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ABSTRACT

The effect of different heat-treatment temperatures (70, 80, 90 and 100 °C) on the foaming properties (foam capacity and stability) and the physicochemical characteristics (surface hydrophobicity, ζ-potential, and interfacial tension values) of camel milk proteins was investigated. Overall, the results showed that while increasing the temperature, greater foamability was measured for camel milk proteins (up to 165%). This behaviour was linked to the heat denaturation and aggregation of camel milk proteins, which led to an increase in the surface hydrophobicity and a decrease in the electronegative charge and interfacial tension. Likewise, our results indicated that the highest β-casein amount in camel milk (~44% of total proteins) as well as its secondary structure (β-sheet conformation) and its high hydrophobicity, regulated the foaming mechanism of camel milk proteins.

1. Introduction

Known in the arid countries as a culturally important drink, camel milk is gaining popularity internationally due to its claimed biological values and its therapeutic properties such as anti-diabetic (Mudgil, Kamal, Yuen, & Maqsood, 2018; Nongonierma, Paolella, Mudgil, Maqsood, & FitzGerald, 2017, 2018), hypo-allergic and anti-cancer properties (Kamal et al., 2018). Consequently, the production of this milk has substantially increased on large commercial scales from modern farms (El-Agamy, Nawar, Shamsia, Awad, & Haenlein, 2009).

Camel milk is different from cow milk in its protein composition and the structure of its proteins, leading to different techno-functional and biological properties (Hailu et al., 2016; Lajnaf et al., 2016). As for milk of other milk-producing mammalian species, camel milk can be classified into two major components of proteins, i.e., caseins and whey proteins. Caseins constitute the main protein fraction of the camel milk representing 52–87% (w/w) of the total milk proteins. β-Casein is the most abundant casein in camel milk followed by α_{S1}-casein, constituting about 65% and 22% of the total caseins content, respectively, compared with 39% and 38% in bovine caseins, respectively. Only 3.3% of the total camel milk casein corresponds to κ-casein, compared with bovine κ-casein being 13% of the total casein in cow milk (El-Agamy, 2006; Ereifej, Alu'datt, Alkhalidy, Alli, I., & Rababah, 2011; Hailu et al., 2016; Kappeler, Farah, & Puhan, 1998).

Whey proteins are the second main protein fraction of camel milk, constituting 24.5% (w/w) of the total proteins (Ereifej et al., 2011). However, whilst in cow milk β-lactoglobulin (β-Lg) is the main component of the whey proteins (72%) followed by α-lactalbumin (α-La) (24%), β-Lg is totally absent in camel milk (Lajnaf et al., 2018; Laleye, Jobe, & Wasesa, 2008; Maqsood et al., 2019; Merin et al., 2001; Omar, Harbourne, & Oruna-concha, 2016).

Thus, the α-La is the major camel whey protein with an average concentration of 2.3 g L^{-1}, a significantly higher value than that for its bovine counterpart (1.1 g L^{-1}; Chatterton, Smithers, Roupas, & Brodkorb, 2006; El-Hatmi, Girardet, Gaillard, Yahyaoui, & Attia, 2007).

Milk foams are defined as colloidal systems where the air-bubbles created are stabilised by a matrix composed of surface-active agents of milk that are mainly proteins (Dickinson, 2003). As for other techno-functional properties of proteins, foaming behaviour is determined by the type of protein and environmental factors including ionic strength, pH and the heat-treatment temperature (Lorenzen, 2000). For bovine milk, the thermal denaturation of proteins has been reported as the elementary step of the reactions leading to the heat-induced aggregation of disulphide-linked proteins. Thus, under a heat-treatment of 60 °C, β-Lg dissociates from a dimeric structure to native monomers. The thermal denaturation of this protein leads to thiol-disulphide exchange reactions and thus heat-induced association of whey proteins and their aggregates with caseins (Kazmierski & Corredig, 2003; Roefs & De Kruif, 1994). Therefore, the resulting foaming properties will be a complex competitive adsorption phenomena between proteins in their native, non-aggregated denatured, and aggregated states (Schmitt, Bovay, Rouvet, Shojaei-Rami, & Kolodziejczyk, 2007).

The foaming properties of bovine milk have been widely studied and reported since the beginning of the last century (Borcherding, Lorenzen, Hoffmann, & Schrader, 2008; Hatakeyama et al., 2019; Kamath, Huppertz, Houlihan, & Deeth, 2008). For camel milk, previous studies noted that that it is greatly frothy, even if when it is shaken slightly (Shalash, 1979), but little research exists on the foaming properties of camel milk proteins (Al-Shamsi, Mudgil, Hassan, & Maqsood, 2018) and their interfacial properties have not been thoroughly described in the literature. Thus, different behaviours can be suggested for the camel milk, as

the different protein composition would have a great impact on the resulting foaming properties of milk after a heat-treatment.

An investigation of the foaming and interfacial behaviour of camel milk at different heating temperatures compared with that of bovine milk could be a very useful tool to control and predict the functionality of dairy systems in both milks. Despite its high production and consumption, cow milk is associated to different nutritional problems, such as allergy. Thus, camel milk was recently suggested as a food alternative to cow milk. Aerated dairy products of camel milk could be of great interest to foam industry. Indeed, fresh camel milk and its products have unique flavour and good nutritional and therapeutic properties such as anti-carcinogenic, anti-diabetic, and anti-hypertensive properties (Al haj & Al Kanhal, 2010). Furthermore, camel milk has been recommended to be consumed by children who are allergic to bovine milk as reported by (El-Agamy et al., 2009).

Camel milk aerated products can compete in the market with those of cow milk as it could have the same attractive textural characteristics of milk foams with important nutritional value, especially to patients allergic to cow proteins. Starting from these considerations, the aim of this work was to investigate the foaming and interfacial properties of bovine and camel milk after different heating temperatures for potential applications of camel milk in the industrial foam production, which could be of great importance to dairy industry.

2. Materials and methods

2.1. Milk samples

Fresh camel milk was collected from 20 different healthy female camels of the same breed (*Camelus dromedarius*), ranging between 2 and 12 months in lactation stage, and belonging to a farm located in the south of Tunisia. Fresh cow milk was derived from a local breed located in the region of Sfax in Tunisia.

Both milk samples were directly transported to the laboratory using cooler bags (at 4 °C). They were then systematically skimmed by centrifugation (Thermo Scientific Heraeus Megafuge centrifuge, Germany) at 3000 × *g* for 20 min at 4 °C (Felfoul, Lopez, Gaucheron, Attia, & Ayadi, 2015a). Both milk types samples were then freeze dried (Bioblock Scientific Christ ALPHA 1-2, IllKrich-Cedex, France) to obtain powders and stored at −20 °C for further use.

2.2. Heat-treatment experiments

Lyophilised milk samples were dissolved in deionised water (Milli-Q system, Millipore, USA) at a level of 1 g L^{-1}. Camel and bovine milk (1g L^{-1} of protein concentration) were heated using water bath at 70 °C, 80 °C 90 °C and 100 °C for 30 min as reported in previous studies (Felfoul, Lopez, Gaucheron, Attia, & Ayadi, 2015b; Laleye et al., 2008). After heating, beakers containing milk camel and bovine milk were put on ice to stop milk protein denaturation. The control milk sample was at 20 °C corresponding to native conditions without heating (Lajnaf et al., 2018; Laleye et al., 2008).

2.3. Foaming properties

Ten millilitres of camel and bovine milk at a protein concentration of 1 g L^{-1} were poured in a glass measuring cylinder (radius 1.5 cm × length 7.5 cm) and whipped using an

appropriate mixer (Ultra Turrax T25, IKA Labortechnik, Staufen Germany) at 13,500 rpm for 2 min at room temperature (~25 °C) (Lajnaf et al., 2016; Marinova et al., 2009). Immediately after whipping, the volume of the created foam was read from the cylinder. Afterwards, foam capacity (FC) was calculated using equation Eq. (1):

$$FC = (V_F/V_0) \times 100 \quad (1)$$

where V_F is the volume of the created foam and V_0 the volume of the initial milk before mixing.

The time for the draining of the volume of foam to half was also measured to determine the foam stability (FS) as described by Marinova et al. (2009).

2.4. Purification and structural characteristics of camel β-casein

2.4.1. Purification of camel β-casein

The bovine and camel β-caseins were extracted using the technique described by Huppertz, Hennebel, Considine, Kelly, and Fox (2006) and modified by Lajnaf et al. (2016).

After defatting camel milk, casein fraction was separated from the whey by rennet coagulation in the presence of 1.4 mL rennet enzyme per litre of camel milk (*Mucor miehei*, strength = 1:10,000, Laboratories Arrazi, Parachimic,Sfax, Tunisia) at 37 °C for 1–2 h followed by centrifugation at 5000 × *g* for 20 min at 20 °C (Fig. 1). The rennet content added was four times higher in camel milk than for bovine milk (0.35 mL L^{-1}), as reported in previous works (Felfoul et al., 2015b; Lajnaf et al., 2018; Lajnaf, Trigui, Samet-Bali, Attia, & Ayadi, 2019; Ramet, 2001). This behaviour was attributed to the differences in the size of casein particles due to reduced κ-casein content (Al haj & Al Kanhal, 2010).

Afterwards, a volume of preheated demineralised water (80 °C) equal to that of the discarded whey was added to the curd and the mixture was kept at 80 °C for 5 min to disable

the action of rennet enzyme, then centrifuged for 5000 × *g* for 15 min at 20 °C. After discarding the supernatant, the curd was kept, macerated and suspended in demineralised water (5 °C) (volume equal to that of the discarded whey previously). Finally, the protein suspension was kept at 5 °C for up to 24 h and centrifuged at 5000 × *g* for 15 min at 5 °C. The supernatants obtained after centrifugation containing the camel and bovine β-caseins were kept at –18 °C then lyophilised (Bioblock Scientific Christ ALPHA 1-2) for further analysis.

The purified freeze dried camel β-casein was used without further modifications or heating treatment.

2.4.2. Infra-red spectroscopic analysis

The absorption spectra of the native lyophilised camel and bovine β-caseins were obtained by FT-IR spectroscopy (Perkin Elmer®, Spectrum™ 100, Singapore) equipped with attenuated total reflection (ATR) accessory containing a diamond/ZnSe crystal. The FT-IR spectra were recorded in the 4000 and 600 cm^{-1} range at room temperature.

2.4.3. Nuclear magnetic resonance analysis

^{1}H nuclear magnetic resonance (NMR) analysis of purified camel and bovine β-caseins in their native state was carried out according to the method of Fernández et al. (2012) with some modifications. Twenty milligrams of lyophilised β-casein sample was dissolved in 500 µL D_2O and put into NMR tube. One-dimensional ^{1}H NMR spectra were recorded at 400 MHz and 25 °C on a Bruker 600M spectrometer (Rheinstetten, Germany).

