



Acid Gelation Properties of Camel Milk—Effect of Gelatin and Processing Conditions

Thao M. Ho^{1,2} · Jiadi Zhao² · Nidhi Bansal²

Received: 30 May 2022 / Accepted: 30 July 2022 / Published online: 11 August 2022
© The Author(s) 2022

Abstract

This study investigated the effects of glucono-delta-lactone (GDL) concentrations (0.8–1.2%, w/w), gelatin content (0.6–1.0%, w/w) and processing conditions on the properties of camel milk acid gels. Although the pH of camel milk reduced to 4.3 within 4 h of acidification at 1.0% GDL, it was unable to form a suitable gel for a yoghurt-like product unless gelatin was added. At 0.8% gelatin, camel milk gels had similar hardness, lower viscosity and rheological strength, and higher water holding capacity as compared to cow milk gels. Heating of camel milk (85 °C/15–20 min), 2-stage homogenization (150/50 bar) or their combination did not significantly affect the water holding capacity, hardness, viscosity, rheological strength and microstructure of camel milk gels. These processing conditions did not affect protein integrity as confirmed by sodium dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis.

Keywords Glucono-delta-lactone · Gel microstructure · Heat treatment · Gelatin · Homogenization

Introduction

Camel milk has high nutritional value with many components and properties homologous to human milk. Camel milk has a satisfactory balance of essential amino acids required for human diet. It lacks β -lactoglobulin and contains a higher percentage of β -casein and lesser amount of α -casein than cow milk (El-Agamy, 2009; Hinz et al., 2012; Kappeler et al., 2003; Lara-Villoslada et al., 2005). Camel milk has a high vitamin and mineral content and contains many protective proteins (e.g. immunoglobulins, lactoferrin, lysozyme and lactoperoxidase) that exhibit anti-cancer, anti-diabetic and anti-bacterial properties (Barłowska et al., 2011; Konuspayeva et al., 2009). These proteins are also responsible for the extended shelf-life of the camel milk at ambient temperature (Kumar et al., 2021). However, the global supply of camel milk is very limited. According to FAO (2019), African countries (e.g. Somalia, Sudan, Nigeria, Kenya, Chad, Mauritania, Ethiopia and Mali) account

for approximately 90% of the fresh whole camel milk global production, followed by Asian countries (e.g. India, Yemen, Saudi Arabia, United Arab Emirates, China and Afghanistan) with approximately 8% share. To increase the distribution and consumption of camel milk, the production of camel milk products that have a longer shelf-life, are easy to transport, preserve the functional properties of camel milk components and are well accepted by the consumers is required.

Yoghurt is one of the most widely consumed fermented dairy products due to its well-known potential health benefits (Mckinley, 2005). It is generally produced by fermenting bovine milk using acidulants, enzymes, or bacterial cultures to produce a firm gel structure (Robinson et al., 2006). For yoghurt, the most important property that determines the appearance, mouthfeel, and overall acceptability by consumers is the gel texture. However, the production of camel milk yoghurt is challenging, due to poor coagulation of camel milk, which results in thin consistency and weak product structure. The weak firmness of camel milk coagulum is primarily associated with the lack of κ -casein and β -lactoglobulin interactions (Ho et al., 2021). Other reasons are the high ratio of whey proteins (WP) to caseins (CN), and the large casein micelle size in the camel milk (Berhe et al., 2017). The WP/CN ratio in cow milk varies in a range from 15:85 to 25:75 depending on the season, diet, breeding and genetic polymorphism while this ratio in camel milk is 24:76–27:73

✉ Nidhi Bansal
n.bansal@uq.edu.au

¹ Department of Food and Nutrition, University of Helsinki, P.O. Box 66, 00014 Helsinki, Finland

² School of Agriculture and Food Sciences, University of Queensland, Brisbane, QLD 4072, Australia

(Roy et al., 2020). It was reported that casein micelle size in raw camel milk was about 173 nm, while that in raw cow milk was approximately 143 nm (Omar et al., 2018). Therefore, traditional approaches to produce yoghurt from bovine milk are not applicable to camel milk. Fermentation of camel milk via starter cultures (e.g. *Streptococcus thermophilus* and *Lactobacillus delbruckii* subsp. *bulgaricus*, 2.5%) and incubation at 37 °C for up to 16 to 18 h did not form the desired curd structure, but instead formed fragile and heterogeneous dispersed flakes with a watery texture (El Zubeir et al., 2012). Despite these challenges, many attempts have been made to improve the firmness and consistency of camel milk gels and prevent syneresis of the product during processing and storage. The reported approaches included mixing camel milk with other mammalian milk, such as buffalo milk (Ibrahem & El Zubeir, 2016), and using various gelation agents such as hydrocolloids (carboxymethyl cellulose, pectin, gum acacia, arabic gum, guar gum, xanthan gum, alginate, κ -carrageenan, sodium carboxymethyl cellulose), skim/non-fat dry milk powder, (polymerized) whey protein isolate, stabilizers (e.g. Grindstred ES255), modified starch, Na₂EDTA, and even mono- and di-glyceride fatty acids (Al-Zoreky & Al-Otaibi, 2015; Galeboe et al., 2018; Hashim et al., 2009; Ibrahim & Khalifa, 2015; Jasim et al., 2018; Kavas, 2016; Khalifa & Ibrahim, 2015; Mudgil et al., 2018; Sakandar et al., 2014). The effects of gelatin powder on the texture and rheological properties of camel milk yoghurts were also investigated (Galeboe et al., 2018; Hashim et al., 2009; Mudgil et al., 2018). However, in these studies, the products were produced using the yoghurt culture, and/or gelatin powder was used in the combination with other components such as CaCl₂, bovine skim milk powders and fatty acids. There are very few studies focusing on the effects of gelatin powder on the acid gelation properties of camel milk. In yoghurt production, pre-heating of milk plays a crucial role, due to its ability to modify the properties of milk proteins and to form a stable structure (Lucey et al., 1999). In addition, heating not only inactivates harmful microorganisms in milk, but also generates desirable properties in the final product, such as increased yoghurt viscosity (Singh, 1993).

Another important processing step in the production of yoghurt is homogenization that reduces the size of the fat globules to less than 2.0 μ m, resulting in formation of new fat globules with increased total surface area. Homogenization has a major effect on the quality and acceptability of yoghurt, as it prevents fat separation during the fermentation process and storage, decreases whey separation, improves whiteness and consistency, enhances mouthfeel and strengthens the gel network (Trujillo et al., 2016). However, to the best of our knowledge, there is limited evidence on the effects of heating and homogenization on

the properties of camel milk gels. In this study, the effects of glucono-delta-lactone (GDL) concentrations (0.8, 1.0, and 1.2%) on pH reduction rate and those of gelatin concentrations (0.6, 0.8 and 1.0%, w/w) and processing conditions (heating, homogenization or both) on the properties of camel milk gel were also investigated. It is noted that we did not investigate the effect of these processing factors on the properties of cow milk gels. We employed cow milk gels as a reference for evaluating camel milk gel properties, aiming to investigate if these factors enabled camel milk gel the properties to be similar to those of cow milk gels. Therefore, we used the commercial cow milk to produce gels and same procedure to prepare gels from both cow and camel milk was followed as reported by Pang et al. (2017).

