International Dairy Journal 141 (2023) 105627

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Heat-induced changes in blends of skimmed buffalo and bovine milk

Carolyn T. Mejares [a, b, *], Thom Huppertz [c, d], Jayani Chandrapala [a]

[a] School of Science, STEM College, RMIT University, Melbourne, VIC, 3083, Australia
[b] School of Technology, University of the Philippines Visayas, Miagao, Iloilo, 5023, Philippines
[c] FrieslandCampina, Amersfoort, the Netherlands
[d] Food Quality and Design Group, Wageningen University & Research, Wageningen, the Netherlands

ARTICLE INFO

Article history:
Received 5 January 2023
Received in revised form
16 February 2023
Accepted 17 February 2023
Available online 24 February 2023

ABSTRACT

This study investigated the physical, chemical, and structural changes in mixtures of buffalo and bovine milk (0:100, 25:75, 50:50, 75:25, 100:0) induced by heating at 80, 85, 90, and 95 °C for 5 min. No significant changes in particle size, zeta potential, and calcium activity were observed in heated buffalo milk and its mixtures with bovine milk, irrespective of the heating temperature, but heating at ≥ 85 °C induced a significant decrease in pH. The increase in viscosity with heating was dependent on the ratio of buffalo to bovine milk and the heating temperature. The variation in casein dissociation, whey proteins denaturation and their association with themselves and casein micelles, and the alteration in salt balance were key factors that contribute to significant heat-induced changes in pH and viscosity of milk blends.

1. Introduction

Milk is one of the most complex foods, consisting mostly of water and nutrients such as proteins, fat, lactose, and minerals (Dumitraşcu et al., 2020). Most milk and dairy products available for consumption are derived from bovine milk, which accounts for 81% of global milk production (OECD/FAO, 2022). However, milk of non-bovine origin can also be utilised for developing high value specialised dairy products due to the perceived nutritional benefits compared with bovine milk (Roy, Ye, Moughan, & Singh, 2020a). The largest portion of non-bovine milk production is contributed by buffalo milk, providing 15% of world milk production (OECD/FAO, 2022).

Buffalo milk is widely produced in Asia, with India and Pakistan being the largest producers. Dairy products from milk mixtures are commonly marketed since these countries produce both bovine and buffalo milk. For instance, UHT-treated milk in India, and Pakistan, but also Egypt, is often produced from a mixture of buffalo milk and bovine milk (Deeth & Lewis, 2017). Mixing milk from different species is a way of improving the quality of dairy products or developing new products with specific sensory, nutritional, and rheological properties. Improved nutritional and organoleptic profile of dairy products made from mixture of buffalo and cow milk had been reported for cream cheese (Fangmeier, Kemerich, Machado, Maciel, & De Souza, 2019), Indian cottage cheese (Chakraborty, Singh, Shivhare, & Basu, 2020), Brazilian semi-hard cheese (Rekowsky, Monteiro, Silva, Conté-Júnior, & Costa, 2022), and stirred yoghurt (Petridis, Dimitreli, Vlahvei, & Deligeorgakis, 2014). This mostly relates to variation in milk composition where buffalo milk offers advantages over bovine milk in terms of physicochemical, compositional and nutritional properties as reported in various studies (Claeys et al., 2014; Fangmeier et al., 2019; Mane & Chatli, 2015; Renhe, Perrone, Tavares, Schuck, & De Carvalho, 2019). Its higher fat, protein and energy contents make buffalo milk more economical for producers, processors and consumers (Sindhu & Arora, 2011). This could allow expansion of the dairy industry in many regions which could eventually strengthen the non-bovine production chain, particularly the buffalo milk-producing countries in Asia.

Liquid milk is generally subjected to thermal treatment to guarantee its microbial safety and stability. Although shelf life is extended, thermal processing also affects the physicochemical and functional properties of milk. This is due to various competitive and interdependent reactions such as heat-induced calcium phosphate precipitation, acid development, modifications of caseins, and denaturation of whey proteins (Fox, Uniacke-Lowe, McSweeney, & O'Mahony, 2015). Studies on thermal processing of milk are widely focused on bovine milk although an increasing number of studies on the effects of low temperature long time pasteurisation (62–65 °C for at least 30 min) and high temperature short time

* Corresponding author.
E-mail address: carolyn.mejares@rmit.edu.au (C.T. Mejares).

https://doi.org/10.1016/j.idairyj.2023.105627

pasteurisation (72–82 °C for 15–30 s) on buffalo milk can be found (Elagamy, 2000; Haq et al., 2013; Ismail, Mashaley, & Sirry, 1969; Khalifa & Ghanimah, 2013; Nasr & Elshaghabee, 2019; Ziyaina, Govindan, Rasco, Coffey, & Sablani, 2018), which were recently reviewed by Mejares, Huppertz, and Chandrapala (2022). Pasteurisation temperatures >85 °C had also been applied to buffalo and bovine milk mixtures used for the manufacture of cultured products such as yoghurt (Çetinkaya, 2019; Petridis et al., 2014) and chhana (Chakraborty et al., 2020). However, these studies dealt with either ingredient modifications or thermal effects on the sensory and nutritional properties of the final product rather than their milk counterpart.

It is often falsely assumed that the scientific information generated on bovine milk can be extrapolated to buffalo milk (Khedkar, Kalyankar, & Deosarkar, 2016) or blends of buffalo milk and bovine milk, but compositional variation necessitates a systematic research work based on molecular level understanding of the influence of heat treatment on buffalo milk and mixtures with bovine milk. Hence, this research investigated the impact of high pasteurisation temperatures (80–95 °C) on the physicochemical and structural properties of buffalo skim milk and mixtures with bovine skim milk at levels equivalent to 25, 50, and 75%. This will enable understanding and subsequently control the effect of thermal processing on the functional properties of products made from buffalo milk or mixtures with bovine milk.