2.5. High performance liquid chromatography analysis

The effect of the different heat-treatments (70, 80, 90, and 100 °C for 30 min) on camel milk proteins was examined by high performance liquid chromatography (HPLC; Agilent 1260 Infinity quaternary LC, Germany) using the method of Yüksel and Erdem (2010). Milk proteins were separated on a C18 column RP-HPLC column (Zorbax Eclipse Plus C18, 250 mm length × 4.6 mm, particle size 5 µm, Packing Lot: B14292) and then analysed using a Shimadzu SPD6A-UV detector measuring the optical density at 220 nm for 40 min.

Overall, 500 µL of milk sample were added to 3.7 mL of a solution containing solvent A (acetonitrile, water, and trifluoroacetic acid, 100:900:1, by vol) and solvent B (acetonitrile, water, and trifluoroacetic acid in a ratio 900:100:1 by vol) in a 70:30 ratio (v/v) (Jafar, Kamal, Mudgil, Hassan, & Maqsood, 2018). The mixture was vortexed for 10 s and then filtered through 0.45 µm nylon filter before injection into the column with a volume of 20 µL.

When the sample was injected, a gradient was generated immediately after sample injection by increasing linearly the proportion of solvent B as function of time from 20% to 46% at the end of the run (30–40 min). The column temperature was maintained at 25 °C and the mobile flow rate was fixed at 1.0 mL min^{-1}.

Standard individual bovine proteins (β-casein, α_S-casein, κ-casein, β-Lg and α-La) were purchased from Sigma Aldrich. Individual standards were diluted in solvent A and solvent B mixture (70:30, v/v), separately. For camel milk, chromatograms of camel casein fraction and whey were used for the determination of each camel protein of camel milk. Thus, 500 µL of camel milk caseins and whey fractions were added to 3.7 mL of the solution containing solvent A and solvent B mixture (70:30, v/v) as milk and bovine standards, separately. Furthermore, a quantitative estimation of each camel milk proteins percentages and the comparison of the data with those of previous works was used to confirm the camel

milk composition (Felfoul, Jardin, Gaucheron, Attia, & Ayadi, 2017; Hailu et al., 2016; Omar et al., 2016). Quantitative estimation of major camel and bovine milk protein percentages was performed by calculating the area of the peak of each protein.

2.6. Thiol group concentration and denaturation rate

The free thiol group concentration was determined as described by Ellman (1959). First, 300 µL of camel and bovine milk at a protein concentration of 1g L^{-1} were mixed with 50 µL 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) solution (consisting of 2 mM DTNB and 50 mM sodium acetate in deionised water), 100 µL 1 M Tris, pH 8.0, and 550 µL deionised water. The mixture was then incubated at 37 °C for 5 min and finally the optical density was measured at 412 nm using a UVmini-1240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

The concentration of thiols in milk (C_{SH}, M) was calculated using Eq. (2):

$$C_{SH} = (OD_{412nm} / \varepsilon_{412}) \times (1000/300) \quad (2)$$

where OD_{412nm} is the absorbance at 412 nm; ε_{412} is the molar extinction coefficient of the DTNB (13,600 $M^{-1}cm^{-1}$) at 412 nm, 300 µL is milk volume (at a protein concentration of 1 g L^{-1}) and 1000 µL is the total volume of the cuvette.

After determining the concentration of thiols in milk (C_{SH}), the milk protein denaturation rate (DR) values were calculated using Eq. (3):

$$DR\ (\%) = [C_{SH}\ (\text{heated milk}) - C_{SH}\ (\text{native milk})] / C_{SH}\ (\text{native milk}) \times 100 \quad (3)$$

2.7. SDS-PAGE

SDS-PAGE (15% acrylamide gel) experiments were carried out using the technique described by Laemmli (1970) and Ereifej et al. (2011). Lyophilised milk samples were dissolved in deionised water at a concentration of 7 g L^{-1}. After applying heat treatment (70, 80, 90 or 100 °C for 30 min) at this protein concentration, 5 µL of this protein solution (containing 35 µg protein) were taken and added to 5 µL sample buffer [0.5 M Tris-HCl pH 6.8, 10% (w/w) SDS, 2% (w/w) glycerol, 0.5 M β-mercaptoethanol, 0.1% (w/w) bromophenol blue] and heated at 95 °C for 5 min. Electrophoresis was performed at constant current 120 V for 2 h (Mini Protean Tetra Cell, BioRad laboratories, USA) and the gel was stained for 12 min with 2.5% (w/v) Coomassie blue R-250 in a mixture of 50% ethanol and 10% acetic acid (v/v). Then, the gel was detained in water solution containing 10% ethanol and 14% citric acid. A mixture of the pre-stained marker proteins (14.4–94 kDa) was subjected to the same procedure described above. The molecular masses of the different milk proteins were estimated by comparing their electrophoretic mobilities with those of marker proteins.

2.8. ζ-potential measurements

The ζ-potential values of camel and bovine milk at a protein concentration of 0.5 g L^{-1} were determined at 25 ± 1 °C using the Zetasizer Nano-ZS90 apparatus (Malvern Instruments, Westborough, MA) as suggested by Magnusson and Nilsson (2011). The ζ-potential value (mV) was determined using Henry's equation (Eq. 4):

$$U_E = (2\varepsilon\zeta f(k\alpha))/3\eta \quad (4)$$

where U_E is the electrophoretic mobility, f(kα) is the function related to the ratio of particle radius (α, nm) and the Debye length (k, nm^{-1}), ε is the permittivity (Farad m^{-1}) and η is the dispersion viscosity (mPa s) (McClements, 2015).

2.9. Determination of protein hydrophobicity

Hydrophobicity for each milk protein solution was determined by the method described by Chelh, Gatellier, and Santé-Lhoutellier (2006) and Al-Shamsi et al. (2018). Briefly, 1 mL of milk sample (at a protein concentration of 1 g L^{-1}) and 200 µL of 1 mg mL^{-1} bromophenol blue (BPB) solution in distilled water were added and mixed well.
A control consisted of the addition of 200 µL of BPB solution (1 g L^{-1}) to 1 mL 20 mM Tris-HCl buffer, pH 8.0, instead of milk sample.

Milk samples and controls were kept under agitation during 10min at room temperature and then centrifuged at 2000 × *g* at 25 °C for 15min (Thermo Scientific Heraeus Megafuge). Supernatants were diluted 1:10 with distilled water and the optical density was measured at 595 nm using UVmini-1240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

The amount of bound-BPB molecules was calculated using Eq. (5):

$$\text{Bound-BPB } (\mu g) = [(OD_{595} \text{ (control)} - OD_{595} \text{ (sample)}]/OD_{595} \text{ (control)} \times 200\ \mu g \quad (5)$$

2.10. Interfacial tension

The interfacial tension for each camel and bovine milk at a protein concentration of 1 g L^{-1} was measured using a TSD 971 Tensiometer (Tensiometry System Digital Gibertini Elettronica, Italia) using The Du Noüy methodology as described by Lam and Nickerson (2015a). Thus, for the determination of the interfacial tension of camel and bovine milk at the air-water interface, 20 mL of 1 g L^{-1} milk protein solution were added within a 40 mm diameter glass sample beaker, followed by the immersion of the Du Nüoy ring (20 mm diameter). Finally, the ring was pulled upwards to stretch the air-water interface of the protein

solution to measure the maximum force (F_{max}) and then to calculate the interfacial tension value (γ) using Eq. (6)

$$\gamma = F_{max}/(4\pi R\beta) \quad (6)$$

where γ is the interfacial tension (mN m^{-1}), R is the radius of the used ring (20 mm), F_{max} is the maximum force (mN), β is a correction factor which depends on two main factors: the dimensions of the ring and the density of the liquid. All interfacial tension measurements (γ) were carried out at 25 °C.

2.11. Statistics

All measurements of each experiment in this work were performed at least in triplicate and mentioned as the mean value ± one standard deviation. Analysis of variance, ANOVA, was performed to test for significance in the main effects of the milk (camel and bovine milk) and heat-treatment conditions (70 °C, 80 °C, 90 °C and 100 °C), along with their associated interactions on the foaming properties and physico-chemical characteristics of milk (surface tension measurements, ζ-potential, DR and hydrophobicity). Statistical analyses were carried out using an appropriate software (IBM SPSS statistics, Version 19, IBM SPSS, USA).

3. Results and discussion

3.1. Foaming properties of milk

Fig. 2 shows the average values of foam capacity (FC) and foam stability (FS) respectively of camel and bovine skim milk as function of the temperature of the heat-

treatment. The same milk protein concentration (1 g L^{-1}) was chosen for both milk samples to make a significant comparison of foaming properties under native conditions (20 °C) and after heating (70, 80, 90 and 100 °C for 30 min).

Fig. 2a shows that camel milk gives better foam than bovine milk regardless of heating temperature value. Under native conditions, i.e., at 20 °C, camel milk was found to give better FC values (~138%) than that of bovine milk (~115%) suggesting that in these conditions, camel milk proteins coat the air bubbles better than bovine milk proteins. This behaviour can be explained by greater amount of β-casein in camel milk when compared with bovine milk as reported by Kappeler, Farah, and Puhan (2003).

These results are in agreement with those of Brooker, Anderson, and Andrews (1986) and Ward, Goddard, Augustin, and McKinnon (1997) who reported that the foamability of milk increased with an increasing content of the β-casein up to a certain degree and then stayed constant. Indeed, this protein is considered as the most surface-active milk protein due to its particular unordered structure and its high hydrophobicity. Thus, the β-casein is the first adsorbed and predominant protein at the air-water interface when compared with other milk proteins (Dickinson, 1998; Zhang et al., 2004). For camel milk, β-casein was found to play the main role in the creation of camel milk foams; the foamability increased with the β-casein amount in bovine and camel proteins mixtures (Lajnaf et al., 2016).

Fig. 2a also shows that, for bovine milk, a thermal treatment during 30min at 70 °C, 80 °C or 90 °C induced a significant increase of the FC from 140% to 154% and then to 169% ($P < 0.05$), respectively. No significant increase was observed in FC values between 90 °C and 100 °C. For the camel milk, statistical analysis showed that heating improved significantly the foamability ($P < 0.05$) in comparison with that of native milk, with better foaming achieved after a heat-treatment at 90 °C and 100 °C. However, no significant

differences in the FC values (165%) could be found when heat-treatment was applied at 70 °C and 80 °C.

