



Low-moisture part-skim mozzarella cheese made from blends of camel and bovine milk: Gross composition, proteolysis, functionality, microstructure, and rheological properties

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ABSTRACT

Camel (CM) milk is used in variety of ways; however, it has inferior gelling properties compared with bovine milk (BM). In this study, we aimed to investigate the physicochemical, functional, microstructural, and rheological properties of low-moisture part-skim (LMPS) mozzarella cheese, made from BM, or BM mixed with 15% CM (CM15%) or 30% CM (CM30%), at various time points (up to 60 d) of storage at 4°C after manufacture. Low-moisture part-skim mozzarella cheeses using CM15% and CM30% had high moisture and total Ca contents, but lower soluble Ca content. Compared with BM cheese, CM15% and CM30% LMPS mozzarella cheese exhibited higher proteolysis rates during storage. Adding CM affected the color properties of LMPS mozzarella cheese manufactured from mixed milk. Scanning electron microscopy images showed that the microstructure of CM15% and CM30% cheeses had smooth surfaces, whereas the BM cheese microstructures were rough with granulated surfaces. Low-moisture part-skim mozzarella cheeses using CM15% and CM30% showed significantly lower hardness and chewiness, but higher stringiness than BM cheese. Compared with BM cheese, CM15% and CM30% cheeses showed lower $\tan \delta$ levels during temperature surges, suggesting that the addition of CM increased the meltability of LMPS mozzarella cheese during temperature increases. Camel milk addition affected the physicochemical, microstructural, and rheological properties of LMPS mozzarella cheese.

Key words: meltability, free oil, viscoelastic properties, proteolysis, hardness

INTRODUCTION

Although cheese occupies an essential position in dairy products, cheese from camel milk remains a challenge under existing conditions (Mbye et al., 2020). The utilization of camel milk for cheese manufacture can preserve the nutrients and enhance the therapeutic properties of camel milk during ripening (Baig et al., 2022). There is no commercial cheese from camel milk (CM) available, and most of the research studies thus far are limited to soft, unripened cheeses prepared with high cooking temperatures, bovine chymosin, and mesophilic cultures (Baig et al., 2022). One particular issue in making cheese from CM is the weak gels that are formed. Due to the large casein micelles, low κ -CN, and high β -CN contents in CM, the acid- and rennet-induced CM gels are fragile (Hailu et al., 2016b). Few attempts have investigated making cheeses from CM (Mbye et al., 2020). Unfortunately, the resulting cheese had a labneh-like structure (Al-zoreky and Almathen, 2021). This particular difficulty renders CM unattractive to dairy manufacturers for use in yogurt and cheese production at present, despite the increasing customer interest due to health benefits associated with CM.

The weak gel formation of CM needs to be investigated to include CM in gelled products and allow changing the functional characteristics of current dairy products, such as cheese. This can enable CM to be used as an ingredient in dairy products (Elbarbary and Saad, 2019), which would create significant economic value for CM farms and the industry. Blending CM with bovine milk (BM) affected the low-fat Akawi (LFA) cheese properties (Abdalla et

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al., 2022). Low-fat Akawi is a white-brined cheese with a moisture content of around ~55% and a pH of ~6.0. Abdalla et al. (2022) found that LFA made from blended CM and BM had better flowability, as compared with that made from BM only. Moreover, LFA cheese from CM:BM blend has greater indicators of potential health benefits LFA cheese from BM only (Ayyash et al., 2021a).

Mozzarella cheese, known as *pasta filata* cheese, is one of the most widely consumed cheese varieties as it is easy to slice and melt. About ~75% of the mozzarella cheese produced in the United States is used as a pizza ingredient (Sutariya et al., 2022). Low-moisture part-skim (LMPS) mozzarella cheese is a preferable type for pizza industries (McMahon and Oberg, 2017). Generally, mozzarella cheese is made in some regions using traditional methods from water buffalo milk, but significant production is from BM (McMahon and Oberg, 2017). Goat milk was also employed to produce high-moisture mozzarella cheese (Faccia et al., 2021). Few attempts have been made to investigate the characteristics of mozzarella made from blended milk, such as bovine-ovine milk blends (Shaker et al., 2012) and buffalo-BM blends (Hussain et al., 2012). To the best of our knowledge, no information is available about using CM in a mixture with BM to produce LMPS mozzarella cheese. Therefore, the objective of this study was to investigate the rheological properties, texture profile, and microstructural characteristics of the LMPS mozzarella cheese made from blends of BM and CM at different ratios. This study was not designed to develop a new product, and thereby the characteristics of the new product development (e.g., sensory evaluation) were out of the scope of this study.

MATERIALS AND METHODS

All chemicals used were of analytical grade and were obtained from Sigma-Aldrich unless otherwise mentioned. No human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

Cheesemaking

Low-fat pasteurized CM (2.7% protein, 1.0% fat, and 4.2% carbohydrates) and BM (3.2% protein, 1.0% fat, and 4.6% carbohydrates) were obtained from local manufacturer (Al-Ain Dairy Farm, Al-Ain, United Arab Emirates). Preliminary works were done to determine the percentage of CM to be mixed with BM as detailed in Abdalla et al. (2022) and based hereon;

thus, the milk samples that were chosen were 100% BM, and blends containing 15% (CM15%) and 30% (CM30%) CM. The cheesemaking procedure was described by Ayyash et al. (2021b) and is presented in Figure 1. Briefly, low-fat milk (12 L) was tempered at 36°C for 30 min in a 13-L temperature-controlled cheese vat. A mozzarella cheese starter culture, consisting of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Chr. Hansen Holding A/S), was added (0.3% wt/wt), followed by incubation for 60 min until pH dropped by 0.1. Subsequently, the double-strength chymosin (Chr. Hansen Holding A/S) was added (60 international milk clotting units/L), and curd was allowed to be formed for 45 min at 36°C. The curd was subsequently cut into ~1-cm³ cubes, and the curds/whey mixture was cooked to 41°C and allowed to settle at pH 6.1. After whey drainage, the curd was milled when the pH reached ~5.2, dry salted at a level of 2%, and mellowed for 20 min. The salted curds (2 kg) were heated to ~60°C in hot water (80°C), manually plasticized, and molded into a ~1.8-kg block. The block was immersed in cooled chilled water at ~2°C to a surface temperature of ~24°C. The cooled blocks were cut into ~500-g portions, vacuumed-packaged, and stored at 4°C. Low-moisture part-skim mozzarella cheeses were sampled on d 1, 30, and 60 of storage.

Chemical Composition

The moisture content was determined by the oven-drying method at 105°C (AOAC International, 1995), ash content by the muffle furnace method (AOAC International, 1995), fat content by the Gerber method (AOAC International, 1995), and protein content by the Kjeldahl method (AOAC International, 1995). For pH measurement, grated cheese (25 g) was homogenized with 25 mL of deionized-distilled (dd)-water homogenized at 12,000 rpm for 1 min at room temperature with an Ultra-Turrax homogenizer (T25; IKA Labor Technik), and the pH was measured using a digital pH meter STATER3100 (OHAUS Corporation; Ayyash and Shah, 2011a).

Total and Colloidal Calcium

The total and soluble Ca contents were assessed according to Metzger et al. (2001). The soluble Ca content was assessed in cheese extract prepared by homogenizing 5 g of cheese with 50 mL of dd-water at 60°C for 30 s. Calcium contents in the whole cheese, and then soluble phase, were analyzed using inductively coupled plasma-optical emission spectrometry (Ayyash and Shah, 2011a).