2. Materials and methods

2.1. Materials

Raw whole bovine and buffalo milk were provided by Saputo Dairy Australia (Laverton North, VIC, Australia) and Floridia Cheese (Thomastown, VIC, Australia) respectively. Sodium azide (CAS No. 26628-22-8; N_3Na) was purchased from Sigma–Aldrich (Australia) and added to raw milk (0.02%, w/v) to minimise microbial growth. Milk was then stored overnight under refrigerated conditions.

2.2. Sample preparation

Skim milk samples were prepared by centrifugation of raw milk using an Easycream 1 solid-wall disc separator (GEA Westfalia Separator GmbH) equipped with 72 discs and running at a bowl speed of 10,000 rpm. Milk was warmed to 45 °C and transferred to the supply tank of cream separator for skimming. After skimming, milk was divided into portions for preparation of samples as follows: 100% bovine milk; blends of buffalo and bovine milk at ratios of 25:75, 50:50, and 75:25; and 100% buffalo milk.

2.3. Thermal treatment

Aliquots (100 mL) of milk samples were transferred into aluminium containers and heated in water baths set at 80, 85, 90, and 95 °C for 5 min. Come-up times between 5.5 (at 80 °C) and 10.0 (at 95 °C) min were recorded. Milk samples were immediately immersed in an ice bath following the heat treatment and transferred to plastic containers upon reaching a temperature <10 °C.

2.4. Physicochemical analysis of unheated and heated milk samples

2.4.1. Measurement of particle size and zeta potential

The particle size and ζ-potential of unheated and heated milk samples were measured at 20 °C using Malvern Zetasizer Nano ZS (ZEN3600, Malvern Instruments Ltd., Malvern, UK). Milk samples were diluted four-fold in Milli-Q water (Merck Millipore, Australia) and placed into folded capillary cell (DTS1070) for analysis. Five readings from an individual sample were collected.

2.4.2. Measurement of pH and calcium activity

The pH of raw and heated skim milk was measured in triplicates using an automatic temperature compensation (ATC) InLab® Max Pro-ISM probe connected to a digital pH meter (SevenCompact pH/Ion, Mettler Toledo) that was calibrated at 20 °C using buffers at pH 4, 7, and 10.

The calcium activity ($a_{Ca^{2+}}$) of skim milk at 20 °C was measured in triplicates using a perfectION™ combined calcium ion selective electrode (Mettler Toledo) equipped with a reference electrolyte connected to a pH/ion meter (SevenCompact, Mettler Toledo) as described by Lin, Wong, Deeth, and Oh (2018) with modification according to electrode manufacturer's instructions. A series of aqueous $CaCl_2$ standards ranging from 0.2 to 25 mM was prepared and the voltage of each solution was measured. The standard curve of voltage versus log Ca^{2+} concentration was used to estimate the Ca^{2+} activity of samples using the equation below:

$$a_{Ca^{2+}} = c_{Ca^{2+}} \cdot \gamma_{Ca^{2+}}$$

where $c_{Ca^{2+}}$ is the ionic calcium concentration (mM) and $\gamma_{Ca^{2+}}$ is the activity coefficient equivalent to 0.4 (Nieuwenhuijse & Van Boekel, 2003).

2.4.3. Viscosity measurements

Viscosity measurement was performed using a rheometer (Discovery Hybrid HR-2, TA Instruments) operated by TRIOS software using the parallel plates as described by Chandrapala, Martin, Zisu, Kentish, and Ashokkumar (2012). Triplicate measurements were taken using a 40 mm plate geometry employing a geometry gap of 500 μm. Test parameters of a flow sweep procedure were initially determined where the shear rate was ramped from 0.1 to 500 s^{-1}. The temperature was kept constant at 20 °C. Viscosity was almost constant from a shear rate of 60 s^{-1} up to 500 s^{-1}; therefore, for the final viscosity measurement, shear rate of 100 s^{-1} was chosen as referenced by O'Donnell, Herlihy, and McKenna (1994).

2.5. Heat-induced protein changes by SDS-PAGE

Analysis of heat-induced casein dissociation and reduction in whey protein concentration was done by ultracentrifugation followed by reducing SDS-PAGE as described by Anema and Klostermeyer (1996) and Grewal, Chandrapala, Donkor, Apostolopoulos, and Vasiljevic (2017) with slight modifications. Non-sedimentable proteins were defined as those that remained in the supernatant during skim milk ultracentrifugation at 88,000×*g* for 60 min at 20 °C (Anema & Klostermeyer, 1996) using a Beckman L-80 Optima ultracentrifuge and the associated Kontron TFT 50.13 fixed-angle rotor. The clear supernatant was carefully removed from the pellet.

For SDS-PAGE under reducing condition, 950 μL 2 × Laemmli sample buffer was first mixed with 50 μL 2-mercaptoethanol before adding to supernatant (1:1). Samples were then heated at 95 °C for 5 min and 10 μL sample as well as protein standards (Precision Plus Protein All Blue Standards, 10–250 kDa, Bio-Rad) were loaded onto 4–20% Mini-PROTEAN TGX gels. The gels were run for 50 min at 150 V then rinsed in Milli-Q water for 15 min before staining with Bio-Safe Coomassie G-250 stain. The gels were left soaked in the staining solution overnight with continuous agitation followed by rinsing with Milli-Q water. Protein bands were visualised using ChemiDoc MP Imaging System (Bio-Rad). The intensities of the major protein bands were determined using the ImageJ software

(version 8) and protein quantity was calculated from their respective band intensity.