Materials and Methods

Materials

Raw camel milk was obtained from a local camel farm (Summer Land Camels, Queensland, Australia). After acquisition, raw camel milk was either immediately processed or kept at 4 °C for a maximum of 3 days. Gelatin powder (beef skin, 220 bloom) was bought from The Melbourne Food Depot (Victoria, Australia). GDL was obtained from Sigma-Aldrich, Brisbane, Australia. Pasteurised full cream cow milk was obtained from a local supermarket (Coles, Queensland, Australia). As advised by the manufacturer, the cow milk was homogenized at 150 bars in the first stage and 50 bars in the second stage, and then pasteurized at 75 °C/30 s.

For SDS-PAGE analysis, precast polyacrylamide gels (4–20%), sample buffer, Precision Plus Protein™ Dual Xtra molecular weight standard, and the Mini Protean Tetra Cell system used to run the gels were obtained from Bio-Rad Laboratories Pty. Ltd, NSW, Australia.

Effect of GDL Concentration on Rate of pH Reduction

The effect of GDL at different concentrations on the rate of pH reduction was investigated for both commercial pasteurized full cream cow milk and raw camel milk. GDL at 0.8, 1.0, and 1.2% (w/w) was added to milk that was heated in a water bath to 40 °C with gentle stirring using an overhead stirrer (400 RPM, Heidolph RZR 2050, Kelheim, Germany). The mixture was stirred well for 60 s and left undisturbed for approximately 4.5 h. During this time, pH of the mixture was measured every 10 min.

Effect of Gelatin Concentration on Gelation Properties of Camel Milk

The results of the previous experiment [Effect of GDL concentration on rate of pH reduction](#) indicated that the optimized GDL concentration (based on pH drop rate) for cow milk and camel milk was 1.0% (w/w), and camel milk did not form a gel despite pH reduction to 4.3. In this experiment, the effects of gelatin at different concentrations (0.6, 0.8, and 1.0%, w/w) on gelation properties of camel milk were investigated. Gelatin powder was added to camel milk that was heated in a water bath to 40 °C with gentle stirring. The mixture was then stirred for 30 min to dissolve the gelatin completely into the camel milk. The dissolution of gelatin did not affect the pH of camel milk. After GDL was added (1.0%), the mixture was mixed well for 60 s and left undisturbed for 4 h. The mixture was then kept at 4 °C for 18 h for gel formation.

For reference samples, gels from cow milk were also prepared by the method reported by Pang et al. (2017), with a slight modification. Cow milk was heated at 85 °C with a dwell time for 15–20 min and cooled to 40 °C. While maintaining this temperature, 0.4% (w/w) gelatin was added and mixed for 30 min. After addition of GDL at 1.0%, the mixture was stirred well for 60 s and left undisturbed for 4 h to form a gel. The gels were kept at 4 °C for 18 h prior to analyzing final pH, hardness, water holding capacity and rheological properties.

Effect of Processing Conditions on Gelation Properties of Camel Milk

The effects of different processing conditions, including heating, homogenization or their combination, on gelation properties of camel milk were studied. For heating, camel milk was heated in a water bath at 85 °C with a dwell time for 15–20 min and then cooled to 40 °C. For homogenization, camel milk was pasteurized in a water bath at 75 °C for 30 s due to the presence of lipase. Lipase activity may lead to lipolysis in camel milk during or after homogenization (or both) as fat globule membranes are ruptured (Lorenzen et al., 2011). After cooling to 40 °C, pasteurized camel milk was homogenized at 150 bars in the first stage and at 50 bars in the second stage using a 2-stage Twin Panda homogeniser (NS2002H, Twin Panda 400, GEA Niro Soavi, Parma, Italia). For combination of heating and homogenization, after heating to 85 °C with a dwell time for 15–20 min, camel milk was cooled to 40 °C and subjected to homogenization at similar conditions as described above. After treatment with heat, homogenization, or both, gelation of camel milk was progressed as described in [Effect of gelatin concentration on gelation properties of camel milk](#) using 0.8% gelatin and 1.0% GDL. Gels formed after being stored at 4 °C for 18 h were analyzed for final pH, hardness, water holding capacity and rheological properties.

Analytical Methods

Camel Milk Composition

The composition of camel milk in terms of protein, fat and lactose content was determined by the Kjeldahl method (AOAC, 2005), Gerber method (AOAC, 2005) and titrimetric method (AS, 1994), respectively.

pH of Milk and Gels

The pH of milk samples and gels was determined using an Aqua-pH meter (TPS 121112, TPS Pyt Ltd., Queensland, Australia). The meter was calibrated with buffers before each measurement.

Flow Behaviour of Camel and Cow milk during gelation

The formation of gels prepared from cow and camel milk during the acidification process with 1% GDL was determined following the method reported by Chen et al. (2019) and Kamal et al. (2017) using a rheometer (ARG2, TA Instruments, Elstree, UK) with cup (70[L] × 28[D] mm) and bob (41.92[L] × 28[D] mm) geometry. The gap between the bob and base of the cup was adjusted to 11.80 mm. After addition of GDL (1.0%) and stirring for 60 s, the mixture was added to the rheometer cup at 42 °C. Dynamic time sweep was performed at a frequency of 1 Hz and strain of 0.5%. The time-sweep experiments were performed at 42 °C for 4.5 h to observe changes in storage modulus (G') and loss modulus (G'') with time.

Rheology Properties of Gels

The viscoelastic properties and flow behaviour of gels were measured using a stress-controlled rheometer (ARG2, TA Instruments, Elstree, UK), as described by Pang et al. (2017), with modifications. For viscosity, approximately 2.5 g of gels after storage at 4 °C for 18 h were placed on the stationary plate of rheometer. A sandblasted and flat plate geometry (40 mm in diameter) was used with a gap of 15 mm from the stationary plate. Any overfilled sample was removed before measurement. The measurements were performed at 25 ± 0.1 °C in the shear rate range of 0 to 100 s^{-1} . The rheological parameters (shear stress, shear rate, and apparent viscosity) were obtained from the software provided by TA Instruments.

The flow behaviour of gels after storage at 4 °C for 18 h was determined by frequency sweep from 0 to 15 Hz and using 0.5% strain, which was within the linear viscoelastic region of the samples. G' and G'' were determined to understand the rheological behaviour of the gels.

Texture Analysis of Gels

The hardness of gels was measured by following method reported by Pang et al. (2014) with a modification using Brookfield texture analyser (ThermoFisher Scientific, Massachusetts, USA). Gels were prepared in small Sarstedt containers (high density polyethylene, 70 mL, 55(H) × 44(D) mm). After camel milk was mixed with GDL as described in [Effect of gelatin concentration on gelation properties of camel milk](#) and [Effect of processing conditions on gelation properties of camel milk](#), it was immediately distributed into 50-mL Sarstedt containers, acidified at 40 °C for h and stored at 4 °C for 18 h before measurement. A flat base cylindrical probe of 12.7 mm diameter was used to measure the hardness. The operating speed of probe was at 1 mm/s, and the trigger force was 0.05 N. The probe penetrated 10 mm into the gel. The hardness (N) was determined based on the force versus time curve.