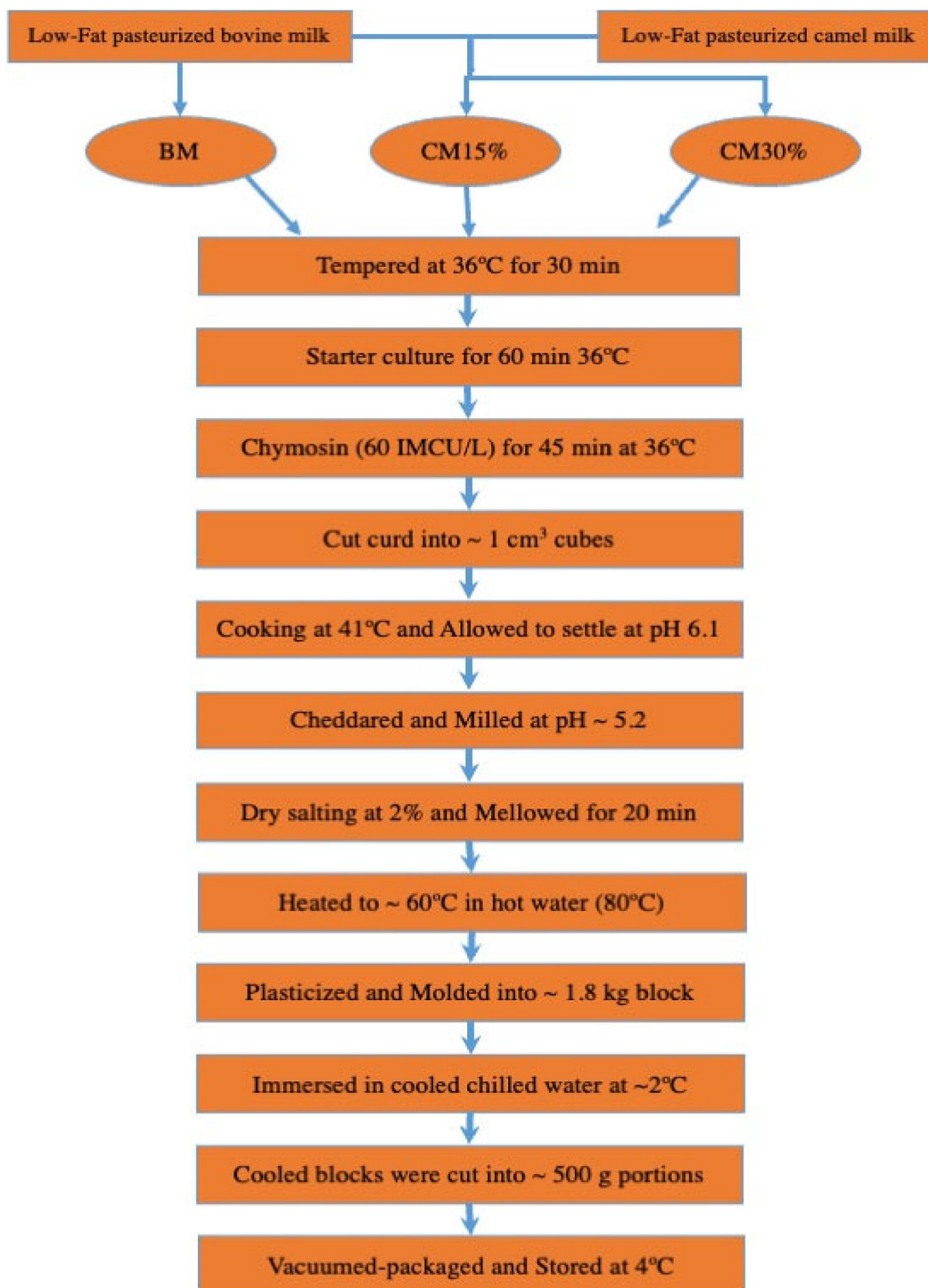


Figure 1. Experimental design and low-moisture part-skim mozzarella cheese manufacturing diagram. BM = bovine milk; CM15% and 30% = BM mixed with 15% or 30% camel milk.

Proteolysis Assessment

pH 4.6-Water-Soluble Nitrogen. Water-soluble extracts (WSE) from the cheese samples were prepared according to Kuchroo and Fox (1982) by homogenizing

grated cheese with dd-water at a ratio of 1:2. The pH of the slurries was adjusted to 4.6, followed by centrifugation at $6,000 \times g$ for 15 min at 4°C. The nitrogen content of the WSE, that is, the water-soluble nitrogen (WSN), was assessed using the Kjeldahl method

(AOAC International, 1995), and the level of pH 4.6-WSN was expressed as a percentage of total nitrogen.

Trichloroacetic Acid-Soluble Nitrogen (12%). To assess the 12% trichloroacetic acid-soluble nitrogen (TCA-SN), a 24% TCA was mixed with an equal volume of pH 4.6-WSE, followed by vortexing for 30 s. The mixture was kept for 30 min at room temperature (20°C), followed by centrifugation at $4,000 \times g$ for 15 min at 4°C. The trichloroacetic acid-soluble nitrogen was assessed by the Kjeldahl method (AOAC International, 1995) and expressed as a percentage of total nitrogen.

o-Phthaldialdehyde Absorbances. o-Phthaldialdehyde (OPA) analysis was performed according to Al-Dhaheri et al. (2017). Briefly, 50 μ L of pH 4.6-WSE was placed into a 1.5-mL cuvette and mixed with 1 mL of OPA reagent prepared freshly according to Al-Dhaheri et al. (2017). The absorbance was measured at 340 nm using a UV-spectrophotometer (Epoch Microplate Spectrophotometer, Agilent).

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of the precipitates resulting from the pH 4.6-WSE preparation (stored at -20°C) was performed according to Ong and Shah (2009). Dithiothreitol was used as the reducing agent, and separation was carried out on a 12% acrylamide separating gel. After electrophoresis, the gels were fixed for 1 h in a solution of 40 mL of ethanol/100 mL of dd-water and 10 mL of acetic acid/100 mL of dd-water, and subsequently stained for 20 h using the QC Quick Coomassie stain (Bio-Rad Laboratories), followed by destaining for 3 h by changing the distilled water 3 times. Gel imaging was performed using Gel Doc XR+ and Chemidoc XRS+ Imaging Systems (Bio-Rad Laboratories).

LMPS Mozzarella Functionality

Meltability. Low-moisture part-skim mozzarella meltability was assessed according to Ayyash and Shah (2011a). Ten grams of shredded cheese was placed in a test tube (32 mm \times 200 mm) and was packed to form a plug at the bottom. The test tube was sealed with a rubber stopper, ventilated with small hole for the hot gas to escape during heating. The test tube was placed vertically in a refrigerator at 4°C for 30 min, and then horizontally in an oven and heated at 104°C for 100 min. Meltability was measured in centimeters from the bottom of the test tube to the point at which the cheese had stopped flowing.

Free Oil. The free oil content of cheeses was determined according to Wadhvani et al. (2011) with

minor modifications. Grated cheese (18 g) was put in a 50-mL Falcon tube and immersed in boiling water for 15 min to melt the cheese. Methanol diluted in distilled water (1:2, 20 mL at 57°C) was immediately added to the bottle, and then the bottle was centrifuged at $6,000 \times g$ at room temperature for 10 min. The free oil content of the cheese was expressed as a percentage as follows:

$$\text{Free oil (\%)} = \text{reading of fat/cheese weight.}$$

Browning. Browning of LMPS mozzarella was determined according to Ayyash and Shah (2011a). Shredded cheese samples were weighed (20 g) into an aluminum pan (7 cm in diameter and 3 cm high) and allowed to temper at room temperature (20°C) before heating. The pans containing the samples were placed into a preheated, forced-air oven at 100°C for 1 h. Cheese samples were cooled to room temperature. The color was measured using Minolta Chroma-meter CR-300 (Minolta Corporation Ltd.), which was calibrated before testing. Three color indices, L^* (light to dark), a^* (red to green), and b^* (yellow to blue) values, were taken for each sample in triplicate. The browning index (BI) was calculated based on the values of L^* , a^* , and b^* parameters (Felix da Silva et al., 2018), using the equations as follows:

$$\text{BI} = [100(x - 0.31)]/0.172,$$

$$\text{where } x = (a^* + 1.75L^*)/(5.645L^* + a^* - 3.012b^*).$$

Microstructure by Scanning Electron Microscopy

The microstructure of cheese samples was studied by scanning electron microscopy according to Ayyash et al. (2018). Small pieces of cheese were fixed on an aluminum holder and coated with a thin layer of gold using a Cressington 108 Auto Sputter Coater (Ted Pella Inc.). Scanning electron microscopy analysis of the gold-coated cheese samples was conducted using a JEOL JSM-6010LA scanning electron microscopy (Akishima), operating at an accelerating voltage of 20 kV. Scanning electron microscopy images were collected in the secondary electron imaging mode, and images were recorded at various magnifications.

Texture Profile Analysis

Cylindrical samples (25 mm diameter \times 20 mm height) were cut, in duplicate, from the center of LMPS mozzarella cheese blocks. Texture profile analysis on these samples was performed according to Ayyash et al.

(2018). Hardness (the force necessary to attain a given deformation), cohesiveness (a measurement of how well the structure of the cheese withstands compression), springiness (the return rate of deformed cheese to its original shape), and chewiness (chewiness = gumminess \times springiness = second area/first area \times hardness \times springiness) of all cheese specimens were tested on the same day of sampling using a Texture Analyzer CT3 (Brookfield AMETEK).

Rheological Properties

Rheological analyses of cheese samples were carried out according to Ayyash et al. (2018). Briefly, samples were cut from at least 3 mm deep into the cheese blocks. These samples were immediately placed in small, airtight plastic containers and equilibrated at room temperature ($25 \pm 1^\circ\text{C}$) for at least 20 min. Small oscillatory amplitude measurements were performed with a Discovery Hybrid Rheometer HR-2 (TA Instruments). The measuring geometry consisted of 2 parallel plates with a diameter of 40 mm, operating at a 2.6-mm gap size with a sample thickness of 3 mm. Excessive cheese was trimmed carefully, and the sample was allowed to rest for 60 s on the rheometer to allow the stresses induced during sample handling to relax. All rheological properties were measured in duplicate.

