Effect of Calcium on Microstructure and Meltability of Part Skim Mozzarella Cheese*

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ABSTRACT

The role of calcium in the microstructure of part skim Mozzarella cheese was evaluated using scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM). Part skim Mozzarella cheeses with 4 calcium levels (control 0.65%, T₁ 0.48%, T₂ 0.42%, and T₃ 0.35%) were manufactured and stored at 4°C. Microstructure and meltability of cheeses were studied on d 1 and 30. The micrographs were analyzed for numbers, area, perimeter, roundness, and size of the fat particles. Reduced calcium cheeses had greater meltability and more hydrated protein matrix with greater number of fat particles (control = 125, T₁ = 193, T₂ = 184, and T₃ = 215 with SEM and control = 86, T₁ = 87, T₂ = 125, and T₃ = 140 with CLSM). Further, area and perimeter of these fat particles were also greater in reduced calcium cheeses. Area, perimeter, and size of fat particles increased and their roundness decreased upon storage of 30 d. Decrease in free serum in the protein matrix of all cheeses upon refrigerated storage was evident from the CLSM. Hydrated protein network and better emulsified fat in low calcium cheeses might have improved melt properties of Mozzarella cheese.

(Key words: calcium, Mozzarella cheese, microstructure, confocal laser scanning microscopy)

Abbreviation key: CCP = colloidal calcium phosphate, CLSM = confocal laser scanning microscopy, SEM = scanning electron microscopy.

INTRODUCTION

Microstructure is one of the major controlling factors of texture and functional properties of cheese. The rela-

tive proportions of casein and calcium together with moisture and fat establish the basic structure of cheese (Lawrence et al., 1984). Understanding the way microstructure is formed would allow us to develop strategies for controlling and improving functional properties of cheese (McMahon, 1995).

Scanning electron microscopy (SEM) has been used to study the microstructure of cheese for many years. While observing the microstructure through SEM, a stream of electrons is fired at the surface of the sample, and an image obtained is based on electrons that are scattered back from the surface (McMahon, 1995). Most studies on cheese microstructure are based on 2-dimensional examination; however, for microstructure evaluation of cheese, a 3-dimensional analysis is required (Gunasekaran and Ding, 1999). Confocal laser scanning microscopy (CLSM) is considered a powerful tool since the laser scanning penetrates the cheese surface to visualize thin optical sections to obtain 3-dimensional analysis of cheese microstructure without disturbing the internal structure. In confocal microscopy, the specimen is observed within a plane both transverse to and along with the optical axis. Briefly, SEM has the advantage of high-resolution visualization and CLSM allows us to observe a cheese sample without disturbing the internal structure and to measure the size and shape of intact fat globules.

Mozzarella is a major cheese variety produced and consumed in the United States. Although the basic curd structure in all cheese varieties is almost the same up to the cooking stage, the stretching in hot water or brine (pasta filata process) imparts peculiar structural components to Mozzarella cheese. Stretching transforms the amorphous 3-dimensional protein matrix into a network of parallel-aligned protein fibers. Serum and fat droplets accumulate in the open channels that separate the bundles of protein fibers, resulting in partial alignment of the fat and serum phases of the cheese. Fat globules normally act as filler between the protein fibers so that protein-protein interaction within the matrix are reduced. Such cheese requires less energy to melt when heated. Upon heating, the melting of fat helps the cheese to flow (Paulson et al., 1998). Primar-

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ily, protein conformation stabilized by weak (noncovalent) interactions such as hydrophobic interactions and disulfide linkages. Hydrophobic interactions controlled by the entropy of the system, and these interactions become stronger at higher temperature. Hence, any functional characteristic based upon hydrophobic interaction becomes more apparent when the curd is heated. When cheese is heated, many proteins unfold and move past each other, causing the cheese to deform and flow (Paulson et al., 1998). However, not all proteins are disrupted and separated from fat upon heating the cheese because of the presence of stronger hydrophobic interactions. McMahon and Oberg (1999) reported changes in Mozzarella cheese structure during manufacturing and storage. Several other reports also include various aspects of Mozzarella cheese microstructure (Paquet and Kalab, 1988; Kiely et al., 1993; Tunick et al., 1993; Poduval and Mistry, 1999).

