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Roua Lajnaf[a,b*], Laetitia Picart-Palmade[b], Hamadi Attia[a], Sylvie Marchesseau[b], M.A. Ayadi[a]

[a]*Alimentary Analysis Unit, National Engineering School of Sfax, BPW 3038, Sfax, Tunisia*

[b]*IATE, Univ Montpellier, INRAE, Institut Agro, Montpellier, France*

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* Corresponding author: Roua Lajnaf

National Engineering School of Sfax, Sfax Tunisia

University of Montpellier

Tel: +216 74 675761

E-mail address: roua_lajnaf@yahoo.fr; roua.lajnef@enis.tn

Abstract

The objective of this research was to explore the foaming properties of camel and bovine milk and their derived proteins fractions including sodium caseinates, sweet whey, β-casein, α-lactalbumin and β-lactoglobulin. First, camel and bovine milk proteins were identified by the reversed-phase high-performance liquid chromatography (RP-HPLC) and foaming properties (Foam capacity (FC) and stability (FS)) were analyzed. Afterwards, competitive adsorption of proteins to the air-water interface for both milk protein fractions was characterized using pendant-drop tensiometry parameters and was compared to intrinsic fluorescence results of pure proteins. Experimental results indicated that the maximum FC values were found for camel skim milk, sodium caseinates and β-casein with higher FS values for bovine β-casein. Differences in the stability and the highest tensioactive properties of camel β-casein were explained with the different molecular structure and its higher hydrophobicity when compared to its bovine counterpart. Thus, milk proteins adsorbed layers are mainly affected by the presence of β-casein which is the first adsorbed and the most abundant protein at the air-water contrary to whey proteins (α-lactalbumin and β-lactoglobulin). These globular proteins are involved in the composition of protein layers at air-water interface, giving higher viscoelastic modulus values, but could not compact well at the interface because of their rigid molecular structure. For camel milk, foaming properties and interfacial behavior are mainly maintained by camel β-casein due to its higher hydrophobicity compared to bovine β-casein and the greater exposure of tyrosine residues despite the absence of tryptophan in consistence with the intrinsic fluorescence results. Furthermore, the absence of the β-lactoglobulin leads to the dominance of the α-lactalbumin at the air-water interface which is characterized by lower hydrophobicity than its bovine counterpart leading to lower viscoelastic modulus values than those of bovine whey, and hence to weaker rheological properties of camel milk protein layer at the air-water interface.

Keywords: Camel and bovine proteins, foaming properties, surface tension, viscoelastic modulus, intrinsic fluorescence

1. Introduction

Foams are important in various types of food products, such as ice creams, whipped creams meringues and chocolate mousses. Thus, the research of new stabilizing and foam-forming agents of natural origin continues to develop promising food ingredients that provide health benefits and functional properties (Li et al., 2020; Murray, 2020). Milk foams are defined as colloidal systems in which the created air bubbles are stabilized by the surface-active components of milk which are mainly milk proteins (Dickinson, 2003; Borcherding, Lorenzen, Hoffmann & Schrader, 2008). Indeed, milk proteins are among the most common commercial foaming ingredients due to their ability to absorb and to spread at the air-water interface after orienting their hydrophilic and hydrophobic groups at the aqueous and non-aqueous phases respectively, leading to a rapid decrease of the surface tension (Nicorescu et al., 2011). Overall, milk proteins are divided into two classes: coagulable caseins (~80%) and soluble serum proteins (~20%). Caseins are often considered intrinsically as unstructured phosphoproteins without specific secondary structures. Compositionally, they consist of four sub-fractions including α_{S1}-, α_{S2}-, β-, and κ-caseins with molar ratio approximately being 4:1:4:1 in bovine milk (Bo-M) (De Kruif & Holt, 2003; Liang & Luo, 2020). These proteins are amphiphilic, with molecular weights ranging between 19 and 25 kDa and isoelectric point (pI) between 4.1 and 5.3. On the other hand, globular whey proteins are composed of four main individual proteins components characterized by different structures, including β-lactoglobulin (53.6%), α-lactalbumin (20.1%), bovine serum albumin (BSA) (6.2%) and immunoglobulins (3.5%) (Table 1) (Hailu et al., 2016).

Caseins and whey proteins are characterized by different surface rheological properties a flexible and disordered structure allowing changes of their conformation more rapidly and easily once adsorbed at the interface, whereas, globular whey proteins, stabilized by intra disulphide bridges, conserve their molecular shape after adsorption (Dickinson, 2001; Rouimi,

75 Schorsch, Valentini & Vaslin, 2005; Marinova et al., 2009; Seta, Baldino, Gabriele, Lupi &
76 Cindio, 2014).

77 Thus, the foaming properties may vary depending on the protein composition of the milk,
78 as observed in bovine and camel milk. Camel milk (Ca-M), known to be a good nutritional
79 source for the people living in various countries of the world (Li et al., 2020), has also a good
80 ability to generate foam (Shalash, 1979; Lajnaf, Zouari, Trigui, Attia & Ayadi, 2020 a).