Water Holding Capacity (WHC) of Gels

The WHC of gels was quantified using centrifugation technique according to Farnsworth et al. (2006). Gels were formed in 50-mL falcon tubes. After camel milk was mixed with GDL as described in [Effect of gelatin concentration on gelation properties of camel milk](#) and [Effect of processing conditions on gelation properties of camel milk](#), it was immediately distributed into falcon tubes, acidified at 40 °C for h, and stored at 4 °C for 18 h before centrifugation at 2,000 rpm for 10 min. The weight of the separated serum on the top was then determined. The WHC was calculated using the formula below.

$$WHC(\%) = \frac{\text{Weight of gel} - \text{Weight of serum separated}}{\text{Weight of gel}} * 100$$

SDS-PAGE Analysis

SDS-PAGE analysis was performed under reducing (R-SDS-PAGE) and non-reducing (NR-SDS-PAGE) conditions, following the method reported by Singh et al. (2019). Samples were mixed 1:1 with 2X sample buffer for NR-SDS-PAGE analysis. For R-SDS-PAGE analysis, samples were mixed 1:1 with 2X sample buffer containing 10% β-mercaptoethanol, and heated at 95 °C for 5 min. A total of 10 µg protein was loaded into each well. This value was calculated based on the protein content in milk samples determined by Kjeldahl method. Electrophoresis was performed at 80 V for 30 min and then at 100 V using Bio-Rad Mini Protean Tetra Cell system (Bio-Rad Laboratories Pty. Ltd, NSW, Australia). The gels were stained with a solution of 0.04% Coomassie Brilliant blue G250, 25% methanol, and

10% acetic acid in water overnight. The gels were scanned and visually analysed and compared to the control and each other, using a Bio-Rad GS-800 Calibrated Densitometer (Bio-Rad Laboratories Pty. Ltd, NSW, Australia).

Gel Microstructure

Gel microstructure was determined with a scanning electron microscope as reported by Pang et al. (2014) with modifications. Gels were cut into small pieces (1 mm³) and placed in 2.5% (v/v) glutaraldehyde solution and fixed using a Pelco Biowave Microwave (Ted Pella, Inc., California, USA) at 80 W for two cycles of 2 min-on, 2 min-off, and 2 min-on. The gels were then rinsed twice with 0.1 M sodium cacodylate and microwaved at 80 W for 40 s. The gels were then dehydrated two times in an ethanol series (concentration of 50%, 70%, 90%, and 100%). For each ethanol concentration, the gels were subjected to microwave at 250 W for 40 s. The gels were then subjected to infiltration with 1/1 100% ethanol/hexamethyldisilazane (HMDS) and then 100% HMDS twice. Gels were biowaved at 150 W for 3 min (without vacuum) for each infiltration. Gels were then dried in a fume hood for at least 1 h.

Dried and fixed gels were mounted on aluminium stubs and coated with iridium using a Quorum Q150T metal coater (Quorum Technologies Ltd, Lewes, UK) for three cycles. The microstructure of the gels was examined using a scanning electron microscope (Hitachi SU3500 SEM, Hitachi High-Technologies Europe GmbH, Krefeld, Germany) at an acceleration voltage of 5 kV, an emission current of 5 µA, a working distance of 10 mm, and magnification of 5000X.

Experimental Design and Statistical Analysis

Experiments were performed following a completely randomized design with three replicates of gels produced from three separate milk batches. All physicochemical analyses were then conducted in duplicate. Results were expressed as the mean ± standard deviation. Experimental data were subjected to analysis of variance (ANOVA) using post-hoc Turkey test to differentiate the gel properties among samples at significance level p = 0.05 using Minitab 16.0 statistical programme (Minitab Inc., USA).

Results and Discussion

Effect of GDL Concentration on pH Reduction Rate

From analysed results, camel milk contains 2.53 ± 0.10% (w/v) fat, 2.97 ± 0.07% (w/w) protein, and 4.31 ± 0.05% (w/w) lactose, which is much lower than those of cow milk.

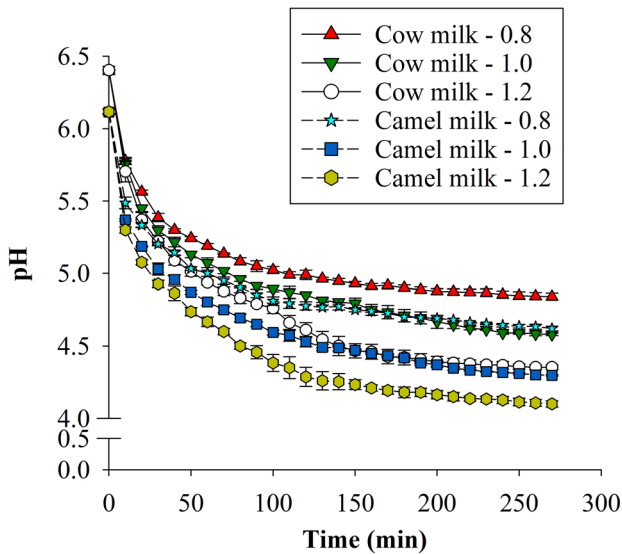


Fig. 1 pH reduction in cow milk and camel milk acidified with 0.8, 1.0 and 1.2% GDL. After 4 h of acidification, for each GDL concentration, the pH of cow milk was significantly higher than that of camel milk ($p < 0.05$), as determined by analysis of variance (ANOVA) using post-hoc Turkey test (Minitab Version.16 statistical programme, Minitab Inc., USA). The results of three replicates were very similar. Average values of three replicates of gels produced from three separate milk batches were used to plot the graph

According to the manufacturer, the fat, protein, and lactose content of cow milk are 3.4% (w/v), 3.3% (w/v), and 5.1% (w/v), respectively. A lower concentration of the main components in camel milk than cow milk was also reported in other studies (Al haj & Al Kanhal, 2010; Kamal et al., 2017).

As indicated in Fig. 1, for both cow and camel milk, the added GDL induced a pH reduction, and the changes in pH was dependent on its concentration. Once GDL is in contact with the water in milk, its ring-shaped molecule opens up and is hydrolysed to gluconic acid, which is a main factor for pH reduction (Feiner, 2016). When the concentration of GDL was increased, the pH value of milk decreased faster. After 4 h of acidification, the pH of cow milk with 0.8, 1.0, and 1.2% GDL was 4.9, 4.6, and 4.4, respectively, being significantly higher than ($p < 0.05$) the corresponding values of camel milk (4.6, 4.3, and 4.1, respectively). A dependence of the acidification rate of camel milk on GDL content was also previously reported (Fetahagić et al., 2002; Zouari et al., 2018). The rate of milk acidification by GDL was not only influenced by its concentration, but also acidification temperature and heat treatment conditions applied to milk prior to acidification (Fetahagić et al., 2002). Visual observation revealed marked differences in the structure of gels produced from cow and camel milk at the same GDL concentration. Unlike cow milk gels, which are a continuous network of proteins, camel milk gels at

three gelation concentrations consisted of groups of protein flakes completely devoid of firmness. Such differences in acidified cow and camel milk gel structures were also reported by Attia et al. (2000). By investigating the changes in the microstructure of camel casein micelles during acidification by GDL, these authors reported that during acidification, the casein micelles experienced three different stages depending on pH. In the first stage, as pH dropped from its initial value (approximately 6.2) to 5.5, the casein micelles maintained their integrity. However, within a pH range of 5.5 to 5.0, the casein micelles significantly lost their microstructure due to their dissociation, resulting in viscoelastic behaviour. Below pH 5.0, the micellar structure collapses and a network of completely demineralized casein aggregates (e.g. groups of casein flakes) totally devoid of firmness was formed. There are many known factors associated with weak texture and thin consistency of camel milk gels, including lack of β -lactoglobulin and low amount of k-casein, high whey protein to casein ratio, and large micelle size (Berhe et al., 2017).

