, and calcium contents were unaffected ($P > .05$) by milling pH under the conditions used in this study (Table 2).

Changes During Refrigerated Storage

Cheese pH and Soluble Nitrogen. Cheese pH increased slightly for all cheeses during storage, but the initial differences in pH among cheeses made using different milling pH were maintained (Figure 1). The amounts of nitrogen soluble in pH 4.6 acetate buffer and 12% TCA were unaffected by the differences in milling pH (Figure 2, A and B, respectively), but soluble nitrogen increased with storage time for all cheeses.

Electrophoresis. The changes in SDS-PAGE patterns of Mozzarella cheese during 50 d of refrigerated storage are shown in Figure 3. Zone 1 contains α_{s1}- and α_{s2}-caseins, zone 2 contains β-casein, and zone 3 contains para-κ-casein. Proteolytic breakdown products are shown in the areas between zones 2 and 3 and below zone 3.

The amount of α_{s1}- and α_{s2}-caseins as a percentage of total protein decreased significantly during refrigerated storage (Figure 4A). However, β-casein remained relatively constant during the refrigerated storage (Figure 4B). Interestingly, breakdown of α_{s1}- and α_{s2}-caseins during storage was typical of observations for Cheddar cheese when calf rennet was used as a coagulant (18). The differences in milling pH did not influence the α_{s1}-, α_{s2}- and β-caseins remaining after various times of refrigerated storage.

DISCUSSION

"No-Brine" Cheese-Making Method

The pH decreased from 6.6 to the desired milling pH during cheese making. The pH at

TABLE 2. Initial chemical composition (n = 3) of Mozzarella cheeses made using three different milling pH.

Component	Milling pH			SEM	LSD[1]
	5.40	5.25	5.10		
pH	5.22[a]	5.16[ab]	5.09[b]	.01	.08
TA,[2] %	.53[a]	.58[ab]	.65[b]	.04	.08
Moisture, %	44.74	44.61	43.84	.21	1.25
Fat, %	21.67	21.25	21.96	.15	.91
FDB,[3] %	39.20	38.33	39.10	.36	2.19
Protein, %	27.84	27.99	28.53	.16	.99
M:P[4]	1.61	1.59	1.54	.02	.09
Salt, %	1.44	1.45	1.43	.03	.15
S in M,[5] %	3.22	3.26	3.26	.07	.43
Calcium, %	.85	.83	.82	.01	.08
Ca (% of P),[6] %	3.04	2.97	2.89	.03	.19

[a,b]Means within same row not sharing common superscripts are different ($P < .05$).

[1]$P < .05$.

[2]Titratable acidity.

[3]Fat content on a dry weight basis.

[4]Ratio of moisture to protein.

[5]Ratio of salt to moisture in the cheese.

[6]Calcium as a percentage of protein content of the cheese.

drawing whey for our cheese making was 6.40 ± .02, which may be higher than average commercial conditions. The pH at drawing whey is higher in our process because 1) milk pH did not drop initially upon addition of a large amount of bulk culture, as occurs commercially, because we used a small amount of direct-to-vat frozen cultures and 2) the direct-to-vat frozen cultures produce acid more slowly at the beginning of cheese making than do bulk starters.

Curd that was salted before stretching was firmer than the curd that was not salted. Therefore, stretching of salted curd required more energy input. Two extra sets of clamps on the Mozzarella mixer were necessary to fasten the plexiglass cover over the screws to prevent cheese from squeezing out through the gap between the cover and body of the mixer. Cheese temperature exiting the mixer ranged from 55 to 57°C. Fat loss in our cheese making was low compared with that of most commercial plants. We achieved a fat on a dry weight basis of 39%, starting with milk containing 2.25% fat. Most commercial plants start with milk containing a higher fat content to obtain a similar percentage for fat on a dry weight basis.

As milling pH decreased from 5.40 to 5.10, moisture of the cheese ranged from 44.7 to 43.8% (Table 2), which is slightly below the legal minimum for low moisture part-skim Mozzarella cheese (5). Moisture content of curd prior to salting was about 50%. Much of the loss in moisture (about 4%) occurred during dry salting. Moisture loss during stretching ranged from 1 to 2%. Further work is being conducted to modify the cheese-making method to achieve higher moisture. The fat on a dry weight basis ranged from 38.3 to 39.2% (Table 2), which is within the legal range of 30 to 45% (5). Salt content of the cheese was well controlled in this process (Table 2).