These results are in agreement with those reported by Kamath et al. (2008) in a study carried out with skim and whole bovine milk. Indeed, the high foam of heated milk at temperature values up to 85 °C is mainly due to the decrease in viscosity of heated milk with increasing temperature leading consequently to a faster adsorption of proteins on the interface. Heated milk has better foaming properties due to the partial unfolding state of globular proteins after heating, leading to the exposure of their reactive functional groups. Therefore, the overall hydrophobicity and flexibility of milk proteins increased resulting a higher foam volume (Bals & Kulozik, 2003; Borcherding et al., 2008; Graham & Phillips, 1979).

Fig. 2a shows also that heating camel and bovine milk at 100 °C significantly reduced their foamability ($P < 0.05$) in comparison with the FC values of 90 °C with a better foaming achieved with the camel milk (195%). These results are in agreement with those of Lam and Nickerson (2015) who reported that a higher heating temperature of milk proteins at neutral pH level leads to greater surface hydrophobicity caused by protein unravelling. This behaviour could explain the reduction of the ability of whey proteins to create emulsions (Lam & Nickerson, 2015).

In the case of camel milk, Lajnaf, Picart-Palmade, Attia, Marchesseau, and Ayadi (2017) found that a thermal treatment at 70 °C or 90 °C for 30min induced a significant increase of the foam volume of the purified camel α-La solutions. This behaviour was explained by the charge repulsion forces of the camel α-La molecules leading to a better adsorption at the air-water interface, which preserve this protein from thermal aggregation after heating.

Fig. 2b shows the foaming stability (FS), i.e., the half-time of liquid drainage from the foams created by bovine and camel milk (at a concentration of 1 g L^{-1}) in response to thermal treatment. First, foams made with bovine milk proteins were found to be more stable than those made with camel milk regardless of heating temperature. This behaviour can be explained by the highest amount of the κ-casein in bovine milk (El-Agamy, 2006; Kappeler et al., 1998). Closs et al. (1990) reported that the stability of milk foams is determined by κ-casein predominantly due to its particular structured form as compared with other caseins. Furthermore, Lajnaf et al. (2016) found that bovine β-casein is characterised by a higher ability to stabilise foams when compared with its camel counterpart due to the difference in their molecular structures. β-Lg could also play an important role in the stability of bovine milk. Indeed, the stability of β-Lg foams is higher than those obtained with both camel and bovine α-La (Lajnaf et al., 2016).

The stability of foam formed from camel and bovine milk increased significantly with increasing preheating temperature up to 90 °C (P <0.05), above which less stable foams were formed (at 100 °C). For bovine milk, an increase of heating temperature from 20 °C to 90 °C, greatly increased the FS value to 720 s, which represents an increase of 600 s (Fig. 2b). For camel milk, the FS values increased significantly from 60 s to 690 s in this temperature range (P < 0.05). This is in agreement with Dickinson (2003) and Borcherding et al. (2008) who reported that heating induces an increase in stability of milk foam due to an increase in the adsorption velocity and the diffusion of heated milk proteins at the interface and a decrease in the apparent viscosity of milk. On the other hand, Tosi, Canna, Lucero, and Ré (2007) observed that heating could significantly improve the stability of foams formed by sweet whey proteins (2–4 times more stable) when compared with native whey. However, temperature must not be higher than 85 °C for 750 s to avoid an excessive denaturation of proteins reducing their ability to stabilise foam.

For camel milk, Lajnaf et al. (2018) reported that the stability of foam formed by the acid camel whey greatly increased; they found that the acid camel whey presented better properties than bovine whey to create and stabilise foams, with a significant increase of these properties after heating (at 70 °C and 90 °C for 30 min) due the extensive aggregation of the α-La. Indeed, the α-La aggregates contributed to improve the foam stability of camel whey solutions (Lajnaf et al., 2017).

3.2. Structural characteristics of camel milk protein: β-casein

3.2.1. Infra-red spectroscopic analysis

Fig. 3a shows the FT-IR spectrum of the native β-caseins obtained from camel and bovine milk without further modification. If the protein fractions obtained of both β-caseins are compared, it is evident that both proteins have a very similar spectrum. Such behaviour can be explained by the sequence similarity and identity between these two proteins which are 84.5% and 67.2%, respectively (Barzegar et al., 2008).

Both β-casein sample spectra showed the same bands in the region of 3400–3100 cm^{-1}, which corresponded to the N–H stretch of amide A and primary amine. Furthermore, the peaks in the region of 2800–2960 cm^{-1} corresponded to the stretching vibration of C–H which are mainly found in aliphatic side chain of proteins (Santoni & Pizzo, 2013; Siu, Ma, & Mine, 2002). The amide I band (1600–1700 cm^{-1}), which is the band of the C=O stretching vibrations, is the most important vibrational bands of the protein skeleton. Indeed, this band was associated with the secondary structure of β-casein as reported by Cao et al. (2019).

The peak positions of amide I bands were 1642 cm^{-1} and 1627 cm^{-1} for camel and bovine β-caseins, respectively, indicating different secondary structure of both β-caseins, especially in the β-sheet structure in accordance with Cao et al. (2019). Indeed, the amide I

band is the most sensitive spectral region of the secondary structure of protein. The amide I band frequency assignments for secondary structures are especially α-helix (1654–1658 cm^{-1}) and β-sheet (1624–1642 cm^{-1}) (Kong & Yu, 2007). This result could explain the higher foam stability of bovine milk when compared with camel milk as the β-casein plays the main role in stabilising milk foams (Lajnaf et al., 2016).

3.2.2. NMR spectroscopy data

The representative ^{1}H NMR spectra obtained from the native purified camel and bovine β-caseins without further modification are illustrated in Fig. 3b. The ^{1}H NMR spectra showed the sharp proton resonance and the chemical shifts variations of protein in the range of 0–1 ppm. In the spectrum of both proteins, methyl signals are observed in the range 0.7 to 0.9 ppm. The intensity of these signals was more pronounced for camel β-casein when compared with its bovine counterpart. Indeed, this is the region where most amino acids containing methyl groups are, and in particular those in random coil regions of the protein (Fernández et al., 2012).

Therefore, the results indicate higher methyl groups, which could be attributed to the higher content of Ile in camel β-casein primary sequence; the amount of Ile in camel β-casein (5.55%) is significantly higher when compared with bovine β-casein (4.12%) (Salmen, Abu-Tarboush, Al-Saleh, & Metwalli, 2012). These results lead to note that camel β-casein is suggested to be more hydrophobic than bovine β-casein. These findings are in agreement with the highest FC values observed with camel milk and with the findings of Lajnaf et al. (2016) and Fernández et al. (2012).

3.3. Milk protein denaturation

3.3.1. Denaturation rate

Denaturation Rate (DR, %) values of camel and bovine milk proteins under different heating temperatures (70, 80, 90, and 100 °C for 30min) are shown in Fig. 4a. After heating, the free –SH group concentration of bovine and camel milk rose significantly ($P < 0.05$) as a function of temperature, with higher contents for camel milk. DR values reached their maximum at 90 °C with values of 75.2 ± 3% and 174.4 ± 15% for bovine and camel milk, respectively. At 100 °C, DR values started to decline to 22.5 ± 5% and 136.9 ±5% for bovine and camel milk, respectively.

These results could be explained by the whey protein denaturation that occurred during the first 30 min of heating at a temperature of 90 °C, regardless of the milk origin. Furthermore, camel whey proteins are characterised by a higher thermal sensitivity than bovine proteins as reported by Felfoul et al. (2015). β-Lg plays the main role in the aggregation phenomenon of the bovine whey proteins after heating. Indeed, after heating at 70 °C, the free –SH groups of the β-Lg monomers were exposed, leading to the reactivity of this protein toward thiol/disulphide interchange reactions during the heat-treatment. Then, the β-Lg reacts with the α-La, which contains four buried disulphide bridges, forming reactive β-Lg–α-La dimers that react with the other whey molecules leading to the creation of the heat induced aggregates at higher temperatures (> 90 °C) (De la Fuente, Singh, & Hemar, 2002). For camel milk, Lajnaf et al. (2018) noted that the camel whey proteins are characterised by a higher thiol group's concentration after heating at 90 °C for 30min. This behaviour was explained by the denaturing temperature of camel α-La (~73.8 °C) and the presence of camel serum albumin (CSA) whose molecular structure is characterised by the presence of 7 disulphide bridges (Lajnaf et al., 2018).

3.3.2. HPLC analysis

The protein composition of camel and bovine milk under native conditions (20 °C) and after different heat-treatments (70, 80, 90, and 100 °C for 30 min) is presented in Fig. 5. HPLC chromatograms of unheated bovine and camel milk protein fractions (Fig. 5a and 5b, respectively, 20 °C) showed that for cow milk, six major peaks (RT: 19.22 min, 23.92 min, 25.74 min, 27.47 min, 27.76 min and 30.37 min) were identified as κ-casein (~7.1%), α-casein (~24.7%), β-casein (~37.4%), α-La (~4.7%), protein fraction F (~1.2%) and β-Lg (~24.9%). Protein fraction (F) is suggested to be dimers of β-Lg (Felfoul et al., 2017).

For the camel milk chromatograms (Fig. 5b, 20 °C), five major protein peaks with retention time (RT) of 19.50 min, 21.63 min, 22,68 min, 25.27 min and 26.93 min were identified. The identification of camel proteins was determined using the chromatograms of camel caseins and whey as it cannot be realised using bovine proteins standards. The proteins with RT of 19.50 and 26.93 min were identified as α-casein (~29.1% of total milk proteins) and β-casein (~44% of total milk proteins), respectively, as the β-casein is the main camel protein in camel milk and caseins fractions (Fig. 5c) and in agreement with previous work (Felfoul et al., 2017; Kappeler et al., 1998). On the other hand, the peak with RT of 21.63 min is the α-La representing 18.8% of total camel milk proteins. Indeed, the α-La is the main whey protein (Fig. 5c) in camel milk as β-Lg is totally absent (Ereifej et al., 2011; Felfoul et al., 2017; Lajnaf et al., 2018). Finally, both peaks with RT of 22.68 min, 25.27 min were specific protein fractions of camel milk whey fractions representing respectively 3.1% (F1) and 4.7% (F2) of the total amount of camel milk proteins. These protein fractions are suggested to be identified as the peptidoglycan recognition protein (PGRP) and CSA for F1 and F2 respectively in agreement with El-Hatmi et al. (2007), Ereifej et al. (2011) and Felfoul et al. (2017).

