

Functionality and Physico-Chemical Characteristics of Bovine and Caprine Mozzarella Cheeses During Refrigerated Storage

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ABSTRACT

Low moisture part-skim Mozzarella cheeses (MC) were manufactured using fresh bovine and caprine milk to study melting, physico-chemical, textural, and microstructural properties of the cheeses during 8 wk of refrigerated storage. Structural changes in cheese matrix were evaluated by scanning electron microscopy and by proteolytic patterns using nitrogen solubility, SDS-PAGE, and Gel-pro analyzer. Meltability of ripened cow and goat MC were not different when fat content of both milks were standardized, whereas bovine MC formed a significantly larger amount of free oil throughout the experiment. The results of the proteolytic patterns, texture attribute (cohesiveness), and microstructure revealed that bovine MC had a greater structural degradation of cheese matrix than caprine MC during the storage. Elevated protein degradation in bovine MC led to more intense brown color formation than the goat counterpart when the cheeses were baked. The melting characteristics showed high positive correlation ($r = 0.51$ to 0.80) with proteolysis, whereas it was negatively correlated with textural characteristics. Among textural attributes, cohesiveness was highly inversely correlated with melting characteristics ($r = -0.69$ to -0.88). High negative correlations were also observed between proteolytic parameters and textural attributes ($r = -0.48$ to -0.81).

(Key words: Mozzarella cheese, functionality, texture, storage)

Abbreviation key: MC = Mozzarella cheese.

INTRODUCTION

Consumption of Mozzarella cheese (MC) has greatly increased in Korea because of gains in the popularity

of pizza and related foods. Considering the ever-increasing popularity of pizza among children and teenagers in Korea, MC consumption is expected to grow. As an ingredient for pizza, MC has unique functionalities in both unmelted and melted states. In unmelted state, shredability to uniform size and overall texture are important functionalities, whereas meltability, free oil formation, stretchability, and browning are considered as major functionalities in melted MC (Kindstedt, 1991). These functionalities critically affect consumer preference. The functional aspects of MC were well reviewed by Kindstedt (1993), and the relationships between functionalities and processing parameters were extensively studied by Yun et al. (1993a, 1995).

The functionalities of MC are governed by various factors such as composition and manufacturing factors and are substantially changed during storage because curd structure is continuously altered. During refrigerated storage of 1 to 3 wk, MC attained optimal functionalities. Typically, melting characteristics of low moisture and low moisture, part skim are improved, and texture was softened during appropriate aging (Rowney et al., 1999). Changes in moisture content and proteolytic breakdown of casein have been suggested as implicating factors associated with functional change during aging (Yun et al., 1993b).

Many types of MC are manufactured from various sources including cow, goat, and buffalo milk. Among these, cheese production from goat milk has been gradually increased in United States, and it may partly be due to a healthy image of goat milk and the continued shift in consumer tastes to specialty cheeses (Park, 1990). Although the nutritional and economical values of goat milk products have considerable impact on dairy industry and consumers, only limited information is available for goat milk cheese. Because goat milk has a different protein composition than cow's milk, the integrity of the protein matrix varies and also influences functional characteristics of MC.

The objective of this study was to systemically compare functional, textural, physico-chemical, and micro-

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structural characteristics of bovine milk MC with those of caprine counterparts by evaluating the changes in these aging properties and their correlations during 8 wk of refrigerated storage.

MATERIALS AND METHODS

Milk, Culture, and Rennet

Fresh cow milk was obtained from dairy plant at Samyook University (Seoul, Korea), and goat milk was kindly provided by the Jorongol dairy goat farm (Hongchon, Korea). Each batch was standardized to 3.2% fat before pasteurization at 65°C for 30 min and cooled to 4°C. Freeze-dried starter culture (MY087) containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* was obtained from Culture Systems (Mishawaka, IN). Blue brand chymosin powder was purchased from Marschall-Rhône-Poulenc Inc. (Madison, WI).

Manufacture of Cheese

Low moisture, part-skim MC were manufactured from bovine milk or caprine milk as described by Kosikowski and Mistry (1999a). After overnight storage at 4°C, the milk (40 kg/each manufacture) was heated to 32°C in a cheese vat. The starter culture (0.01% of milk weight) was added and ripened for 30 min, then chymosin powder was added (0.003%). The milk was agitated for 1 min and set for 30 min. The coagulum was cut with a 0.6-cm wire knife and allowed to cure for 5 min. The curds were then stirred gently for 15 min followed by cooking at 39°C for 40 min. At the end of cooking, the whey (pH 6.3 to 6.4) was drained. When the pH was declined to 5.3, the curd was stretched and kneaded in multidirection for 5 min in water at 70 to 80°C. The plasticized curd was molded into blocks, cooled to 20°C, and brine salted (140 g of NaCl/L) for 1 h. After salting, the curd blocks were dried with paper towels, vacuum packed, and stored at 4°C up to 8 wk.