2.6. Data and statistical analysis

The experiment was carried out in two separate milk batches. Statistical analysis of data for effects of pasteurisation on properties of skim milk was performed by one-way Analysis of Variance (ANOVA) using SPSS statistical software version 28.0 (IBM, New York, USA). Mean differences were analysed using Tukey's probability pairwise comparison test at $p < 0.05$.

3. Results

3.1. Physicochemical changes as affected by heating

Unheated bovine skim milk had a significantly lower Z-average diameter (187.3 nm) compared with buffalo skim milk (202.2 nm) and blending bovine and buffalo skim milk affected the particle size proportionally to the proportion of buffalo milk (Table 1). The particle sizes of bovine and buffalo milk were within the ranges of 165–211 nm and 176–250 nm, respectively, in line with previous reports (Bijl, Vries, van Valenberg, Huppertz, & van Hoojidonk, 2014; Hussain, Bell, & Grandison, 2013; Roy, Ye, Moughan, & Singh, 2020b; Thompkinson & Sabikhi, 2012). There was no significant change in particle size after heating, irrespective of the ratio of buffalo and bovine milk. The result is in contrast with Anema and Li (2003) and Perveen, Butt, Huma, and Nawaz (2015), who reported increases in particle size of reconstituted bovine skim milk and 3% solution of buffalo phosphocaseinate, respectively, after heating at 75–90 °C for various times. Similarly, heat treatment did not affect the ζ-potential of buffalo milk and buffalo:bovine milk blends (Table 1), whereas Patil et al. (2021) and Quant, Albis, and Perez (2019) reported significant ζ-potential increases with heating >85 °C for buffalo skim milk concentrates and whey protein concentrates, respectively. However, there was a significant increase (more negative) in ζ-potential for bovine milk with temperatures ≥85 °C (Table 1). These differences are due to the variation in heat treatment used, mode and duration of heating, and most importantly, the milk composition.

The pH of unheated skim buffalo milk was higher (pH 6.93) compared with unheated skim bovine milk (pH 6.83, Table 1). These values were about 0.07–0.15 pH units higher than those reported pH by Ahmad et al. (2008), Ahmed, Elahi, Salariya, and Rashid (2014), and Patil et al. (2019). Unheated buffalo skim milk had significantly lower Ca^{2+} activity than unheated bovine skim milk (Table 1) and these values are also lower than the reported Ca^{2+} activity values of bovine milk (Tanaka, Suzuki, Kotb, & Kamiya, 2011) and buffalo milk (Kashwa, 2016). The variation between our results and previously reported values may be due to the differences on the composition of milk owing to different animal breeds, diet, environmental and geographical changes. Heat treatment at ≥85 °C resulted in a significant pH reduction, albeit by < 0.1 pH unit, for all ratios of buffalo and bovine milk, but no significant heat-induced change was observed for Ca^{2+} activity (Table 1). The significant pH reduction is mostly attributed to heat-induced changes in the mineral equilibria, as reported previously for bovine milk (Anema, 2009; Chandrapala, McKinnon, Augustin, & Udabage, 2010; Singh, Chandrapala, Udabage, McKinnon, & Augustin, 2015) and buffalo milk (Adhikari & Mathur, 1993). Correlation between pH and Ca^{2+} activity was high in bovine skim milk and milk blends ($R^2 = 0.95$ to 0.99) but slightly low ($R^2 = 0.86$) in buffalo skim milk. Interestingly, the magnitude of the pH reduction increased with the increase in buffalo milk proportion in the samples (Table 1), indicating greater alteration in the salt equilibrium of buffalo milk compared with bovine milk.

The viscosity of unheated buffalo skim milk was significantly higher than unheated bovine skim milk (Table 1), which aligns well with previous studies on individual milks (Ismail & El Deeb, 1973; Khalifa & Ghanimah, 2013; Sindhu & Arora, 2011; Tambat &

Table 1
Physicochemical properties of buffalo skim milk and mixtures with bovine skim milk after heat treatment.[a]

