Effects of ultrafiltration followed by heat or high-pressure treatment on camel and bovine milk cheeses

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Abstract

The aim of this study was to assess the effects of ultrafiltration (UF) combined with high-pressure processing (HPP) or heat treatment on the quality of soft cheese produced from camel milk (CM) or bovine milk (BM). Milk was concentrated by UF (0, 1, and 2-fold) before treatment with HPP at 350 MPa or 550 MPa for 5 min at 4°C or by pasteurization at 65°C for 30 min or at 75°C for 30 s. Cheeses were produced using starter cultures and camel chymosin and pH, yield, proximate composition, texture profile, rheological properties, and protein profiles were determined. The highest yield of BM cheese (26%) was observed under the treatment with 2-fold UF combined with HPP at 550 MPa. CM cheese had the highest storage and loss moduli as well as the total solid and protein content under this treatment. According to SDS-PAGE electrophoresis, CM cheeses were more susceptible to proteolysis and had a higher number of low-molecular-weight bands, indicating the involvement of some active enzymes compared with BM cheeses. In conclusion, UF combined with HPP can enhance the cheese total solid content and gel structure in CM cheese products compared with heat treatment.

1. Introduction

The production and consumption of camel milk (CM) and its products such as cheese and yougurt have increased considerably over recent years. The global market for camel milk and products, estimated at US$10.6 billion in 2020, is projected to reach US$15.5 billion by 2027, increasing at a compound annual growth rate of 5.6% throughout the analysis period 2020–2027 [1]. The increased popularity of CM is due to its nutritional and therapeutic properties [2,3], including antidiabetic [4–6], anti-allergic [7], anti-autistic [8], anti-carcinogenic [9], antimicrobial [10], and anti-hypertensive [11] properties.

CM is difficult to process into hard and semi-hard cheese compared with other types of milk [12–14]. The fragile and poor structural quality of CM cheeses is associated with their larger casein micelle size averaging 260–300 nm) versus BM (100–140 nm) [15,16], low concentrations of κ-casein [17,18], and small size of fat globules [19] compared with bovine milk (BM). These characteristics prolong the coagulation time [20] and affect the textural quality of coagulum, which is an important attribute affecting cheese quality. Recently, both the scientific community and manufacturers have become increasingly interested in improving CM coagulation property. Thus, various attempts have been made to improve the quality of CM cheese by mixing CM with sheep milk [21], adding microbial transglutaminase [22], and using direct acidification with citric and acetic acid [20,23], camel chymosin and starter cultures for acidification [24–26], and ultrafiltration (UF) [27]. The UF of milk can increase the yield and textural quality of cheeses by promoting increases in the retention of non coagulable protein, fat, and total solids [28].

The purpose of pasteurization in the dairy industry is primarily to reduce the bacteria load in milk below the permissible level. As a result of heat treatment, milk has a longer shelf-life and microorganisms that are detrimental to cheese processing are less likely to thrive and multiply [29,30], but curd formation can be adversely affected by higher temperatures, resulting in longer coagulation times and softer gels [31]. Milk

pasteurization alters milk proteins and impairs their coagulation properties [32,33]. The deterioration of milk coagulation properties of heat-treated milk is due to the denaturation of β-lactoglobulin (β-LG) and formation the of complexes with κ-casein (κ-CN) [34], which restrict the ability of rennet enzymes hydrolyze κ-CN [35]. Thus, longer milk coagulation times and weaker curds are expected when dealing with pasteurized rather than raw milk [26] with high temperature short time (HTST) having a greater impact on milk cogulation time than low temperature long time (LTLT) pasteurization [26].

An alternative to thermal treatments is high pressure processing (HPP), which is increasing in popularity despite that it is more expensive and uses batch technology [36]. HPP disrupts and reduce casein micelle size, dissolves colloidal calcium phosphate, and denature whey proteins [37]. HPP also reduces the concentration of viable food bacteria by disrupting their cell membranes and protein-synthesizing ribosomes [38]. Unlike heat treatments, HPP preserves the quality of fresh food without affecting the flavor of the food or nutrients such as vitamins [39]. HPP treatments wer shown to reduce the coagulation time and produce firmer BM cheeses [40,41]. HPP treatment has also been shown to increase the total solids content and positively affect the textural quality of CM cheese [26,31].

To the best of our knowledge, the effects of HPP following UF treatment on the properties of CM cheese have not yet been studied. We hypothesized that HPP treatment following the UF of milk can enhance the coagulation properties of milk and the physico-chemical properties of cheese. Three UF concentration factors (0, 1, and 2), two HPP processes (350 and 550 MPa for 5 min at 4°C), and two heat treatments LTLT (65°C for 30 min) and HTST (75°C for 30 sec) were used in our experiments. The effects of UF, HPP, or UF-heat treatment on the physico-chemical, rheological, and proteolytic activities of cheeses derived from CM and BM were compared.

2. Materials And Methods

2.1 Materials

Al-Ain Dairy farm provided pooled camel and bovine milk. Milk was transported in refrigerated coolers (4°C) to the Department of Food Science at United Arab Emirates University. Raw CM and BM milk proximate composition which was determined by near-infrared multipurpose analyzer (Ettlingen, Germany)[42] it comprised of the following: CM vs BM (pH, 6.6 and 6.7; total solids, 12.2 and 12.8%; protein, 2.9 and 3.2% fat, 3.1 and 3.2%), respectively. The starter culture comprised a 1:1 mixture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp bulgaricus*, and commercially-prepared camel chymosin (50 IMCU/mL) from Asia and Africa Chr. Hansen (Dubai, UAE). All other chemicals and reagents, including TEMED Ultra (N,N,N',N'-tetramethylethylenediamine, > 99%), protein (molecular weight marker), 10% glycerol, 1% lithium dodecyl sulfate, 62.5 mM Tris-HCl (pH 6.8, 0.005% bromophenol blue), 4× Laemmli sample buffer stacking gel buffer, resolving gel buffer and APS (ammonium persulphate) were obtained from Sigma-Aldrich (St. Louis, Missouri, USA).

2.2 UF, HPP, and heat treatment of milk

UF processes were performed at 30°C on the milk samples with concentration factors (CFs) of 0, 1, and 2 which was followed by pressure (350 or 550 MPa for 5 min at 4°C) or heat processing (LTLT: 65°C for 30 min, or HTST: 75°C for 30 s) according to Mbye et al. [26]. Milk was ultrafiltrated with an HFK-131 membrane at 40°C (molecular weight cut-off of 10 kDa; Koch Membrane Systems, Wilmington, MA, USA) prior to any treatment. The milk CF was calculated based on volume reduction (i.e., until an estimated permeate volume was collected). For HPP treatments, CM and BM were filled into plastic bottles (330 mL) and then sealed in polyethylene bags before HPP treatment. The pressurization was conducted using an IsoLab high hydrostatic pressure food processor (Essex, United Kingdom). The pressure machine operates at an inlet temperature of 4°C with a maximum pressure of 700 MPa, a pressure rate of 5 MPa/s, and a heating rate of (0.5°C/100 MPa). Cold water is circulated into the chamber *via* a water jacket to maintain the set temperature. In the pressure chamber, the plastic bottles containing samples of milk were immersed for 5 min at 350 MPa/4°C or 5 min at 550 MPa/4°C. For heat treatments, milk samples were pasteurized with (FT75 Laboratory Pasteuriser, Hampshire, England).

2.3 Cheese preparation

UF-treated CM or BM samples (2L) followed by HPP-treatments or pasteurization were processed into cheeses immediately after manufacturing. The milk was heated at 65°C/30 min or 75°C/30 s or pressurized at 350 MPa/5 min or 550 MPa/5 min at 4°C. The milk samples were then cooled to 43°C and inoculated with 3% (w/v) of Direct Vat Set cultures of (*Streptococcus thermophilus and Lactobacillus delbrueckii subsp bulgaricus*) (1:1) for 60 min until the pH of the milk reached 6.2 in order to enhanced the rate of coagulation. Then, CHY-MAX®M recombinant camel chymosin (50 IMCU per L) was added to each sample and the samples were incubated at 30°C until the milk coagulates [26]. Cheesecloth was used to drain the curd [43].