Compositional Analysis and Yield

The moisture and ash contents of the MC were determined by the oven drying method (method 926.08) and Gravimetric method (method 935.42), respectively (AOAC, 1990). The protein and fat contents were determined by the Kjeldahl method and modified Babcock method, respectively (Kosikowski and Mistry, 1999b). All analyses were done at least in triplicate. Moisture in nonfat substance (MNFS) and fat in dry matter (FDM) were calculated as follows: $MNFS = \% \text{ moisture} \times 100 / (100 - \% \text{ fat})$; $FDM = \% \text{ fat} \times 100 / (100 - \% \text{ moisture})$.

Meltability

Changes of meltability in MC during storage were determined in triplicate by the method of Savello et al. (1989). A cheese plug (15.0 ± 0.1 g, 22 mm height) was placed at one end of Pyrex glass tube (30 mm i.d., 250 mm long) and incubated at 30°C for 2 h. The melting tubes were heated at 110°C for 50 min in a horizontal position and flow distance from reference line was measured in centimeters.

Free Oil

Changes of free oil formation in MC during storage were determined in triplicate by the method of Kindstedt and Rippe (1990) and expressed as percentage in cheese fat.

Texture Profile Analysis

Textural characteristics of cheese were determined using Texture analyzer (TA/XT2 Stable Micro System, Godalming, UK). The specimens were prepared in rectangular shape ($25 \times 30 \times 10$ mm) after each storage time at 4°C. Immediately after preparation, specimens were wrapped in plastic film and were tempered at 10°C for 20 min before analysis. The specimens were submitted to two successive compressions to 50% of their initial height using flat-headed plunger (20 mm diameter) at a constant rate of 1 mm/s. Textural characteristics such as hardness, springiness, and cohesiveness were calculated by Texture analyzer software version 3.7. Averages of six measurements were reported.

Proteolysis

Protein degradation was determined by the amounts of soluble nitrogen in pH 4.6 acetate buffer and in 12% TCA for each storage time using the method of Bynum and Barbano (1985). The results were expressed as percentage of total nitrogen in cheese. The breakdown of major casein components during storage was monitored using SDS-PAGE according to Yun et al. (1993b). The intensity of individual casein bands ($\alpha_{s1} + \alpha_{s2}$ and β) remaining at designated storage time was calculated using Gel-Pro analyzer (Media Cybernetics, Silver Spring, MD) and expressed as a percentage of casein existed in the first-day cheese (100%).

Microstructure

The specimens at each storage time were prepared by the method of Savello et al. (1989). The specimens were fixed using 2% glutaraldehyde solution at 22°C for 4 h and sliced to $1 \times 5 \times 5$ mm. They were dehydrated

Table 1. Mean composition of Mozzarella cheese (MC) produced either from caprine or bovine milk.¹

Milk source for MC	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	MNFS ² (%)	FDM ³ (%)
Caprine	47.55 (0.43)	21.50 (0.35)	27.25 (0.42)	3.70 (0.13)	60.57 (0.33)	40.99 (0.29)
Bovine	48.42 (0.34)	21.13 (0.40)	26.07 (0.27)	4.38 (0.18)	61.39 (0.25)	40.97 (0.19)

¹Values in parentheses indicate standard error of the mean.

²Moisture in nonfat.

³Fat in dry matter.

in a graded ethanol series to 100% ethanol and defatted in chloroform. The dehydrated specimen was dried in Critical Point Drying equipment (Hitachi Ltd., Tokyo, Japan) and mounted on an aluminum stub. The specimens were coated for 60 s with gold-palladium in an E-1010 ion sputter coater (Hitachi Ltd.) and observed under Hitachi S-2380 scanning electron microscope at 15 keV.

Browning Test

A browning test of cheeses was carried out using Minolta chromameter (model CR-300, Minolta Camera Co., Osaka, Japan). Duplicate cheese samples were uniformly distributed on dough and were baked in a 240°C oven (National, ME-M610, Matsushita Ltd., Osaka, Japan) for 2 min. The cooked color of sample was measured at the center and at four points. The averages of three color indices (CIE L*, a*, and b*) were recorded.