Physicochemical property	Heat treatment	Milk sample				
		Bovine skim milk	25:75 milk blend	50:50 milk blend	75:25 milk blend	Buffalo skim milk
Particle size (nm)	Unheated	187.3 ± 3.5^{Aa}	193.8 ± 6.4^{Ba}	199.3 ± 6.3^{BCa}	201.1 ± 4.8^{Ca}	202.2 ± 4.2^{Ca}
	80 °C	187.4 ± 2.4^{Aa}	194.2 ± 5.7^{Ba}	198.1 ± 3.6^{BCa}	201.9 ± 5.2^{Ca}	203.3 ± 5.4^{Ca}
	85 °C	186.9 ± 2.9^{Aa}	195.2 ± 2.7^{Ba}	197.7 ± 4.1^{BCa}	201.7 ± 3.9^{Ca}	205.1 ± 3.9^{Ca}
	90 °C	190.6 ± 3.4^{Aa}	194.7 ± 5.7^{ABa}	199.5 ± 5.4^{BCa}	203.3 ± 4.2^{Ca}	204.6 ± 1.6^{Ca}
	95 °C	189.9 ± 5.0^{Aa}	195.9 ± 5.6^{Ba}	202.7 ± 3.4^{Ca}	203.6 ± 4.1^{Ca}	204.9 ± 4.5^{Ca}
Zeta potential (mV)	Unheated	-19.4 ± 2.1^{Aa}	-20.3 ± 1.9^{Aa}	-20.6 ± 1.5^{Aa}	-21.1 ± 1.3^{Aa}	-21.3 ± 1.4^{Aa}
	80 °C	-21.1 ± 1.5^{Aab}	-21.3 ± 1.4^{Aa}	-21.4 ± 1.6^{Aa}	-21.4 ± 1.1^{Aa}	-21.7 ± 1.2^{Aa}
	85 °C	-21.5 ± 1.3^{Ab}	-21.7 ± 1.3^{Aa}	-21.8 ± 1.5^{Aa}	-21.9 ± 1.5^{Aa}	-22.0 ± 1.1^{Aa}
	90 °C	-21.5 ± 1.5^{Ab}	-21.7 ± 1.4^{Aa}	-22.0 ± 1.3^{Aa}	-22.0 ± 1.7^{Aa}	-22.2 ± 1.7^{Aa}
	95 °C	-21.7 ± 1.6^{Ab}	-22.0 ± 1.5^{Aa}	-22.3 ± 1.5^{Aa}	-22.2 ± 1.7^{Aa}	-22.3 ± 1.6^{Aa}
pH	Unheated	6.83 ± 0.02^{Aa}	6.86 ± 0.03^{ABa}	6.89 ± 0.03^{BCa}	6.91 ± 0.04^{CDa}	6.93 ± 0.03^{Da}
	80 °C	6.81 ± 0.03^{Aab}	6.83 ± 0.01^{ABa}	6.86 ± 0.03^{BCa}	6.89 ± 0.02^{CDa}	6.91 ± 0.02^{Dab}
	85 °C	6.81 ± 0.02^{Aab}	6.82 ± 0.01^{ABb}	6.84 ± 0.02^{BCb}	6.88 ± 0.01^{CDb}	6.90 ± 0.02^{Db}
	90 °C	6.80 ± 0.02^{Ab}	6.81 ± 0.01^{Ab}	6.83 ± 0.02^{Ab}	6.87 ± 0.01^{Bbc}	6.89 ± 0.03^{Bb}
	95 °C	6.79 ± 0.02^{Ab}	6.80 ± 0.01^{Ab}	6.81 ± 0.01^{ABb}	6.84 ± 0.00^{Bc}	6.84 ± 0.01^{Bc}
Calcium activity (mM)	Unheated	1.02 ± 0.07^{Aa}	0.96 ± 0.09^{ABa}	0.92 ± 0.06^{BCa}	0.89 ± 0.06^{BCa}	0.84 ± 0.07^{Ca}
	80 °C	0.97 ± 0.06^{Aa}	0.93 ± 0.06^{ABa}	0.92 ± 0.07^{ABa}	0.87 ± 0.07^{Ba}	0.85 ± 0.07^{Ba}
	85 °C	0.95 ± 0.05^{Aa}	0.92 ± 0.05^{ABa}	0.90 ± 0.04^{ABa}	0.86 ± 0.06^{ABa}	0.84 ± 0.07^{Ba}
	90 °C	0.94 ± 0.05^{Aa}	0.91 ± 0.06^{ABa}	0.88 ± 0.05^{ABa}	0.85 ± 0.06^{ABa}	0.83 ± 0.07^{Ba}
	95 °C	0.91 ± 0.06^{Aa}	0.90 ± 0.06^{Aa}	0.87 ± 0.05^{Aa}	0.84 ± 0.07^{Aa}	0.82 ± 0.06^{Aa}
Viscosity (mPa s)	Unheated	1.48 ± 0.20^{Aa}	1.61 ± 0.28^{ABa}	1.80 ± 0.15^{BCa}	1.98 ± 0.04^{CDa}	2.24 ± 0.09^{Da}
	80 °C	1.54 ± 0.18^{Aa}	1.66 ± 0.14^{Aa}	1.80 ± 0.08^{ABa}	1.99 ± 0.06^{Ba}	2.24 ± 0.09^{Ca}
	85 °C	1.60 ± 0.15^{Aab}	1.67 ± 0.15^{Aa}	1.88 ± 0.04^{ABa}	2.11 ± 0.09^{Ba}	2.52 ± 0.04^{Cb}
	90 °C	1.67 ± 0.17^{Aab}	1.69 ± 0.14^{Aa}	1.79 ± 0.15^{Aa}	2.33 ± 0.05^{Bb}	2.53 ± 0.06^{Bb}
	95 °C	1.78 ± 0.09^{Ab}	1.75 ± 0.15^{Ba}	1.88 ± 0.11^{Ba}	2.33 ± 0.10^{Cb}	2.61 ± 0.01^{Cb}

[a] Blend refers to ratio of buffalo skim milk to bovine skim milk. Heat treatment was for 5 min. Values are means ± standard deviation; at each heat treatment, different upper case superscript letters indicate significant difference ($p < 0.05$) and within the same physicochemical property, mean of samples in a column with different lowercase superscript letters differ significantly.

Srinivasan, 1979). This is most likely due to the higher protein determined content (4.2%) in buffalo milk than bovine milk (3.2%). The heat-induced changes in viscosity depended on both the ratio of buffalo:bovine milk, as well as heating temperature (Table 1). For instance, there were significant increases in viscosity for bovine milk, buffalo milk, and the 75:25 buffalo:bovine milk blend when heated at ≥95 °C, 85 °C and 90 °C respectively, although no significant change in viscosity was found for buffalo:bovine milk blends of 25:75 and 50:50 irrespective of the heating temperature (Table 1).