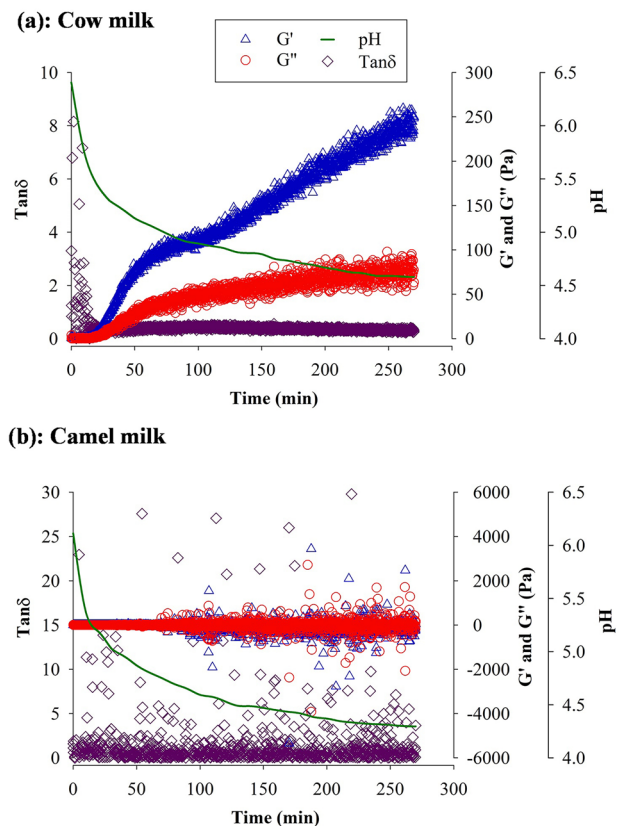


Fig. 2 Changes in storage modules (G' , blue triangle symbols); loss modules (G'' , red circle symbols); $\tan\delta$ (violet diamond symbols); and pH (green lines) during acidification of cow milk **a** and camel milk **b** with 1.0% GDL. The results of three replicates were very similar. Average values of three replicates of gels produced from three separate milk batches were used to plot the graphs

As reported above, visual observation revealed a similarity in gel formation of camel milk with three levels of GDL. To understand the gelation of camel milk during acidification, rheological changes of camel milk with 1% GDL was chosen for further determinations, and compared with those of cow milk. The results are shown in Fig. 2. G' and G'' represent solid-like and liquid-like properties of milk, respectively, during acidification. Loss tangent ($\tan\delta$), which is the ratio of G'' to G' , represents the type of the viscoelastic properties of materials by which those with higher loss tangent exhibit more liquid-like behaviour. The initiation of gel formation (also known as gelation point) is indicated by an increase of G' due to the formation of new interactions among proteins and rearrangement of the protein network; the pH at this point is known as gelation pH (Lucey, 2017). As shown in Fig. 2, only cow milk showed signals of gel formation, as G' started to increase after about 17 min of acidification, corresponding to pH 5.5. During that time, $\tan\delta$ rapidly dropped to less than 0.4. After 17 min, G' of cow milk gels kept increasing and was always higher than G'' during investigated acidification time, indicating the highly structured cow milk gels. In contrast, camel milk did not show any indication of gel formation despite pH reduction to 4.3. During acidification, G' and G'' values of camel milk gels were overlapped and did not experience any significant change. Fluctuation of G' and G'' values was observed after 2 h of acidification could be due to the formation of protein flakes. However, a study by Zouari et al. (2018) revealed that at higher GDL concentration (e.g. 1.75–2.5%), raw and reconstituted spray-dried camel milk formed weak gels, as the gelation process (e.g. the increase of G' value) was observed at pH 4.4 to 4.6 depending on GDL concentration, fat content, and acidification temperature. In summary, although camel milk was unable to form gels at all GDL

concentrations, the concentration of 1.0% (w/w) reduced the pH of cow and camel milk to a desirable value (pH 4.5 for cow milk and pH 4.3 for camel milk) within 4 h of acidification. In addition, as indicated in Fig. 1, at 0.8% GDL, pH of camel milk could not reach the desirable value while at 1.2% GDL, pH of camel milk was reduced too fast during acidification. Accordingly, 1% GDL was used for both cow and camel milk for the following experiments.

Effect of Gelatin Concentration on Gel Properties

Hardness, WHC, final pH, viscosity (at shear rate of 50 s^{-1}), G' and G'' of the gels prepared from camel milk with 0.6, 0.8, and 1.0% gelatin in comparison with those of cow milk with 0.4% gelatin are shown in Table 1 and Fig. 3. The curves for viscosity changes of cow and camel milk gels at different shear rates are shown in Fig. S1 (Supplementary material). Cow milk gel was used as reference sample, and was prepared by following the optimal conditions published by Pang et al. (2017). The hardness of camel milk gels increased approximately seven folds as the added gelatin concentration increased from 0.6 to 1.0%. Camel milk gels with 0.8% gelatin had a similar hardness value ($p > 0.05$) as cow milk gels (Table 1). Regarding WHC, all camel milk gels with added gelatin were similar to each other, and had significantly greater WHC than that of cow milk gels, which had lower gelatin concentration ($p < 0.05$). The high water-binding ability of gelatin is due to changes in its conformation from coil to helix, and to its interactions with casein matrices, which help retain the aqueous phase, prevent water drainage, and result in mechanical reinforcement effects (Andiç et al., 2013). These may be the reasons for the high WHC in camel milk gels and for the greater hardness of camel milk gels with higher gelatin