2.4 CM and BM cheese yields, physicochemical properties, and rheological properties

Cheese yield was calculated as the percentage of amount of cheese produced from the mass of processed milk. A digital pH meter was used to measure the pH of the cheese samples (OHAUS, Starter 3100, New Jersey, USA). Titratable acidity was determined following the standard method described in Mbye et al. [20].

A CT III texture analyzer (Brookfield, Middleborough, Massachusetts, USA) was used to determine cheese texture (hardness, gumminess, and chewiness) at room temperature with a target distance of 5 mm and a speed of 1 mm/s as described in Mbye et al. [20]. A rheometer (Discovery Hybrid Rheometer, TA Instruments, Delaware, USA) with flat plate was used to determine the linear viscoelastic region and frequency sweep. The horizontal plate was loaded with the samples, which were spread across its surface. The plate was then used to cover the sample to a 1-mm gap. A stress from 0.01 to 100% at 0.01–10 Hz was used [20]. The linear viscoelastic region was carried out at 1 Hz to characterize the linear

viscoelastic region. The equipment software obtained data on the elastic modulus (G'), viscous modulus (G"), and tan delta (δ).

2.5 Electrophoresis of camel and bovine milk cheeses

Sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE) electrophoresis was carried out on camel and bovine cheeses following the methods of Laemmli [44]. The cheese samples were homogenized for 2 min, using T 25 digital Ultra-Turrax (IKA-Werke GmbH and Co., KG, Staufen, Germany). To dissociate caseins, the urea-cheese dispersion was incubated in a temperature-controlled water bath at 37°C for 2 h and then defatted by centrifugation at 9,150 g at 4°C for 35 min and filtered through Whatman no. 1 filter paper (pore size, 11µm). Portions of the

filtered sample, (10 µL) were added to 30 µL of dithiothreitol (freshly added to the solution) in a 4× Laemmli buffer solutionand heated for 5 min at 90°C in a temperature-controlled water bath. The samples (6 µL) were loaded onto polyacrylamide gels (thickness of 1 mm) prepared using Bio-Rad's Mini-PROTEAN Tetra cell (Hercules, California, USA) resolving gel (12%) was prepared using 3.75 mL of 1.5 M Tris-HCl (pH 8.8), 6 mL of 30% acrylamide/Bis solution 29:1, 5.03 mL of deionized water, 150 µL of 10% SDS solution, 7.5 µL of TEMED, and 75 µL of 10% APS (ammonium persulphate). To prepare the stacking gel (4%), 3.78 mL of 0.5 M Tris-HCl (pH 6.8), 1.98 mL of 30% acrylamide/Bis solution 29:1, 9 mL of deionized water, 75 µL of 10% APS, 15 µL of TEMED, and 150 µL of 10% SDS solution were added. A power supply from Bio-Rad was used to conduct electrophoresis at 200 V. To fix the protein bands, gels were soaked in 40% alcohol and 10% acetic acid for 1 h. The gel was stained with colloidal Coomassie stain for 20 h. The distilled water was changed three times during the 3-h destaining period. Images were acquired and densitometry was performed using the ChemidocTM XRS + and Gel DocTM XR + Imaging Systems (Bio-Rad Laboratories Inc., Hercules, California, USA). Proteins peaks were integrated and their densities were calculated using Imaging Lab software (version 6).

2.6 Statistical analysis

Analytical measurements and cheese preparation experiments were conducted in triplicate, and the means of the sample values were calculated. Differences in yield, physical properties, and chemical properties were determined by two-way analysis of variance (ANOVA) using Minitab software to analyze the data. We used least significant difference tests to compare mean values between groups, and the threshold for statistical significance was $p \leq 0.05$.

3. Results And Discussion

3.1 CM and BM cheese yields

Figure 1 displays the effects of UF-HPP and UF-pasteurization on CM and BM cheese yields. An increase in UF concentration factors from zero to one and from one to two and an increase in HPP from 350 MPa to 550 MPa resulted in significant increases in the cheese yield ($P < 0.05$). Increases in yield, from 10.9 to

13.6% of CM cheese and from 20.6 to 26.4% of BM cheese, were observed from 2 UF-HPP-treated milk at 550 MPa (Fig. 1). Similarly, a 17% increase increase in cheese yield and 2 times higher protein content were described for UF-concentrated sheep milk [45]. This might be explained by the fact that the UF process retains proteins, fats, and the total solids in the concentrated milk[46,47] leading to increased cheese yield. UF has also been reported to reduce the casein micelle size in CM [27], which enhances coagulation and increases cheese yield [48]. A shorter gelation time was observed in UF processed milk presumably due to its higher casein content [49]. While HPP treatments up to 200 MPa for 10–60 min were able to reduce the coagulation time, treatments at 400 MPa and above produced a longer coagulation time when applied for more than 10 minutes [50]. Studies of CM have shown that the use of pressures over 400 MPa causes a delay in CM coagulation and resulted in soft cheese texture with higher moisture content, the extent of moisture increases as the pressure increases [26,31]. Mild HPP treatments seem to cause denaturation of whey proteins and the formation of complexes with casein micelles, thereby increasing cheese yield [51]. CM cheese yield increased from 11.5 to 14.7% when the pressure was increased from 350 to 550 MPa for 5 min. A similar increasing trend was observed by Mbye et al. [26]. In other studies, Huppertz et al.[52] reported a higher yield for cheeses made from HPP-treated cows' milk (100–400 MPa) stored for 24 h at 20°C than those produced immediately after HPP milk treatment. HPP-treated ewes' milk was also reported to achieve an 11% higher curd yield than non-HPP-treated ewes' milk pressurized at 200 MPa/30 min; while a considerable increase of (15%) in curd yield was achieved after milk was HPP processed at 400 MPa/30 min [53]. These increments in curd/cheese yields may also be enhanced by greater moisture retention and incorporation of some denatured ß-lactoglobulin [54].

3.2 pH, titratable and composition CM and BM cheeses

Table 1 shows the pH and titratable acidity of UF-pasteurized and UF-HPP-processed milk cheeses. The pH was 5.9 ± 0.02 and 6.5 ± 0.3 for CM and BM treated at 550 MP for 5 min, compared to 4.3 ± 0.01 and 4.9 ± 0.01 for milks heated at 75°C for 30 s, respectively, with the pH of HPP-treated milk cheeses being significantly higher than those of heat-treated cheeses ($P < 0.05$) in agreement with previous studies [26,41]. The pH values of heat and HPP-treated 4.7 ± 0.01 and 6.6 ± 6.3 ± 0.01 were lower than those of raw CM and BM, which were 6.6 ± 0.03 and 6.7 ± 0.02, respectively. The extent of the pH change depends on the pressure and temperature levels, the presence of microorganisms, and the milk composition [55,56]. The higher pH of HPP than temperature-treated cheeses can be attributed to the solubilization of micellar calcium phosphate, which increases ionized calcium levels in milk [41,57,58]. CM casein micelles, which have higher buffering capacity than BM micelles [59], resist the change in acidity and the pH levels post HPP-treatment [56](Itturmendi et al. 2020). Yang et al.[55] observed a small, but significant, increase in pH in skimmed milk after 600-MPa/5-min pressure treatment.