3.2. Changes in non-sedimentable proteins of heat-treated milk

Unheated bovine skim milk had the highest level of non-sedimentable caseins, which decreased with increase in buffalo skim milk concentration (Fig. 1A). The proportion of casein liberated into the serum phase increased during heat treatment, but such increases were a function of both buffalo:bovine milk ratio and temperature of heating wherein the maximum increases occurred at higher temperature and as the proportion of buffalo milk increases. For instance, the level of non-sedimentable caseins in bovine skim milk and 25:75 blend of buffalo:bovine milk was highest at 80 °C while 50:50 and 75:25 buffalo:bovine milk blends had the maximum dissociation at 85 °C (Fig. 1A). Similarly, after heating at this temperature, non-sedimentable caseins in buffalo skim milk increased to almost ninefold of its initial quantity. In contrast, the reduction of non-sedimentable β-lactoglobulin and α-lactalbumin was higher in bovine skim milk compared with buffalo skim milk (Fig. 1B and C). Furthermore, the reduction of non-sedimentable β-lactoglobulin (Fig. 1B) was found to be higher than α-lactalbumin (Fig. 1C), irrespective of the thermal treatment. Mixed milk had higher reduction in non-sedimentable whey proteins compared with their individual milk counterparts. For instance, the highest reduction in non-sedimentable β-lactoglobulin and α-lactalbumin reduction (>50% and >25% respectively) was measured in 25:75 and 75:25 blends of buffalo:bovine milk heated at ≥90 °C (Fig. 1B and C).

4. Discussion

The physical, chemical, and structural changes observed were a function of buffalo:bovine milk ratio and/or heat treatment conditions. The ζ-potential significantly increased (more negative) for bovine milk heated at ≥85 °C without a significant change in particle size, whereas buffalo skim milk and buffalo:bovine milk blends did not exhibit any significant changes on particle size nor charge (Table 1). The change in ζ-potential is mostly due to the association of whey proteins with casein micelles and association of heat-precipitated calcium phosphate with the micelles as previously reported by Singh (2004) and Anema and Klostermeyer (1996). In this study, the significant increase (more negative) in ζ-potential of bovine milk suggests increased formation of whey protein-casein complexes in bovine milk as compared with that in buffalo milk or buffalo:bovine milk blends. This is supported by SDS-PAGE results (Fig. 1B) where marked reduction in β-lactoglobulin was measured in bovine skim milk heated at ≥85 °C. So, it clearly confirms the fact that whey protein denaturation and subsequent associations occur with bovine milk more readily as compared in buffalo milk. When these milks are mixed, there was a higher reduction in non-sedimentable whey proteins compared with their individual milk counterparts probably because of (a) higher amount of denatured whey proteins due to presence of more heat-sensitive bovine milk whey proteins, and (b) higher surface area for adsorption afforded by buffalo milk caseins. As bovine milk whey proteins denature at a faster rate, the adsorption of denatured whey proteins on casein micelles would occur faster in partially substituted milk samples through a new and reinforced covalently driven interactions that created aggregates large enough to sediment with the casein micelles on ultracentrifugation.

pH is one of the dominant factors governing heat-induced dissociation of caseins (Anema, 2021; Huppertz, 2016; Singh, 2004). With increasing temperature, the solubility of calcium phosphate decreases (Huppertz, 2016; Nieuwenhuijse & Huppertz, 2022), resulting in the precipitation of calcium phosphate, partially onto the casein micelles, leading to liberation of H^+ hence a decrease in milk pH (Anema, 2009; Fox et al., 2015; Kelly, Datta, & Deeth, 2012). The pH of the samples significantly decreased after heating at ≥85 °C irrespective of the ratio of buffalo milk to bovine

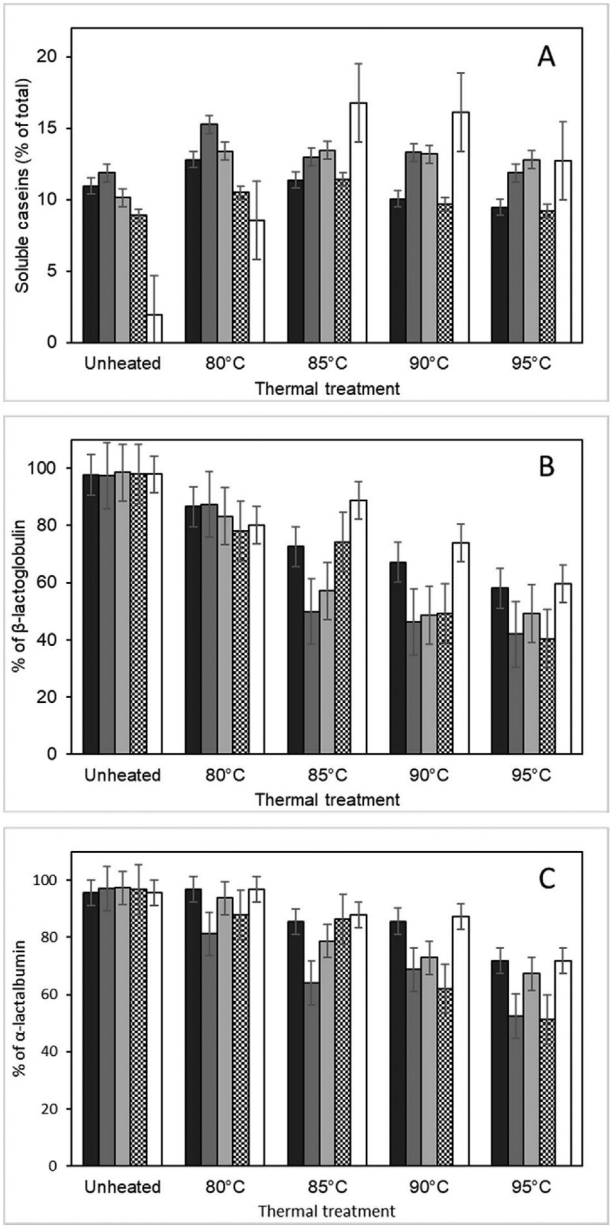

Fig. 1. Changes in the level of non-sedimentable proteins obtained from skim milk samples (■, bovine milk; ■, 25% buffalo milk:75% bovine milk blend; ■, 50% buffalo milk:50% bovine milk blend; ▩, 75% buffalo milk:25% bovine milk blend; □, buffalo milk) following heat treatment at 80–95 °C for 5 min: A, total caseins; B, β-lactoglobulin; C, α-lactalbumin.

milk although the magnitude of pH reduction increased with the increase in buffalo milk concentration (Table 1). The diffusible (or soluble) fraction of calcium and inorganic phosphate of buffalo milk was found to be similar to that of cow milk (Abd El-Salam & El-Shibiny, 2011; Ahmad et al., 2008) but the conversion of soluble calcium and phosphate to the colloidal phase during heating had been found to occur faster in buffalo milk (Adhikari & Mathur, 1993) which could explain the greater pH reduction with increase in buffalo milk concentration in this study.