Calcium plays an important role in manufacturing as well as functional properties of Mozzarella cheese. The majority (63.7%) of total calcium in milk is present as a colloidal calcium phosphate (CCP). This CCP functions as a bridge to bind submicelles in the casein micelles. The terminals of CCP are connected with calcium and phosphate. Another terminal of phosphate is bound to serine of casein (Schmidt, 1982). Solubilization of calcium during cheese making occurs as a function of pH reduction; as a result, the CCP dissociates from the casein micelle, leaving calcium and phosphate at the terminals of casein. This decrease in calcium binding to casein is attributed to a decrease in hydrophobic binding sites of submicelles, which results in weakening of the extent of binding strength between submicelles (Kimura et al., 1992). Decrease in calcium content is also associated with decrease in numbers of binding sites for casein particles in cheese (Parker and Dalglish, 1977) and allows transformation of the tough and hard, 3-dimensional structure of curd into soft and stretchable texture of cheese. It is believed that hydrophobic interactions in cheeses are reduced due to calcium reduction. Too much casein associated calcium and phosphate results in tough curd that tears and fractures during stretching, whereas too little calcium and phosphate results in complete loss of structure and stretch (Kindstedt et al., 1999). A typical concentration of calcium is indeed important for controlling interactions of proteins in the matrix of Mozzarella cheese. The concentration of calcium and phosphate, particularly distribution between casein-associated calcium and soluble calcium is crucial in deciding the functionality of Mozzarella cheese. Our recent study (Joshi et al., 2002) revealed that micellar calcium plays an important role in improving melt and other related functional properties of part skim Mozzarella cheese. It was also revealed

that reduction of approximately 35% calcium in the cheese almost doubled its melt area and also caused a reduction in time-temperature required for softening and melting of part skim Mozzarella cheese on d 1 (Joshi et al., 2003).

It was postulated that significant changes might be occurring in the microstructure of low calcium cheeses, which would ultimately lead to improved meltability. This study is an attempt to evaluate the changes taking place in the microstructure of part skim Mozzarella cheese as a function of calcium.

MATERIALS AND METHODS

Cheese Manufacture

Four vats of part skim Mozzarella cheeses were made at the South Dakota State University dairy plant on the same day using standardized (1.8% fat) and pasteurized (63°C for 30 min) milk. Milk at 4°C taken in each of 4 stainless steel vats. Vat 1 served as a control. For the other 3 vats, 15 g of USP grade citric acid followed by a required solution of 10% (vol/vol) acetic acid were added to bring the pH of cold milk to 6.2 (T₁), 5.9 (T₂), and 5.6 (T₃), respectively. Milk was warmed to 35°C (control) and 32°C (T₁, T₂, and T₃). Diluted (1:20 in distilled water) double strength chymosin (Chymax, Chr. Hansen Inc., Milwaukee, WI) was added (0.1 mL/kg of milk) to each vat and the curd was set, cut, allowed to heal for 10 to 15 min, and stirred gently to facilitate syneresis. When a sufficient amount of whey was generated, 50% of the original milk volume was drained as whey, and glucono- δ -lactone was added until the pH of the curd dropped to 5.1. The amount of glucono- δ -lactone varied depending on the treatment. Final whey draining was followed by dry salting (2%, wt/wt) of the curd. Cooking time-temperatures and final draining of whey from all the curd were adjusted to obtain uniform moisture in all cheeses. Curd from control and experimental vats (T₁, T₂, and T₃) was weighed before salting and allowed to drain for a longer time if required, so that a similar weight for all curds was achieved. Cheese curd was then hand stretched in 5% salt brine at 77°C, filled in small wooden molds, and immersed in an ice water bath for 1 h. The cold cheese blocks were cut into pieces, individually vacuum packaged using a Spiro Mac vacuum packaging machine (Sogevac, France) in a barrier bag, and stored at 4°C until analysis on d 1 and 30.