The linear viscoelastic range was determined by performing a strain sweep at a frequency of 1.0 Hz, with the strain values varying from 0.1 to 10%. A strain in the linear viscoelastic range (0.1–1%) was then selected for a frequency sweep test, where the strain was set at 0.5%, and the frequency was varied from 0.1 to 20 Hz at 25°C . The dynamic parameters storage modulus (elastic component; G'), loss modulus (viscous component; G''), and the loss tangent ($\tan \delta$) were documented. The rheological properties of the LMPS cheeses as a function of temperature were determined according to Guinee et al. (2002). The cheese samples were heated from 20 to 85°C , at a heating rate of $3^\circ\text{C}/\text{min}$, with a strain of 0.5% and a frequency of 1.0 Hz. The dynamic parameters G' , G'' , and $\tan \delta$ were recorded.

Statistical Analysis

All cheesemaking was repeated in duplicates and sampled twice ($n = 4$). A one-way ANOVA was carried out to investigate the effect of cheese type on parameters at the same storage time. For the same cheese type, a one-way ANOVA was carried out to investigate the effect of the storage period on cheese parameters. Means comparisons at the same storage period or the same cheese type were performed using Tukey's test.

All statistical analyses were carried out using Minitab 20.0 software (Minitab Inc.).

RESULTS AND DISCUSSION

Gross Composition of LMPS Mozzarella Cheeses

Table 1 shows the composition of LMPS mozzarella cheese prepared from BM and CM-BM blends. Compared with BM cheese, CM15%, and CM30% cheese had significantly ($P < 0.05$) lower DM, protein, and fat content, but higher moisture content. The CM15% and CM30% cheeses had significantly ($P < 0.05$) greater ash, colloidal Ca, and total Ca contents than BM cheese. No significant ($P < 0.05$) differences were found in the NaCl content between cheeses. The higher moisture content in mixed-milk cheeses could be attributed to the greater water retention capacity of CM proteins compared with BM proteins (Ipsen, 2017). In agreement with this finding, Abdalla et al. (2022) found that Akawi cheese manufactured from blends of CM and BM had greater moisture content than the Akawi cheese made from BM.

The higher total Ca concentration in CM15% and CM30% cheese than in BM cheese (Table 1) might be due to the higher Ca content in CM milk than in BM milk (Hailu et al., 2016a). On d 1, 30, and 60, the colloidal Ca concentrations in CM15% and CM30% cheese were higher ($P > 0.05$) than in BM cheese (Table 1). This implies that increasing the CM milk ratio could increase the total and colloidal Ca contents. The decrease in colloidal Ca contents during storage could be attributed to the continuous proteolysis in LMPS mozzarella cheese, as shown later in the proteolysis section (Figure 2; Lucey et al., 2003). pH of all LMPS mozzarella cheese changed insignificantly, although Ca content differences were significant. We assume that protein buffering capacity contributed to the insignificant changes in pH between the experimental treatments or during storage.

Proteolysis Assessment

Figures 1A, 1B, and 1C present the pH 4.6-WSN (%), OPA as absorbances (nm), and TCA-SN (%) contents in LMPS mozzarella cheeses at d 1, 30, and 60 of storage. For all time points, the cheeses made from mixed milk showed significantly higher ($P < 0.05$) pH 4.6-WSN, OPA, and TCA-SN values compared with BM cheese (control). The highest values were noticed with CM30%, followed by CM15% cheese (Figure 2). The greater proteolytic levels seen in LMPS mozzarella cheeses prepared from mixed milk might be due to primary protein hydrolysis caused by the activity of residual chymosin (Ardö et al., 2017). This may suggest

Table 1. Chemical composition, calcium contents, and pH values of low-moisture part-skim mozzarella cheeses made from bovine milk (BM) or blends of BM and 15% (CM15%) or 30% (CM30%) camel milk¹

Parameter	BM	CM15%	CM30%
Moisture content (g/100 g)	50.9 ± 0.49 ^b	53.6 ± 0.30 ^a	53.8 ± 0.55 ^a
Protein content (g/100 g)	30.6 ± 0.61 ^a	29.0 ± 1.28 ^b	29.0 ± 0.59 ^b
Fat content (g/100 g)	15.1 ± 1.08 ^a	13.6 ± 1.75 ^b	13.9 ± 0.44 ^b
DM (g/100 g)	49.1 ± 0.49 ^a	46.3 ± 0.24 ^b	46.3 ± 0.42 ^b
Ash content (g/100 g)	3.1 ± 0.31 ^b	3.7 ± 0.32 ^a	3.4 ± 0.21 ^a
Total Ca (mg/100 g)	504.6 ± 13.83 ^c	612.0 ± 14.42 ^b	745.4 ± 23.68 ^a
Salt content (g/100 g)	1.0 ± 0.29 ^a	1.1 ± 0.24 ^a	1.1 ± 0.08 ^a
Colloidal Ca, d 1 (mg/100 g)	305.9 ± 13.8 ^b	419.3 ± 17.8 ^a	469.6 ± 8.7 ^a
Colloidal Ca, d 30 (mg/100 g)	293.8 ± 7.6 ^c	375.5 ± 20.8 ^b	471.1 ± 1.5 ^a
Colloidal Ca, d 60 (mg/100 g)	252.5 ± 14.6 ^c	383.0 ± 38.8 ^b	436.6 ± 19.2 ^a
pH, d 1	5.2 ± 0.15 ^a	5.3 ± 0.24 ^a	5.3 ± 0.35 ^a
pH, d 30	5.2 ± 0.37 ^a	5.3 ± 0.20 ^a	5.3 ± 0.51 ^a
pH, d 60	5.5 ± 0.25 ^a	5.4 ± 0.71 ^a	5.4 ± 0.12 ^a

^{a-c}Means with different superscripts in the same row differed significantly ($P < 0.05$).

¹Values are means ± SD ($n = 4$).

that CM proteins are more vulnerable to proteolytic degradation than BM proteins, probably due to the variations in casein content and structure. Additionally, the significantly higher ($P < 0.05$) moisture contents in cheeses prepared from mixed milk (Table 1) may contribute to higher proteolysis (pH 4.6-WSN, TCA-SN, OPA) values noticed in these cheeses (Ardö et al., 2017).

Figure 3 displays the proteolysis pattern by SDS-PAGE of the cheese pellets resulting after the preparations of pH 4.6-WSE of the experimental cheeses on d 1, 30, and 60 of storage. The intensities of the bands of β -CN and the α_s -CN showed minor reductions by d 30 for all of the cheeses (Figure 3). During storage, new (polypeptide) bands appeared in the 20 to 6.4 kDa region (Figure 3). Additionally, the proteolytic patterns were clear in CM15% and CM30% cheeses compared with BM. This supports our explanation that CM caseins could be more susceptible to hydrolysis by residual chymosin and proteases than their bovine counterpart. The action of residual chymosin in cheese, and perhaps plasmin on caseins, could explain this observation (McSweeney, 2017).

Functional Properties

Meltability and Oiling-Off. Cheese meltability is an essential indicator of the level to which a cheese melts when heated. Meltability results for LMPS mozzarella cheeses made from BM, CM15%, and CM30% on d 1, 30, and 60 are presented in Figure 4A. As shown in Figure 4A, all LMPS mozzarella cheeses showed an increase in meltability with the progress of maturation. On d 1 and 30, CM30% cheeses had a significantly higher meltability compared with CM15% and BM cheeses. At d 60, CM15% and CM30% cheeses exhibited significantly ($P < 0.05$) greater melting degrees

relative to BM cheese. This could be attributed to the higher moisture content in CM15% and CM30% cheeses relative to BM cheese, which is associated with melting ability (McMahon and Oberg, 2017). Moreover, the high β -CN and large micelles size could cause a weak protein network formed by CM caseins during cheese processing, and could consequently improve the meltability of the LMPS mozzarella cheeses made from blended milk. Furthermore, the higher proteolysis rates in LMPS mozzarella cheeses made from blended milk (Figures 1 and 2) could also contribute to the high meltability in these cheeses (Ayyash and Shah, 2011b). The hydrolysis of the charged peptides could decrease the total number of protein-protein bonds and thereby increase meltability during storage (Lucey et al., 2003). Concerning oiling-off (tendency of free oil to separate from melted cheese; Ah and Tagalpallewar, 2017), there were no differences in the amount of free oil released between the melted cheeses (Figure 4B).