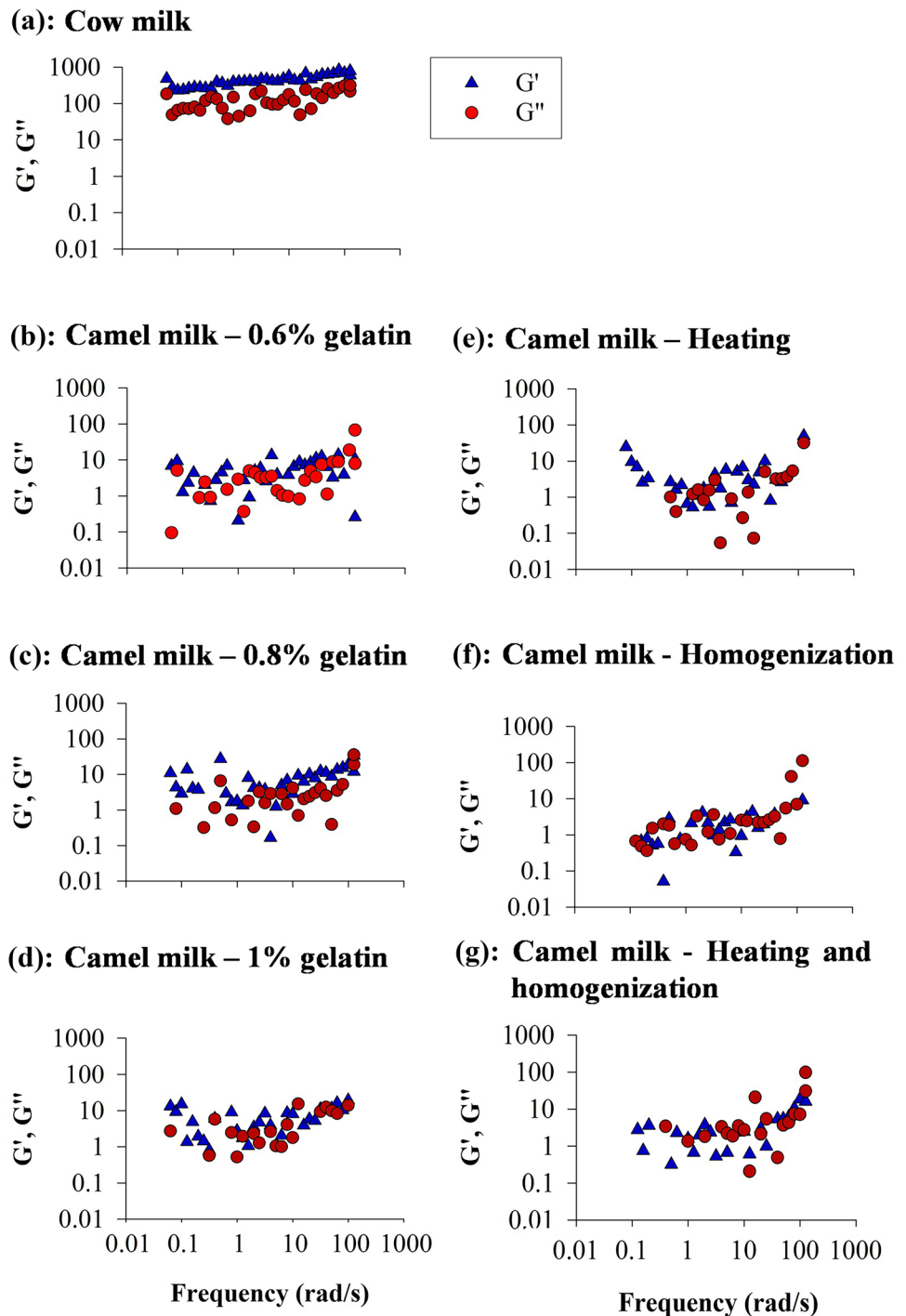
Table 1 Hardness, water holding capacity (WHC), final pH and viscosity (at a shear rate of 50 s^{-1}) of gels produced from cow milk (reference sample*) and camel milk added with different gelatin concentrations (%) and subjected to heating and/or homogenization

Samples	Gelatin (%)	Treatment	Hardness (N)	pH	WHC (%)	Viscosity (Pa.s) [#]
Cow milk	0.4	Heating	4.72 ± 0.12^{bc}	4.42 ± 0.05^a	98.56 ± 0.16^a	0.237 ± 0.066^a
Camel milk	0.6	NA	1.67 ± 0.40^d	4.51 ± 0.04^{ab}	99.68 ± 0.37^b	0.042 ± 0.002^b
	0.8	NA	4.19 ± 0.51^{bc}	4.71 ± 0.04^b	99.67 ± 0.36^b	0.043 ± 0.002^b
	1.0	NA	7.11 ± 1.42^a	4.65 ± 0.01^b	99.92 ± 0.14^b	0.049 ± 0.005^b
	0.8	Heating	5.24 ± 0.39^b	4.60 ± 0.06^b	99.96 ± 0.07^b	0.029 ± 0.003^b
		Homogenization	3.38 ± 0.31^{cd}	4.75 ± 0.34^b	99.96 ± 0.03^b	0.033 ± 0.005^b
		Heat + Homogenization	3.41 ± 0.34^{cd}	4.67 ± 0.12^b	99.99 ± 0.01^b	0.025 ± 0.001^b

*Cow milk gel is used as reference sample for comparison with camel milk gels, and was prepared by following the method reported by Pang et al. (2017). NA – not applicable. Heating in a water bath at $85\text{ }^\circ\text{C}$ with a dwell time of 15–20 min. Homogenization: 150 bars in the first stage and 50 bars in the second stage. All samples are added with 1% GDL. Results were expressed as the mean of three replicates of gels produced from three separated milk batches \pm standard deviation. Means with different letters (e.g. a, b, c) in the same column indicated significant differences among samples ($p < 0.05$), which was obtained from analysis of variance (ANOVA) using post-hoc Turkey test (Minitab Version.16 statistical programme, Minitab Inc., USA)

[#]Viscosity of gels after stored at $4\text{ }^\circ\text{C}$ for 18 h

Fig. 3 Frequency sweeps of gels produced from cow and camel milk. **a:** cow milk was added with 0.4% (w/w) gelatin (Pang et al., 2017) and was used as reference sample; **b, c** and **d:** camel milk was added with 1% GDL and gelatin at concentrations of 0.6, 0.8 and 1.0%, respectively; **e, f** and **g:** camel milk added with 1% GDL and 0.8% gelatin was subjected to heating, homogenization and combination of heating and homogenization, respectively. The results of three replicates were very similar. Average values of three replicates of gels produced from three separate milk batches were used to plot the graphs. Unit of G' and G'' was Pascal (Pa).



concentrations. Similarly, the improvement of gel hardness and WHC of gelatin was also reported for cow milk (Fizman et al., 1999), and already in previous studies on camel milk (Hashim et al., 2009). In addition, the similarities in WHC of all camel milk gels indicated that 0.6% gelatin was sufficient to prevent serum separation.

The viscosity of camel milk gels with 0.6 to 1.0% gelatin measured at a shear rate of 50 s^{-1} was not significantly

different, and fluctuated between 0.042 to 0.049 mPa.s (Table 1). These values were almost six-fold lower than that of cow milk gel (approximately 0.237 mPa.s). In addition, the frequency dependence of gels (obtained by plotting $\log(G')$ and $\log(G'')$ versus $\log(\omega)$; Fig. 3) showed that only cow milk gels exhibited viscoelastic characteristics, with G' being higher than G'' during the entire frequency range. Moreover, at a certain frequency, the G' and G'' values of

cow milk gel were markedly higher those of all camel milk gels, indicating that camel milk gels formed with gelatin were very weak, when compared with cow milk gels. From these results, it can be concluded that, although the added gelatin assisted in formation of camel milk gels, concentrations of 0.6 to 1.0% gelatin did not enhance their rheological strength. In our study, the rheological gel strength did not correlate with the gel hardness obtained from the gel texture analysis, as there were differences in sample deformation mechanisms between the two techniques. A lack of correlation in gel structure as measured by rheometer and texture analyzer was also reported previously (Pang et al., 2017). Considering all gel properties, 0.8% gelatin was chosen for investigating the effects of processing conditions as this concentration provided the camel milk gels with a similar hardness to cow milk gels.

Effect of Processing Conditions on Gel Properties

When compared with camel milk gels with 0.8% gelatin (without heating and homogenizing), either heating at 85 °C with a dwell time for 15–20 min, 2-stage homogenization (150 bars in the first stage, 50 bars in the second stage) or their

combination, did not significantly affect the WHC, hardness, viscosity and rheological strength of camel milk gels (Table 1 and Fig. 3). It is well reported that heating and homogenization improve the structure, rheological properties, and acceptability of cow milk gels (Lucey et al., 1999; Singh, 1993). However, due to the lack of β -lactoglobulin, low amount of k-casein and small fat globule size (e.g. D[4,3] values of raw camel and cow milk are 2.56 μm and 4.16 μm , respectively, Ho et al., 2021) of camel milk, the heating or homogenization conditions (or both) used in this study did not induce similar changes in proteins as in cow milk. This was supported by the SDS-PAGE results, which indicated that camel milk proteins preserved their integrity during heating and homogenization. Further investigation about effects of fat globule size induced by varied homogenisation pressure levels on the properties of camel milk gels is necessary.