Chemical Analysis

All cheese samples were analyzed in duplicate, and average results were expressed. Moisture was determined gravimetrically using the Mojonnier method

(Atherton and Newlander, 1987) and fat by modified Babcock method (Marshall, 1992). To determine protein content, total nitrogen content of the cheese was measured by the Kjeldahl method (AOAC, 2000) and a factor of 6.38 was used to convert nitrogen content to protein content. Ash content was determined by using a muffle furnace (AOAC, 2000) and salt by the Volhard method (AOAC, 2000). For calcium determination, cheese samples were dried and ashed, and the residues were dissolved and diluted in acidified aqueous solution of 2.5% hydrochloric acid. A portion was diluted in 0.5 % lanthanum oxide (La_2O_3) solution and analyzed by atomic adsorption spectroscopy at 422.7 nm (AOAC, 2000).

Scanning Electron Microscopy

Samples for SEM were cut from the interior of each cheese with a razor blade into a rectangular strip of $1 \times 1 \times 10$ mm size. The cheese strips were fixed by immediately immersing in a freshly prepared 2.8% glutaraldehyde solution and stored for 6 h at refrigerated temperature (4 to 6°C). Samples were dehydrated by subsequent transfer, for 10 min each, in a series of ethanol concentrations (10, 20, 40, 60, 80, 90, and 100%), extracted 3 times in chloroform for 30 min each and stored in a glass tube containing ethanol. The ethanol solution was changed every week during storage. When all the samples were ready, they were freeze-fractured in liquid nitrogen, thawed in ethanol, and finally dried in a critical point carbon dioxide drying apparatus (Denton Vacuum, Inc., Cherry Hill, NJ). The dried pieces were mounted on aluminum stubs using silver paint or 2-sided sticky tape and coated with a thin layer of gold and palladium in a Hummer VI sputter coater (Techniques Electron Microscopy Systems Inc., Munich, Germany). Microstructure examination of these specimens was done using an ISI Super IIIA scanning electron microscope (International Scientific Instruments Inc.) operated at 15 kV and 1000 $\times$ magnification. Micrographs of at least 3 fields were taken on black and white instant sheet film (Type 55 PN Polaroid, Cambridge, MA).

Confocal Laser Scanning Microscopy

Confocal laser scanning microscopy study was done to observe the aqueous background of protein matrix distinct from the fat globules and to study the orientation of fat particles in the cheese. A plug was drawn from a cheese block using a cork borer of 4 mm diameter in a perpendicular direction to the stretching, and a slice of less than 1 mm was cut using 2 parallel scalpels. The sample at 4°C was immediately stained by immersing in 0.1% solution of rhodamine B fluorescent dye

(Sigma Chemical Co., St. Louis, MO) for 2 min, placed on a glass micro slide (7.5×2.5 mm), and covered by a #1 cover slip. Cheese temperature was maintained at 4°C to avoid the problem of smearing of fat globules during sample preparation and subsequent observations. All the samples were viewed under a 20 $\times$ magnification lens with the aperture set to 3 and laser intensity of 50 in an Olympus confocal microscope (Leeds Precision Instrument Inc., Minneapolis, MN). A krypton-argon laser in dual beam fluorescent mode at 568 nm was used to excite the dye. A neutral density filter was set to 100% transmittance, and the separation between observations planes was kept to 1 μ . For each sample, a depth up to 30 μ m was viewed, which resulted in 31 adjacent layers for observation. The confocal images were observed using a fluoview software version 2.0.32 (Olympus Optical Co. Ltd.) and data saved in .tif (tagged image file) format for further analysis.

Image Analysis

Micrographs of SEM were scanned using a Scan Jet 5300 C scanner to obtain images in bit map (.bmp) format. The images were edited to differentiate between voids of fat globules and cracks and background of protein matrix using Adobe Photoshop. The fat particles, which appeared light in the micrographs, were darkened, and the cracks were eliminated before image analysis to eliminate artifact effect and truly examine the fat particles. The images obtained from SEM micrographs and CLSM analyses were analyzed using software HL Image ++ (HL Image++, Western Vision Software, USA), and parameters such as numbers, roundness (i.e., deviation from a perfect circle), area, perimeter and size of the fat globules were obtained.