As expected, HPLC chromatograms showed that camel milk proteins exhibited a higher amount of β-casein which can be considered as the main protein of camel milk

(representing 44% of the total camel proteins). The results are consistent with the data previously reported by other works (Felfoul et al., 2017; Hailu et al., 2016; Omar et al., 2016). No peaks were detected for the κ-casein, probably due to its low concentration that makes it obscured by other caseins, in agreement with the results of Farah, Rettenmaier, and Atkins (1992).

Furthermore, chromatograms showed that the α-La was the major whey protein in camel milk and represents ~18.8% of the total milk proteins, which was significantly higher than the content of the α-La in bovine milk (~4.7%) in agreement with Ereifej et al. (2011) who reported that the α-La had the greatest content in soluble camel milk fraction ranging between 5.2 and 19.36%.

To reveal the denaturation and aggregation phenomena of the proteins in both camel and bovine milk, HPLC results (Fig. 5a,b) showed that caseins (peaks corresponding to κ-casein, α-casein and β-casein) remained almost intact after heating both camel and bovine milk (Felfoul et al., 2017). However, the main camel and bovine whey proteins were significantly affected upon heating as function of the themal-treatment temperature. Indeed, the peaks of the α-La and the β-Lg started immediately diminished after the heat-treatment of bovine milk at 80 °C for 30 min. The β-Lg peak totally disappeared after heating at 90 °C and 100 °C during 30 min, unlike the β-Lg dimer peak that increased due to the creation of heat-induced disulphide-bonded dimers as intermediates in the β-Lg aggregation (Manderson, Hardman, & Creamer, 1998).

For heat-treated bovine milk (Fig. 5a), a new protein fraction peak appeared(F2, RT = 26.65 min). This peak could be attributed to β-Lg multimers. Indeed, after heating at 80 °C, the β-Lg in its monomeric form can associate with other proteins and aggregate. Thus, the quantities of β-Lg monomers decreased and the amount of aggregates larger than trimers increased (Felfoul et al., 2017; Moro, Báez, Busti, Ballerini, & Delorenzi, 2011).

The heating temperatures chosen (70, 80, 90 and 100 °C for 30 min) were based on the work of Felfoul et al. (2015b) and Laleye et al. (2008). These parameters correspond to different stages of denaturation of milk proteins. Indeed, it has been shown by previous studies that at 70 °C, the β-Lg molecules are reduced from dimers to monomers and begin to unfold. Furthermore, the denaturation temperature of the β-Lg in sweet bovine whey is around 80 °C (79.6±0.7 °C) as reported by Felfoul et al. (2015b). On the other hand, the denaturation temperature values of both bovine and camel α-La are near 70 °C. Felfoul et al. (2015b) reported also that 90 °C is the temperature of the total denaturation and aggregation of whey proteins. Finally, we have chosen the heating temperature of 100 °C to make sure that all proteins are already denatured. Heating up time is 30 min was chosen according to previous work, especially that of Lam and Nickerson (2015).

Fig. 5b indicates that thermal treatment of camel milk at 70, 80, 90 and 100 °C for 30 min had no any significant effect on the camel casein fraction and on the whey proteins as CSA and PGRP except for the camel α-La. Indeed, Fig. 5b shows that the peak of the α-La began to decline after the heat-treatment at 70 °C during 30min. Afterwards, it decreased more with further increase of the heating temperature from 80 °C to 100 °C for 30min. The reduction of the chromatograms peaks could be the consequence of the proteins denaturation and/or aggregation under heating. Felfoul et al. (2017) confirmed that camel α-La completely disappeared in camel milk heat-treated at 80 °C for 60 min. Atri et al. (2010) showed greater thermal stability of the camel α-La than the bovine α-La in its both states: holo-α-La (with calcium) and apo-α-La (without calcium) due to the lack of the β-Lg in camel milk (Elagamy, 2000).

3.3.3. Electrophoresis patterns of camel and bovine milk

For the native bovine (Fig. 6a, lane 20 °C), seven major proteins bands of 80 kDa, 66 kDa, 35kDa, 30 kDa, 28 kDa, 18 kDa and 14 kDa were identified, corresponding to lactoferrin, BSA (bovine serum albumin), α_S-casein, β-casein κ-casein, β-Lg and α-La, respectively. On the other hand, Fig. 6b (lane 20 °C) shows that six major protein bands (80 kDa, 66 kDa, 35kDa, 30kDa, 25kDa and 14 kDa) were identified in camel milk as lactoferrin, CSA, β-casein, α_S-casein, κ -casein and α-La. As expected, the most abundant whey protein in bovine milk is β-Lg, whereas this protein is not detected in camel milk. These results are in agreement with previously reported findings (Ereifej et al., 2011; Felfoul et al., 2017; Lajnaf et al., 2018) and with HPLC results (section 3.3.2). Furthermore, camel milk also contains three protein fractions (F) of 60 kDa (F1), 22 kDa (F2) and 19 kDa (F3). These fractions are suggested to be specific components of camel milk whey as reported by Lajnaf et al. (2018). They are not comparable with any protein in bovine milk. Therefore, F3 is suggested to be identified as the PGRP (19.1 kDa) (Kappeler, Heuberger, Farah, & Puhan, 2004).

Pasteurisation temperature (70 °C) caused no visible modification in both camel and bovine protein gel patterns (Fig. 6, Lane 70 °C) in agreement with the results of Felfoul et al. (2015b). Some faint bands in the region between BSA and caseins appeared to increase in intensity after heating at 80 °C, 90 °C and 100 °C. According to Havea, Singh, and Creamer (2002), these bands were probably intermediate protein species (β-lactoglobulin dimers, trimers) that were formed during milk heating.

Camel α-La band remained constant after heating milk at both temperature values 70 and 80 °C, whereas at 90 °C, α-La, F1 and κ-casein bands decreased, in agreement with the findings of Felfoul et al. (2015b). For bovine milk, the β-Lg and α-La bands remained constant at 70 °C and 80 °C, but started to disappear after the heat treatment temperature of 90 °C in agreement with Felfoul et al. (2015b).

3.4. Surface characteristics of milk proteins

3.4.1. Surface hydrophobicity

The impact of various thermal treatments (70, 80, 90 and 100 °C for 30 min) on skimmed bovine and camel milk was studied through the changes in the overall surface hydrophobicity of camel and bovine milk protein solutions at the same protein concentration (1g L^{-1}; Fig. 4b). Under native conditions (20 °C), the surface hydrophobicity of camel milk protein solution was significantly higher than that of its bovine counterpart ($P < 0.05$); the bound-BPB amounts at 20 °C were 1.49 ± 0.51 µg mL^{-1} and 3.40 ± 0.72 µg mL^{-1} for bovine and camel milk, respectively. These results are consistent with those of Kappeler et al. (1998). The differences in the protein proportions in both milk samples and the presence of highly hydrophobic proteins as camel β-casein and α-La in camel milk could explain the higher surface hydrophobicity of the camel milk proteins relative to those in bovine milk (Atri et al., 2010; Lajnaf et al., 2016).

The surface hydrophobicity of bovine milk proteins increased when the temperature of the heat-treatment rose from 20 °C to 80 °C (bound-BPB = 6.32±0.40 µg mL^{-1}), and then it decreased with further increase of temperature from 90 °C to 100 °C (Fig. 4b). After heating bovine milk at 90 °C and 100 °C for 30min, bound-BPB values reached 1.91 ± 0.70 and 0.71± 0.61 µg mL^{-1}, respectively. As was the case for bovine milk, the surface hydrophobicity of camel milk greatly increased after heating at 70 °C for 30 min and achieved a maximum value of bound-BPB values of 11.16 ± 0.34 µg mL^{-1} at 80 °C. Finally, it significantly decreased to 5.95 ± 0.62 µg mL^{-1} at 90 °C and 2.48 ± 0.29 µg mL^{-1} at 100 °C.

In support of these results, Borcherding et al. (2008) found that higher temperature during heating leads to an increase of the hydrophobic interactions due to an exposure of hydrophobic groups, which are buried inside the globular structure of whey proteins.

Moreover, Lam and Nickerson (2015) noted that the hydrophobic moieties of whey proteins would be buried within the molecular structure of the created aggregates leading to the reduction of the overall hydrophobicity rate of milk proteins. These findings are in agreement with the lowest surface hydrophobicity observed with bovine milk at 90 °C and 100 °C and with the results of HPLC (section 3.2.2).

The highest surface hydrophobicity of camel milk proteins after heating can be explained by the lack of the β-Lg in this milk and also the exceptional hydrophobicity of camel α-La in agreement with the highest FC values of camel milk (section 3.1) and with the results of Atri et al. (2010), who found that the purified camel α-La was characterised by the higher surface hydrophobicity that given by its bovine counterpart. Indeed, the primary structure of camel α-La shows greater hydrophobicity of the α-La amino-acid sequence 25–35 in the hydrophobic core.

3.4.2. Determination of ζ-potential

Surface charge (or ζ-potential) of bovine and camel milk (at a protein concentration of 1 g L^{-1}) as a function of temperature pre-treatments (20, 70, 80, 90, and 100 °C for 30 min) were measured and are given in Fig. 7a. Overall, the ζ-potential values of camel milk were significantly lower than that of their bovine counterparts (P <0.05) regardless of heating temperature value except after heating at 90 °C and 100 °C for 30min.

Under native conditions, the ζ-potential values were –22.6 ± 0.8 mV and –19.9 ± 0.6 mV for bovine and camel milk, respectively. This is consistent with the results of Momen et al. (2018) and Lajnaf et al. (2018). These authors found that the ζ-potential values of camel whey were significantly lower than those of bovine whey proteins isolate solutions and suggested that this difference can be associated with the difference the protein composition of both wheys, the presence of highly basic protein in camel whey, lactoferrin (isoelectric point,

pI, = 8.8), and the difference in the pI of 5.01 and 4.2 for camel and bovine α-La (5.01 and 4.2, respectively).

Fig. 7a also shows that camel and bovine milk appeared less negatively charged after a heat-treatment at 70, 80, 90 and 100 °C for 30 min. Thermal treatment at 70 °C for 30min induced a significant decrease of the electronegative charge from –22.6 ± 0.8 mV to –19.6 ± 0.8 mV and from –19.9 ± 0.6 to –18 ± 0.5 mV bovine and camel milk respectively ($P < 0.05$).

No significant difference was observed between camel and bovine milk after heating at 90 °C and 100 °C for 30min (ζ-potential –16.5 mV). This behaviour can be explained by the denaturation and aggregation of the different milk proteins that may play an important role in this change of electronegative charge.