Statistical Analyses

A randomized completely block design in two blocks was used in this experiment (Montgomery, 1991). There were six replicate trials for each cheese and changes in functional characteristics including free oil formation, meltability, proteolysis, texture attributes, browning, and microstructure of the cheese samples were determined. All data were analyzed by analysis of variance using the general linear model procedure (SAS system, version 6.12). Duncan's multiple comparison test was used as a guide for pair comparison of the treatment means at the significance level of $P < 0.05$. Pearson's correlation coefficients between functional characteristics were also calculated using the CORR procedure in SAS system.

RESULTS AND DISCUSSION

Cheese Composition

The chemical compositions of MC made either from bovine and caprine milk are compared in Table 1. The

results indicated that there were no significant differences in the composition between bovine and caprine milk cheeses. This indicates that any functional differences between bovine and caprine MC found in later analyses are mainly due to the nature of each MC rather than absolute contents of each chemical component. The composition of cheeses fitted within the US definitions for low moisture, part-skim MC (Kindstedt, 1993).

Meltability

The substantial increase in meltability occurred at 1 wk refrigerated storage for both bovine and caprine MC as shown in Figure 1. There was no significant difference in meltability between bovine and caprine MC during the experimental period. The amount and distribution of fat in protein matrix probably affects flow property of cheese upon heating. McMahon et al. (1999) reported that changes of water partitioning in cheese influenced meltability by causing re-arrangement of protein matrix. As revealed in Figure

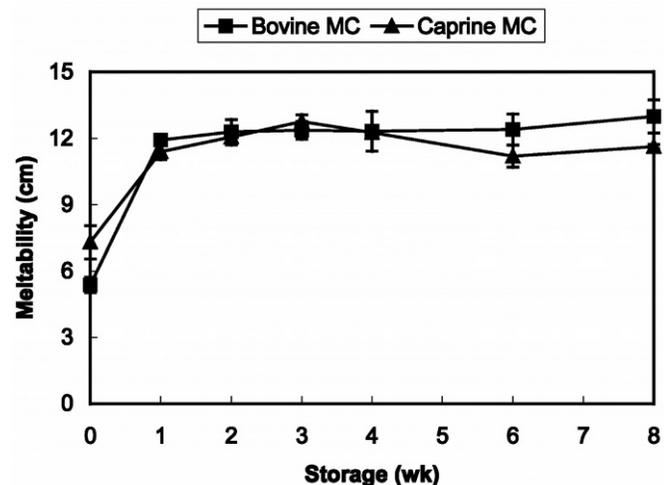


Figure 1. Changes in meltability of bovine (■) and caprine (▲) Mozzarella cheese during storage.

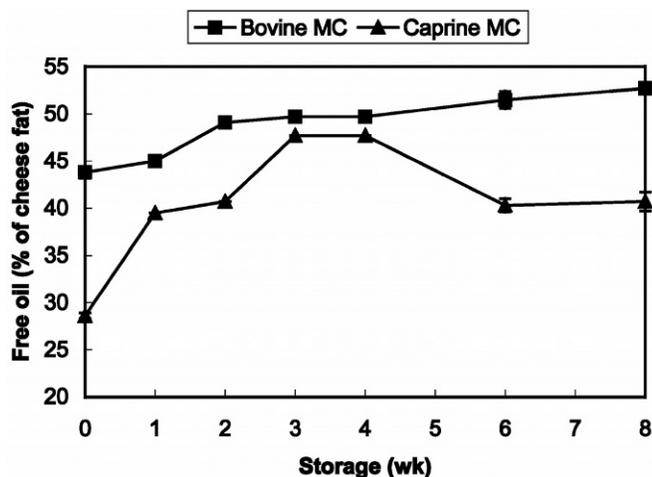


Figure 2. Changes in free oil formation of bovine (■) and caprine (▲) Mozzarella cheese during storage.

1, fresh MC tended to have poor meltability, and meltability was improved during refrigerated storage.

The major structural rearrangement in protein matrix of both cheeses could occur during early storage period up to 1 wk. The meltability of bovine and caprine MC was similar at 2 to 4 wk storage when MC generally develops desirable functionality (Kindstedt, 1993). Thus, the meltability of matured cheeses was not significantly different when fat content of bovine and caprine milk was standardized.

Free Oil

Although the changing pattern of meltability and free oil formation was very similar throughout the storage period, free oil formation showed slightly larger difference between bovine and caprine MC than meltability test. Bovine MC formed significantly larger amounts of free oil than that of caprine MC throughout the storage. Formation of free oil in bovine MC gradually increased as the storage continued. The first significant increase of free oil was observed at 2 wk of storage, and it increased further to more than 6 wk of storage in bovine MC. In the case of caprine MC, the significant increase of free oil occurred at 1 and 3 wk of storage and then decreased more than 4 wk of storage (Figure 2).