81 The physico-chemical composition of camel and cow milk (fat, lactose and total solids
82 content) was relatively close to that of Bo-M (Al haj & Al Kanhal, 2010; Lajnaf et al., 2019)
83 with a similar total solids content (13.01 ± 0.12 % for Bo-M and 12.95 ± 0.17 % for Ca-M) a
84 lipid content around 34.1 ± 0.5 g L^{-1} and 35.4 ± 0.6 g L^{-1} for both Bo-M and Ca-M, respectively,
85 and a lactose concentration of 46.1 ± 2.2 g L^{-1} and 43.5 ± 1.1 g L^{-1} in Bo-M and Ca-M,
86 respectively. On the other hand, the total mineral content ranges between 6 and 9 g L^{-1} of milk
87 with an average value of 7 g L^{-1} and 7.9 g L^{-1} for Bo-M and CaM, respectively (Al haj & Al
88 Kanhal, 2010).

89 For the protein concentrations, the main values in Ca-M (22.06 g L^{-1}) were significantly
90 lower than those in Bo-M (28.36 g L^{-1}) (Lajnaf et al., 2019) and represent 75.4% (w/w) of the
91 camel proteins (Ereifej, Alu'datt, Alkhalidy, Alli & Rababah, 2011). The specificity of Ca-Milk
92 is its high proportion in β-casein (65% of the total camel caseins compared to 36% (w/w) for
93 Bo-M) with a concentration of 15.6 g L^{-1} (Table 1) instead of 9.5 g L^{-1} in Bo-M (Davies & Law,
94 1980; Kappeler, Farah & Puhan, 2003; Ereifej et al., 2011; Omar, Harbourne & Oruna-Concha,
95 2016) and its low concentration in κ-casein (3.3% of the total camel caseins instead of 13% of
96 the total casein in Bo-M (Table 1). Camel β-casein is composed of 217 amino-acid residues,
97 which are more numerous than those of bovine β-casein residues (209 amino-acid residues).
98 Therefore, the similarity and identity between camel and bovine β-caseins are 84.5% and
99 67.2%, respectively (Table 1). Protein similarity measures the similarities between aminoacid
100 sequences including residues with similar biochemical properties while, protein sequence

identity is defined by the ratio of the number of identical residues in a pair of aligned protein sequences to the length of the shorter one (Table 1) (Kanduc, 2012). Camel β-casein has a molecular weight (MW) of 24.65 kDa, while its bovine counterpart has a MW of 23.58 kDa. The isoelectric point (pI) is about 4.66 and 4.49 for camel and bovine β-casein, respectively. Hence, in milk (pH ~ 6.5), these proteins are negatively charged (Eigel et al., 1984; Kappeler, Farah & Puhan, 1998; Barzegar et al., 2008).

Whey proteins are the second main protein component in Bo-M and Ca-M representing 20% and 24.5% of the total cow and camel proteins, respectively (Ereifej et al., 2011; Madureira et al., 2010). In bovine whey proteins, the average β-lactoglobulin fraction accounted for 53.6% of total whey with a concentration of 3.1 g L^{-1} (Table 1), followed by the α-lactalbumin (20.1%) (Hailu et al., 2016). However, the β-lactoglobulin is absent in camel whey (Merin et al., 2001; Omar et al., 2016; Lajnaf et al., 2018, Lajnaf, Trigui, Samet-Bali, Attia & Ayadi, 2019; Lajnaf et al., 2020 a) and the α-lactalbumin is the major protein in the soluble fraction of Ca-M with an average concentration of 3.5 g L^{-1} which is significantly higher when compared to the α-lactalbumin content in Bo-M (1.1 g L^{-1}) (Table 1). Camel α-lactalbumin has 123 amino acids, a MW of 14.43 kDa and a pI of 4.87. This protein shares some main molecular characteristics with its bovine counterpart in terms of number of amino-acid residues with a MW of 14.18 kDa and a pI of 4.65 (El-Agamy, 2009). Thus, camel and bovine α-lactalbumin show 82.9% similarity and 69.1% identity (Atri et al., 2010).