Meltability

Cheese meltability was measured by a modified Schreiber test (Muthukumarappan et al., 1999). Cheese samples (28.5 mm diameter and 5 g of weight) in triplicate were placed on an aluminum plates and heated in an air convective oven (Gallenkamp Plus Oven, UK) at 90°C for 5 min. The plates were cooled at room temperature, and the area of the melted cheese was measured using image-processing software (HL Image++, Western vision Software).

Statistical Analysis

The experiment was replicated 4 times to make 4 vats of cheese (control, T_1 , T_2 , and T_3) each time. Thus, a total 16 vats of cheese were made during the experiment. The data were analyzed using PROC GLM of

Table 1. Average ($n = 4$) composition of part skim Mozzarella cheeses.

Parameter (%)	Treatment			
	Control ¹	T ₁ ²	T ₂ ³	T ₃ ⁴
Moisture	53.62	54.52	54.58	54.00
Fat	16.75	17.50	17.50	17.50
Protein	26.14	26.22	24.96	25.48
Ash	3.03 ^a	2.90 ^{ab}	2.65 ^{ab}	2.44 ^b
Salt	1.47	1.79	1.72	1.82
Ca	0.65 ^a	0.48 ^b	0.42 ^c	0.35 ^c

^{a,b,c}Means within same row not sharing common superscript are different ($P < 0.05$)

¹Control = Cheese made from milk without preacidification.

²T₁ = Cheese made from milk preacidified to pH 6.2.

³T₂ = Cheese made from milk preacidified to pH 5.9.

⁴T₃ = Cheese made from milk preacidified to pH 5.6.

SAS software (SAS, Version 6.1, SAS Inst. Inc., Cary, NC) to find significant differences among the treatments. The difference was considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

Cheese Composition

The major composition of all cheeses was similar ($P > 0.05$); however, a difference ($P < 0.05$) in calcium and ash contents of these cheeses (Table 1) was observed. Our aim was to study the effect of various levels of calcium. Hence, we attempted to achieve uniform moisture in all the cheeses during manufacturing. Due to uniform moisture content in the cheeses, variation was also minimized in fat, protein, and salt contents.

Microstructure

The SEM images of fresh (d 1) cheeses containing different levels of calcium are shown in Figure 1. It is difficult to see an elongation of fat globules and alignment of the casein matrix that has been reported by other researchers in previous studies. This could be due to the hand stretching used in our study, and, as a result, our cheeses did not receive as extensive stretching as typical mechanical stretching used on an industrial scale to manufacture Mozzarella cheese. Also, the fat serum channels are only organized during storage (McMahon and Oberg, 1999). The proteins formed a continuous plane in which fats were trapped as discrete voids (globules) or as elongated pools. The protein matrix in the control appeared as different planes adjacent to each other and maintaining their identity. The fat globules were fewer and scattered throughout the protein matrix of the control cheese. As the calcium was lowered, the protein matrix became smoother and more

homogeneous. Reduction of calcium caused inclusion of greater numbers of fat globules within the same area of protein matrix, as the control, and those appeared denser within the protein matrix. Some of these observed differences in the microstructure could be related to the differences in meltability between the treatments, which would cause the low calcium cheeses to be stretched to a greater extent, and this would have caused some of the observed differences in fat globule size and distribution. Table 2 indicates that the average maximum void number (i.e., visible empty spaces occupied earlier by serum and fat that were removed due to sample preparation during SEM) in T₃, T₂, T₁, and control cheeses were 215, 184, 193, and 125, respectively. An increase ($P < 0.001$) in the number, area, and perimeter of fat globules in the cheese matrix was observed with reduction in calcium content in cheese (Table 3). However, calcium reduction had no effect ($P > 0.05$) on size (radius) and roundness of fat particles in fresh cheeses (Table 3). Further, such effect was the maximum in the lowest calcium cheese (T₃), and it was evident that the most fat particles numbers and the maximum area, perimeter, and radius occurred in T₃ cheese (Table 2).