Free oil formation of MC depended on various factors such as fat content, size of fat globules, profiles of fatty acids, and proteolysis (Tunick, 1994). Several researchers have shown that the size of fat globules of bovine milk is larger than that of caprine milk (Park, 1994; Attaie and Richter, 2000). Because the release of free oil can be facilitated from the MC with larger fat globules, the difference of fat globule sizes might be one of

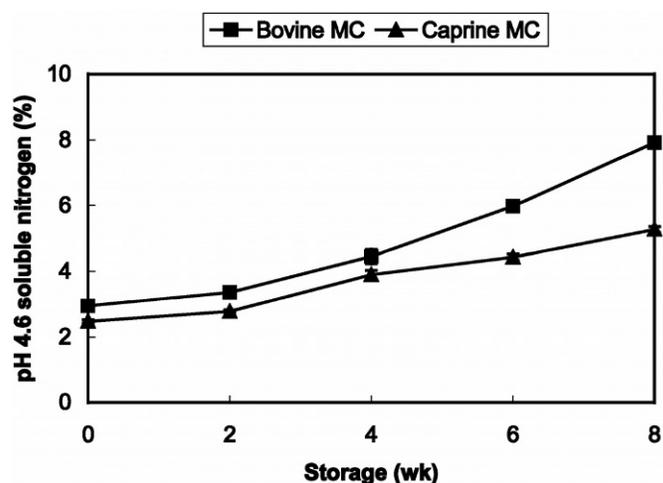


Figure 3. Changes in pH 4.6 soluble nitrogen content of bovine (■) and caprine (▲) Mozzarella cheese during storage.

the factors explaining higher free oil formation in bovine MC. In relation to fatty acid profiles, the difference of polymorphic fat structure between bovine and caprine milk might affect free oil formation.

Proteolysis

Proteolysis is an indicator of the integrity of the protein matrix, and the difference in the extent of proteolysis between bovine and caprine MC probably reflects the easiness of structural degradation of protein matrix. Bovine MC showed a significantly larger amount of soluble nitrogen and TCA soluble proteins than those of caprine MC throughout the storage (Figures 3 and 4). Moreover, the rate of increase for both soluble nitro-

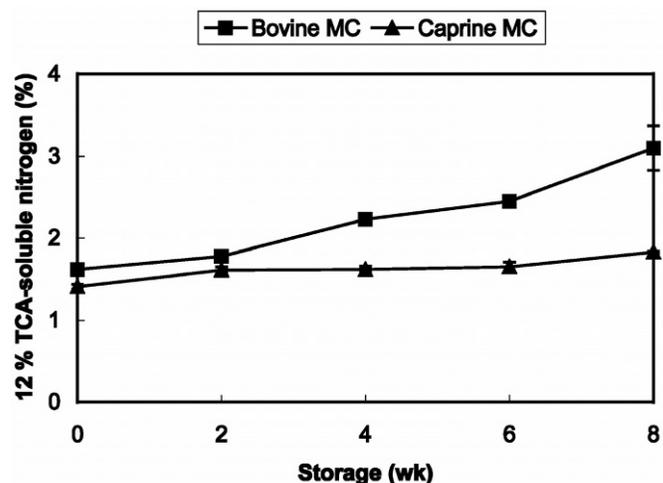


Figure 4. Changes in 12% TCA soluble nitrogen content of bovine (■) and caprine (▲) Mozzarella cheese during storage.

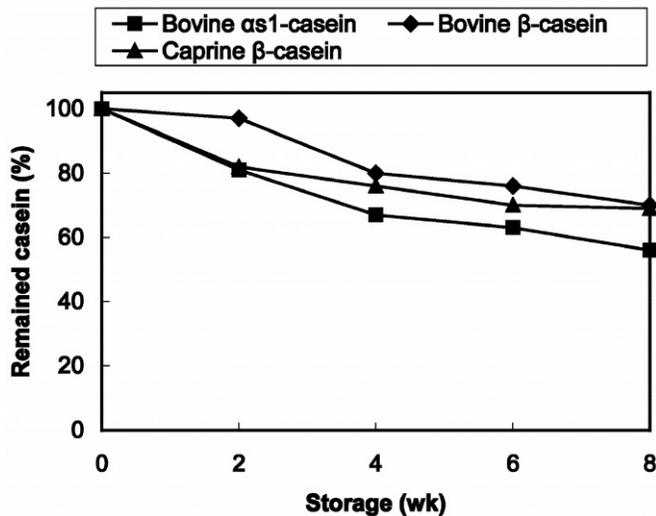


Figure 5. Degradation of major caseins (■: bovine α_{s1} -casein, ◆: bovine β -casein, and ▲: caprine β -casein) in Mozzarella cheese during storage.

gens in bovine MC was much faster than that of caprine MC. These results indicated that a greater extent of proteolysis occurred in bovine MC during storage. The decreased strength of protein matrix by proteolysis allowed fat globule to coalesce and to facilitate release of free oil as shown above.