Browning. The browning of LMPS mozzarella cheese caused by the Maillard reaction is closely associated with its baking. Also, browning and blistering are regarded as critical quality characteristics for the pizza baking performance of LMPS mozzarella (Ah and Tagalpallewar, 2017). Regarding BI and color characteristics of LMPS mozzarella cheeses after heating, Table 2 presents color parameters; namely, L^* , a^* , b^* , and BI, indicating whiteness, redness, yellowness, and browning index, respectively. The results indicated significant differences ($P < 0.05$) in color parameters (especially a^* and b^*) among the experimental cheeses after heating (Table 2). On d 1, mixed-milk cheeses (CM15% and CM30%) showed significantly ($P < 0.05$) lower a^* and b^* values, but higher L^* in comparison to BM cheese as cheese ripening progressed (at d 30 and 60); additionally, mixed-milk cheeses showed an increase in a^* and b^* values but a decrease in L^* value as

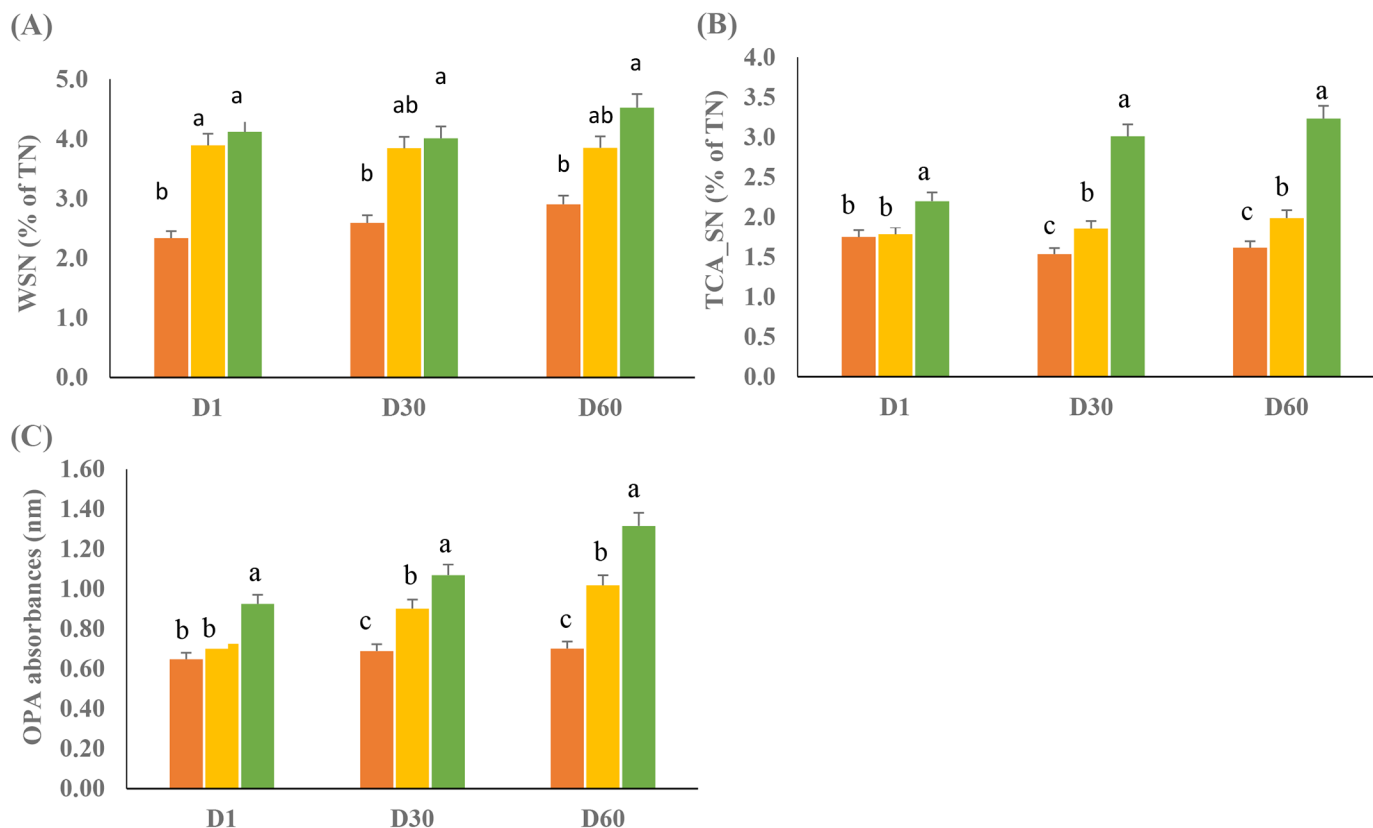


Figure 2. Proteolysis parameter including pH 4.6-water-soluble nitrogen (WSN; A), trichloroacetic acid-soluble nitrogen (TCA_SN; B), and o-phthalaldehyde (OPA; C) of low-moisture part-skim mozzarella cheese, during storage, made from bovine milk (orange bar), or blends of bovine milk and 15% (yellow bar) or 30% (green bar) camel milk. Values are mean \pm standard deviation ($n = 4$). Bars with different lowercase letters (a–c) at the same storage time differed significantly ($P < 0.05$). TN = total nitrogen.

compared with BM cheese. These results are consistent with the BI results (Table 2), where mature (at d 60) LMPS mozzarella cheeses made from mixed milk had greater BI values relative to BM cheese. This indicates a higher concentration of nonenzymatic browning products in mature cheeses made from mixed milk, which could be due to the greater proteolytic levels seen in LMPS mozzarella cheeses prepared from mixed milks (Figure 2). Proteolysis progress may result in a greater concentration of accessible amino groups capable of participating in the Maillard browning process (Ah and Tagalpallewar, 2017). Furthermore, color formation in mozzarella is generally influenced by starter culture, sugar consumption, and production techniques that favor sugar elimination.

Texture Profile Analysis. Figure 5 exhibits the texture profile analysis for LMPS mozzarella cheeses prepared from BM and BM-CM mixes on d 1, 30, and 60. We detected significant ($P < 0.05$) differences in texture properties between the experimental cheeses. For all storage periods, CM15% and CM30% cheeses had lower ($P < 0.05$) hardness values than BM cheese.

This could be attributed to the weaker protein network formed in the presence of CM caseins compared with BM. Moreover, the lower hardness in CM15% and CM30% coincided with the higher moisture content in the same cheeses (Table 1) and proteolysis (Figure 2) detected in CM15% and CM30% cheeses. The negative association between hardness, proteolysis, and moisture content has been reported in the literature (Lucey et al., 2003). The hardness and chewiness of all LMPS mozzarella cheeses decreased noticeably after 30 and 60 d of storage (Figure 5). This result concurs with the result reported by Guinee et al. (2001). Low-moisture part-skim mozzarella cheeses made of CM15% and CM30% cheeses had lower ($P < 0.05$) hardness and chewiness values than BM (Figure 5A and 4D). However, springiness values showed the opposite trend (Figure 5B). This springiness trend may be attributable to greater protein-protein interactions during storage time with prolonged proteolysis. The cohesiveness values of LMPS mozzarella cheeses made of blended milk were slightly higher than BM only (Figure 5C).

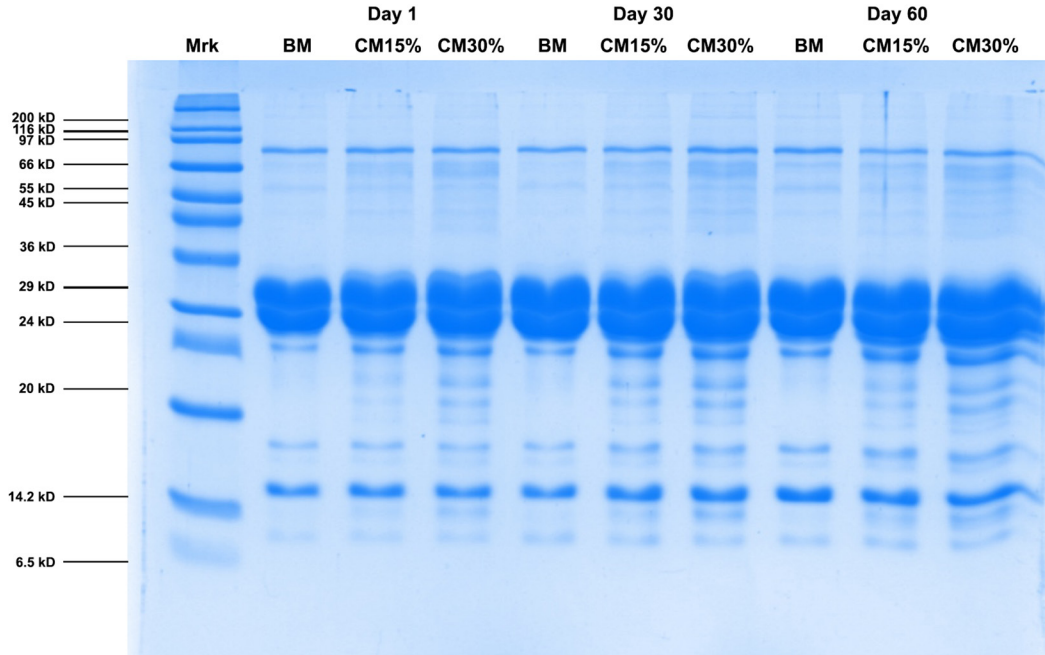


Figure 3. The SDS-PAGE image of low-moisture part-skim mozzarella cheeses, during storage, made from bovine milk (BM) or blends of BM and 15% (CM15%) or 30% (CM30%) camel milk.