The high pH at drawing whey (i.e., pH 6.40) in our process may decrease chymosin retention (19), which may reduce proteolysis during storage. The high pH at drawing should cause more calcium retention in the cheese, which may make cheese texture firmer and make stretching more difficult, particularly if the cheese pH is high (13). However, once the extra clamps were attached to the pilot-scale mixer, even the cheeses milled at pH 5.40 were not difficult to stretch. The calcium content of cheese made by our method ranged

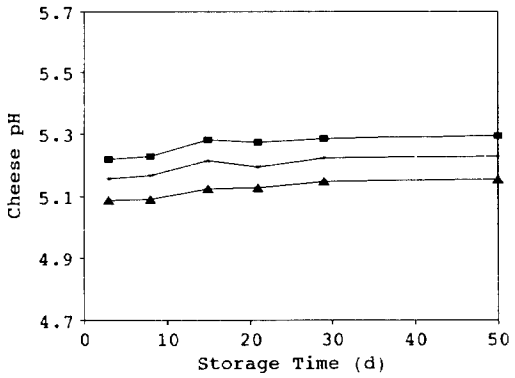

Figure 1. Impact of milling pH on pH of Mozzarella cheese during storage at 4°C for milling pH 5.40 (■), 5.25 (*), and 5.10 (▲). SEM = .017.

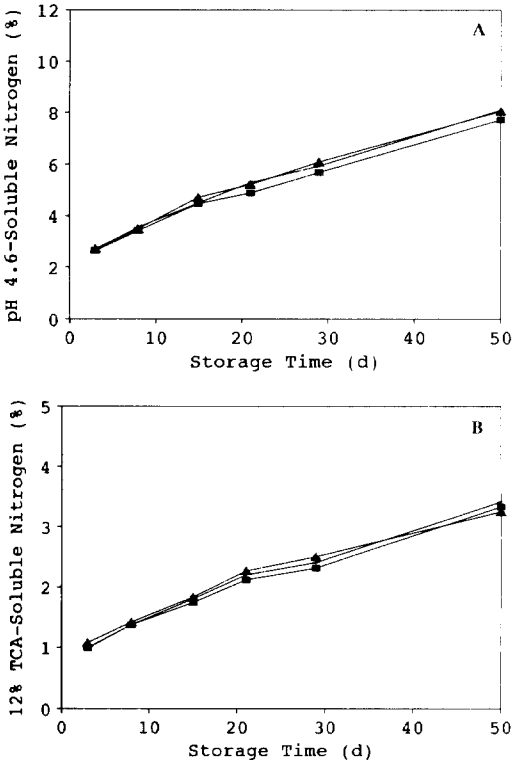

Figure 2. Impact of milling pH on pH 4.6-soluble nitrogen (A; SEM = .16%) and 12% TCA-soluble nitrogen (B; SEM = .83%) of Mozzarella cheese during storage at 4°C for milling pH 5.40 (■), 5.25 (*), and 5.10 (▲).

from .82 to .85%, which is comparable with that of commercial low moisture part-skim Mozzarella cheese (16, 26). The normal calcium content of our cheese, in spite of high pH at drawing whey, may be due to a larger amount of calcium loss into the hot salt water during stretching in our process. When unsalted curd is stretched in hot water, less calcium may be removed from the cheese.

The cooking temperature (in the vat) of 41°C at pH 6.5 to 6.4 would not be expected to inactivate chymosin. The stretching temperature used in our cheese making was 57°C for water with 55°C for curd, which may be lower than other reported values, i.e., 85°C for water with 55 to 80°C for curd (6) and 82°C water without curd temperature provided (23). However, the cheese temperature exiting the mixer in commercial cheese plants in the United States usually ranges from 57 to 65°C. Lower stretching temperature may permit greater enzyme survival, particularly at curd pH < 5.4 when coagulants are more heat stable. The amount of proteolysis is determined by the sum of those factors plus cooling rate and storage time and temperature.

The "no-brine" Mozzarella cheese-making method was developed to reduce experimental error by improving uniformity of cheese composition. Preliminary experiments indicated that the cheese produced by this "no-brine" cheese-making method had a more homogeneous chemical composition within and among blocks than cheese made using brine salting. This homogeneity allowed more consistent and representative sampling. Without salt and moisture gradients from the outside to the center of each block, the proteolysis and functional properties of the cheese were more uniform.