Protein Profiling by Electrophoresis

SDS-PAGE protein profiles of camel milk subjected to heating and/or homogenization were visually analyzed to identify if selected processing conditions affected the integrity of camel milk proteins. Non-reducing and reducing

Fig. 4 Non-reducing SDS-PAGE and reducing SDS-PAGE analyzes of raw and processed camel milk. Lane (S) is for molecular weight standards (Precision Plus Protein™ Dual Xtra Prestained Protein Standards, Bio-Rad Laboratories Pty Ltd., NSW, Australia). The standards are a mixture of 12 recombinant proteins (2–250 kD) including nine blue-stained bands and three pink reference bands (2, 25 and 75 kD), and the 2 kD band is not visible on fluorescent blots. Lane (1) is for raw camel milk. Lane (2) is for homogenized camel milk (150 bars in the first stage and 50 bars in the second stage). Lane (3) is for heated camel milk (85 °C in a water bath with a dwell time of 15–20 min). Lane (4) is for heated and homogenized camel milk

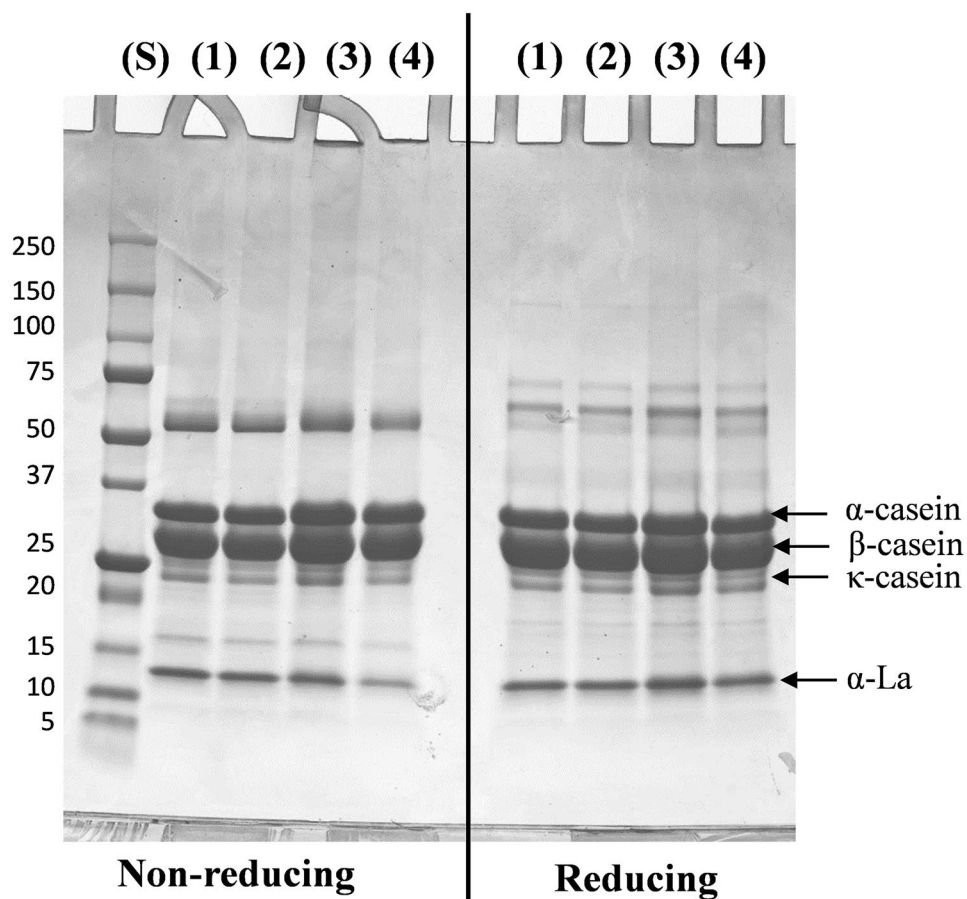
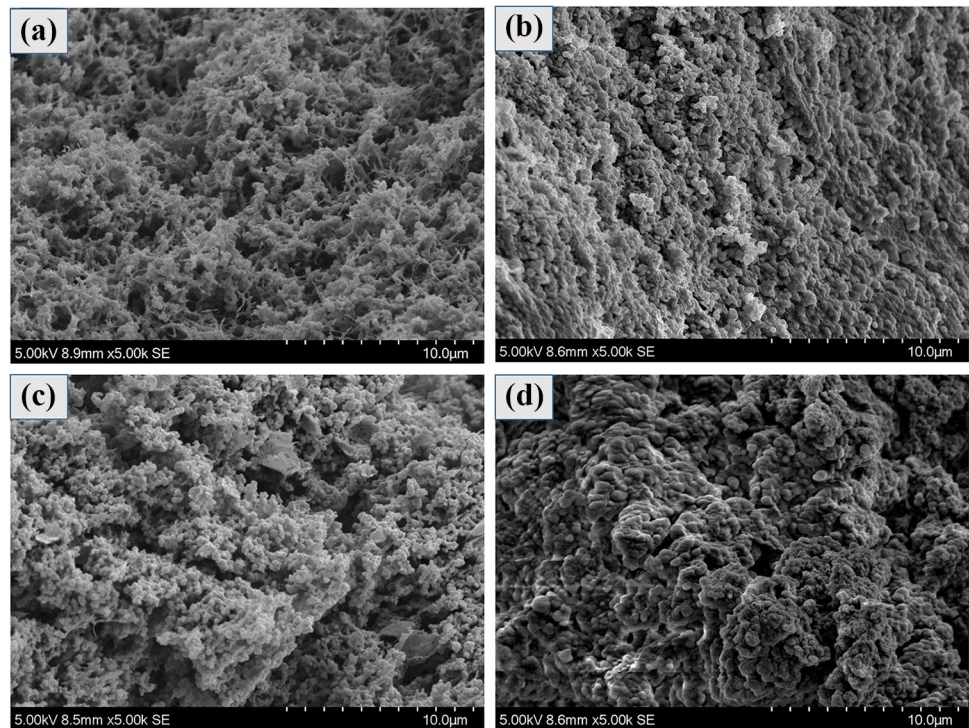


Fig. 5 SEM of gels prepared from cow and camel milk gels. **a:** cow milk was added with 0.4% (w/w) gelatin (Pang et al., 2017) and was used as reference sample; **b:** camel milk without heating and homogenizing; **c:** camel milk heated at 85 °C in a water bath with a dwell time of 15–20 min; and **d:** camel milk homogenized at 150 bars in the first stage and 50 bars in the second stage. All camel milk samples were added with 0.8% (w/w) gelatin. All samples are added with 1% GDL



SDS-PAGE results of raw camel milk proteins are shown in lane (1) in Fig. 4. The results were almost identical to other studies that reported the molecular weights of α -casein (27.6 kDa), β -casein (23.8 kDa), κ -casein (22.4 kDa), and α -lactalbumin (14.4 kDa), and no band for β -lactoglobulin in camel milk (El-Agamy et al., 2009; Hinz et al., 2012; Salmen et al., 2012). In addition, similarities in the bands in lanes (1), (2), (3) and (4) indicated that homogenization (heating at 75 °C for 15 s before homogenization), heating at 85 °C with a dwell time for 15–20 min or the combination of heating at 85 °C with a dwell time for 15–20 min and homogenization did not affect protein integrity. This indicates the high heat stability of camel milk proteins. It was reported that heating of camel whey proteins at 65, 75, and 85 °C for 30 min did not cause any visible changes in electrophoretic patterns (Elagamy, 2000). Similarly, Felfoul et al. (2015) reported that heating below 90 °C for 30 min does not significantly affect the electrophoretic patterns of camel milk casein bands. We were unable to find in the literature electrophoresis patterns of camel milk subjected to homogenization to support our results. However, similar results have been reported for cow milk. Qi et al. (2015) found that homogenization, pasteurization at 72 °C for 15 s, and a combination of both treatments did not significantly alter the relative composition of whey proteins measured by SDS-PAGE.