The same trends were reported for bovine milk by Borcherding et al. (2008) who observed that the heat-treatment of milk induces a decrease in its pH value leading to a lower protein negative charge and therefore a lower electrostatic repulsive forces as a result of heating. On the other hand, Lajnaf et al. (2018) reported that the acid camel whey carried less negative charge after heating at 90 °C for 30 min due to the dominance of the α-La in camel whey and its aggregation after a thermal treatment at this temperature.

3.4.3. Determination of the interfacial tension of milk

The average interfacial tension values (γ) of camel and bovine milk at a protein concentration of 1 g L^{-1} after thermal treatment in the temperature range 70–100 °C for 30 min are shown in Fig. 7b. Camel and bovine milk proteins significantly reduced the interfacial tension of the air-water interface from 72 mN m^{-1} ($P < 0.05$), which was estimated to be close to the surface tension value of pure water. The surface tension values for camel and bovine milk were in good agreement with those obtained by Kamath et al. (2008) and by Bertsch (1983) for raw and pasteurised bovine milk.

The surface tension values of camel milk were significantly lower than that of bovine milk ($P < 0.05$) regardless of heating temperature value (Fig. 7b). Thus, in native conditions (20 °C), the order of effectiveness was camel milk ($\gamma = 43.4 \pm 1.4$ mN m^{-1}) > bovine milk ($\gamma = 32.8 \pm 1.7$mN m^{-1}).

Fig. 7b shows also that the heat-treatments in the temperature range 70 °C to 100 °C for 30 min was found to improve the ability to reduce the interfacial tension at the air-water interface for both camel and bovine milk. Furthermore, a greater efficiency to reduce the surface tension values was attributed to the milk treated at higher temperature values. Indeed, a thermal treatment at 70 °C for 30 min induced a significant decrease of the surface tension from 43.4 to 36.8 mN m^{-1} and from 32.8 to 28.9 mN m^{-1} for bovine and camel milk, respectively ($P < 0.05$).

After a heat-treatment of 90 °C for 30 min, both milk samples retained the best interfacial properties when compared with the other thermal treatments. Hence, the order of effectiveness at this temperature value was camel milk ($\gamma = 21.2 \pm 0.6$ mN m^{-1}) > bovine milk ($\gamma = 31.2 \pm 1.2$ mN m^{-1}). No significant change was found on the evolution of the surface tension values of camel and bovine milk between 90 °C and 100 °C. This behaviour was explained by the increase of the number of hydrophobic interactions after heating due to an exposure of proteins hydrophobic groups, which are buried inside the globular whey proteins at temperature values below 40 °C (Borcherding et al., 2008).

The behaviour of native camel and bovine milk can be explained by the higher content of β-casein, which is in agreement with previous foaming properties (section 3.1). Purified camel β-casein is more efficient in reducing the surface tension at the air-water interface than its bovine counterpart because of the difference in the amino-acid residue composition (Lajnaf et al., 2016). Heat-treatment of the isolated camel α-La at 90 °C for 30 min and at pH 6.5 also significantly improved the ability of this protein to reduce the

interfacial tension at the air-water interface, contrary to acidic pH values (pH 4.3), where the α-La was found to aggregate under heating leading to the reduction of its tensioactive properties (Lajnaf et al., 2017).

4. Conclusion

This study investigated the foaming and physicochemical properties of camel milk proteins as a function of heating temperature (70, 80, 90 and 100 °C). Our findings confirmed the exceptional foaming properties of camel milk and highlighted the importance of the protein composition and their denaturation and/or aggregation state. These observations were related to the high β-casein amount in camel milk as well as its different secondary structure, especially in the β-sheet conformation and its high hydrophobicity as confirmed by FT-IR and ^{1}H-NMR results. On the other hand, the camel milk proteins were found to have the lowest values of foam stability because of the lowest amount of the κ-casein and the absence of β-Lg in camel milk. In addition, the results of this study indicated that heat-treatment improved the foaming capacity and stability of camel milk proteins. Indeed, the heat-treatment affected the physicochemical properties of camel milk proteins with an increase of surface hydrophobicity and a slight decrease of their negative ζ-potential and the interfacial tension values. These findings can be justified by the denaturation and/or aggregation of camel milk proteins after heating in consistence with the DR, RP-HPLC and SDS-PAGE results.

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References

Al-Shamsi, K. A., Mudgil, P., Hassan, H. M., & Maqsood, S. (2018). Camel milk protein hydrolysates with improved technofunctional properties and enhanced antioxidant potential in in vitro and in food model systems. *Journal of Dairy Science*, *101*, 47–60.

Al haj, O. A., & Al Kanhal, H. A. (2010). Compositional, technological and nutritional aspects of dromedary camel milk. *International Dairy Journal*, *20*, 811–821.

Atri, M. S., Saboury, A. A., Yousefi, R., Chobert, J., Haertle, T., & Moosavi-Movahedi, A. A. (2010). Comparative study on heat stability of camel and bovine apo and holo α-lactalbumin. *Journal of Dairy Research*, *77*, 43–49.

Bals, A., & Kulozik, U. (2003). Effect of pre-heating on the foaming properties of whey protein isolate using a membrane foaming apparatus. *International Dairy Journal*, *13*, 903–908.

Barzegar, A., Yousefi, R., Sharifzadeh, A., Dalgalarrondo, M., Chobert, J. M., Ganjali, M. R., et al. (2008). Chaperone activities of bovine and camel β-caseins: Importance of their surface hydrophobicity in protection against alcohol dehydrogenase aggregation. *International Journal of Biological Macromolecules*, *42*, 392–399.

Bertsch, A. J. (1983). Surface tension of whole and skim-milk between 18 and 135C. *Journal of Dairy Research*, *50*, 259–267.

Borcherding, K., Lorenzen, P. C., Hoffmann, W., & Schrader, K. (2008). Effect of foaming temperature and varying time/temperature-conditions of pre-heating on the foaming properties of skimmed milk. *International Dairy Journal*, *18*, 349–358.

Brooker, B. E., Anderson, M., & Andrews, A. T. (1986). The development of structure in whipped cream. *Food Structure*, *5*, Article 12.

Cao, X., He, Y., Kong, Y., Mei, X., Huo, Y., He, Y., et al. (2019). Elucidating the interaction mechanism of eriocitrin with β-casein by multi-spectroscopic and molecular simulation methods. *Food Hydrocolloids*, *94*, 63–70.

Chatterton, D. E. W., Smithers, G., Roupas, P., & Brodkorb, A. (2006). Bioactivity of β-lactoglobulin and α-lactalbumin – Technological implications for processing. *International Dairy Journal*, *16*, 1229–1240.

Chelh, I., Gatellier, P., & Santé-Lhoutellier, V. (2006). A simplified procedure for myofibril hydrophobicity determination. *Meat Science*, *74*, 681–683.

Closs, B., Courthaudon, J., & Lorient, D. (1990). Effect of chemical glycosylation on the surface properties of the soluble fraction of casein. *Journal of Food Science*, *55*, 437–439.

De la Fuente, M. A., Singh, H., & Hemar, Y. (2002). Recent advances in the characterisation of heat-induced aggregates and intermediates of whey proteins. *Trends in Food Science and Technology*, *13*, 262–274.

Dickinson, E. (1998). Proteins at interfaces and in emulsions. Stability, rheology and interactions. *Journal of the Chemical Society, Faraday Transactions*, *94*, 1657–1669.

Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids*, *17*, 25–39.

Elagamy, E. 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.

El-Agamy, E. I. (2006). Camel milk. In Y. W. Park, G. F. W. Haenlein, & W. L. Wendorff (Eds), *Handbook of milk of non-bovine mammals* (pp. 297–344). Holboken, NJ, USA:

Wiley Blackwell.

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-Hatmi, H., Girardet, J. M., Gaillard, J. L., Yahyaoui, M. H., & Attia, H. (2007). Characterisation of whey proteins of camel (*Camelus dromedarius*) milk and colostrum. *Small Ruminant Research*, *70*, 267–271.

Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, *82*, 70–77.

Ereifej, K. I., Alu'datt, M. H., Alkhalidy, H. A., Alli, I., & Rababah, T. (2011). Comparison and characterisation of fat and protein composition for camel milk from eight Jordanian locations. *Food Chemistry*, *127*, 282–289.

Farah, Z., Rettenmaier, R., & Atkins, D. (1992). Vitamin content of camel milk. *International Journal for Vitamin and Nutrition Research*, *62*, 30–33.

Felfoul, I, Jardin, J., Gaucheron, F., Attia, H., & Ayadi, M. A. (2017). Proteomic profiling of camel and cow milk proteins under heat treatment. *Food Chemistry*, *216*, 161–169.

Felfoul, I, Lopez, C., Gaucheron, F., Attia, H., & Ayadi, M. A. (2015a). A laboratory investigation of cow and camel whey proteins deposition under different heat treatments. *Food and Bioproducts Processing*, *96*, 256–263.

Felfoul, I, Lopez, C., Gaucheron, F., Attia, H., & Ayadi, M. A. (2015b). Fouling behavior of camel and cow milks under different heat treatments. *Food and Bioprocess Technology*, *8*, 1771–1778.

Fernández, E., Artiguez, M. L., de Marañón, I. M., Villate, M., Blanco, F. J., & Arboleya, J.-C. (2012). Effect of pulsed-light processing on the surface and foaming properties of β-lactoglobulin. *Food Hydrocolloids*, *27*, 154–160.

Graham, D. E., & Phillips, M. C. (1979). Proteins at liquid interfaces: I. Kinetics of adsorption and surface denaturation. *Journal of Colloid and Interface Science*, *70*, 403–414.

Hailu, Y., Hansen, E. B., Seifu, E., Eshetu, M., Ipsen, R., & Kappeler, S. (2016). Functional and technological properties of camel milk proteins: a review. *Journal of Dairy Research*, *83*, 422–429.

Hatakeyama, S., Akiyama, M., Yoneyama, R., Watanabe, K., Koizumi, R., Miyaji, K., et al. (2019). Effects of manufacturing conditions on the foaming properties of milk and sensory characteristics of foamed milk. *LWT-Food Science and Technology*, *99*, 555–561.

Havea, P., Singh, H., & Creamer, L. K. (2002). Heat-induced aggregation of whey proteins: comparison of cheese WPC with acid WPC and relevance of mineral composition. *Journal of Agricultural and Food Chemistry*, *50*, 4674–4681.

Huppertz, T., Hennebel, J.-B., Considine, T., Kelly, A. L., & Fox, P. F. (2006). A method for the large-scale isolation of β-casein. *Food Chemistry*, *99*, 45–50.

Jafar, S., Kamal, H., Mudgil, P., Hassan, H. M., & Maqsood, S. (2018). Camel whey protein hydrolysates displayed enhanced cholesteryl esterase and lipase inhibitory, anti-hypertensive and anti-haemolytic properties. *LWT-- Food Science and Technology*, *98*, 212–218.