Impact of Milling pH

Cheese pH, TA, and Composition. The times from culture addition to milling were ($\bar{X}$ ± SD) 201 ± 5, 210 ± 7, and 244 ± 6 min for milling pH 5.40, 5.25, and 5.10, respectively. Only 9 min were required to develop enough

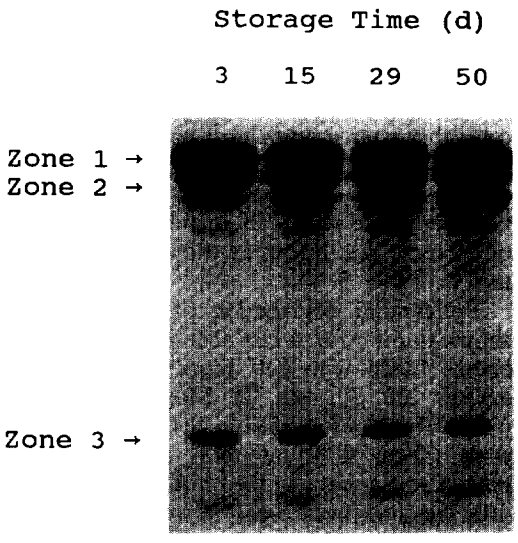

Figure 3. The SDS-PAGE gel of Mozzarella cheese (milling pH 5.25) sampled at 3, 15, 29, and 50 d of storage at 4°C; zone 1 = α_{s1}- and α_{s2}-caseins; zone 2 = β-casein; and zone 3 = para-κ-casein.

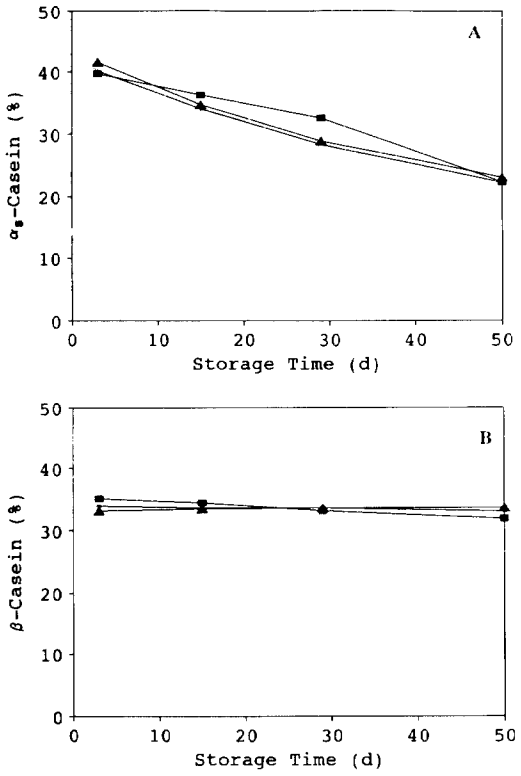

Figure 4. Impact of milling pH on relative percentage of intact α_{s1}- and α_{s2}-caseins (A; SEM = 1.07%) and intact β-casein (B; SEM = .95%) in total protein in Mozzarella cheese at 3, 15, 29, and 50 d of storage at 4°C for milling pH 5.40 (■), 5.25 (*), and 5.10 (▲).

acidity to reduce pH of the curd from 5.40 to 5.25, but 34 min were needed between pH 5.25 and pH 5.10, probably because of the increase in the buffer index of milk components (i.e., the molar quantity of acid or base required to change the pH by one unit) as the pH of the system changes from 7.0 to 5.0 (29).

Thus, progressively more acid production and more time are required to achieve a unit lowering of the pH of the cheese curd as the pH decreases. The rate of acid development did not differ for cheeses made using all three milling pH. The variation in milling pH was achieved by changing cheddaring time; i.e., the cheddaring time was shortened or extended to reach the desired milling pH. When milling pH were varied from 5.40 to 5.10, the resulting final cheese pH ranged from 5.22 to 5.09, respectively (Table 2). All cheese pH were within the range observed in a survey of commercial cheeses (22). The TA of cheese at 3 d ranged from .53 to .65%. The lower the milling pH (and the resulting cheese pH), the higher was the TA.

Cheese composition was not affected by the differences in milling pH from 5.40 to 5.10 (Table 2), but milling pH had a significant ($P < .05$) impact on cheese pH. Although not significant ($P > .05$), moisture content tends to be lower with lower milling pH because of the longer total time of making. For large vats of cheese (i.e., 2000 kg of cheese), 30 to 40 min may be required to mill the entire batch of curd. Over that period, curd pH at milling differs considerably from the beginning to the end of the vat. Because this commercial practice is common, the influence of milling pH within and among vats on composition is probably small relative to larger vat to vat variations caused by other factors.

Proteolysis. The amounts of nitrogen soluble in pH 4.6 acetate buffer and 12% TCA (Figure 2, A and B) and the amounts of residual α_{s1}-, α_{s2}-, and β-caseins (Figure 4, A and B) were not significantly affected by the differences in milling pH. Little of the variation in indices of proteolysis among treatments could be explained by differences in milling pH, as indicated by the low proportion of the total sum of mean squares accounted for by the milling pH term of the model (Table 3).