Microstructure of Gels

The microstructure of the gels prepared from cow milk (used as reference sample) and camel milk subjected to heating and/or homogenization was investigated, and the results are illustrated in Fig. 5. Cow milk gels were characterized by a porous and homogeneous structure in which protein aggregates were distributed among the protein network and connected by thin strands and sheets. It appeared that there was some degree of fusion between casein micelle particles, between casein and whey proteins, or both. It was reported that the filamentous structures and protein aggregates of cow milk gels were caused by heating at 85 °C for 30 min (similar to our study) before acidification (Sanchez et al., 2000). Heat treatment at a temperature greater than 80 °C induces denaturation of whey proteins (e.g. β -lactoglobulin), which results in formation of complexes with κ -casein located at the surface of casein micelles to form such filamentous structures (Davies et al., 1978). All camel milk gels had a similar structure with a coarse protein network and a few linked protein aggregates (Fig. 5b–d). The structure of camel milk gels was dense with small voids. It is likely that the structure of camel milk gels was dominated by that of gelatin gels. As camel milk proteins preserved their integrity during heating and homogenization (Fig. 4), the microstructure of camel milk gels was not affected by heating and homogenization.

Conclusion

We observed that it was not possible to produce firm gels from camel milk without use of additives, which is consistent with previous studies. After acidification by GDL to pH 4.3, camel milk was in the form of flakes instead of firm gel. The addition of gelatin improved the WHC, hardness, rheological strength, and viscosity of camel milk gels. However, when compared with cow milk gels, the rheological strength and viscosity in camel milk gels were much lower. Accordingly, the use of other hydrocolloids or their combination with gelatin should be further investigated to improve the desirability of camel milk gels. In addition, conventional heating and homogenization conditions, which are essential for the preparation of cow milk gels, were unnecessary for the preparation of camel milk gels as they did not exhibit any significant effects on the properties.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11947-022-02890-5>.

Acknowledgements The authors acknowledge the facilities and the scientific and technical assistance of the School of Agriculture and Food Sciences at The University of Queensland. We would like to extend special thanks to Dr. Jaspal Singh for assistance with SDS-PAGE analysis. The authors also acknowledge the facilities and the scientific and technical assistance of the Australian Microscopy & Microanalysis Research Facility at the Centre for Microscopy and Microanalysis, The University of Queensland.

Author Contribution T.M.H and J.Z. conducted the experiment to collect the data and interpreted the results. T.M.H drafted and revised the manuscript. N.B. as a corresponding author supervised the experiments and corrected the manuscript. All authors reviewed the manuscript.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions.

Data Availability Research data are not shared.

Declarations

The authors have no relevant financial or non-financial interests to disclose.

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Al-Zoreky, N. S., & Al-Otaibi, M. M. (2015). Suitability of camel milk for making yogurt. *Food Science and Biotechnology*, *24*, 601–606.
- Al haj, O. A., & Al Kanhal, H. A. (2010). Compositional, technological and nutritional aspects of dromedary camel milk. *International Dairy Journal*, *20*, 811–821.
- Andiç, S., Boran, G., & Tunçtürk, Y. (2013). Effects of carboxyl methyl cellulose and edible cow gelatin on physico-chemical, textural and sensory properties of yoghurt. *International Journal of Agriculture & Biology*, *15*, 245–251.
- AOAC. (2005). Official methods of analysis. Association of Official Agricultural Chemists, Washington DC., USA.
- AS. (1994). Methods of chemical and physical testing for the dairying industry: Method 4.10: Dried milk and dried milk products - Determination of lactose - Titrimetric method. Standards Australia Committee on Chemical Analysis of Dairy Products, Australia.
- Attia, H., Kherouatou, N., Nasri, M., & Khorchani, T. (2000). Characterization of the dromedary milk casein micelle and study of its changes during acidification. *Le Lait*, *80*, 503–515.
- Barłowska, J., Szwajkowska, M., Litwińczuk, Z., & Król, J. (2011). Nutritional value and technological suitability of milk from various animal species used for dairy production. *Comprehensive Reviews in Food Science and Food Safety*, *10*, 291–302.
- Berhe, T., Seifu, E., Ipsen, R., Kurtu, M. Y., & Hansen, E. B. (2017). Processing challenges and opportunities of camel dairy products. *International Journal of Food Science*, *8*. <https://doi.org/10.1155/2017/9061757>
- Chen, C., Wang, P., Zhang, N., Zhang, W., & Ren, F. (2019). Improving the textural properties of camel milk acid gel by treatment with trisodium citrate and transglutaminase. *LWT*, *103*, 53–59.
- Davies, F., Shankar, P., Brooker, B., & Hobbs, D. (1978). A heat-induced change in the ultrastructure of milk and its effect on gel formation in yoghurt. *Journal of Dairy Research*, *45*, 53–58.
- El-Agamy, E. I. (2009). Bioactive Components in Camel Milk. In Young, W. P. (ed.) *Bioactive components in milk and dairy products*, Iowa: Wiley-Blackwell, *107*, 159–192.
- El-Agamy, E. I., Nawar, M., Shamsia, S. M., Awad, S., & Haenlein, G. F. W. (2009). Are camel milk proteins convenient to the nutrition of cow milk allergic children? *Small Ruminant Research*, *82*, 1–6.
- El Zubeir, I., Basher, M., Alameen, M., Mohammed, M., & Shuipe, E. (2012). The processing properties, chemical characteristics and acceptability of yoghurt made from non bovine milks. *Livestock Research for Rural Development*, *24*, 1–11.
- Elagamy, E. (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.
- FAO, Food and Agriculture Organization of the United Nations. (2019). FAOSTAT database. FAO, Rome, Italy. <http://www.fao.org/faostat/en/#home>
- Farnsworth, J., Li, J., Hendricks, G., & Guo, M. (2006). Effects of transglutaminase treatment on functional properties and probiotic culture survivability of goat milk yogurt. *Small Ruminant Research*, *65*, 113–121.
- Feiner, G. (2016). Additives. In G. Feiner (Ed.), *Salami: Practical science and processing technology, chapter 4* (pp. 59–88). Academic Press.
- Felfoul, I., Lopez, C., Gaucheron, F., Attia, H., & Ayadi, M. A. (2015). Fouling behavior of camel and cow milks under different heat treatments. *Food and Bioprocess Technology*, *8*, 1771–1778.
- Fetahagić, S., Mačej, O. D., Denin-Đurđević, J. D., & Jovanović, S. T. (2002). The influence of GDL concentration on milk pH change during acid coagulation. *Journal of Agricultural Sciences*, *47*, 75–85.