Various authors reported that the dynamic surface tension parameters are the main determining factors which are directly associated with the foamability of proteins (Marinova et al., 2009). For instance, a rapid decrease in surface tension indicates a fast adsorption of proteins at the interface and thus greater foaming capacity and stabilization of the integrated air bubbles to avoid coalescence (Tamm, Sauer, Scampicchio & Drisch, 2012). In different studies on foaming and emulsifying properties of cow proteins (caseins and whey proteins), the surface tension has been analyzed using pendant-drop tensiometry which is considered as a very

127 accurate method (Mellema & Isenbart, 2004; Cases et al., 2005; Marinova et al., 2009; Tamm
128 et al., 2012; Seta et al., 2014; Zhou, Sala & Sagis, 2020 a). For Bo-M proteins, previous studies
129 have reported that both individual caseins and casein micelles dispersions exhibited good
130 foaming and interfacial properties compared to whey proteins (Cao et al., 2018; Dombrowski
131 et al., 2016; Xiong et al., 2020). For instance, the β-casein adopts immediate equilibrium
132 conformation at the air-water interface due to the higher conformational flexibility. However,
133 the globular proteins with ordered secondary structure, such as α-lactalbumin and β-
134 lactoglobulin rearrange much more slowly than caseins due to strong intra- and inter-molecular
135 interactions including disulphide bridges (Cornec et al., 1999; Zhou, Tobin, Drusch & Hogan,
136 2020 b).

137 Although Ca-M is known to have good foaming properties, comprehensive studies on the
138 foaming and interfacial properties as well as the competitive adsorption of Ca-M proteins are
139 missing. Therefore, the present work aims to provide an in-depth understanding of the
140 mechanisms involved in the interfacial properties of proteins from two different dairy systems
141 (Ca-M and Bo-M) in order to reveal the foaming behavior of camel and bovine milk and which
142 proteins are responsible for the creation and the stabilization of camel and bovine milk foam.
143 Thus, the objective of the current research is to examine the competitive adsorption behavior
144 of protein to the air interfaces in foams made from camel and bovine skim milk, sodium
145 caseinates and whey protein through the individual proteins (β-casein, β-lactoglobulin and α-
146 lactalbumin). Hence, skim milk, sodium caseinates, and whey proteins were chosen as proteins
147 mixed systems in their native states without any heating temperature or pH level modification,
148 meanwhile β-casein, β-lactoglobulin, and α-lactalbumin were chosen as proteins pure systems.
149 For Bo-M, pure β-casein, β-lactoglobulin and α-lactalbumin were used as they represent the
150 major constituents of the air-water interface in Bo-M foams (Brooker, Anderson & Andrews,
151 1986; Borcherding et al., 2008). For Ca-M, purified camel α-lactalbumin and β-casein were
152 chosen as they represent the main proteins in both soluble and micellar fractions of Ca-M

153 respectively, and as β-lactoglobulin is completely absent. The interfacial properties performed
154 with the pendant-drop tensiometer were then correlated with the results obtained for foaming
155 properties (foaming capacity and foam stability). Thus, we consider the clarification of foaming
156 mechanism and the competitive adsorption behavior of camel and bovine milk at the air-water
157 interface as a major novelty of this work.

158 ## 2. Material and methods

159 ### *2.1. Materials*

160 Ca-M samples (*Camelus dormedarius*) were purchased from a camel farm in the Medenin
161 region of Tunisia. Fresh bovine (*Bos taurus*) milk was supplied by a local farmer in the region
162 of Montpellier in France (La ferme de DILHAC, Isabelle et Serge Rayrolles, 12600, Lacroix-
163 Barrez).

164 Purchased samples were systematically cooled to 4 °C and pH values were measured (744-
165 pH meter, Metrohm, Herisau, Switzerland). For both milk samples, fat was removed by
166 centrifugation at 1,000g for 20 min at 4 °C (centrifuge Beckman CO-LE80K, Coulter, Fullerton,
167 CA) and skim milk was stored at -18 °C for further experimental analysis (Kappeler,
168 Ackermann, Farah & Puhan, 1999).

169 ### *2.2. Bovine protein fractions*

170 Sweet bovine whey and Na-cas were extracted using rennet coagulation and acid
171 precipitation. Bovine sodium caseinates (hereafter noted as Na-cas) were extracted after acid
172 precipitation according to the method of Thompson, Boland and Singh (2009). First, skim Bo-
173 M was acidified using HCl solution (1M) to the pI of bovine caseins (pH~ 4.6), followed by a
174 centrifugation at 5,000 g for 20 min at 20 °C using Beckman centrifuge CO-LE80K (Coulter,
175 Fullerton, CA). Afterwards, casein fraction was resolubilized in an amount of deionized water
176 (provided from Milli-Q system Millipore, USA) equal to that of the discarded acid whey.
177 Finally, pH value was adjusted to that of milk using 1M NaOH, yielding bovine Na-cas
178 (Thompson et al., 2009).