Impact of Age

Cheese pH. A small but significant ($P < .05$) increase in cheese pH (about .07) occurred during 50 d of refrigerated storage for all cheeses, but the initial differences (at d 3) in cheese pH were maintained throughout (Figure 1). Changes in pH during storage depend on the type of cheese. According to Lawrence et al. (19), the pH of Cheddar decreases slightly in the first 14 d of ripening as the residual lactose is metabolized. Thereafter, Cheddar cheese pH increases slightly (ca. .1 in 6 mo).

Proteolysis. Increases in the soluble nitrogen contents (in pH 4.6 acetate buffer, from 2.7 to 7.9% of total nitrogen; in 12% TCA, from 1.0 to 3.3% of total nitrogen) from 3 d to 50 d of storage at 4°C were highly significant (Figure 2, A and B; Table 3). Overall, these values are lower than those for Cheddar cheese (18) or Gouda cheese (30). However, the storage temperature for Mozzarella cheese is lower than that for Cheddar or Gouda.

Even though both linear and quadratic terms of age were significant ($P < .01$), the mean square for the linear term of age was much greater than the mean square for the quadratic term of age in the model (Table 3), indicating that the increase in soluble nitrogen contents of cheese with age was predominantly linear.

The amounts of residual α_{s1}- and α_{s2}-caseins in the cheese decreased significantly (from 40 to 22% of total protein, representing 45% reduction) during the 50 d of refrigerated storage (Figure 4A). This decrease clearly indicates that a large amount of proteolysis occurs with time. The amount of residual α_{s1}- and α_{s2}-caseins in Mozzarella was higher than in Cheddar made with calf rennet (18). The rate of decrease was linear, as shown in Figure 4A and in Table 3.

Preferential degradation of α_s-casein was shown in our Mozzarella cheese, as in Cheddar cheese made with calf rennet (18, 19). The amount of β-casein in Mozzarella cheese (made from pasteurized milk) remained constant (about 33% of total protein) during refrigerated storage in the present study (Figure 4B). The amount of β-casein was different ($P < .05$) depending on the day of cheese making because of differences among batches in milk composition, which was a block in the experimental design to separate the treatment effect (i.e., milling pH).

TABLE 3. Mean squares and probabilities for cheese pH and indices of proteolytic changes of Mozzarella during 50 d of storage at 4°C.

Factors	Cheese pH		pH 4.6-Soluble nitrogen		12% TCA-Soluble nitrogen		α_{s1}- and α_{s2}-Caseins		β-Casein	
	MS	P	MS	P	MS	P	MS	P	MS	P
Whole-plot factor										
Milling pH	.05*	.04	.24	.43	.05	.16	17.0	.19	.01	.99
Day of cheese making (blocked)	.01	.16	1.60	.10	.45	.02	11.0	.29	18.07*	.04
Order of cheese making (blocked)	<.01	.42	.27	.41	.07	.12	3.7	.55	2.62	.24
Error	<.01		.19		.01		8.9		1.61	
Subplot factor										
Age	.03*	<.01	110.61*	<.01	21.71*	<.01	1400.6*	<.01	5.87	.16
Age × age	.01*	.01	1.51*	<.01	.36*	<.01	3.6	.32	.03	.92
Interaction of milling pH × age	<.01	.94	.08	.35	.01	.75	3.5	.38	5.68	.15
Interaction of milling pH × (age × age)	<.01	.90	.03	.69	.02	.40	14.8*	.03	.02	.99
Error	<.01		.07		.02		3.5		2.72	
R^2	.877		.983		.975		.958		.524	

*Statistically significant ($P < .05$).

In a study of Cheddar cheese (18) made from pasteurized milk using calf rennet, little breakdown of β-casein occurred after 1 mo of storage at 8°C. In the same study (18), about 18% of the β-casein in Cheddar cheeses was degraded at 3 mo. Thus, the proteolysis of β-casein in Mozzarella seems to be comparable with that of Cheddar during the first 50 d of refrigerated storage when calf rennet is used to make both types of cheese. Apparently, overall proteolysis in Mozzarella during storage at 4°C may be slightly slower than for Cheddar stored at 8°C, but the general trends in proteolysis are the same in both types of cheese when they are made using calf rennet.

CONCLUSIONS

Differences in milling pH affected cheese pH and TA but did not affect the initial chemical composition or proteolysis. During 50 d of refrigerated storage, the differences in cheese pH were maintained. Proteolysis occurred during storage of Mozzarella cheese at 4°C because of the residual activity of coagulant or because of enzymes from milk and starter bacteria. The amounts of residual intact α_{s1}- and α_{s2}-caseins significantly decreased, but the amount of residual intact β-casein remained constant during 50 d of refrigerated storage.

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