Table 1
The physicochemical properties of CM and BM cheeses*

Sample	pH		Titratable Acidity	
	Camel	Bovine	Camel	Bovine
0 F 65	4.3 ± 0.02^{i}	4.9 ± 0.06^{e}	2.7 ± 0.12^{b}	1.5 ± 0.05^{a}
1 F 65	4.3 ± 0.01^{i}	4.8 ± 0.06^{g}	2.8 ± 0.13^{b}	1.4 ± 0.05^{b}
2 F 65	4.2 ± 0.02^{h}	5.1 ± 0.03^{d}	2.6 ± 0.12^{b}	1.3 ± 0.05^{c}
0 F 75	4.1 ± 0.02^{j}	4.7 ± 0.01^{g}	3.1 ± 0.15^{a}	1.5 ± 0.05^{a}
1 F 75	4.3 ± 0.05^{i}	4.8 ± 0.01^{f}	2.7 ± 0.11^{b}	1.4 ± 0.01^{b}
2 F 75	4.4 ± 0.01^{g}	4.9 ± 0.01^{e}	2.5 ± 0.07^{c}	1.3 ± 0.15^{c}
0 F 350	4.9 ± 0.01^{f}	6.5 ± 0.01^{b}	2.5 ± 0.05^{c}	0.9 ± 0.05^{d}
1 F 350	4.9 ± 0.01^{f}	6.6 ± 0.01^{a}	2.4 ± 0.05^{d}	0.8 ± 0.05^{e}
2 F 350	5.2 ± 0.02^{d}	6.6 ± 0.02^{a}	2.1 ± 0.15^{e}	0.8 ± 0.05^{e}
0 F 550	5.8 ± 0.70^{b}	6.3 ± 0.01^{c}	2.1 ± 0.10^{e}	0.9 ± 0.18^{d}
1 F 550	5.8 ± 0.03^{b}	6.5 ± 0.05^{b}	1.9 ± 0.05^{f}	0.9 ± 0.07^{d}
2 F 550	5.9 ± 0.02^{a}	6.5 ± 0.03^{b}	1.2 ± 0.10^{g}	0.9 ± 0.04^{d}

The data are presented as mean + standard deviation (n = 3). Significantly different values within a row are indicated by different superscripts ($p < 0.05$).

The cheeses composition of CM and BM is presented in Fig. 2. The results showed that the UF process significantly affected the cheeses' solid, fat, and protein composition. Thus a significant ($p < 0.05$) difference was observed between unfiltered milk cheeses and UF processed cheeses regarding fat, protein, and total solids. The highest total solid was (42%) recorded in CM cheese made with 2UF-HPP 350 Mpa. The value was higher than the value found in soft CM cheese made with UF- fortified Allium roseum powder (AR) reported by El Hatmi et al.[27] and soft CM made with STL-12 culture found by Bekele et al.[60], which was (39.4%), but lower than the value reported by Hailu et al.[61] for soft brined CM cheese (45.42%). CM 2UF-HPP 350 Mpa had the highest protein content (16%), which is similar to the values reported by Bekele et al.[60] for CM cheese made with STL-12 culture. Variations in cheese chemical composition might be caused by the original composition of milk and the cheese manufacturing process.

3.3 Textural properties of CM and BM cheeses

UF had a significant effect on the textural properties of both CM and BM cheese samples ($P < 0.05$), and the highest level of UF had the most pronounced effect compared with control samples (Fig. 3). Increased hardness and chewiness were observed for both CM and BM cheeses when the UF concentration increased from 1 to 2-fold. The hardest cheeses were the UF-LTLT (1524 g) in BM and the 2UF-HPP at 350 MPa (795 g) in CM (Fig. 3). Due to its smaller average size (100–140 nm) the casein micelles in BM milk are less hydrated than those in CM milk [15], explaining the higher hardness of ultrafiltered BM cheese retentate samples compared to CM UF cheeses. UF-processed milk might produce cheeses with different textural properties due to changes in casein micelles and the content of total solids (protein and fat). These cheeses attain greater ionic strength and increased protein–protein interactions with a lower degree of electrostatic repulsion between the proteins [62]. UF-treated cheeses may also have higher hardness due to a higher total solid content of the retentate and higher calcium retention in the milk [63,64]. CM gel hardness has been reported to increase following HPP treatments at 350 MPa for 5 min at 4ºC and LTLT at 65°C for 30 min [26,31].

HPP of milk above 400 MPa yielded cheeses with lower hardness and chewiness values than non-HPP-treated milk. This can be due to whey protein denaturation, wherein the proteins prevent the excessive dissociation of the casein micelles and the reformation of casein aggregates during curd formation [65].

3.4 Rheological properties of CM and BM cheeses

A frequency sweep test was used to measure the dynamic viscoelastic behavior of the cheeses to determine how different strain rates and levels of stress affect the storage and loss modulus behavior of cheese. The storage modulus (G’), loss modules, (G”), and tan (δ) of CM and BM cheeses are shown in Fig. 4 and Fig. 5, respectively. In each sample of this study, the storage modulus was greater than the loss modulus, and the tan delta was greater than 1, indicating that the elastic nature of cheese was more pronounced than the viscous nature of the cheeses [66]. Figures 4 and 5 illustrate how UF-HPP treatment considerably affected the rheological properties of cheese samples having greater impact on CM cheeses and negligible effects on BM cheeses. It appears that UF has increased the elastic behavior of proteins as well as their bonding interaction as reported by Pires et al. [67]. The highest G' and G" were observed under the 2UF-HPP treatment CM at 350 Mpa (Fig. 4). While, highest modulus for BM was observed on heat-treated at 65°C, and the lowest modulus was observed for cheese from CM health treated at 75°C (Fig. 4), BM cheese HPP treated at 550 Mpa had lower modulus which may be due to weakened hydrophobic interactions. According to previous studies HPP treatment weakens hydrophobic interactions and decreases connecting bonds in cheese [68,69]. The firmness and viscoelasticity of cheese increase as the protein content increases [66], and may increased casein cross-linking. The protein content and compactness were higher for the cheese produced via the 2UF-HPP treatment at 350 MPa, indicating a strengthening of some type of protein interactions [70].

3.5 Proteolysis in CM and BM cheeses

The extent and rate of proteolysis of cheese depend on the presence and activity of specific proteolytic enzymes (e.g., starters, nonstarters, residual chymosin, plasmin, and other proteinases), which are affected by several factors including processing technique, salt, pH, and moisture content. Additionally, the structure of cheese and the ease of access to different cleavage sites on the caseins might be affected by these factors and potentially affect the intensity of proteolysis [34]. Figure 6 shows differences in the proteolytic intensity between CM and BM cheeses. CM cheeses produced from HPP-treated milk had a higher level of proteolytic activity and thus possessed more low-molecular-weight peptides than BM cheeses. Several factors might contribute to explaining this observation, including high moisture content, the degradation of β-casein by the enzyme plasmin, which accounts for 65% of CM compared with 40% of BM β-casein of percentage of caseins in the milks respectively [71]. β-casein is highly vulnerable to attack by plasmin but less so to attack by chymosin and bacterial enzymes [72]. Cheeses having high moisture content are mostly subjected to primary proteolysis by the milk endogenous proteases, mainly plasmin, which has a marked activity on β-CN, and by exogenous enzyme, mainly chymosin from residual rennet acting primarily on αS1-casein [73]. Results from this study (Fig. 6) and a previous study [26] provide evidence of increased proteolysis in CM cheeses, probably as a result of plasmin-mediated degradation of the β-casein [74].

HPP has no effect on plasmin activity *per se* but improves the enzyme access towards β-casein, which explains why HPP-treated cheeses hydrolyze β-casein more efficiently [72]. HPP is known to accelerate cheese proteolysis[72] because casein micelle membrane damage due to high pressure increases cellular permeability, which leads to the release of intracellular materials such as peptidases into the medium [75]. Moreover, HPP can alter the structure of casein micelles and make caseins more vulnerable to proteases [76]. The casein micelles of CM are also richer in minerals such as phosphate, calcium, magnesium, and citrate and are more hydrated than BM caseins [59], which make important contributions to the structure of micelles and their vulnerability to proteolytic attack.

4. Conclusions

Our findings revealed that HPP treatment provides an alternative to pasteurization for the production of CM cheese. UF combined with HPP processing can produce high-quality CM cheese by promoting the retention of nutrients. HPP of CM at 350 MPa for 5 min after UF resulted in cheese with a firmer texture and improved protein network compared with HPP at 550 MPa, which might stem from the increase in casein proteolysis at higher pressure. The CM cheeses were more susceptible to proteolysis than BM cheeses, possibly due to the increase in plasmin activity and mineralization. Additional research is needed to further optimize the production of camel cheeses using HPP technology.

Declarations

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Authors' contributions

Afaf Kamal-Eldin conceived the study, acquired funding, and oversaw all activities.