The images obtained from CLSM study of these cheeses (Figure 2) also showed that as the calcium level in cheeses was reduced, there was increase in number of fat particles compared with the control. The fat particles appeared brighter against a dark background of continuous protein matrix. The hazy surrounding of the fat particles was considered as free serum in the protein matrix. Analysis of the CLSM images using HL Image++ software indicated that the protein matrix of the control had 86 fat particles per micrograph followed by 87, 125, and 140 for T₁, T₂, and T₃ cheeses, respectively (data not shown). The number of fat particles observed in SEM and CLSM vary because a different size of cheese area was viewed under the microscope. However the major outcome of both techniques confirmed the trend; i.e. increase in number of fat particles within the protein matrix upon reduction in calcium content of cheese. Figure 3 illustrates images taken at different depths (1, 10, 20, and 30 μ) of cheeses. At the beginning, (1 μ depth), there were very few fat particles in the control, whereas in the experimental cheeses some fat particles already appeared. As the laser intensity reached to deeper layers, an increase in numbers of fat particles in all the cheeses was apparent. However, reduced calcium cheeses had greater increase in number of fat particles compared with the control. Figure 2 also shows that a free serum surrounded the fat particles especially in the control cheese. A greater amount of free serum was visible in control, which was observed as absorbed in the protein matrix as the calcium was

Figure 1. Scanning electron micrographs (1000 $\times$) of part skim Mozzarella cheese with different levels of calcium (control = 0.65%, T_1 = 0.48%, T_2 = 0.42%, and T_3 = 0.35%) on d 1. Black arrow indicates cavity of fat and white bold arrow shows smooth protein matrix. Bar indicates 10 μ .

reduced in the subsequent cheeses. Reduction in calcium led to a decrease in the number of pools of expressible serum and increase in volume fraction of protein matrix. During preacidification of milk as the pH of milk drops from 6.0 to 5.6, the micelles appear to swell

and a maximum dissociation of casein takes place (van Hooydonk et al., 1986). Due to protonation of carboxyl groups, the net negative charge diminishes and all amino groups are almost fully charged. Solubilization of micellar calcium phosphate diminishes the interaction

Table 2. Effect of calcium content on size, distribution, and arrangement of fat particles in microstructure of part skim Mozzarella cheese analyzed by SEM.

Parameter	Treatment							
	Control ¹		T_1 ²		T_2 ³		T_3 ⁴	
	d 1	d 30	d 1	d 30	d 1	d 30	d 1	d 30
Number	125	129	193	201	184	204	215	207
Area (μ^2)	149	236	231	369	212	376	343	287
Perimeter (μ)	529	767	876	1260	802	1290	1064	1062
Roundness (%)	0.777	0.621	0.718	0.657	0.741	0.603	0.744	0.687
Radius (μ)	0.525	0.671	0.520	0.646	0.522	0.650	0.602	0.574

¹Control = Cheese with 0.65% calcium.

² T_1 = Cheese with 0.48% calcium.

³ T_2 = Cheese with 0.42% calcium.

⁴ T_3 = Cheese with 0.35% calcium.

Table 3. Mean square and probability of size, distribution and arrangement of fat particles in microstructure of part skim Mozzarella cheese analyzed by SEM.

Source	df	Number	Area	Perimeter	Roundness	Radius
Treatment (T)	3	8412***	30744***	273752***	0.0028	0.0025
Error-1	6	91	1355	5347	0.0020	0.0013
Storage (D)	1	535	98935***	625695***	0.0629***	0.1012***
T × D	3	164	18325**	55714*	0.0034	0.0108**
Error-2	6	347	3337	26978	0.0014	0.0019
R ²	0.93	0.93	0.92	0.92	0.93	

* $P < 0.05$.** $P < 0.01$.*** $P < 0.001$.