Milk with smaller micelles, such as BM, forms a more compact and harder gel network than milks with bigger micelles, such as CM (Li and Zhao, 2019; Ayyash et al., 2022). As a result, the bigger camel micelles may disrupt the consistency of the BM-dominant para-CN network, causing brittle points in the matrix and decreasing hardness. The current study reveals that the inclusion of CM had a substantial influence on the textural characteristics of the LMPS mozzarella cheese. Ramírez-López and Vélez-Ruiz (2018) have reported similar hardness results in fresh panela cheese made

from goat-bovine blended milk. The interaction between the BM and CM micelles and structural arrangements of the cheese network remains unknown and requires further investigation.

Cheese Microstructure. Microstructural characteristics of the cheeses on d 1, 30, and 60 are shown in Figure 6. At d 1, the scanning electron microscopy image of the BM only (control; Figure 1A) showed no elongated fat globules or proper alignment of the casein matrix reported by previous studies. This may be attributed to the hand plasticizing technique employed in

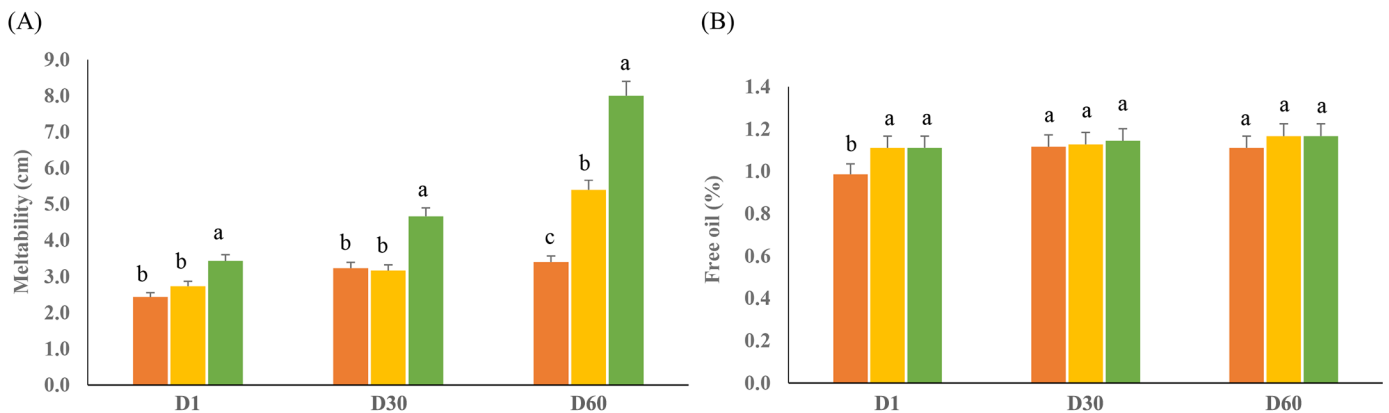


Figure 4. Meltability (A) and free oil (B) of low-moisture part-skim mozzarella cheese, during storage, made from bovine milk (orange bar) or blends of BM and 15% (yellow bar) or 30% (green bar) camel milk. Values are mean ± standard deviation (n = 4). Bars with different lowercase letters (a-c) at the same storage time differed significantly ($P < 0.05$).

Table 2. Color parameters after heating at 100°C of low-moisture part-skim mozzarella cheeses made from bovine milk (BM) or blends of BM and 15% (CM15%) or 30% (CM30%) camel milk, and ripened for 1, 30, or 60 d at 4°C¹

Storage	Cheese	Color parameter ²			
		L*	a*	b*	Browning index
d 1	BM	52.9 ± 0.50 ^a	13.7 ± 0.73 ^a	31.6 ± 0.69 ^a	104.2
	CM15%	56.4 ± 3.98 ^a	9.1 ± 3.26 ^b	28.5 ± 1.15 ^b	79.2
	CM30%	56.3 ± 4.63 ^a	8.1 ± 5.18 ^b	27.2 ± 3.74 ^b	73.9
d 30	BM	53.9 ± 1.48 ^a	11.5 ± 0.87 ^{ab}	30.8 ± 0.79 ^b	95.5
	CM15%	54.8 ± 1.69 ^a	12.2 ± 1.43 ^a	33.1 ± 0.43 ^a	103.0 ^b
	CM30%	55.4 ± 1.08 ^a	10.1 ± 0.96 ^b	30.0 ± 1.04 ^b	87.3
d 60	BM	51.0 ± 0.96 ^a	9.6 ± 0.94 ^c	31.1 ± 0.91 ^b	101.5
	CM15%	47.9 ± 0.26 ^b	14.8 ± 1.22 ^a	35.4 ± 1.11 ^a	140.5
	CM30%	49.0 ± 1.34 ^b	12.3 ± 1.16 ^b	31.9 ± 1.71 ^b	115.4

^{a-c}Means with different superscripts in the same column and same storage time differed significantly ($P < 0.05$).

¹Values are mean ± SD (n = 16).

²Color characteristics of low-moisture part-skim mozzarella cheese after heating; L* = whiteness; a* = redness; b* = yellowness.

this study. Similar results were reported by Joshi et al. (2004). The protein matrix of the BM cheese consisted of different planes attached to each other. This is also similar to the report by Joshi et al. (2004).

The scanning electron microscopy images of cheese samples on d 1, 30, and 60 revealed clear differences in cheese microstructure between BM cheese and cheeses prepared from CM-BM mixes (Figure 6). The scanning electron microscopy images from d 1 revealed that the structures in CM15% and CM30% cheeses had a smooth surface, whereas in BM cheese, slightly rough structures with granular surfaces were apparent. On d 30 and 60, the BM cheese developed a dense network of aggregated caseins and finer pores, most likely due to the larger protein proportion, whereas pores were lacking in the CM15% and CM30% cheese structures (Figure 3). This finding implies that CM addition affected the microstructural characteristics of LMPS mozzarella cheese manufactured from CM-BM milk, which is consistent with previous findings (Abdalla et al., 2022). Hence, such observation requires additional investigation, including examination of the pertinent fundamentals.

Rheological Properties

Linear Viscoelastic Region. The strain sweep test results for the LMPS mozzarella on d 1 and 60 are presented in Figure 7 (A–F). Because all the LMPS mozzarella cheeses displayed viscoelastic behavior in the linear viscoelastic range of 0.1 to 1.0% strain, the strain of 0.5% was chosen for further rheological testing. On d 1 and 60, the variations in rheological characteristics between cheeses were noticeable. The results revealed that experimental cheeses had $G' > G''$, which is consistent with the structures described by Abdalla

et al. (2022) in LFA cheese made from blended bovine and CM.

Viscoelastic Properties. The frequency sweep testing is a quantitative method to evaluate the viscoelastic properties of cheese at different maturity stages, at different utilization temperatures, or both (Tunick and Van Hekken, 2010). Figure 8 (A–D) reveals that on d 1 and 60, all of the LMPS mozzarella cheeses had $G' > G''$ within the examined frequency range (0.1–10 Hz). On d 1, the G' and G'' values of the CM30% cheese were lower than that of CM 15% and BM cheeses, which could be due to the possible disruptive effect of camel micelles on the bovine matrix, resulting in a cheese with a weak texture, a poor protein network of CM, and high-moisture content (Table 1). On d 60, the G' of the CM30% cheese was higher than at d 0 storage (Figure 8D). This trend might be attributed to greater proteolysis rates (Figure 2) in LMPS mozzarella cheeses produced from mixed milk during storage. The increased proteolysis could increase the intermediate peptides, which increase the water holding capacity of the proteins, and then influence rheological characteristics (Fox et al., 2017). Furthermore, proteolysis could increase the hydrophobic interactions, which are considered, by researchers, the main factor that affects the cheese structure (Lucey et al., 2003; Stankey et al., 2017).