Based on the SDS-PAGE followed by gel scanning, α_{s1} -CN was preferentially degraded during storage in bovine MC. Degradation of β -CN was also detected more than 2 wk storage of bovine MC and decreased to 70% after 8 wk of storage (Figure 5). In accordance with previous report (Fox, 1989), the rennet retained some activity after cooking step and contributed to the proteolysis of α_{s1} -CN. The degradation of β -CN suggested that plasmin could be involved in proteolysis during the storage. Farkye et al. (1991) reported similar results and demonstrated the evidence that plasmin caused hydrolysis of β -CN in MC.

The degradation of α_s -CN in caprine MC could not be differentiated because caprine milk contained a much smaller amount of α_s -CN than its β -CN. Although the rate of degradation was different, the extent of β -CN degradation was similar in both bovine and caprine MC after 8 wk of storage. Mulvihill and Fox (1979) reported that there was no significant difference in the proteolytic activity and specificity of chymosins on β -CN between bovine and caprine species. They also postulated that β -CN of these species have very similar amino acid sequences based on almost identical proteolytic patterns. Trujillo et al. (1995) also confirmed using SDS-PAGE that polypeptides produced from bovine and caprine β -CN by calf rennet were identical.

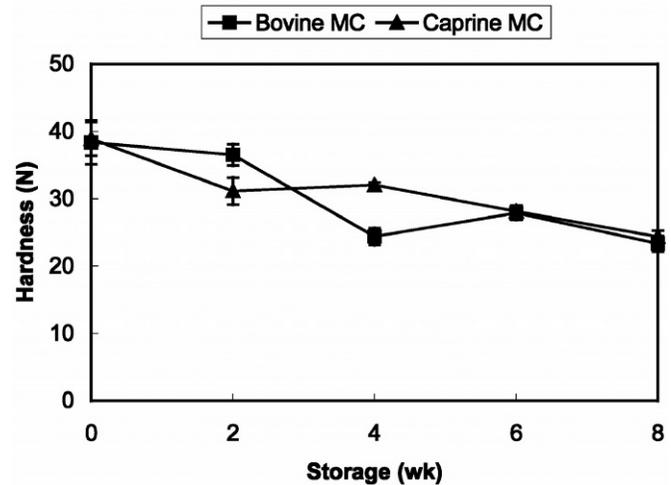


Figure 6. Changes in TPA hardness of bovine (■) and caprine (▲) Mozzarella cheese during storage.

Texture

Figure 6 depicts the changes of hardness values for bovine and caprine MC during storage. The hardness of bovine and caprine MC decreased with age. Significant hardness changes in bovine MC were found after more than 2 wk of storage, whereas hardness of caprine MC significantly decreased at 1 wk of storage ($P < 0.05$). No further decrease was observed for caprine MC up to the 8 wk of storage.

Springiness of bovine MC was not changed until 4 wk, but a decrease in springiness was apparent after 4 wk of storage (Figure 7). Similar to the pattern of hardness change, springiness of caprine MC decreased

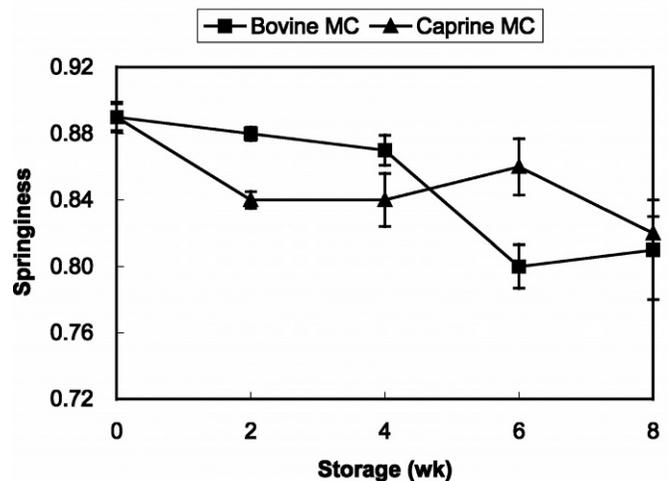


Figure 7. Changes in TPA springiness of bovine (■) and caprine (▲) Mozzarella cheese during storage.