between the proteins, which may have caused swelling and dissociation of casein in form of submicelles (van Hooydonk et al., 1986). Kindstedt and Guo (1997) also indicated that solubilization of micellar calcium can promote hydration of para casein in cheese. Our experimental cheeses were made from milk preacidified at pH 6.2, 5.9, and 5.6, in which part of the calcium was solubilized. Thus hydration and swelling of the protein matrix in our experimental cheeses is justified. McMahon and Oberg (1999) observed incompletely fused pro-

tein strands and numerous serum pockets in fat free cheese containing 0.6% calcium; however, the cheese containing 0.3% calcium had relatively homogeneous structure and no serum pockets. Cheese with high calcium contents has increased protein-protein interaction, causing micro aggregation and micro syneresis within the protein matrix. Such cheeses are considered less hydrated and have firm body but poor melting. Guinee et al. (2002) observed a greater degree of protein swelling with greater degree of casein hydration in re-

Figure 2. Confocal laser scanning microscopy images of part skim Mozzarella cheese containing different amounts of calcium (control = 0.65%, T_1 = 0.48%, T_2 = 0.42%, and T_3 = 0.35%) on d 1. Large arrow indicates protein matrix containing free serum and small arrow shows fat particles.

Figure 3. Confocal laser scanning microscopy layered images of part skim Mozzarella cheese with different levels of calcium (control = 0.65%, T_1 = 0.48%, T_2 = 0.42%, and T_3 = 0.35%) on d 1.

duced calcium Mozzarella cheeses on d 1. Rowney et al. (1999) suggested that solubilization of calcium may affect the binding sites on the casein molecules and influence the structure of casein matrix. Thus calcium is important for controlling interactions of proteins in the cheese matrix. Addition of salt and hydration of protein cause a decrease in hydrophobic interactions between protein molecules. Such proteins are less highly aggregated in the matrix and require less energy to disrupt when heated, resulting in better melt (Paulson et al., 1998). Lowering calcium content of the cheese acts in a similar manner to that of increasing the salt concentration (McMahon and Oberg, 1998). Low calcium in serum phase of fresh pizza cheese along with salt has been reported to create an environment that favors solvation, swelling, and partial solubilization of casein fibers that weaken the curd matrix and define the cheese microstructure (Guo and Kindstedt, 1995). Thus, lowering calcium has an overall effect on arrangement of fat particles and amount of free serum sur-

rounding the protein matrix and extent of hydration and swelling of protein matrix in the cheese.

Scanning electron microscopy images of the microstructure of cheeses containing various concentrations of calcium after 30 d of storage (Figure 4) showed that the protein matrix transformed into a homogeneous mass, and the fat particles merged and occurred as distinct pools squeezed between the protein fibers. Image analysis of the micrographs indicated that there was an increase ($P < 0.001$) in area, perimeter, and radius and decrease ($P < 0.001$) in roundness of fat particles (Tables 2 and 3). However, the number of fat particles remained unchanged ($P > 0.05$). The fat particles transformed into larger size with greater area and perimeter upon storage (except T_3). However, the roundness was reduced during storage, and the fat particles appeared elongated and distorted. Effect of storage was maximum on T_1 , and on T_3 there was no change in number of fat particles or reduction in fat area, perimeter, roundness, and size (Table 2). Presence of elon-

Figure 4. Scanning electron micrographs (1000 $\times$) of part skim Mozzarella cheese with different levels of calcium (control = 0.65%, T_1 = 0.48%, T_2 = 0.42%, and T_3 = 0.35%) after 30 d of storage at 4 $^{\circ}$ C. Black arrow indicates cavity of fat and white bold arrow shows smooth protein matrix. Bar indicates 10 μ .

gated fat pools suggested the coalescence of fat droplets in the weakened protein matrix. Confocal laser scanning microscopy images of d-30 cheese (Figure 5) also revealed a more homogeneous and hydrated protein matrix as compared with fresh cheeses. The free serum in the protein matrix seemed reduced; however, many fat globules in the control and T_1 cheeses were still surrounded by the serum. Guinee et al. (2002) observed smaller-sized fat droplets upon storage of Mozzarella cheese. Loss of identity of protein fibers and swelling and transforming into a more continuous protein matrix, during storage was also observed in their study. Kiely et al. (1992) observed that an intervening para casein matrix separates fat globules or their aggregates. It is likely that proteolytic destruction of the protein barrier facilitates coalescence of nearby fat globules on heating. Paquet and Kalab (1988) provided evidence that neighboring fat globules agglomerate after

collapse of the para casein matrix during heating. Consequently, a proteolytically weakened matrix is likely to have less ability to hold fat during heating. Our experimental cheeses showed a similar tendency at the end of 30 d of refrigerated storage. These cheeses possessed elongated fat pools and had improved melting upon heating.