Mutamed Ayyash and Rabih Kamleh designed the study. Mustapha Mbye performed the laboratory experiments, and Basim Moh'd Ismail Abu-Jdayilanalyzed the results of the rheology. Mustapha Mbye wrote the first draft. All authors reviewed drafts, contributed critically to the manuscript, and gave final approval for submission.

Competing Interest Declaration

There are no competing interests to declare.

Data Availability

All data generated or analysed during this study are included in this published article (and its supplementary information files).

References

1. Camel Dairy Market Size, Growth, Trends & Analysis 2022–2027 | IMARC Group. https://www.imarcgroup.com/camel-dairy-market.
2. Mbye, M., Ayyash, M., Abu-Jdayil, B. & Kamal-Eldin, A. The Texture of Camel Milk Cheese: Effects of Milk Composition, Coagulants, and Processing Conditions. Front Nutr **9**, 779 (2022).
3. Solanki, D. & Hati, S. Fermented camel milk: A Review on its bio-functional properties. Emir J Food Agric **30**, 268–274 (2018).
4. Ejtahed, H. S. *et al.* Effect of camel milk on blood sugar and lipid profile of patients with type 2 diabetes: a pilot clinical trial. Int J Endocrinol Metab **13**, (2015).
5. Agrawal, R. P., Jain, S., Shah, S., Chopra, A. & Agarwal, V. Effect of camel milk on glycemic control and insulin requirement in patients with type 1 diabetes: 2-years randomized controlled trial. Eur J Clin Nutr **65**, 1048–1052 (2011).
6. Mohamad, R. H. *et al.* Camel milk as an adjuvant therapy for the treatment of type 1 diabetes: verification of a traditional ethnomedical practice. J Med Food **12**, 461–465 (2009).
7. Ehlayel, M., Bener, A., Abu Hazeima, K. & Al-Mesaifri, F. Camel Milk Is a Safer Choice than Goat Milk for Feeding Children with Cow Milk Allergy. *ISRN Allergy* 2011, 1–5 (2011).
8. al Shirian, S. & al Dera, H. Descriptive characteristics of children with autism at Autism Treatment Center, KSA. Physiol Behav **151**, 604–608 (2015).

9. Ayyash, M., Al-Dhaheri, A. S., al Mahadin, S., Kizhakkayil, J. & Abushelaibi, A. In vitro investigation of anticancer, antihypertensive, antidiabetic, and antioxidant activities of camel milk fermented with camel milk probiotic: A comparative study with fermented bovine milk. J Dairy Sci **101**, 900–911 (2018).
10. Benkerroum, N., Mekkaoui, M., Bennani, N. & Hidane, K. Antimicrobial activity of camel's milk against pathogenic strains of Escherichia coli and Listeria monocytogenes. Int J Dairy Technol **57**, 39–43 (2004).
11. Shuangquan, Tsuda, H. & Miyamoto, T. Angiotensin I-converting enzyme inhibitory peptides in skim milk fermented with Lactobacillus helveticus 130B4 from camel milk in Inner Mongolia, China. J Sci Food Agric **88**, 2688–2692 (2008).
12. Konuspayeva, G., Faye, B. & Loiseau, G. The composition of camel milk: A meta-analysis of the literature data. Journal of Food Composition and Analysis **22**, 95–101 (2009).
13. Bornaz, S., Sahli, A., Attalah, A. & Attia, H. Physicochemical characteristics and renneting properties of camels' milk: A comparison with goats', ewes' and cows' milks. Int J Dairy Technol **62**, 505–513 (2009).
14. Berhe, T., Seifu, E., Ipsen, R., Kurtu, M. Y. & Hansen, E. B. Processing Challenges and Opportunities of Camel Dairy Products. *Int J Food Sci* 2017, (2017).
15. Ibrahem, S. A. & el Zubeir, I. E. M. Processing, composition and sensory characteristic of yoghurt made from camel milk and camel-sheep milk mixtures. Small Ruminant Research **136**, 109–112 (2016).
16. Kamal, M., Foukani, M. & Karoui, R. Rheological and physical properties of camel and cow milk gels enriched with phosphate and calcium during acid-induced gelation. J Food Sci Technol **54**, 439–446 (2017).
17. Ryskaliyeva, A. *et al.* Combining different proteomic approaches to resolve complexity of the milk protein fraction of dromedary, Bactrian camels and hybrids, from different regions of Kazakhstan. PLoS One **13**, e0197026 (2018).
18. Mohamed, H., Johansson, M., Lundh, Å., Nagy, P. & Kamal-Eldin, A. Short communication: Caseins and α-lactalbumin content of camel milk (Camelus dromedarius) determined by capillary electrophoresis. J Dairy Sci **103**, 11094–11099 (2020).
19. Meena, S., Rajput, Y. S. & Sharma, R. Comparative fat digestibility of goat, camel, cow and buffalo milk. Int Dairy J **35**, 153–156 (2014).
20. Mbye, M. *et al.* Physicochemical properties, sensory quality, and coagulation behavior of camel versus bovine milk soft unripened cheeses. NFS Journal **20**, 28–36 (2020).
21. Abdl Whap M. A. Derar & Ibtisam E. M. El Zubeir. Effect of Fortifying Camel Milk with Sheep Milk on the Processing Properties, Chemical Composition and Acceptability of Cheeses. J Food Sci Eng **6**, (2016).
22. Abou-Soliman, N. H. I. *et al.* The Impact of Microbial Transglutaminase on the Quality and Antioxidant Activity of Camel-Milk Soft Cheese. Food Nutr Sci **11**, 153–171 (2020).

23. Mohamed, A. E., Babiker, I. A. & Mohamed, T. E. Preparation of fresh soft cheese from dromedary camel milk using acid and heat method. Res Opin Anim Vet Sci **3**, 289–292 (2013).
24. Bekele, B., Hansen, E. B., Eshetu, M., Ipsen, R. & Hailu, Y. Effect of starter cultures on properties of soft white cheese made from camel (Camelus dromedarius) milk. J Dairy Sci **102**, 1108–1115 (2019).
25. Al-zoreky, N. S. & Almathen, F. S. Using recombinant camel chymosin to make white soft cheese from camel milk. Food Chem **337**, (2021).
26. Mbye, M. *et al.* Effects of Pasteurization and High-Pressure Processing of Camel and Bovine Cheese Quality, and Proteolysis Contribution to Camel Cheese Softness. Front Nutr **8**, 336 (2021).
27. el Hatmi, H. *et al.* Fortification of soft cheese made from ultrafiltered dromedary milk with Allium roseum powder: Effects on textural, radical scavenging, phenolic profile and sensory characteristics. LWT **132**, 109885 (2020).
28. Li, S., Ye, A. & Singh, H. The effect of ultrafiltration on the acid gelation properties of protein-standardised skim milk systems. Food Res Int **146**, (2021).
29. Rankin, S. A., Bradley, R. L., Miller, G. & Mildenhall, K. B. A 100-Year Review: A century of dairy processing advancements-Pasteurization, cleaning and sanitation, and sanitary equipment design. J Dairy Sci **100**, 9903–9915 (2017).
30. Franzoi, M., Costa, A., Vigolo, V., Penasa, M. & de Marchi, M. Effect of pasteurization on coagulation properties of bovine milk and the role of major composition traits and protein fractions. Journal of Food Composition and Analysis **114**, (2022).
31. Omar, A., Harbourne, N. & Oruna-Concha, M. J. Effects of industrial processing methods on camel skimmed milk properties. Int Dairy J **84**, 15–22 (2018).
32. Britten, M. & Giroux, H. J. Rennet coagulation of heated milk: A review. Int Dairy J **124**, 105179 (2022).
33. Blecker, C., Habib-Jiwan, J. M. & Karoui, R. Effect of heat treatment of rennet skim milk induced coagulation on the rheological properties and molecular structure determined by synchronous fluorescence spectroscopy and turbiscan. Food Chem **135**, 1809–1817 (2012).
34. Foxx, P. F., Guineee, T. P., Cogann, T. M. & Mcsweeney, P. L. H. Fundamentals of Cheese Science Second Edition.
35. Dalgleish, D. G. The Enzymatic Coagulation of Milk. Cheese: Chemistry, Physics and Microbiology 69–100 (1993) doi:10.1007/978-1-4615-2650-6_3.
36. Leite Júnior, B. R. de C., Tribst, A. A. L., Yada, R. Y. & Cristianini, M. Milk-clotting activity of high pressure processed coagulants: Evaluation at different pH and temperatures and pH influence on the stability. Innovative Food Science and Emerging Technologies **47**, 384–389 (2018).
37. Huppertz, T. *et al.* Effects of high pressure treatment on the rennet coagulation and cheese-making properties of heated milk. Innovative Food Science & Emerging Technologies **6**, 279–285 (2005).
38. Agregán, R. *et al.* High-pressure processing in inactivation of Salmonella spp. in food products. Trends Food Sci Technol **107**, 31–37 (2021).