Table 2. Changes in Hunter color values (L^* , a^* , and b^*) of bovine (B-MC) and caprine mozzarella (C-MC) cheese during storage. Color indices were determined after cooking at 240°C for 2 min.¹

Storage (wk)	L^*		a^*		b^*	
	B-MC ¹	C-MC ²	B-MC	C-MC	B-MC	C-MC
0	79.6 ^{abc} (1.74)	81.0 ^a (1.76)	-4.3 ^{bc} (0.09)	-3.7 ^a (0.24)	17.4 ^{bc} (0.29)	14.9 ^d (0.98)
2	79.4 ^{abc} (1.15)	79.9 ^{ab} (1.10)	-4.6 ^{cd} (0.07)	-4.3 ^b (0.15)	18.1 ^a (0.58)	15.9 ^{cd} (0.21)
4	78.5 ^{bc} (0.77)	79.6 ^{abc} (1.39)	-4.8 ^d (0.38)	-4.5 ^{bc} (0.08)	18.2 ^a (1.27)	17.0 ^{bc} (0.91)
6	77.8 ^c (1.09)	78.5 ^{abc} (1.81)	-5.2 ^e (0.22)	-4.8 ^{bc} (0.25)	21.0 ^a (1.04)	16.9 ^c (1.13)
8	74.7 ^d (1.84)	78.2 ^{bc} (0.94)	-5.9 ^f (0.21)	-3.7 ^a (0.20)	22.1 ^a (2.40)	14.9 ^d (1.22)

^{a-f}Means within the same color indices with different superscript differ significantly ($P < 0.05$).

¹Values in parentheses indicate standard error of the mean.

at 1 wk of storage, and no substantial changes were found. The cohesiveness of caprine MC was greater than bovine MC throughout the storage (Figure 8). Tunick et al. (1995) reported that proteolytic breakdown of protein matrix led to decrease in hardness and springiness. Because protein and fat content was similar for both cheeses, the extent of proteolysis could be responsible for the textural differences. The sensitivity to structural breakdown of bovine MC was tended to be higher than caprine MC and greatly weakened the protein network. As shown in Figure 8, the caprine MC retained higher resistance when the external force was applied.

Browning

The whiteness (L -value) of cheese can be affected by fat and protein matrix of cheese and varies by heat-

induced change of serum phase confined in the matrix (Rudan et al., 1999; Metzger et al., 2000). The lightness was consistently higher in caprine MC during the storage, although the differences were not significant (Table 2). Brown and red color formation (b^* and a^* value) of bovine MC steadily increased during storage, and bovine MC resulted in significantly greater browning characteristics than that of caprine MC.

These results reflected greater extent of proteolysis observed in bovine MC.

The degradation of matrix provided substrate (amino group) for amino-carbonyl reaction (Tunick et al., 1993) and higher free oil formation in bovine MC are easily scorched in a high baking temperature, resulting increased a^* value. Based on the result, caprine MC showed relatively lower browning potential during storage.

Microstructure

The changes of microstructure during the storage of MC were visualized by scanning electron microscopy (Figure 9). The small cavities present in cheese at early storage were transformed to elongated fibrous matrix as storage proceeded. The typical fibrous matrix was found at 2 wk for bovine MC and 4 wk for caprine MC. Bovine MC appeared to have a denser protein matrix at early storage, but fusion and aggregation of small cavities occurred faster than caprine MC as aging continued. The enlargement of cavities during storage might suggest weakening of the protein matrix (Podaval and Mistry, 1999). The weakening of protein matrix was reflected in textural characteristics as shown in Figures 6 to 8.

More than 6 wk of storage of bovine MC, the bovine MC lost elastic matrix, indicating excessive softening. The structural changes in caprine MC during storage were slower and less prominent than that of bovine

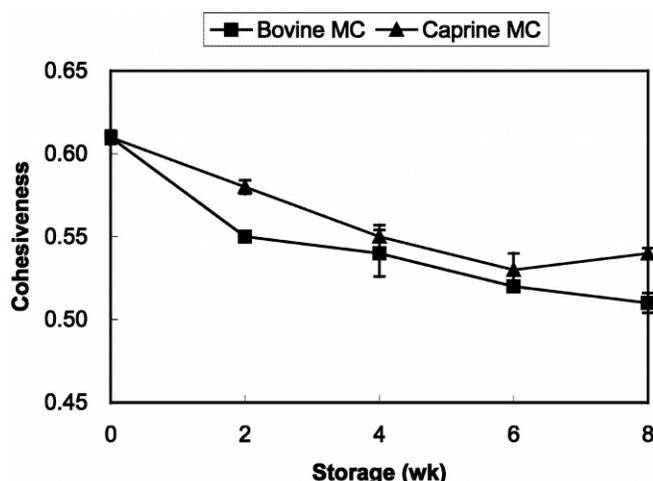


Figure 8. Changes in TPA cohesiveness of bovine (■) and caprine (▲) Mozzarella cheese during storage.