Meltability

As the calcium content of cheese was reduced from 0.65% in the control to 0.48% in T_1 , 0.42% in T_2 and 0.35% in T_3 , an increase ($P < 0.001$) in meltability of the cheeses was observed (Figure 6). The low calcium cheeses (T_3 on d 1) had almost 2.6 times greater melt area compared with the control. These results establish the impact of calcium reduction on meltability of cheese. Reducing the calcium causes increased inter-

Figure 5. Confocal laser scanning microscopy images of part skim Mozzarella cheese with different levels of calcium (control = 0.65%, T_1 = 0.48%, T_2 = 0.42%, and T_3 = 0.35%) after 30 d of storage at 4°C. Large arrow indicates protein matrix containing free serum and small arrow shows fat particles.

Figure 6. Effect of calcium on meltability of part skim Mozzarella cheese on d 1 and 30. C = 0.65 % calcium, T_1 = 0.48 % calcium, T_2 = 0.42 % calcium, and T_3 = 0.35 % calcium. ^{a,b,c,d} Bars within same day not sharing common superscript are different ($P < 0.05$). ^{A,B} Bars not sharing common superscript are different ($P < 0.05$) for storage period.

action of proteins with surrounding serum, causing more hydration of proteins and better melting of the cheese. As indicated from the microstructure of the cheeses containing different levels of calcium (Figures 1 to 5), more free serum is observed around high calcium cheese (control), and is absorbed in the protein matrix with reduction in calcium content of cheese (T_1 to T_3). As moisture is absorbed from fat serum channels into the protein matrix, the proteins become more hydrated. This allows the proteins to flow more easily when heated and results in improved meltability (McMahon and Oberg, 1998). The microstructure of reduced calcium cheeses in our study also showed that such cheeses had more fat particles entrapped in the protein matrix compared with the control cheese, which might have contributed in better melting. Casein in the reduced calcium curd better emulsifies fat so that less fat oozes out when the cheese is heated, resulting into better melting (McMahon et al., 1993).

During storage, an increase in melt area of the cheeses was observed (Figure 6). However, improvement in meltability of high calcium cheeses (e.g., control and T₁) was more noticeable upon storage compared with that of the low calcium cheeses (e.g., T₂ and T₃). Changes in cheese structure due to protein breakdown play an important role in contributing to increased melting of cheese during storage. Proteolysis of casein allows fat globules, which are initially dispersed in the protein matrix, to coalesce when cheese is heated, increasing its meltability (Kiely et al., 1992; Tunick et al. 1997). An increase in the meltability of cheese during storage can be explained in terms of changes in water and protein status within the cheese.

CONCLUSIONS

The influence of calcium on microstructure of part skim Mozzarella cheese was studied by SEM as well as CLSM techniques. Cheeses with different calcium contents, viz. control (0.65%), T₁ (0.48%), T₂ (0.42%), and T₃ (0.35%), were manufactured by the direct acidification method. Microstructure and meltability of these cheeses were evaluated on d 1 and 30. The control cheese at d 1 had large protein aggregates, and its fat particles were surrounded by free serum within the protein matrix. Reduced calcium cheeses had more homogeneous structure, and the protein matrix was more hydrated and had greater number of fat particles, which ultimately resulted in better meltability of these cheeses. The results of the SEM study were supported by CLSM. The role of calcium in governing microstructure and consequently meltability of Mozzarella cheese is established. The refrigerated storage of cheeses up to 30 d caused increases in size, area, and perimeter of the fat particles; however, the numbers of fat particles remained unchanged. Free serum in the cheese matrix and roundness of fat particles reduced upon storage. Lower calcium cheeses have more hydrated protein network and better-emulsified fat. A cumulative effect of all these factors probably results in increased meltability of low calcium cheeses.

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