39. Szczepańska, J., Pinto, C. A., Skąpska, S., Saraiva, J. A. & Marszałek, K. Effect of static and multi-pulsed high pressure processing on the rheological properties, microbial and physicochemical quality, and antioxidant potential of apple juice during refrigerated storage. *LWT* **150**, (2021).
40. Serna-Hernandez, S. O., Escobedo-Avellaneda, Z., García-García, R., Rostro-Alanis, M. de J. & Welti-Chanes, J. High hydrostatic pressure induced changes in the physicochemical and functional properties of milk and dairy products: A review. Foods **10**, (2021).
41. Nassar, K. S. *et al.* Rheological and microstructural properties of rennet gel made from caprine milk treated by HP. J Food Eng **267**, 109710 (2020).
42. Mohamed, H., Nagy, P., Agbaba, J. & Kamal-Eldin, A. Use of near and mid infra-red spectroscopy for analysis of protein, fat, lactose and total solids in raw cow and camel milk. Food Chem **334**, (2021).
43. Mbye, M., Mohamed, H., Raziq, A. & Kamal-Eldin, A. The effects of camel chymosin and Withania coagulans extract on camel and bovine milk cheeses. *Scientific Reports 2021 11:1* **11**, 1–14 (2021).
44. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227**, 680–685 (1970).
45. Faion, A. M. *et al.* Production of Serra da Estrela cheese from ultrafiltered sheep's milk. Emir J Food Agric **32**, 384–390 (2020).
46. Li, S., Ye, A. & Singh, H. The effect of ultrafiltration on the acid gelation properties of protein-standardised skim milk systems. Food Res Int **146**, (2021).
47. Custódio Leite, Z. T., Vaitsman, D. S., Dutra, P. B. & Guedes, A. Milk and some of its derivatives: from antiquity to the present time. Quim Nova **29**, 876–880 (2006).
48. Ferrer, M., Alexander, M. & Corredig, M. Changes in the physico-chemical properties of casein micelles during ultrafiltration combined with diafiltration. LWT - Food Science and Technology **59**, 173–180 (2014).
49. Govindasamy-Lucey, S., Jaeggi, J. J., Martinelli, C., Johnson, M. E. & Lucey, J. A. Standardization of milk using cold ultrafiltration retentates for the manufacture of Swiss cheese: Effect of altering coagulation conditions on yield and cheese quality. J Dairy Sci **94**, 2719–2730 (2011).
50. Inácio, R. S., Barros, R., Saraiva, J. A. & Gomes, A. M. P. Optimization of Raw Ewes' Milk High-Pressure Pre-Treatment for Improved Production of Raw Milk Cheese. Foods **11**, 435 (2022).
51. Kapoor, S., Singh, M. P., Vatankhah, H., Deshwal, G. K. & Ramaswamy, H. S. Production and quality improvement of Indian cottage cheese (Paneer) using high pressure processing. Innovative Food Science and Emerging Technologies **72**, (2021).
52. Huppertz, T., Fox, P. F. & Kelly, A. L. High pressure treatment of bovine milk: effects on casein micelles and whey proteins. J Dairy Res **71**, 97–106 (2004).
53. López-Fandiño, R., Ramos, M. & Olano, A. Rennet Coagulation of Milk Subjected to High Pressures. J Agric Food Chem **45**, 3233–3237 (1997).
54. Lopez-Fandiño, R., Carrascosa, A. v. & Olano, A. The Effects of High Pressure on Whey Protein Denaturation and Cheese-Making Properties of Raw Milk. J Dairy Sci **79**, 929–936 (1996).

55. Yang, S. *et al.* Staff of the Department of Food Science. Innovative Food Science and Emerging Technologies **63**, (2007).
56. Iturmendi, N. *et al.* Influence of high hydrostatic pressure treatments on the physicochemical, microbiological and rheological properties of reconstituted micellar casein concentrates. Food Hydrocoll **106**, (2020).
57. Huppertz, T. & de Kruif, C. G. High pressure-induced solubilisation of micellar calcium phosphate from cross-linked casein micelles. Colloids Surf A Physicochem Eng Asp **295**, 264–268 (2007).
58. Zobrist, M. R., Huppertz, T., Uniacke, T., Fox, P. F. & Kelly, A. L. High-pressure-induced changes in the rennet coagulation properties of bovine milk. Int Dairy J **15**, 655–662 (2005).
59. Attia, H., Kherouatou, N., Nasri, M. & Khorchani, T. Characterization of the dromedary milk casein micelle and study of its changes during acidification. Lait **80**, 503–515 (2000).
60. Bekele, B., Hansen, E. B., Eshetu, M., Ipsen, R. & Hailu, Y. Effect of starter cultures on properties of soft white cheese made from camel (Camelus dromedarius) milk. J Dairy Sci **102**, 1108–1115 (2019).
61. Hailu, Y. *et al.* Rheological and sensory properties and aroma compounds formed during ripening of soft brined cheese made from camel milk. Int Dairy J **81**, 122–130 (2018).
62. Huppertz, T., Fox, P. F. & Kelly, A. L. The caseins: Structure, stability, and functionality. *Proteins in Food Processing, Second Edition* 49–92 (2017) doi:10.1016/B978-0-08-100722-8.00004-8.
63. Kaminarides, S., Moschopoulou, E. & Karali, F. Influence of Salting Method on the Chemical and Texture Characteristics of Ovine Halloumi Cheese. Foods 2019, *Vol. 8, Page 232* **8**, 232 (2019).
64. Ayyash, M. M., Sherkat, F., Francis, P., Williams, R. P. & Shah, N. P. The effect of sodium chloride substitution with potassium chloride on texture profile and microstructure of Halloumi cheese. J Dairy Sci **94**, 37–42 (2011).
65. Huppertz, T., Fox, P. F. & Kelly, A. L. Effects of high pressure treatment on the yield of cheese curd from bovine milk. Innovative Food Science & Emerging Technologies **5**, 1–8 (2004).
66. Soltani, M., Sahingil, D., Gokce, Y. & Hayaloglu, A. A. Effect of blends of camel chymosin and microbial rennet (Rhizomucor miehei) on chemical composition, proteolysis and residual coagulant activity in Iranian Ultrafiltered White cheese. J Food Sci Technol **56**, 589 (2019).
67. Pires, A. F. *et al.* Use of ultrafiltrated cow's whey for the production of whey cheese with Kefir or probiotics. J Sci Food Agric **101**, 555–563 (2021).
68. Messens, W., van de Walle, D., Arevalo, J., Dewettinck, K. & Huyghebaert, A. Rheological properties of high-pressure-treated Gouda cheese. Int Dairy J **10**, 359–367 (2000).
69. Ozturk, M. *et al.* Effect of various high-pressure treatments on the properties of reduced-fat Cheddar cheese. J Dairy Sci **96**, 6792–6806 (2013).
70. Ozturk, M., Govindasamy-Lucey, S., Jaeggi, J. J., Johnson, M. E. & Lucey, J. A. Low-sodium Cheddar cheese: Effect of fortification of cheese milk with ultrafiltration retentate and high-hydrostatic pressure treatment of cheese. J Dairy Sci **98**, 6713–6726 (2015).