This heat-induced alteration in the salt balance of milk could reduce the structural integrity of the casein micelles, leading to dissociation of caseins from the micelles during heating (Anema & Klostermeyer, 1997; Singh & Fox, 1987). This dissociation has been suggested to occur due to the formation of an alternative calcium phosphate form that is less capable of binding casein molecules and maintaining the micelle structure (Anema & Klostermeyer, 1997). Increased hydration and voluminosity of the casein micelles (Anema, Lowe, & Lee, 2004; Walstra, Wouters, & Geurts, 2005) result to an increase in viscosity, which was observed (Table 1). Based on the level of non-sedimentable proteins (Fig. 1), buffalo milk, the 75:25 buffalo:bovine milk blend, and bovine milk have higher levels of non-sedimentable caseins than the 25:75 and 50:50 buffalo:bovine milk blends, which mostly would explain the high viscosities observed with heating for these ratios. The low level of non-sedimentable caseins and higher reduction in whey proteins measured in 25:75 and 50:50 milk blends indicate that denatured whey proteins associated with the micelles and these aggregates co-sedimented with the casein micelles on ultracentrifugation. This is further supported by a higher correlation between heat-induced viscosity changes and casein dissociation ($R^2 = 0.727$ to 0.829) than whey proteins reduction ($R^2 = -0.68$ to -0.79) in these milk samples. So, it can be concluded that the presence of non-sedimentable caseins plays a major role towards viscosity of the pure milk and milk blends of buffalo and bovine milk.

5. Conclusion

Heat-induced changes on the physicochemical and structural properties of skim milk were a function of buffalo to bovine milk ratio and temperature. Significant changes in viscosity and pH were observed at heating $\geq$85 °C and with higher proportion of buffalo skim milk. The variation in the denaturation of whey proteins and their associations with themselves and casein micelles, the heat-induced dissociation of caseins, and the heat-induced alteration in the salt balance of milk systems are the key factors in deciding the physical, chemical, structural, and functional characteristics of blends of buffalo milk and bovine milk. These reasons occur at certain degrees based on the heat treatment temperature. Overall, the results of this study bring a better understanding of the changes observed during heat treatment of buffalo skim milk and mixtures with bovine skim milk.

Credit Author Statement

Carolyn T. Mejares: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft preparation and revision **Thom Huppertz**: Methodology, Writing – review & editing **Jayani Chandrapala**: Conceptualization, Methodology, Resources, Writing – review & editing, Supervision

Declaration of competing interest

None.

Acknowledgments

The authors greatly acknowledge the financial and technical support from Royal Melbourne Institute of Technology, Australia and the RMIT Research Scholarship.

References

Abd El-Salam, M. H., & El-Shibiny, S. (2011). A comprehensive review on the composition and properties of buffalo milk. *Dairy Science & Technology, 91*, 663–699.

Adhikari, A. K., & Mathur, O. N. (1993). Heat induced changes in the microstructure of casein micelles and mineral balance of cows' and buffalos' milk during prolonged boiling. *Japanese Journal of Dairy and Food Science, 42*, A171–A179.

Ahmad, S., Gaucher, I., Rousseau, F., Beaucher, E., Piot, M., Grongnet, J. F., et al. (2008). Effects of acidification on physico-chemical characteristics of buffalo milk: A comparison with cow's milk. *Food Chemistry, 106*, 11–17.

Ahmed, N., Elahi, S., Salariya, A. M., & Rashid, A. A. (2014). Physico-chemical and organoleptic comparison of buffalo, cow and goat milk and their yogurt samples. *Pakistan Journal of Science, 66*, 225–231.

Anema, S. G. (2009). Effect of milk solids concentration on the pH, soluble calcium and soluble phosphate levels of milk during heating. *Dairy Science & Technology, 89*, 501–510.

Anema, S. G. (2021). Heat-induced changes in caseins and casein micelles, including interactions with denatured whey proteins. *International Dairy Journal, 122*, Article 105136.

Anema, S. G., & Klostermeyer, H. (1996). ζ-potentials of casein micelles from reconstituted skim milk heated at 120 °C. *International Dairy Journal, 6*, 673–687.

Anema, S. G., & Klostermeyer, H. (1997). Heat-induced, pH-dependent dissociation of casein micelles on heating reconstituted skim milk at temperatures below 100 °C. *Journal of Agricultural and Food Chemistry, 45*, 1108–1115.

Anema, S. G., & Li, Y. (2003). Association of denatured whey proteins with casein micelles in heated reconstituted skim milk and its effect on casein micelle size. *Journal of Dairy Research, 70*, 73–83.

Anema, S. G., Lowe, E. K., & Lee, S. K. (2004). Effect of pH at heating on the acid-induced aggregation of casein micelles in reconstituted skim milk. *Lebensmittel-Wissenschaft und -Technologie, 37*, 779–787.