Kamal, H., Jafar, S., Mudgil, P., Murali, C., Amin, A., & Maqsood, S. (2018). Inhibitory properties of camel whey protein hydrolysates toward liver cancer cells, dipeptidyl peptidase-IV, and inflammation. *Journal of Dairy Science*, *101*, 8711–8720.

Kamath, S., Huppertz, T., Houlihan, A. V, & Deeth, H. C. (2008). The influence of temperature on the foaming of milk, *International Dairy Journal, 18*, 994–1002.

Kappeler, S., Farah, Z., & Puhan, Z. (1998). Sequence analysis of *Camelus dromedarius* milk

caseins. *The Journal of Dairy Research*, *65*, 209–222.

Kappeler, S. R., Farah, Z., & Puhan, 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.

Kappeler, S. R., Heuberger, C., Farah, Z., & Puhan, Z. (2004). Expression of the peptidoglycan recognition protein, PGRP, in the lactating mammary gland. *Journal of Dairy Science*, *87*, 2660–2668.

Kazmierski, M., & Corredig, M. (2003). Characterization of soluble aggregates from whey protein isolate. *Food Hydrocolloids*, *17*, 685–692.

Kong, J., & Yu, S. (2007). Fourier transform infrared spectroscopic analysis of protein secondary structures. *Acta Biochimica et Biophysica Sinica, 39,* 549–559.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *227*, 680–685.

Lajnaf, R., Picart-Palmade, L., Attia, H., Marchesseau, S., & Ayadi, M. A. (2016). Foaming and adsorption behavior of bovine and camel proteins mixed layers at the air/water interface. *Colloids and Surfaces B: Biointerfaces*, *151*, 287–294.

Lajnaf, R., Picart-Palmade, L., Attia, H., Marchesseau, S., & Ayadi, M. A. (2017). The effect of pH and heat treatments on the foaming properties of purified α-lactalbumin from camel milk. *Colloids and Surfaces B: Biointerfaces*, *156*, 55–61.

Lajnaf, R., Picart-Palmade, L., Cases, E., Attia, H., Marchesseau, S., & Ayadi, M. A. (2018). The foaming properties of camel and bovine whey: The impact of pH and heat treatment. *Food Chemistry*, *240*, 295–303.

Lajnaf, R., Trigui, I., Samet-Bali, O., Attia, H., & Ayadi, M. A. (2019). Comparative study on emulsifying and physico-chemical properties of bovine and camel acid and sweet wheys. *Journal of Food Engineering*, *268,* 109741.

Laleye, L. C., Jobe, B., & Wasesa, A. A. H. (2008). Comparative study on heat stability and functionality of camel and bovine milk whey proteins. *Journal of Dairy Science*, *91*, 4527–4534.

Lam, R. S. H., & Nickerson, M. T. (2015). The effect of pH and temperature pre-treatments on the physicochemical and emulsifying properties of whey protein isolate. *LWT - Food Science and Technology*, *60*, 427–434.

Lorenzen, P. C. (2000). Verfahren zur modifizierung von Milcheiweiß. *Kieler Milchwirtschaftliche Forschungsberichte*, *52*, 47–59.

Magnusson, E., & Nilsson, L. (2011). Interactions between hydrophobically modified starch and egg yolk proteins in solution and emulsions. *Food Hydrocolloids*, *25*, 764–772.

Manderson, G. A., Hardman, M. J., & Creamer, L. K. (1998). Effect of heat treatment on the conformation and aggregation of β-lactoglobulin A, B, and C. *Journal of Agricultural and Food Chemistry*, *46*, 5052–5061.

Maqsood, S., Al-Dowaila, A., Mudgil, P., Kamal, H., Jobe, B., & Hassan, H. M. (2019). Comparative characterization of protein and lipid fractions from camel and cow milk, their functionality, antioxidant and antihypertensive properties upon simulated gastro-intestinal digestion. *Food Chemistry*, *279*, 328–338.

Marinova, K. G., Basheva, E. S., Nenova, B., Temelska, M., Mirarefi, A. Y., Campbell, B., et al. (2009). Physico-chemical factors controlling the foamability and foam stability of milk proteins: Sodium caseinate and whey protein concentrates. *Food Hydrocolloids*, *23*, 1864–1876.

McClements, D. J. (2015). *Food emulsions: principles, practices, and techniques*. Boca Raton, FL, USA: CRC press.

Merin, U., Bernstein, S., Yagil, R., Creveld, C. Van, Lindner, P., & Gollop, N. (2001). Short communication A comparative study of milk serum proteins in camel (*Camelus*

dromedarius) and bovine colostrum, *Livestock Production Science, 67*, 297–301.

Momen, S., Salami, M., Emam-Djomeh, Z., Hosseini, E., Sheibani, N., & Moosavi-Movahedi, A. A. (2018). Effect of dry heating on physico-chemical, functional properties and digestibility of camel whey protein. *International Dairy Journal*, *86*, 9–20.

Moro, A., Báez, G. D., Busti, P. A., Ballerini, G. A., & Delorenzi, N. J. (2011). Effects of heat-treated β-lactoglobulin and its aggregates on foaming properties. *Food Hydrocolloids*, *25*, 1009–1015.

Mudgil, P., Kamal, H., Yuen, G. C., & Maqsood, S. (2018). Characterization and identification of novel antidiabetic and anti-obesity peptides from camel milk protein hydrolysates. *Food Chemistry*, *259*, 46–54.

Nongonierma, A. B., Paolella, S., Mudgil, P., Maqsood, S., & FitzGerald, R. J. (2017). Dipeptidyl peptidase IV (DPP-IV) inhibitory properties of camel milk protein hydrolysates generated with trypsin. *Journal of Functional Foods*, *34*, 49–58.

Nongonierma, A. B., Paolella, S., Mudgil, P., Maqsood, S., & FitzGerald, R. J. (2018). Identification of novel dipeptidyl peptidase IV (DPP-IV) inhibitory peptides in camel milk protein hydrolysates. *Food Chemistry*, *244*, 340–348.

Omar, A., Harbourne, N., & Oruna-Concha, M. J. (2016). Quantification of major camel milk proteins by capillary electrophoresis. *International Dairy Journal*, *58*, 31–35.

Ramet, J. -P. (2001). *The technology of making cheese from camel milk (Camelus dromedarius)*. Rome, Italy: Food and Agriculture Organisation of the United Nations.

Roefs, S. P. F. M., & De Kruif, K. G. (1994). A model for the denaturation and aggregation of β-lactoglobulin. *European Journal of Biochemistry*, *226*, 883–889.

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.

Santoni, I., & Pizzo, B. (2013). Evaluation of alternative vegetable proteins as wood adhesives. *Industrial Crops and Products*, *45*, 148–154.

Schmitt, C., Bovay, C., Rouvet, M., Shojaei-Rami, S., & Kolodziejczyk, E. (2007). Whey protein soluble aggregates from heating with NaCl: physicochemical, interfacial, and foaming properties. *Langmuir*, *23*, 4155–4166.

Shalash, M. R. (1979). The production and utilization of camel milk. In W. R. Cockrill (Ed.), *The Camelid. An all-purpose animal. Proceedings of the Khartoum Workshop on Camels* (Vol. 1, pp. 196–208). Uppsala, Sweden: Scandinavian Institute of African Studies.

Siu, N.-C., Ma, C.-Y., & Mine, Y. (2002). Physicochemical and structural properties of oat globulin polymers formed by a microbial transglutaminase. *Journal of Agricultural and Food Chemistry*, *50*, 2660–2665.

Tosi, E., Canna, L., Lucero, H., & Ré, E. (2007). Foaming properties of sweet whey solutions as modified by thermal treatment. *Food Chemistry*, *100*, 794–799.

Ward, B. R., Goddard, S. J., Augustin, M. A., & McKinnon, I. R. (1997). EDTA-induced dissociation of casein micelles and its effect on foaming properties of milk. *Journal of Dairy Research*, *64*, 495–504.

Yüksel, Z., & Erdem, Y. K. (2010). Detection of the milk proteins by RP-HPLC. *GIDA-Journal of Food*, *35*, 5–11.

Zhang, Z., Dalgleish, D. G., & Goff, H. D. (2004). Effect of pH and ionic strength on competitive protein adsorption to air/water interfaces in aqueous foams made with mixed milk proteins. *Colloids and Surfaces B: Biointerfaces*, *34*, 113–121.

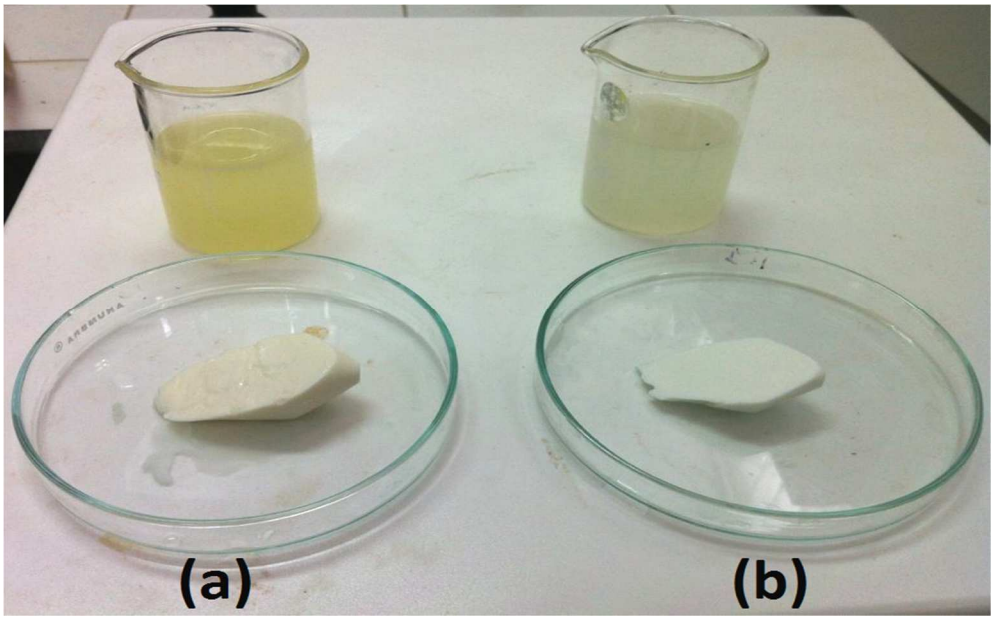
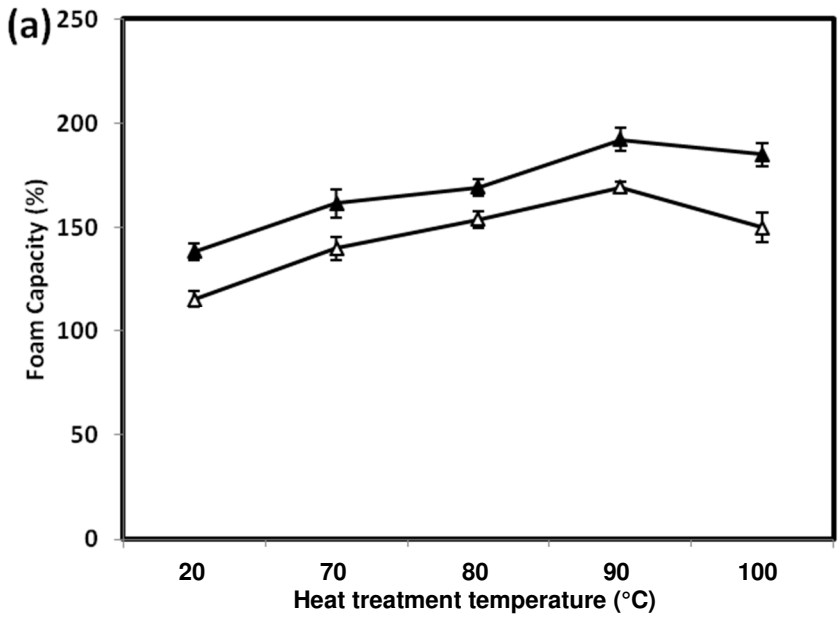