71. Dizon, M., Tatarko, M., Szabo, K. & Hianik, T. Application of high-resolution ultrasonic spectroscopy for detection of the plasmin activity toward β-casein. Food Chem **353**, (2021).
72. Juan, B., Ferragut, V., Buffa, M., Guarnis, B. & Trujillo, A. J. Effects of High Pressure on Proteolytic Enzymes in Cheese: Relationship with the Proteolysis of Ewe Milk Cheese. J Dairy Sci **90**, 2113–2125 (2007).
73. Petrella, G. *et al.* Study of proteolysis in river buffalo mozzarella cheese using a proteomics approach. (2015) doi:10.3168/jds.2015-9732.
74. di Luccia, A. *et al.* Occurrence of β-casein fragments in cold-stored and curdled river buffalo (Bubalus bubalis L.) milk. J Dairy Sci **92**, 1319–1329 (2009).
75. Trujillo, A. J. *et al.* Application of high pressure treatment for cheese production. Food Research International **33**, 311–316 (2000).
76. Huppertz, T., Fox, P. F. & Kelly, A. L. Susceptibility of plasmin and chymosin in Cheddar cheese to inactivation by high pressure. J Dairy Res **71**, 496–499 (2004).

Figures

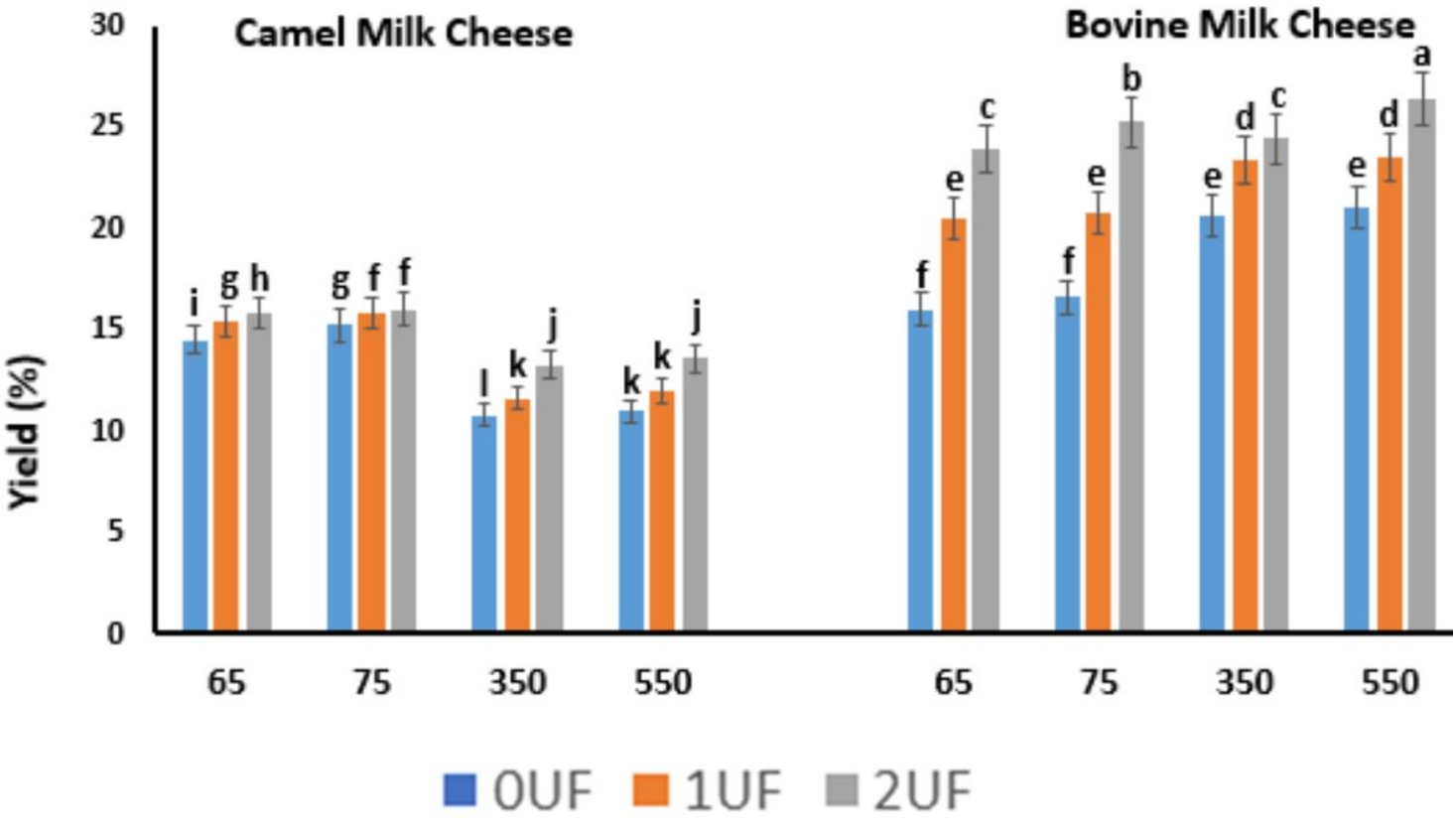

Figure 1

Yield of camel and bovine milk cheeses

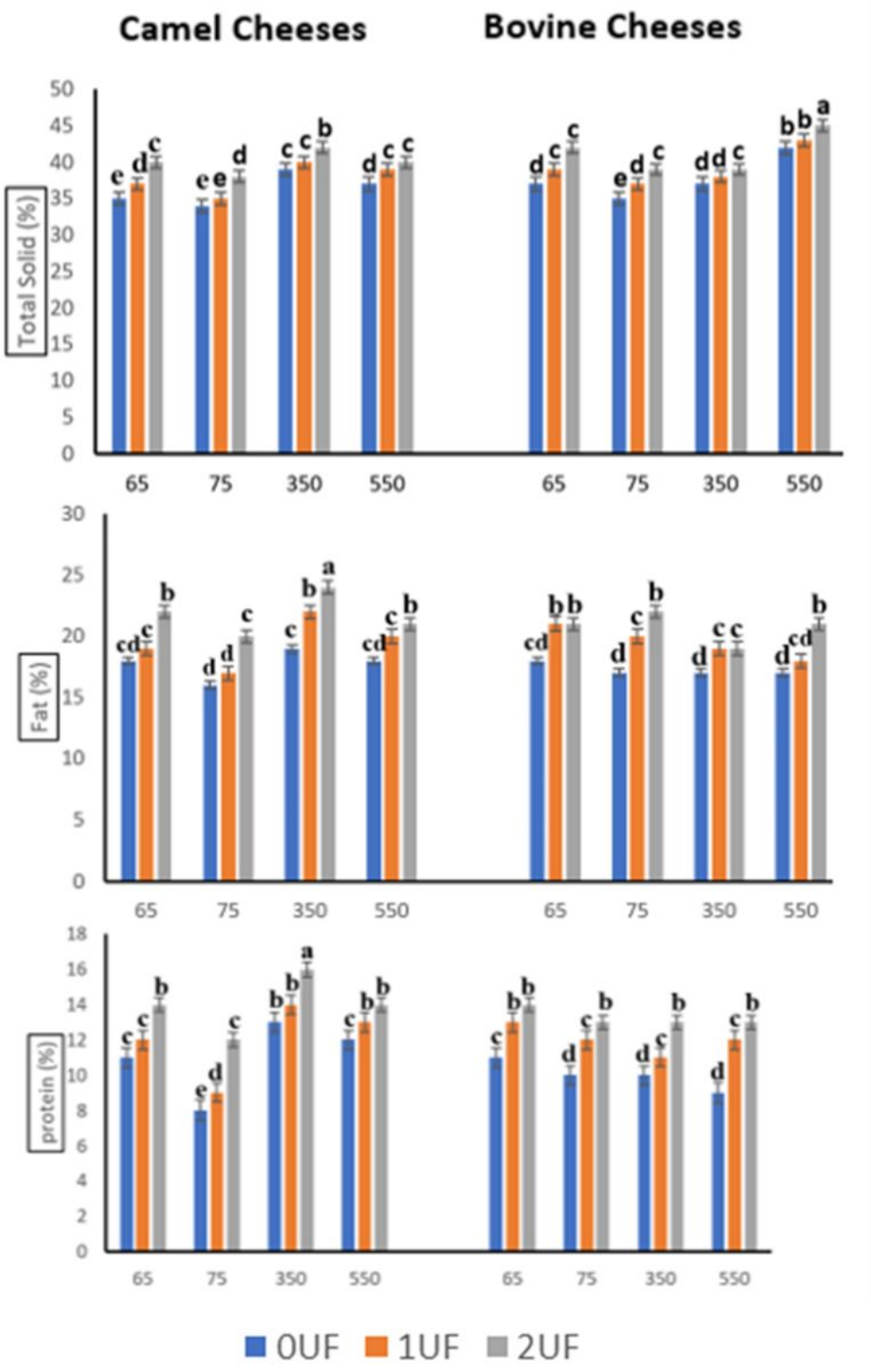

Figure 2

Camel and bovine cheese composition texture

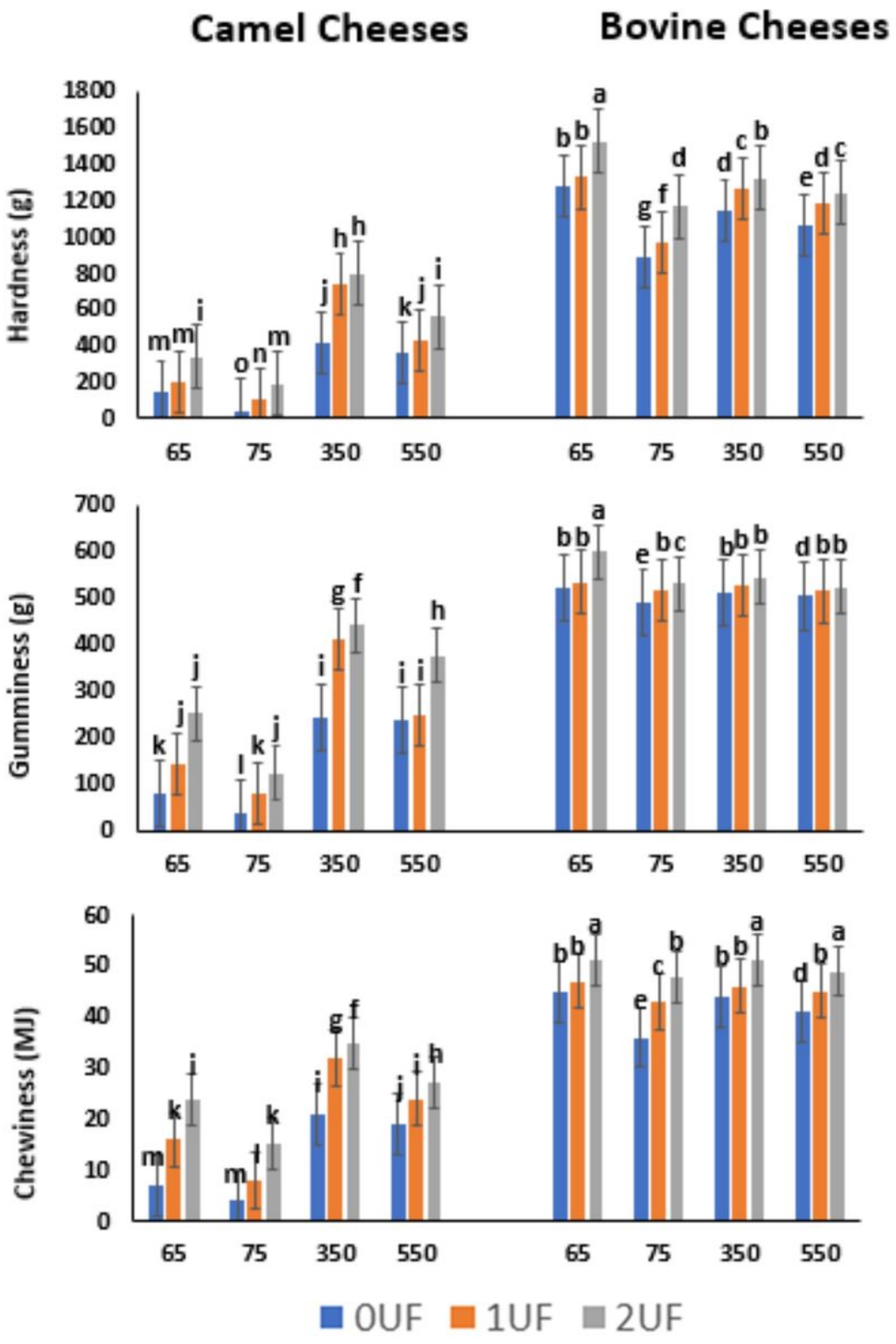

Figure 3

Camel and bovine cheese texture (hardness, gumminess, and chewiness)

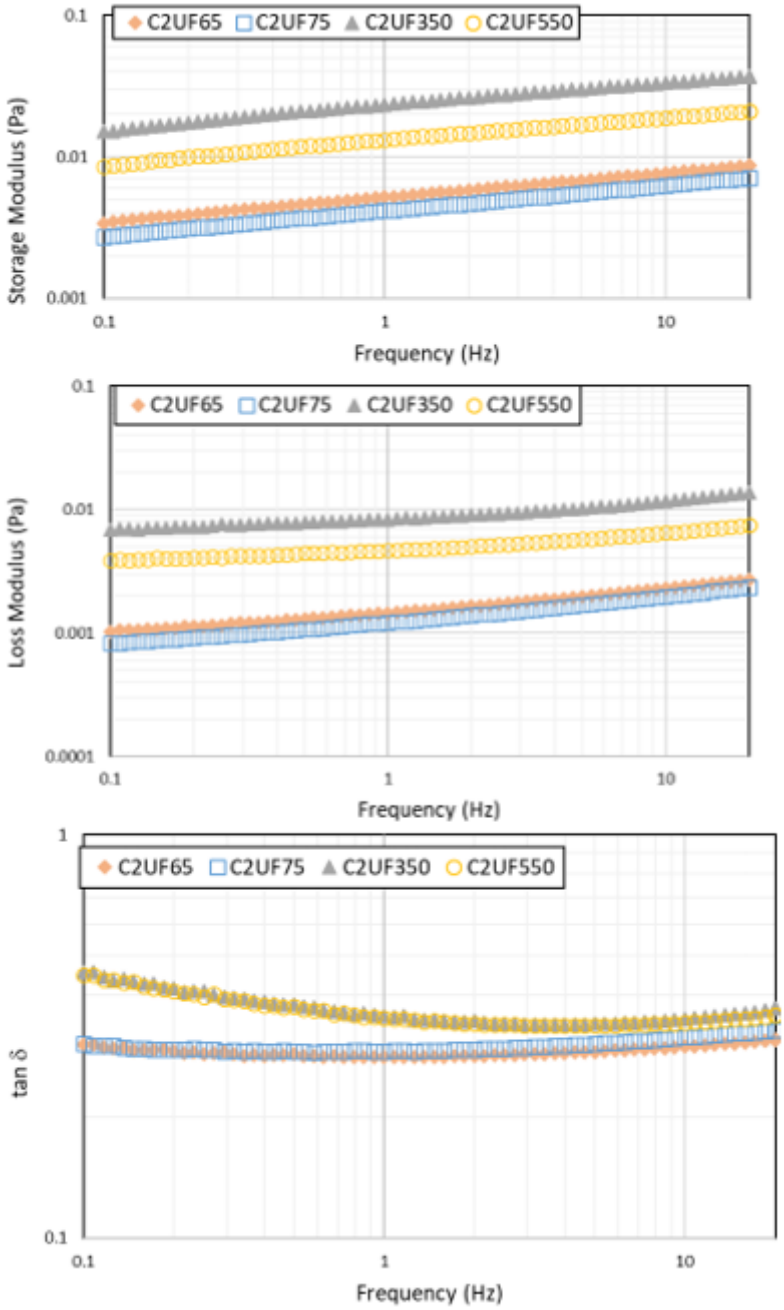

Figure 4

The storage modulus, loss modulus, and tan delta results of two-fold ultrafiltration concentration factor (2UF) of camel milk under various treatments (65°C, 75°C, 350 and 550 Mpa)