Bijl, E., Vries, R., van Valenberg, H., Huppertz, T., & van Hoojidonk, T. (2014). Factors influencing casein micelle size in milk of individual cows: Genetic variants and glycosylation of κ-casein. *International Dairy Journal, 34*, 135–141.

Çetinkaya, A. (2019). Determination of changes during storage in chemical and microbial compositions of yoghurts made from buffalo milk and buffalo and cow milk mixtures. *Archivos Latinoamericanos de Nutricion, 69*, 89–98.

Chakraborty, P., Singh, T., Shivhare, U. S., & Basu, S. (2020). Understanding the effect of milk composition and milking season on quality characteristics of chhana. *Journal of Texture Studies, 52*, 45–56.

Chandrapala, J., Martin, G. J. O., Zisu, B., Kentish, S. E., & Ashokkumar, M. (2012). The effect of ultrasound on casein micelle integrity. *Journal of Dairy Science, 95*, 6882–6890.

Chandrapala, J., McKinnon, I., Augustin, M. A., & Udabage, P. (2010). The influence of milk composition on pH and calcium activity measured in situ during heat treatment of reconstituted skim milk. *Journal of Dairy Research, 77*, 257–264.

Claeys, W. L., Verraes, C., Cardoen, S., De Block, J., Huyghebaert, A., Raes, K., et al. (2014). Consumption of raw or heated milk from different species: An evaluation of the nutritional and potential health benefits. *Food Control, 42*, 188–201.

Deeth, H. C., & Lewis, M. J. (2017). *High temperature processing of milk and milk products*. Chichester, UK: John Wiley & Sons.

Dumitraşcu, L., Râpeanu, G., & Stănciuc, N. (2020). Evaluation of thermal processing in the dairy industry using milk enzymes. In P. P. Srivastav, D. P. Verma, A. R. Patel, & A. R. Al-Hilphy (Eds.), *Emerging thermal and nonthermal technologies in food processing* (pp. 1–24). Palm Bay, FL, USA: Apple Academic Press, Inc.

Elagamy, I. I. (2000). Effect of heat treatment on camel milk proteins with respect to antimicrobial factors: A comparison with cows' and buffalo milk proteins. *Food Chemistry, 68*, 227–232.

Fangmeier, M., Kemerich, G. T., Machado, B. L., Maciel, M. J., & De Souza, C. F. V. (2019). Effects of cow, goat, and buffalo milk on the characteristics of cream cheese with whey retention. *Food Science and Technology, 39*, 122–128.

Fox, P. F., Uniacke-Lowe, T., Mcsweeney, P. L. H., & O'Mahony, J. A. (2015). *Dairy chemistry and biochemistry* (2nd ed.). Cham, Switzerland: Springer International Publishing.

Grewal, M. K., Chandrapala, J., Donkor, O., Apostolopoulos, V., & Vasiljevic, T. (2017). Electrophoretic characterization of protein interactions suggesting limited feasibility of accelerated shelf-life testing of ultra-high temperature milk. *Journal of Dairy Science, 100*, 76–88.

Haq, I., Khaskheli, M., Kiani, F. A., Talpur, A. R., Lochi, G. M., Soomro, A. A., et al. (2013). Effect of heat treatment on physico-chemical characteristics of skimmed milk. *Journal of Agriculture and Food Technology, 3*, 5–13.

Huppertz, T. (2016). Heat stability of milk, 4th ed.. In P. L. H. McSweeney, & J. A. O'Mahony (Eds.), *Advanced dairy chemistry* (pp. 179–196). Cham, Switzerland: Springer Science+Business Media. Proteins: Applied aspects

Hussain, I., Bell, A. E., & Grandison, A. S. (2013). Mozzarella-type curd made from buffalo, cows' and ultrafiltered cows' milk. 1. Rheology and microstructure. *Food and Bioprocess Technology, 6*, 1729–1740.

Ismail, A. A., & El Deeb, S. A. (1973). Effect of heat processing, storing and homogenization on the viscosity, opacity and stability of cow and buffalo milks. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 152*, 202–207.

Ismail, A. A., Mashaley, R. I., & Sirry, I. (1969). Effects of heating at three temperatures on the nitrogen constituents of cow and buffalo skimmilks. *Journal of Dairy Science, 53*, 423–426.

Kashwa, M. (2016). Composition of water buffalo milk during the first period of lactation – Relation to mozzarella cheese properties *(Master's thesis)*. Lund, Sweden: Swedish University of Agricultural Sciences.

Kelly, A. L., Datta, N., & Deeth, H. C. (2012). Thermal processing of dairy products. In D. W. Sun (Ed.), *Thermal food processing: New technologies and quality issues* (2nd ed., pp. 273–306). Boca Raton, FL, USA: CRC Press.

Khalifa, M. Y., & Ghanimah, M. A. (2013). Studies on fluid milk viscosity as affected by some factors. *Journal of Biological Chemistry and Environmental Sciences, 8*, 57–66.

Khedkar, C. D., Kalyankar, S. D., & Deosarkar, S. S. (2016). Buffalo milk. In B. Caballero, P. M. Finglas, & F. Toldrá (Eds.), *Encyclopedia of food and health* (pp. 522–528). San Diego, CA, USA: Academic Press.

Lin, L., Wong, M., Deeth, H. C., & Oh, H. E. (2018). Calcium-induced skim milk gels using different calcium salts. *Food Chemistry, 245*, 97–103.

Mane, B. G., & Chatli, M. K. (2015). Buffalo milk: Saviour of farmers and consumers for livelihood and providing nutrition. *Agricultural Rural Development, 2*, 5–11.

Mejares, C. T., Huppertz, T., & Chandrapala, J. (2022). Thermal processing of buffalo milk – a review. *International Dairy Journal, 129*, Article 105311.