Figure 1


(a)
250
200
150
100
50
0
Foam Capacity (%)
20
70
80
90
100
Heat treatment temperature (°C)

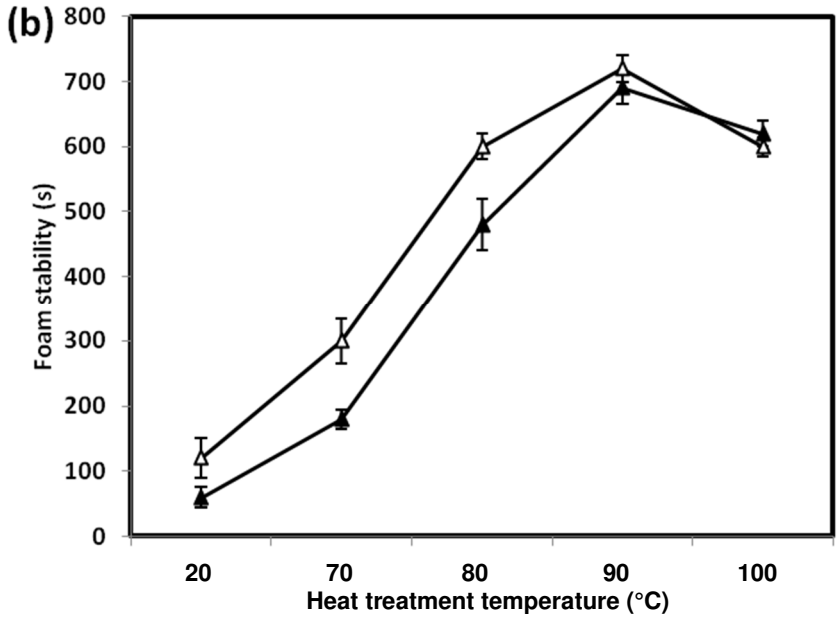

(b)
800
700
600
500
400
300
200
100
0
Foam stability (s)
20
70
80
90
100
Heat treatment temperature (°C)

Figure 2

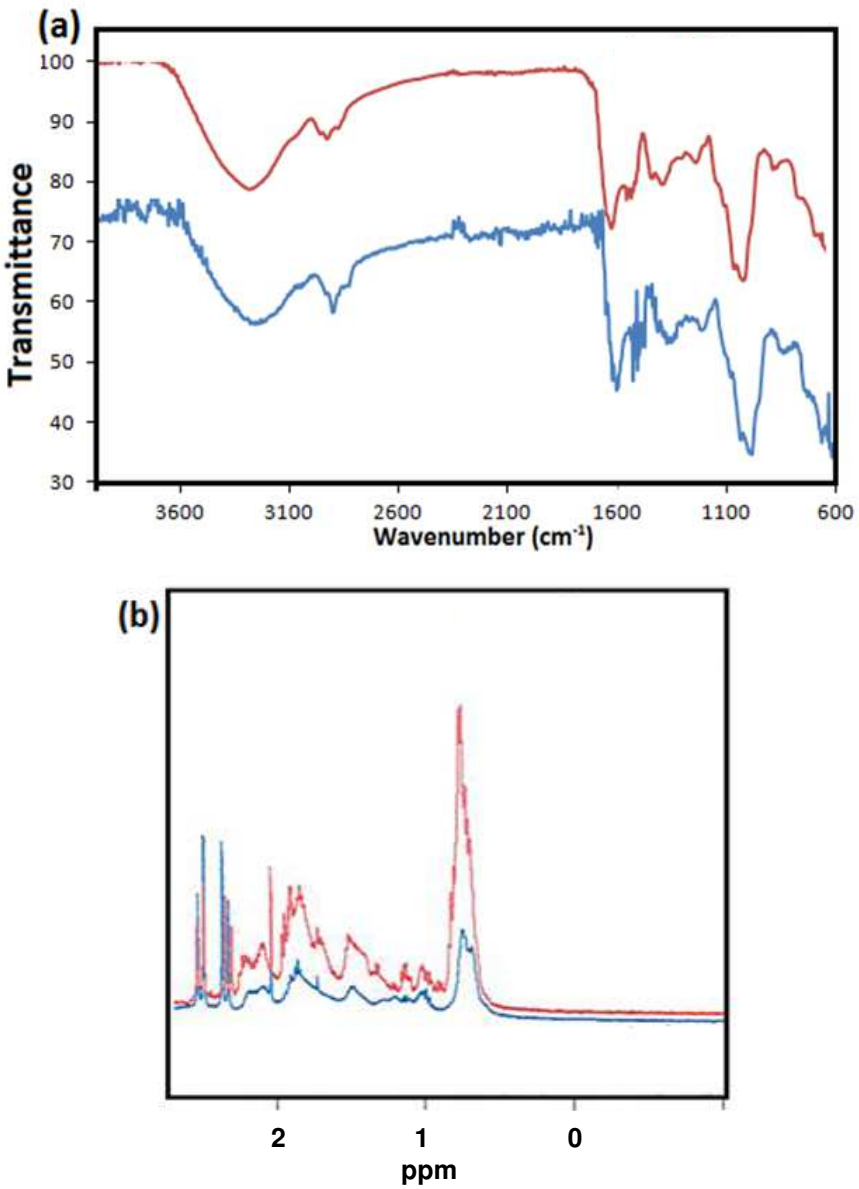
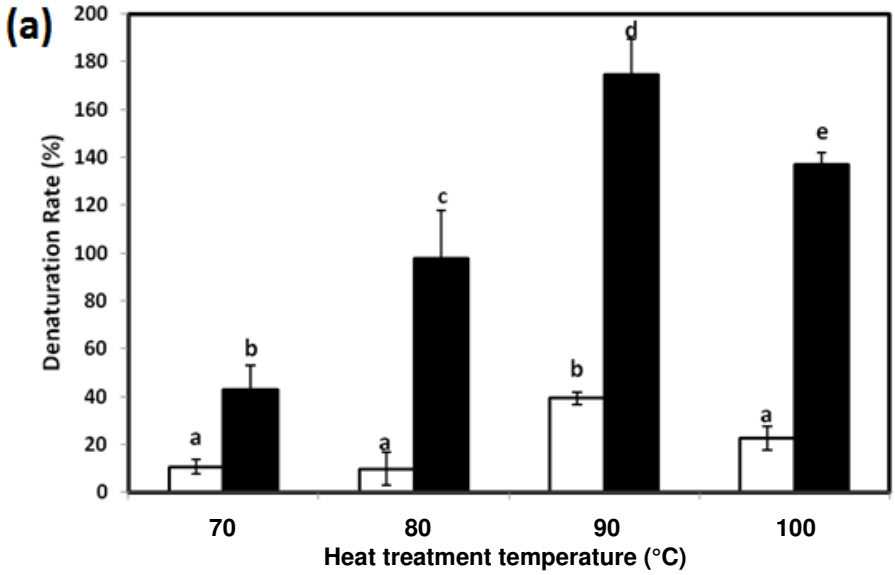

(a)
Transmittance
100
90
80
70
60
50
40
30
3600
3100
2600
2100
1600
1100
600
Wavenumber (cm-1)
(b)
2
1
0
ppm

Figure 3


(a)
Denaturation Rate (%)
200
180
160
140
120
100
80
60
40
20
0
a
b
a
c
b
d
a
e
70
80
90
100
Heat treatment temperature (°C)

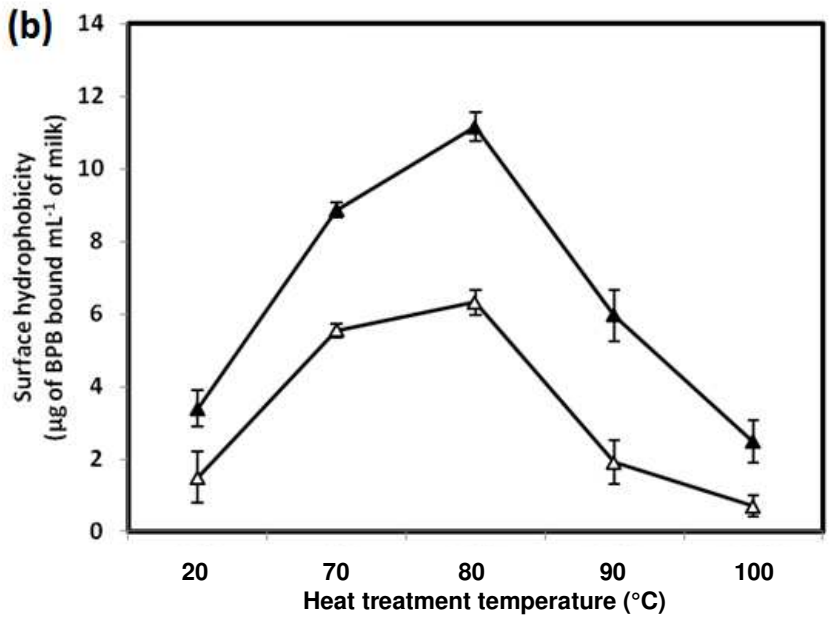

(b)
Surface hydrophobicity
(µg of BPB bound mL-1 of milk)
14
12
10
8
6
4
2
0
20
70
80
90
100
Heat treatment temperature (°C)