On d 1 and at the frequency range of 1 to 10 Hz, CM15% and CM30% cheeses showed lower $\tan \delta$ values than BM cheese (Figure 8E), indicating that LMPS mozzarella cheeses made from mixed milk became more resistant to structural change. However, after 60 d of maturity, these cheeses had noticeably higher $\tan \delta$ values than BM cheese (Figure 8F). The increase in proteolysis rate (Figure 1) during storage may explain the lower resistance to structural conversion in LMPS moz-

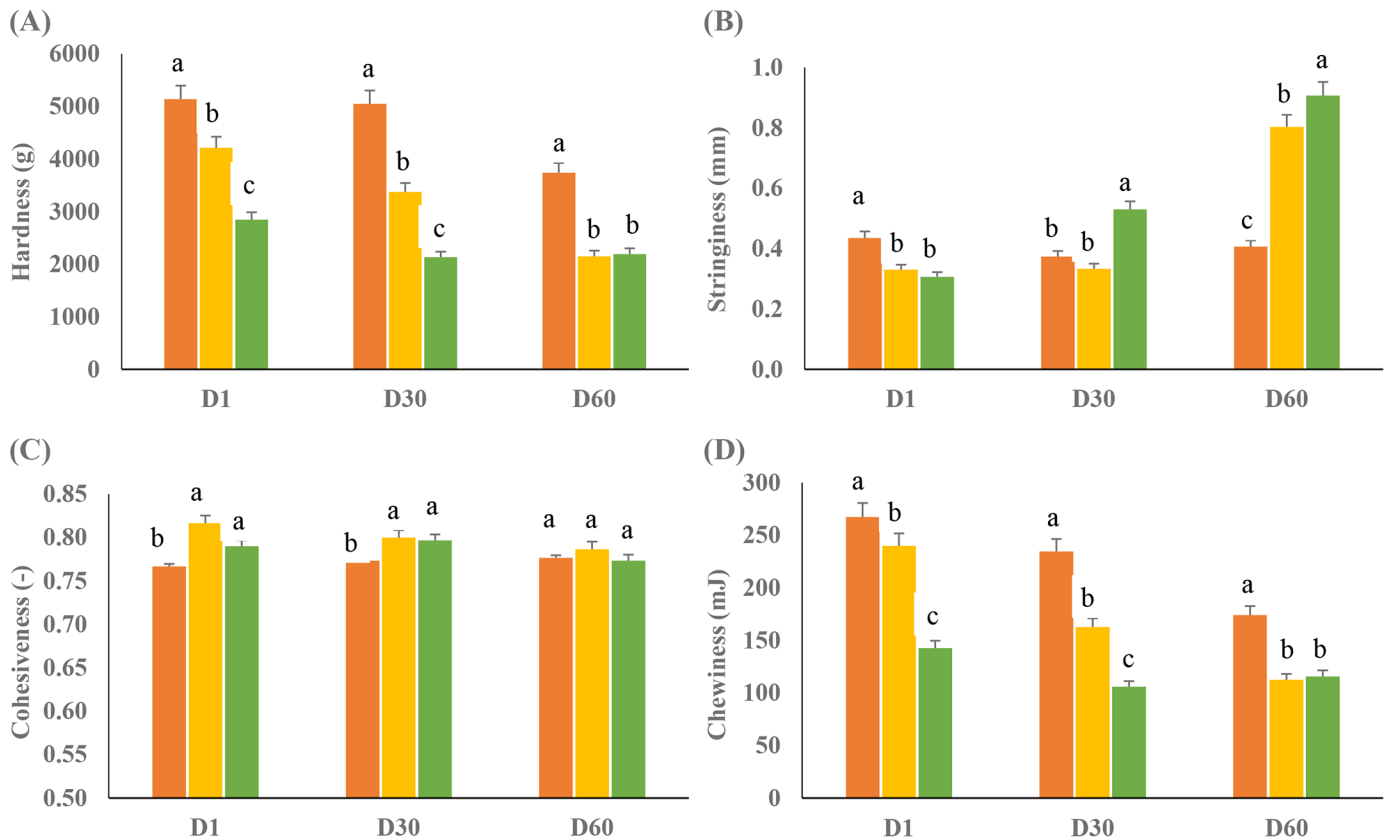


Figure 5. Hardness (A), stringiness (B), cohesiveness (C), and chewiness (D) of low-moisture part-skim mozzarella cheese, during storage, made from bovine milk (orange bar) or blends of bovine milk and 15% (yellow bar) or 30% (green bar) camel milk. Values are mean \pm standard deviation ($n = 12$). Bars with different lowercase letters (a–c) at the same storage time differed significantly ($P < 0.05$).

zarella cheeses. The number and strength of linkages between casein particles and also the particle's makeup, dispersion, and arrangement determine G' , G'' , and $\tan \delta$ (Tunick and Van Hekken, 2010). We assume that the larger size of the camel micelles disturbed the continuity of the para-CN network, causing weak points in the cheese matrix.

Temperature Sweep Test. Figure 9 (A–F) depicts changes in the G' , G'' , and $\tan \delta$ as functioned to temperature increase (25–85°C) on d 1 and 60 of storage for the LMPS mozzarella cheeses. On d 1, when compared with BM cheese, the CM15% and CM30% cheeses exhibited a decreasing trend in G' during temperature elevation from 25 to 55°C (Figure 9A). As the temperature increased from 57°C to 85°C, the G' values increased, with mixed cheeses having lower values than BM cheese (Figure 9A). A similar trend was observed with G'' values (Figure 9C). This increase might indicate structural reformation at temperatures over 60°C. This result requires additional research to fully comprehend the mechanism of the CM impact on the protein network in LMPS mozzarella cheese. On d 60, as compared with BM cheese, CM15% and CM30% exhibited

higher G' and G'' values at $>45^\circ\text{C}$ (Figures 8B and 8D). This result could be attributed to higher hydrophobic interactions that may be developed in blended cheeses (CM15% and CM30%) more than in BM cheese (Lucey et al., 2003; McMahon and Oberg, 2017). This suggests that moisture content and proteolysis are the main factors that affect the viscoelastic properties of LMPS mozzarella made from CM-BM milk. The findings of the present study necessitate further studies for understanding the network arrangements and interactions between caseins from camel and BM.

Tan delta (δ), as a function of temperature, provides a quantitative measure for the cheese gel-sol transition. All the LMPS mozzarella cheeses were principally elastic-like ($\tan \delta \text{ max} < 0.8$) with different degrees (Figure 9E). When the temperature exceeded 40°C, $\tan \delta$ values rose, peaked between 60 to 70°C, and then they decreased (Figure 9E). Melting of milk fat may be accountable for the initial increase in $\tan \delta$ from 20°C to 45°C (Stankey et al., 2017), it represents approximately 20% of the weight of LMPS mozzarella, and when heated, it liquefies, initiating the softening process (McMahon and Oberg, 2017).