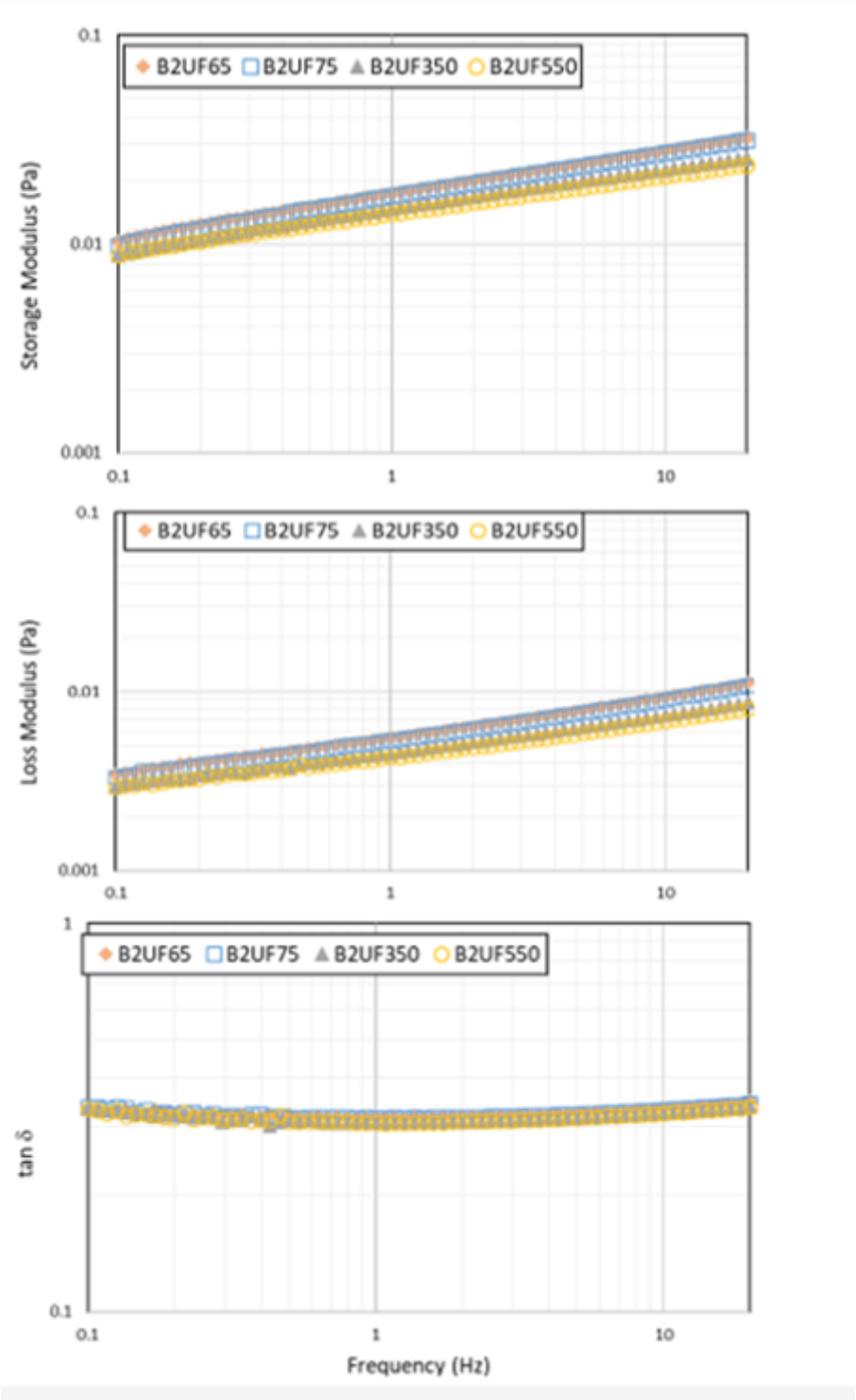

Figure 5

The storage modulus, loss modulus, and tan delta results of two-fold ultrafiltration concentration factor (2UF) of bovine milk under various treatments (65°C, 75°C, 350 and 550 Mpa)

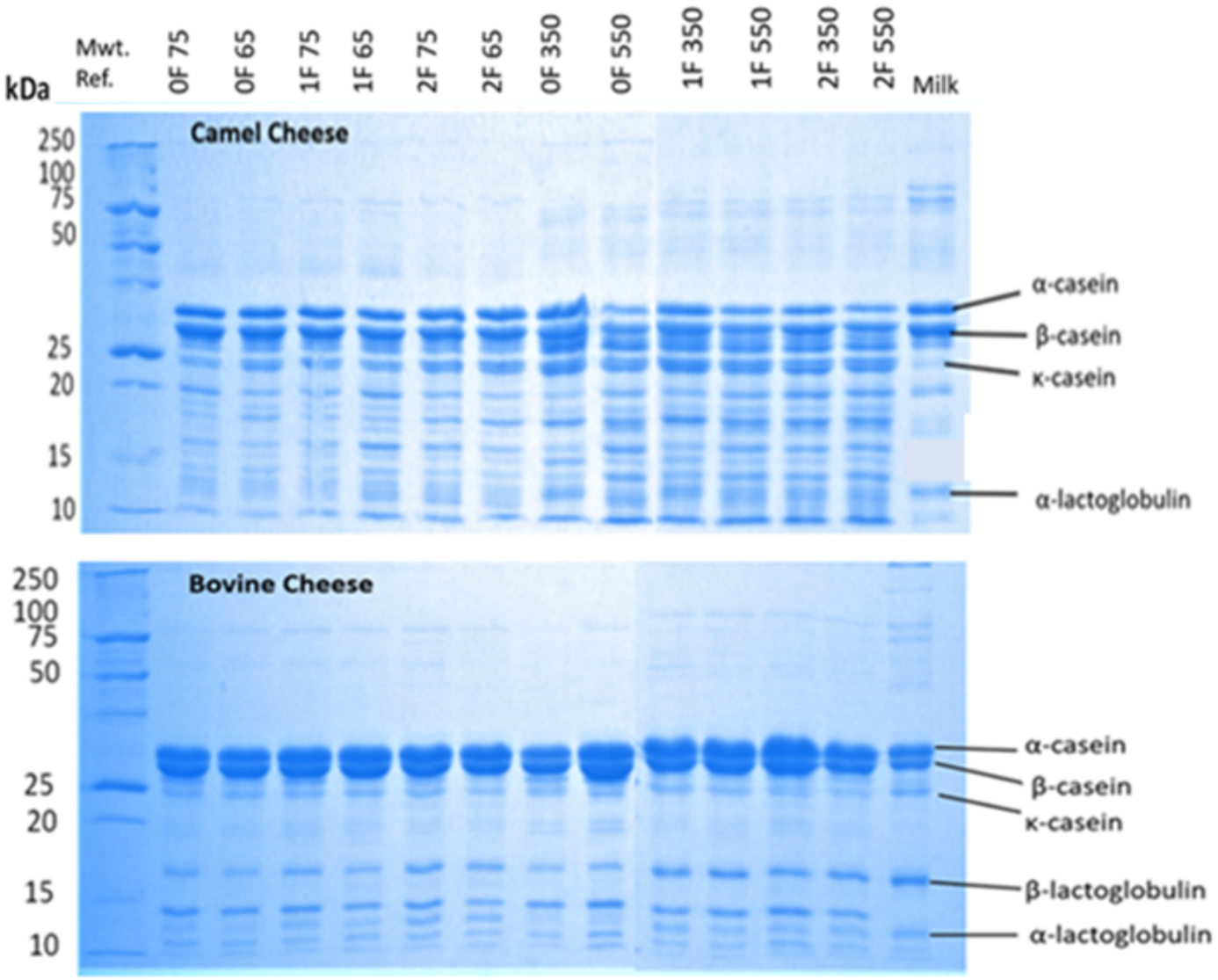

Figure 6

SDS-PAGE electropherograms of camel and bovine milk cheeses

Supplementary Files

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