Figure 4

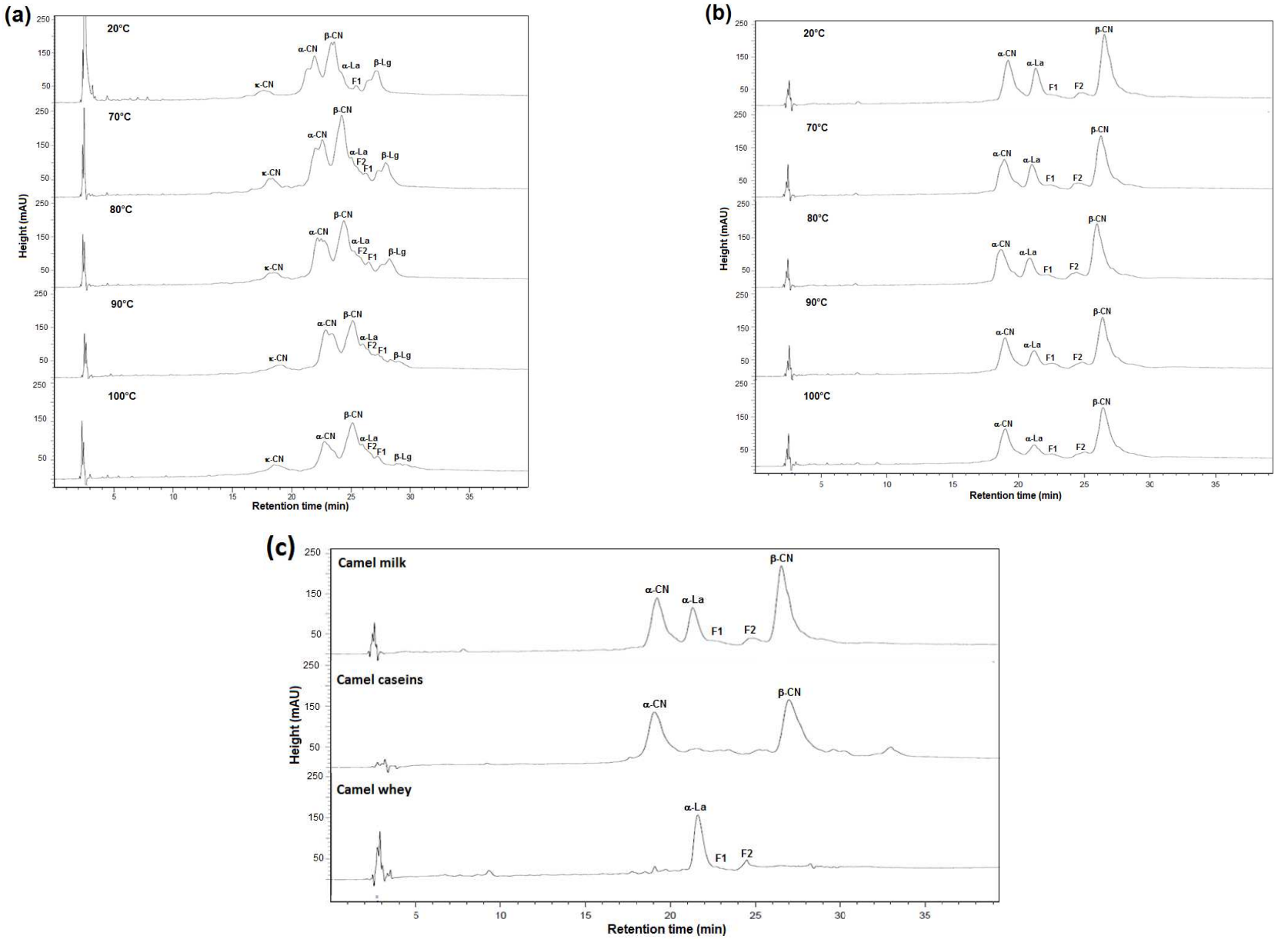

(a)
20°C
70°C
80°C
90°C
100°C
κ-CN
α-CN
β-CN
α-La
β-Lg
F1
F2
Height (mAU)
Retention time (min)
(b)
20°C
70°C
80°C
90°C
100°C
α-CN
α-La
β-CN
F1
F2
Height (mAU)
Retention time (min)
(c)
Camel milk
Camel caseins
Camel whey
α-CN
α-La
β-CN
F1
F2
Height (mAU)
Retention time (min)

Figure 5

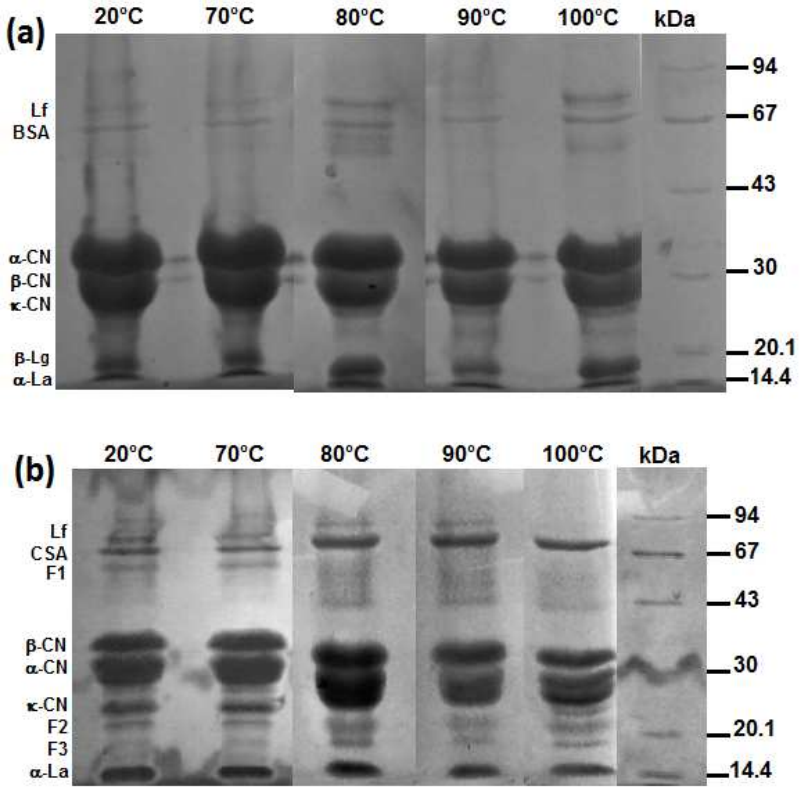

(a)
20°C
70°C
80°C
90°C
100°C
kDa
Lf
BSA
α-CN
β-CN
κ-CN
β-Lg
α-La
94
67
43
30
20.1
14.4
(b)
20°C
70°C
80°C
90°C
100°C
kDa
Lf
CSA
F1
β-CN
α-CN
κ-CN
F2
F3
α-La
94
67
43
30
20.1
14.4

Figure 6

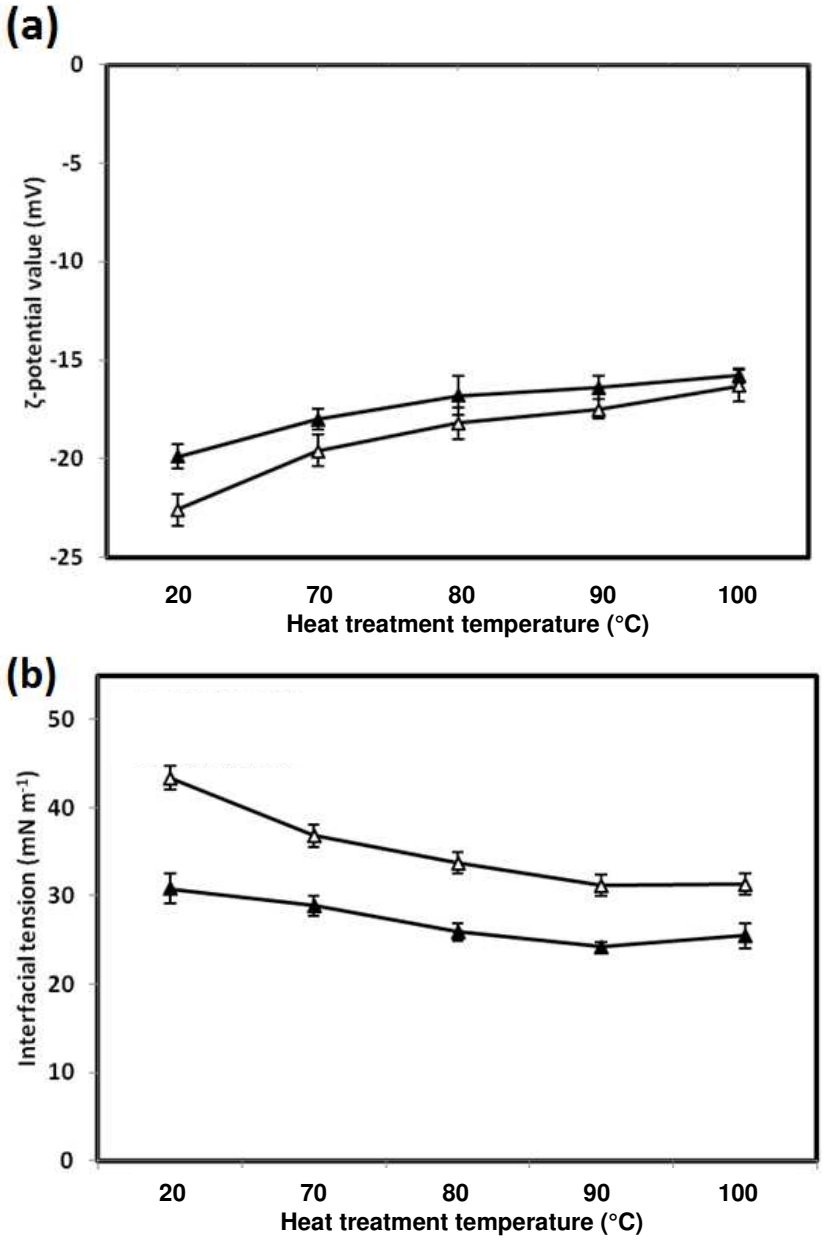

(a)
0
-5
-10
-15
-20
-25
ζ-potential value (mV)
20
70
80
90
100
Heat treatment temperature (°C)
(b)
50
40
30
20
10
0
Interfacial tension (mN m-1)
20
70
80
90
100
Heat treatment temperature (°C)

Figure 7