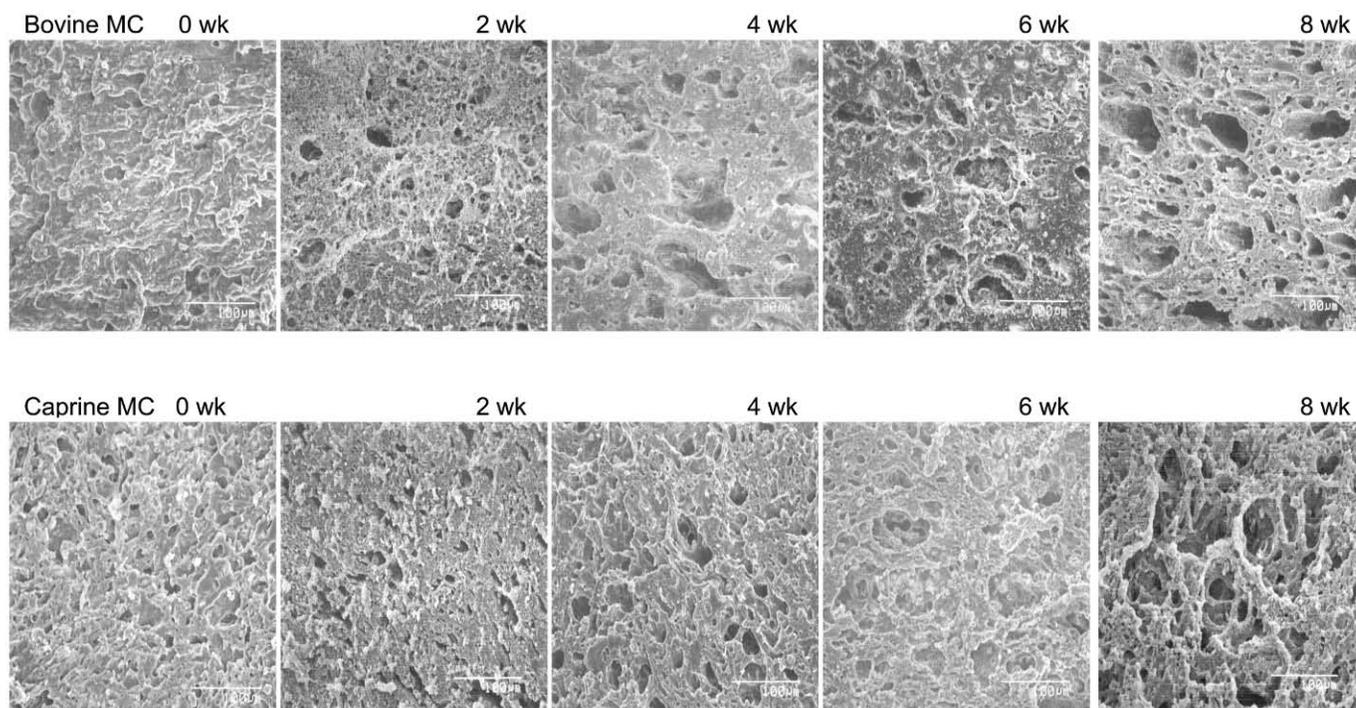


Figure 9. Changes in microstructure of bovine and caprine Mozzarella cheese during storage.

MC. McMahon et al. (1999) reported that redistribution of protein and water rather than changes in fat globules was the main cause for structural change in MC during storage. Therefore, the changes in the protein matrix by various factors including proteolysis affected rearrangement of the protein matrix, resulting in displacement of water in the matrix.

Relationship Between Physico-Chemical Characteristics of Mozzarella Cheese

The unique texture and melting characteristics of MC are closely related to structural characteristics of cheese curd (Kindstedt, 1993), and the structural integrity of cheese curd is continuously changed during stor-

Table 3. Correlation coefficients between physicochemical characteristics of bovine Mozzarella cheese.¹