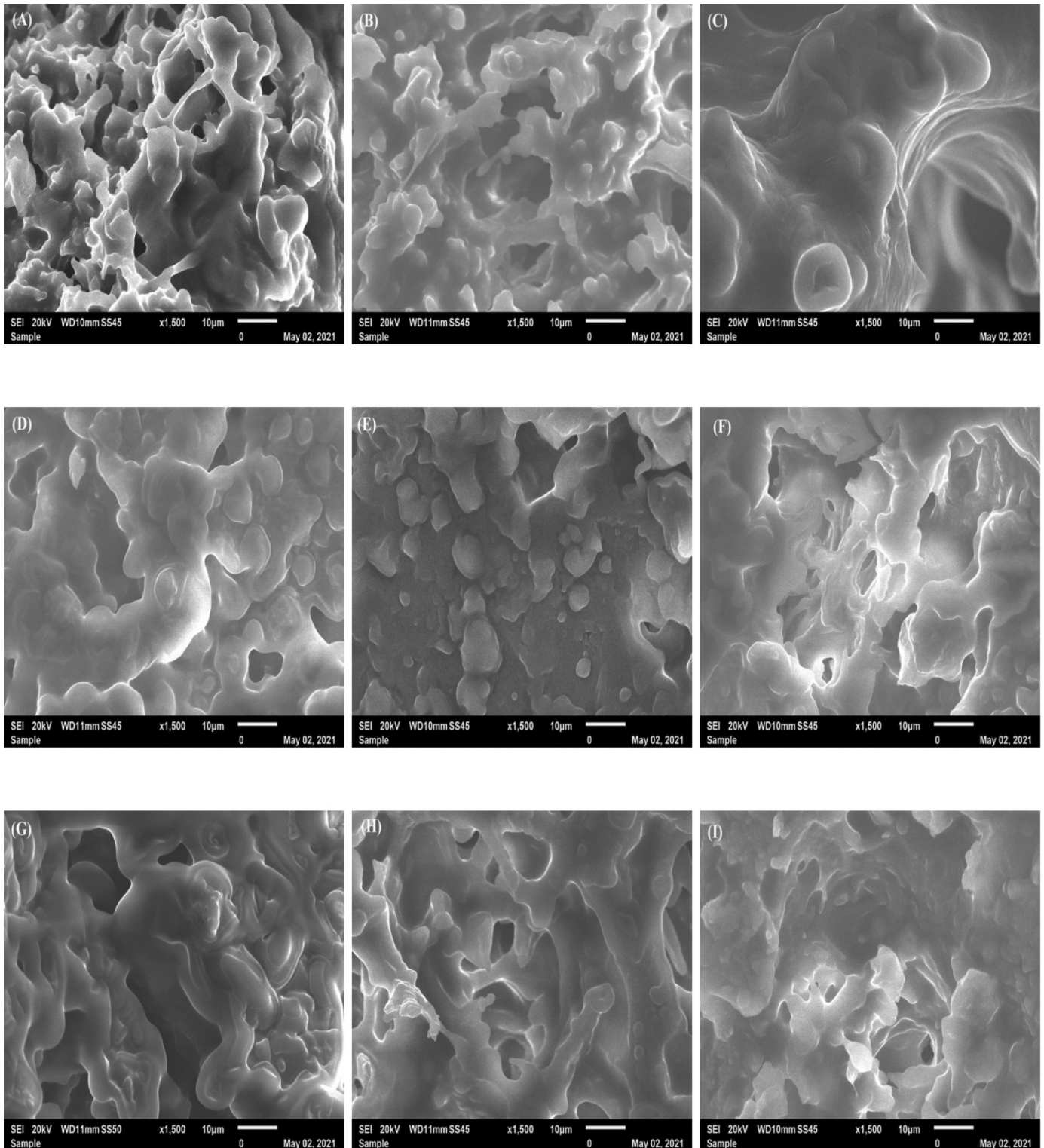


Figure 6. Scanning electron microscopy images of low-moisture part-skim mozzarella cheese during storage. A, B, and C represent bovine milk (BM), and blends of BM and 15% (CM15%) and 30% (CM30%) camel milk, respectively, at d 0. D, E, and F represent BM, CM15%, and CM30%, respectively, at d 30. G, H, and I represent BM, CM15%, and CM30%, respectively, at d 60.

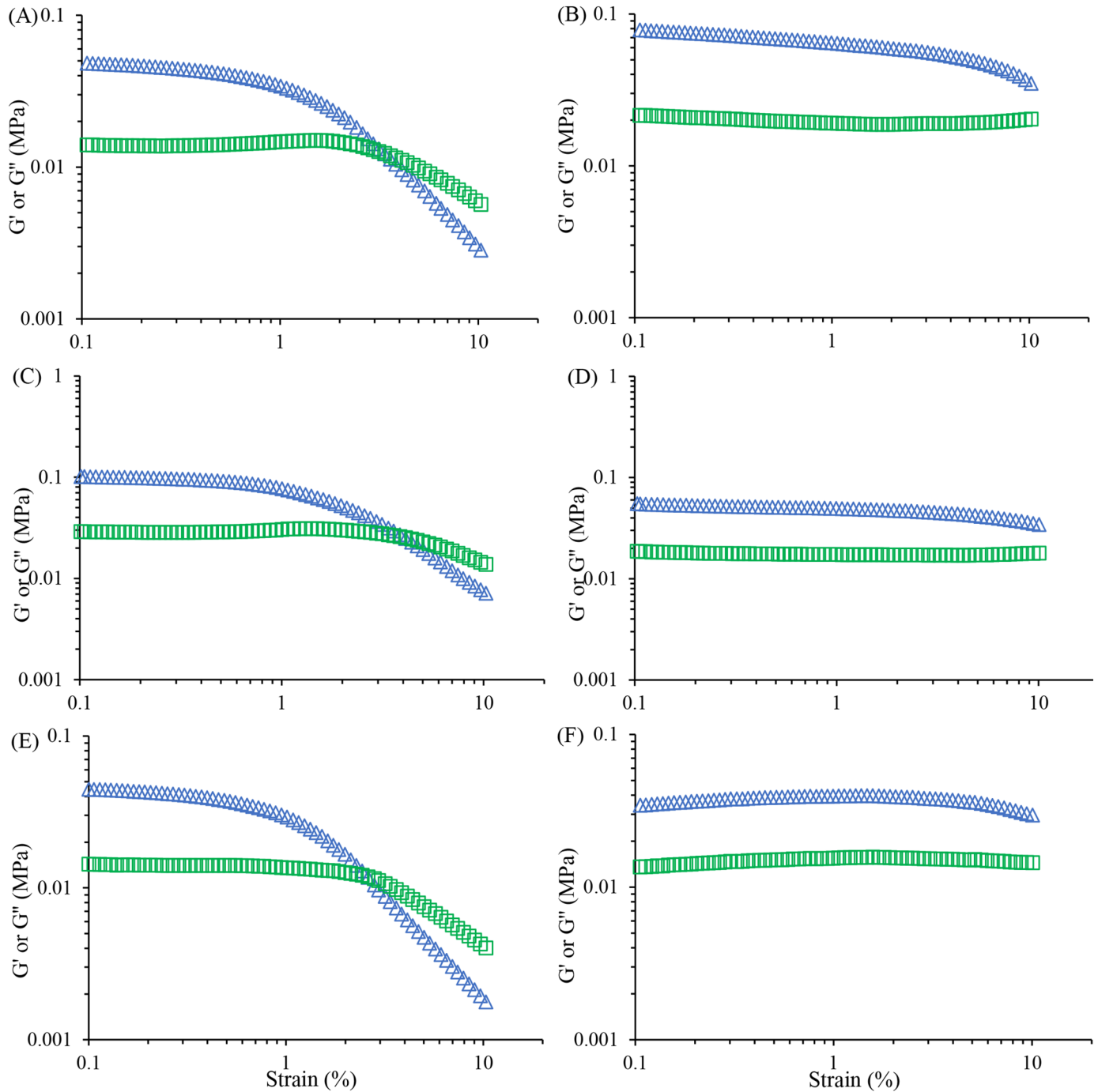


Figure 7. Storage modulus (G' ; blue triangle) and loss modulus (G'' ; green square) of the linear test of low-moisture part-skim mozzarella cheeses during storage. Bovine milk (BM) at d 0 (A) and d 60 (B); blend of BM and 15% camel milk at d 0 (C) and d 60 (D); blend of BM and 30% camel milk at d 0 (E) and d 60 (F).

On d 1, cheeses produced from mixed milk showed higher $\tan \delta$ values at ~ 55 to 65°C , which is a higher temperature than BM cheese milk (Figure 9E). This indicates that the cheese has not developed adequate functional characteristics. Low-moisture part-skim

mozzarella cheese only requires a minimal ripening period (typically a few weeks at 4°C) to develop the desired functional properties for utilization as a pizza ingredient (McMahon and Oberg, 2017). After 60 d of maturation, the variation in transition temperatures