- Fiszman, S., Lluch, M., & Salvador, A. (1999). Effect of addition of gelatin on microstructure of acidic milk gels and yoghurt and on their rheological properties. *International Dairy Journal*, 9, 895–901.
- Galeboe, O., Seifu, E., & Sekwati-Monang, B. (2018). Production of camel milk yoghurt: Physicochemical and microbiological quality and consumer acceptability. *International Journal of Food Studies*, 7, 51–63.
- Hashim, I., Khalil, A., & Habib, H. (2009). Quality and acceptability of a set-type yogurt made from camel milk. *Journal of Dairy Science*, 92, 857–862.
- Ho, T. M., Zou, Z., & Bansal, N. (2021). Camel milk: A review of its nutritional value, heat stability, and potential food products. *Food Research International*, 153, 110870.
- Hinz, K., O'Connor, P. M., Huppertz, T., Ross, R. P., & Kelly, A. L. (2012). Comparison of the principal proteins in bovine, caprine, buffalo, equine and camel milk. *Journal of Dairy Research*, 79, 185–191.
- Ibrahim, S. A., & El Zubeir, I. E. (2016). Processing, composition and sensory characteristic of yoghurt made from camel milk and camel-sheep milk mixtures. *Small Ruminant Research*, 136, 109–112.
- Ibrahim, A. H., & Khalifa, S. A. (2015). The effects of various stabilizers on physicochemical properties of camel's milk yoghurt. *Journal of American Science*, 11, 15–24.
- Jasim, M. A., Salih, G. M., & Hamk, M. L. (2018). The effect of arabic gum on physicochemical and sensory properties of camel milk, yogurt. *Journal of Zankoy Sulaimani, Special Issue, 2nd Int. Conference of Agricultural Sciences*, 97–104.
- Kamal, M., Foukani, M., & Karoui, R. (2017). Rheological and physical properties of camel and cow milk gels enriched with phosphate and calcium during acid-induced gelation. *Journal of Food Science and Technology*, 54, 439–446.
- Kappeler, S. R., Farah, Z., & Puhani, Z. (2003). 5'-Flanking regions of camel milk genes are highly similar to homologue regions of other species and can be divided into two distinct groups. *Journal of Dairy Science*, 86, 498–508.
- Kavas, N. (2016). Yoghurt production from camel (*Camelus dromedarius*) milk fortified with samphire molasses and different colloids. *Mljekarstvo/dairy*, 66, 34–47.
- Khalifa, S. A., & Ibrahim, A. H. (2015). Influence of addition modified starches as stabilizer on physicochemical and textural properties of camel milk yoghurt. *Zagazig Journal of Agricultural Research*, 42, 295–307.
- Kumar, A., Seth, R., & Kumawat, D. (2021). Effect of some processing treatments on shelf life of camel milk in comparison to cow milk. *The Indian Journal of Animal Sciences*, 91(8).
- Konuspayeva, G., Faye, B., & Loiseau, G. (2009). The composition of camel milk: A meta-analysis of the literature data. *Journal of Food Composition and Analysis*, 22, 95–101.
- Lara-Villoslada, F., Olivares, M., & Xaus, J. (2005). The balance between caseins and whey proteins in cow's milk determines its allergenicity. *Journal of Dairy Science*, 88(5), 1654–1660.
- Lorenzen, P. C., Wernery, R., Johnson, B., Jose, S., & Wernery, U. (2011). Evaluation of indigenous enzyme activities in raw and pasteurised camel milk. *Small Ruminant Research*, 97, 79–82.
- Lucey, J., Munro, P., & Singh, H. (1999). Effects of heat treatment and whey protein addition on the rheological properties and structure of acid skim milk gels. *International Dairy Journal*, 9, 275–279.
- Lucey, J. A. (2017). Chapter 7 - Formation, Structural Properties, and Rheology of Acid-Coagulated Milk Gels. In McSweeney, P. L. H., Fox, P. F., Cotter P. D. and Everett, D. W. (eds.) *Cheese*, 4th edition, pp. 179–197 Academic Press, San Diego.
- Mckinley, M. C. (2005). The nutrition and health benefits of yoghurt. *International Journal of Dairy Technology*, 58, 1–12.
- Mudgil, P., Jumrah, B., Ahmad, M., Hamed, F., & Maqsood, S. (2018). Rheological, micro-structural and sensorial properties of camel milk yogurt as influenced by gelatin. *LWT - Food Science and Technology*, 98, 646–653.
- Omar, A., Harbourne, N., & Oruna-Concha, M. J. (2018). Effects of industrial processing methods on camel skimmed milk properties. *International Dairy Journal*, 84, 15–22.
- Pang, Z., Deeth, H., Sopade, P., Sharma, R., & Bansal, N. (2014). Rheology, texture and microstructure of gelatin gels with and without milk proteins. *Food Hydrocolloids*, 35, 484–493.
- Pang, Z., Deeth, H., Yang, H., Prakash, S., & Bansal, N. (2017). Evaluation of tilapia skin gelatin as a mammalian gelatin replacer in acid milk gels and low-fat stirred yogurt. *Journal of Dairy Science*, 100, 3436–3447.
- Qi, P. X., Ren, D., Xiao, Y., & Tomasula, P. M. (2015). Effect of homogenization and pasteurization on the structure and stability of whey protein in milk. *Journal of Dairy Science*, 98, 2884–2897.
- Robinson, R., Lucey, J., & Tamime, A. (2006). Manufacture of yoghurt. In A. Tamime (Ed.), *Fermented milks* (pp. 53–75). Blackwell Publishing Ltd.
- Roy, D., Ye, A., Moughan, P. J., & Singh, H. (2020). Composition, structure, and digestive dynamics of milk from different species—A review. *Frontiers in Nutrition*, 7, 577759.
- Sakandar, H. A., Imran, M., Huma, N., Ahmad, S., Aslam, H. K. W., Azam, M., & Shoaib, M. (2014). Effects of polymerized whey proteins isolates on the quality of stirred yoghurt made from camel milk. *Journal of Food Processing & Technology*, 5, 1–5.
- Salmen, S. H., Abu-Tarboush, H. M., Al-Saleh, A. A., & Metwalli, A. A. (2012). Amino acids content and electrophoretic profile of camel milk casein from different camel breeds in Saudi Arabia. *Saudi Journal of Biological Sciences*, 19, 177–183.
- Sanchez, C., Zuniga-Lopez, R., Schmitt, C., Despond, S., & Hardy, J. (2000). Microstructure of acid-induced skim milk–locust bean gum–xanthan gels. *International Dairy Journal*, 10, 199–212.
- Singh, H. (1993). Heat induced interactions of proteins in milk. In Proceedings of the International Dairy Federation (IDF) Seminar, held in Munich (Germany), 25–28 August 1992, IDF, Brussels (Belgium), 191–204.
- Singh, J., Prakash, S., Bhandari, B., & Bansal, N. (2019). Comparison of ultra high temperature (UHT) stability of high protein milk dispersions prepared from milk protein concentrate (MPC) and conventional low heat skimmed milk powder (SMP). *Journal of Food Engineering*, 246, 86–94.
- Trujillo, A. -J., Roig-Sagués, A. -X., Zamora, A., & Ferragut, V. (2016). 12—High-pressure homogenization for structure modification. In K. Knoerzer, P. Juliano, & G. W. Smithers (Eds.), *Innovative Food Processing Technologies: Extraction, Separation, Component Modification And Process Intensification* (pp. 315–344). Woodhead Publishing.
- Zouari, A., Marchesseau, S., Chevalier-Lucia, D., Raffard, G., Ayadi, M. A., & Picart-Palmade, L. (2018). Acid gelation of raw and reconstituted spray-dried dromedary milk: A dynamic approach of gel structuring. *International Dairy Journal*, 81, 95–103.