Nasr, N. F., & Elshaghabee, F. M. F. (2019). Enhancement of shelf life of pasteurized milk using Nisplin® essential oils emulsion. *International Journal of Current Microbiology and Applied Sciences, 8*, 257–266.

Nieuwenhuijse, H., & Huppertz, T. (2022). Heat-induced changes in milk salts: A review. *International Dairy Journal, 126*, Article 105220.

Nieuwenhuijse, J. A., & Van Boekel, M. A. J. S. (2003). Protein stability in sterilized milk and milk products. In P. F. Fox, & P. L. H. McSweeney (Eds.) (3rd ed.,, *Vol. 1. Advanced dairy chemistry* (pp. 947–974). San Diego, CA, USA: Kluwer Academic/Plenum Publishers. Proteins.

O'Donnell, C., Herlihy, N., & McKenna, B. (1994). Use of an in-line viscometer in the manufacture of skim milk powder. In T. Yano, R. Matsuno, & K. Nakamura (Eds.), Developments in food engineering. Proceedings of the 6th international Congress on Engineering and food, chiba, Japan *(23–27 may 1993* (pp. 927–929). Cham, Switzerland: Springer Science+Business Media.

OECD/FAO. (2022). *OECD-FAO agricultural outlook 2022-2031*. Paris, France: OECD Publishing.

Patil, A. T., Meena, G. S., Upadhyay, N., Khetra, Y., Borad, S. G., & Singh, A. K. (2019). Effect of change in pH, heat treatment and diafiltration on properties of medium protein buffalo milk protein concentrate. *Journal of Food Science & Technology, 56*, 1462–1472.

Patil, A. T., Meena, G. S., Upadhyay, N., Khetra, Y., Singh, A. S., & Borad, S. G. (2021). Buffalo milk protein concentrate 60: Effect of skim milk heat treatment on its reconstitutability and functionality. *Lebensmittel-Wissenschaft und -Technologie, 148*, Article 111638.

Perveen, R., Butt, M. S., Huma, N., & Nawaz, H. (2015). Effect of pH and heat treatment on physicochemical characteristics of sodium phosphocaseinate powder. *The Journal of Animal & Plant Sciences, 25*, 1146–1152.

Petridis, D., Dimitreli, G., Vlahvei, K., & Deligeorgakis, C. (2014). Effects of buffalo and cow milk mixtures enriched with sodium caseinates on the physicochemical, rheological and sensory properties of a stirred yogurt product. *Journal of Food Research, 3*, 54–69.

Quant, A. J. B., Albis, A. R., & Perez, J. A. (2019). Effect of heat treatment on physical properties of whey protein beverages (WPB). *Scientific Study & Research. Chemistry & Chemical Engineering, Biotechnology, Food Industry, 20*, 209–218.

Rekowsky, B. S. S., Monteiro, M. L. G., Silva, T. M., Conté-Júnior, C. A., & Costa, M. P. (2022). Semi-hard buffalo cheese: How cow's milk affects sensory acceptance? *Brazilian Journal of Food Technology, 25*, Article e2022030.

Renhe, I. R. T., Perrone, Í. T., Tavares, G. M., Schuck, P., & De Carvalho, A. F. (2019). Physicochemical characteristics of raw milk. In L. A. Nero, & A. F. de Carvalho (Eds.), *Raw milk: Balance between hazards and benefits* (pp. 29–43). San Diego, CA, USA: Academic Press.

Roy, D., Ye, A., Moughan, P. J., & Singh, H. (2020a). Composition, structure, and digestive dynamics of milk from different species – a review. *Frontiers in Nutrition, 7*, Article 577759.

Roy, D., Ye, A., Moughan, P. J., & Singh, H. (2020b). Gelation of milks of different species (dairy cattle, goat, sheep, red deer, and water buffalo) using glucono-δ-lactone and pepsin. *Journal of Dairy Science, 103*, 5844–5862.

Sindhu, J. S., & Arora, S. (2011). Milk | buffalo milk. In J. W. Fuquay (Ed.), *Encyclopedia of dairy sciences* (2nd ed., pp. 503–511). San Diego, CA, USA: Academic Press.

Singh, H. (2004). Heat stability of milk. *International Journal of Dairy Technology, 57*, 111–119.

Singh, M. J., Chandrapala, J., Udabage, P., McKinnon, I., & Augustin, M. A. (2015). Heat-induced changes in the properties of modified skim milks with different casein to whey protein ratios. *Journal of Dairy Research, 82*, 135–142.

Singh, H., & Fox, P. F. (1987). Heat stability of milk: Influence of colloidal and soluble salts and protein modification on the pH-dependent dissociation of micellar κ-casein. *Journal of Dairy Research, 54*, 523–534.

Tambat, R. V., & Srinivasan, M. R. (1979). Changes in surface tension, viscosity and curd tension of buffalo and cow milk during cheddar cheese manufacture. *Indian Journal of Dairy Science, 32*, 173–176.

Tanaka, M., Suzuki, T., Kotb, S., & Kamiya, Y. (2011). Relation of calcium activity in milk and milk production of Holstein cows in hot season. *Asian-Australasian Journal of Animal Sciences, 24*, 1372–1376.

Thompkinson, D. K., & Sabikhi, L. (2012). *Quality milk production and processing technology*. Delhi, India: New India Publishing Agency.

Walstra, P., Wouters, J. T. M., & Geurts, T. J. (2005). *Dairy science and technology* (2nd ed.). Boca Raton, FL, USA: CRC Press.

Ziyaina, M., Govindan, B. N., Rasco, B., Coffey, T., & Sablani, S. S. (2018). Monitoring shelf life of pasteurized whole milk under refrigerated storage conditions: Predictive models for quality loss. *Journal of Food Science, 83*, 409–418.