	Meltability	TCA soluble N	pH 4.6 soluble N	TPA Hardness	TPA Springiness	TPA Cohesiveness	L*	a*	b*
Free oil	0.67 (<0.01)	0.76 (<0.01)	0.80 (<0.01)	-0.70 (<0.01)	-0.60 (0.02)	-0.88 (<0.01)	-0.63 (0.02)	-0.74 (<0.01)	0.62 (0.02)
Meltability		0.53 (0.04)	0.59 (0.02)	-0.55 (0.03)	-0.32 (0.25)	-0.77 (<0.01)	-0.35 (0.20)	-0.59 (0.02)	0.58 (0.02)
TCA soluble N			0.91 (<0.01)	-0.78 (<0.01)	-0.48 (0.07)	-0.72 (<0.01)	-0.74 (<0.01)	-0.88 (<0.01)	0.71 (<0.01)
pH 4.6 soluble N				-0.76 (<0.01)	-0.55 (0.03)	-0.79 (<0.01)	-0.80 (<0.01)	-0.91 (<0.01)	0.77 (<0.01)
TPA Hardness					0.51 (<0.01)	0.58 (<0.01)	0.41 (0.02)	0.62 (<0.01)	-0.50 (<0.01)
TPA Springiness						0.59 (<0.01)	0.59 (<0.01)	0.67 (<0.01)	-0.59 (<0.01)
TPA Cohesiveness							0.59 (<0.01)	0.75 (<0.01)	-0.64 (<0.01)
								0.71 (<0.01)	-0.49 (<0.01)
									-0.86 (<0.01)

¹Values in parentheses indicate *P*-value of coefficients.

Table 4. Correlation coefficients between physicochemical characteristics of caprine Mozzarella cheese.¹

	Meltability	TCA soluble N	pH 4.6 soluble N	TPA Hardness	TPA Springiness	TPA Cohesiveness	L*	a*	b*
Free oil	0.85 (<0.01)	0.54 (0.04)	0.49 (0.06)	-0.58 (0.02)	-0.65 (<0.01)	-0.69 (<0.01)	-0.11 (0.69)	-0.49 (0.06)	0.39 (0.15)
Meltability		0.65 (<0.01)	0.51 (0.05)	-0.62 (0.01)	-0.66 (0.07)	-0.72 (<0.01)	-0.22 (0.43)	-0.44 (0.10)	0.29 (0.29)
TCA soluble N			0.80 (<0.01)	-0.74 (<0.01)	-0.59 (0.02)	-0.67 (<0.01)	-0.54 (0.04)	-0.11 (0.71)	-0.17 (0.55)
pH 4.6 soluble N				-0.81 (<0.01)	-0.60 (0.02)	-0.81 (<0.01)	-0.45 (0.10)	-0.04 (0.87)	-0.08 (0.78)
TPA Hardness					0.54 (<0.01)	0.68 (<0.01)	0.33 (0.08)	0.02 (0.90)	-0.03 (0.88)
TPA Springiness						0.36 (0.05)	0.25 (0.18)	0.08 (0.69)	-0.03 (0.86)
TPA Cohesiveness							0.41 (0.03)	0.46 (0.01)	-0.42 (0.02)
L*								(-0.06) (0.73)	-0.21 (0.26)
a*									-0.72 (<0.01)

¹Values in parentheses indicate *P*-value of coefficients.

age. The relationships among various physico-chemical characteristics of MC are shown in Tables 3 and 4.

Overall, the pattern of correlations found between parameters was similar in both bovine and caprine MC. The melting characteristics including free oil and meltability showed high positive correlation ($r = 0.51$ to 0.80) with the indicators of proteolysis including TCA soluble N and pH 4.6 soluble N. However, the melting characteristics were negatively correlated with textural characteristics. Among textural attributes, cohesiveness showed a very high inverse relationship with melting characteristics ($r = -0.69$ to -0.88). The high negative relationships were also found between proteolysis and textural attributes ($r = -0.48$ to -0.81). These results confirmed that structural degradation by proteolysis weakened gel matrix of cheese curd with improving melting characteristics.

Although proteolysis parameters showed high negative relationships with all color indices of bovine MC, no significant relationship was found between proteolysis and color indices in caprine MC. As stated previously, relatively lower extent of proteolysis occurred during the storage of caprine MC would reduce the potential of browning and subsequently less prominent color changes might occur by baking.

CONCLUSIONS

There was no difference in meltability between caprine and bovine MC. The free oil formation of caprine MC was improved by aging for 3 to 4 wk. However, free oil formation of caprine MC decreased with further storage. The difference in free oil formation between bovine and caprine MC was ascribed to intrinsic differ-

ence of fat and protein matrix rather than total fat and moisture content present in cheese. The impacts of fat globule size, polymorphic structure, and casein micelle structure on melting properties need to be further studied for clarification. During storage, changes of microstructure and textural characteristics of bovine MC were faster than those of caprine MC. This result was due to a relatively lower level of proteolysis in caprine MC during the storage. The extent of proteolysis also affected cooked color formation.

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