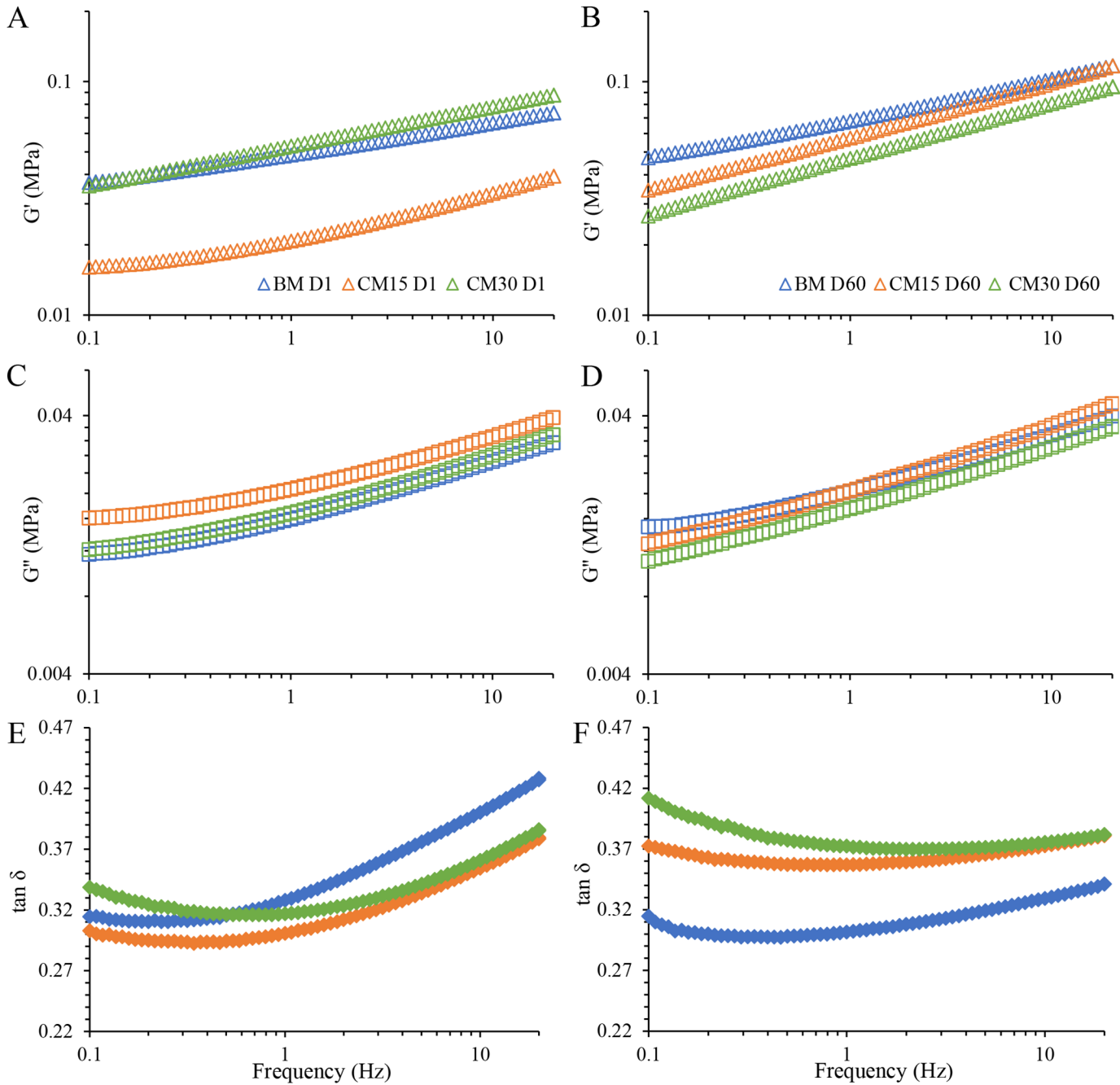


Figure 8. Storage (G') modulus at d 1 (A) and d 60 (B), loss (G'') modulus at d 1 (C) and d 60 (D), and tan delta (δ) at d 1 (E) and d 60 (F) of low-moisture part-skim mozzarella cheeses during storage. Bovine milk (BM), and blends of BM and 15% (CM15%) and 30% (CM30%) camel milk are blue, orange, and green triangle symbols, respectively.

between mixed-milk cheeses and the BM cheese became noticeable (Figure 9F). This may be attributed to the increased proteolysis rate during the storage period (Figure 2).

Melting behavior is generally increased by the conditions that reduce protein-protein interactions in the protein matrix and promote protein-water interactions

(Lucey et al., 2003; McMahon and Oberg, 2017). The addition of CM impaired the cheese protein network and affected the transition temperature. Compared with BM cheese, the CM15% and CM30% showed lower tan δ levels. In contrast to BM cheese, the tan δ in CM15% and CM30% cheeses hardly rose at high temperatures ($>40^{\circ}\text{C}$; Figure 9F). This may be due to

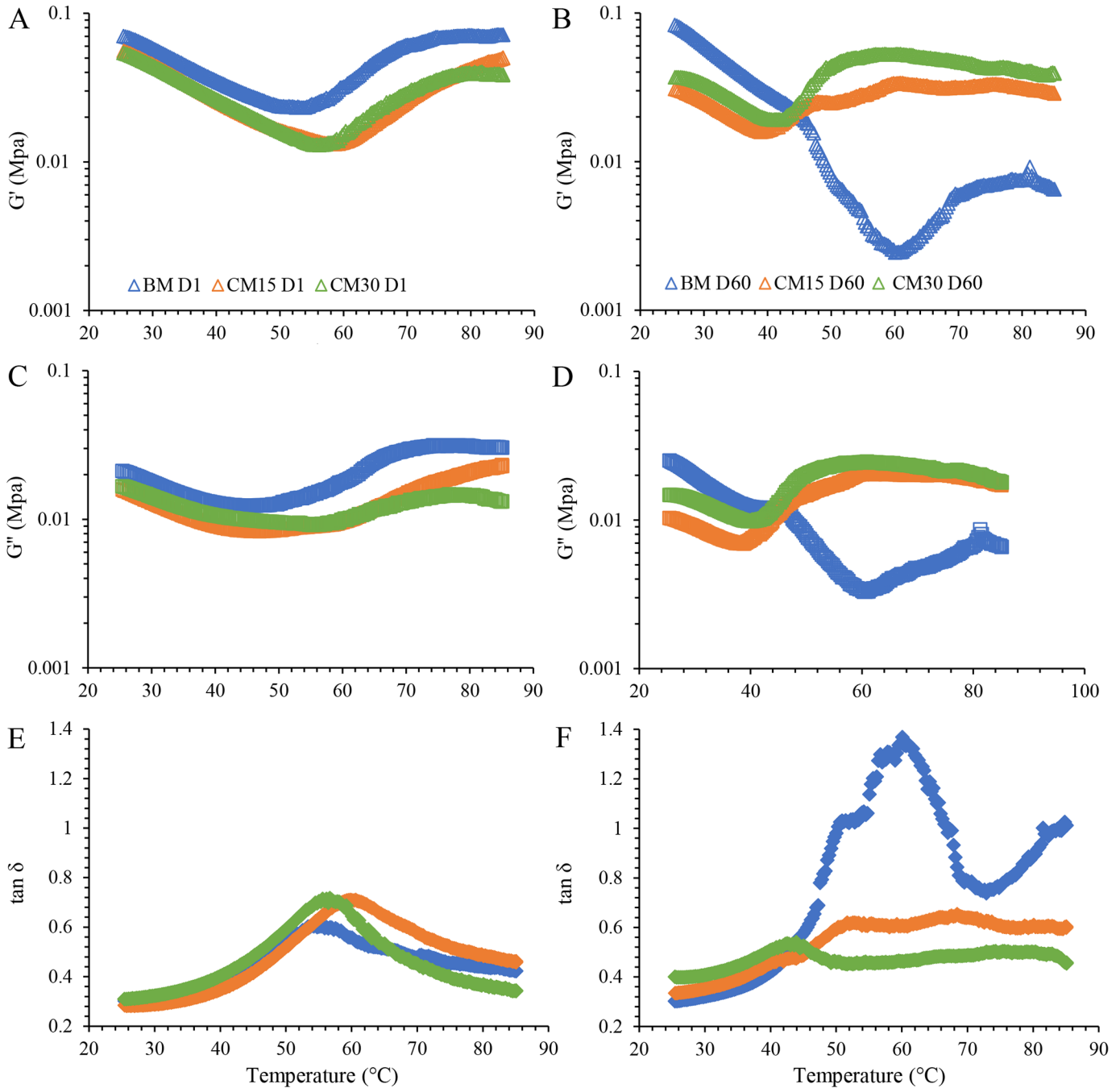


Figure 9. Storage (G') modulus at d 1 (A) and d 60 (B), loss (G'') modulus at d 1 (C) and d 60 (D), and $\tan \delta$ (δ) at d 1 (E) and d 60 (F), as functioned to temperature, of low-moisture part-skim mozzarella cheeses during storage. Bovine milk (BM), and blends of BM and 15% (CM15%) and 30% (CM30%) camel milk are blue, orange, and green triangle symbols, respectively.

altering the type or strength of caseins interactions in the CM15% and CM30% cheeses that could disrupt the normal balance accountable for melting. This might be attributed to the weaker protein network in mixed-milk LMPS mozzarella cheese. Weak gel formation in CM has been widely documented (Boukria et al., 2020; Mbye et al., 2021; Ayyash et al., 2022).

CONCLUSIONS

Based on the findings of this investigation, we conclude that combining BM and CM altered the rheological characteristics of the resulting LMPS mozzarella cheese. Camel milk addition affected the rate of soluble Ca and proteolysis, altering the functional properties of

the LMPS mozzarella cheese. Adding CM affected the color properties of LMPS mozzarella cheese manufactured from mixed milk. The microstructures formed in mixed-milk cheeses had smooth surfaces, whereas the BM cheese microstructures were rough with granulated surfaces. Low-moisture part-skim mozzarella cheeses prepared from mixed milk showed less firmness and chewiness but higher stringiness than BM cheese. To increase the opportunities and to improve the functionality of LMPS mozzarella as an ingredient in another food formula, more research on the interactions of caseins in CM and BM, as well as the way Ca is distributed in mixed-milk cheese (bovine and camel), would be extremely beneficial.

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