Sweet bovine whey was extracted from skim Bo-M after an enzymatic coagulation at 37°C for 1 h in the presence of 0.35 mL microbial rennet enzyme per liter of skim Bo-M (Parachimic, Laboratories Arrazi, Sfax, Tunisia, strength = 1:10,000) (Lajnaf et al., 2019). Afterwards, sweet bovine whey was separated from casein fraction by a centrifugation at 5000g for 20 min at 20 °C.

Pure bovine proteins were provided from Sigma-Aldrich and used without further purification. The purity of β-casein (Product #: C6905, Lot #: SLBH6096V); β-lactoglobulin (Product #: L2506, Lot #: SLBB4325V) and α-lactalbumin (Product #: L5385, Lot #: SLBJ2493V) are ≥ 98%, ≥ 85% and ≥ 85%, respectively.

2.3. Camel protein fractions

Unlike the pure bovine proteins, Ca-M individual proteins were purified according to previous works because pure camel proteins are not yet commercialized. The authors used purification methods for camel proteins which leads to obtain camel proteins in their native form without denaturation (ultrafiltration for camel α-lactalbumin and cold-solubilization for camel β-casein (Huppertz et al., 2006; Salami et al., 2009)).

The experimental procedures for the isolation of camel protein fractions are summarized in Fig. 1.

After milk defatting, the casein fraction of Ca-M was separated from the soluble fraction by rennet addition (1.4 mL L^{-1} of milk) at 37 °C for 1 h (Felfoul, Lopez, Gaucheron, Attia & Ayadi, 2015; Lajnaf et al., 2018, 2019). The rennet coagulum was centrifuged at 5,000g for 15 min at 20 °C (centrifuge Beckman CO-LE80K, Coulter, Fullerton, CA). Afterwards, the curd containing the camel caseins was kept for the β-casein purification and the supernatant representing the soluble fraction of milk identified as sweet camel whey was also retained for α-lactalbumin purification.

Camel α-lactalbumin was purified from sweet whey by ultrafiltration (UF) as described by previous works (Salami et al., 2009; Lajnaf, Picart-Palmade, Attia, Marchesseau & Ayadi, 2017; Lajnaf, Gharsallah, Jridi, Attia & Ayadi, 2020 b). Indeed, sweet camel whey was applied to a UF membrane characterized by a molecular mass cut off of 30 kDa (Amicon-bioseperations model 8050). The UF system was operated at a pressure of 1 bar and room temperature for 3 h. The UF permeate containing purified camel α-lactalbumin was stored at −18 °C for further use.

Camel β-casein was isolated from rennet camel curd obtained previously using the cold-extraction method described by Huppertz et al. (2006). A volume of heated demineralized water (80 °C) equal to that of the removed sweet whey was added to the curd and the mixture was kept at 80 °C for 5 min using a water bath to disable the action of the added rennet enzyme and then centrifuged at 5,000 g for 15 min at 20 °C using a Beckman centrifuge CO-LE80K (Coulter, Fullerton, CA). The curd was stored, macerated and suspended in deionized water (5 °C) at a volume equal to that of the discarded whey. Finally, the protein suspension was kept at 5 °C for up to 24 h and centrifuged at 5,000 g at 5 °C for 15 min. The supernatant obtained containing the isolated camel β-caseins and camel α-lactalbumin and was also stored at -18 °C for further analysis.

Camel sodium Na-cas samples were extracted according to the methods described previously for Bo-M (section 2.2) and using the method of Thompson et al. (2009) with slight modifications. The pH of skim Ca-M was lowered to near the pI of camel caseins (pH~ 4.3) using HCl solution (1M) (Wangoh, Farah & Puhan, 1998; Felfoul et al., 2015). Camel acid coagulum was centrifuged at 5,000 g for 20 min at 20 °C using Beckman centrifuge CO-LE80K (Coulter, Fullerton, CA) and then, resolubilized in deionized water (provided from Milli-Q system Millipore, USA) at a volume equal to that of the discarded whey.

The pH value of camel caseins solution was adjusted to that of milk using 1M NaOH yielding camel Na-cas. The purity of extracted camel α-lactalbumin (~ 91.2%) and β-casein (~

81.5%) was verified by the reversed-phase high-performance liquid chromatography (RP-HPLC) (Yüksel & Erdem, 2010) (Fig. 3B).

2.4. Protein solution preparation

The different protein solutions were prepared by dissolving them in 20 mM Tris-HCl buffer, pH 7 (Atri et al., 2010). The pH value was chosen to approximately correspond to milk conditions, furthermore the adopted buffer is commonly used to control the pH in similar studies (Ibanoglu & Ibanoglu, 1999; Lajnaf, Picart-Palmade, Attia, Marchesseau & Ayadi, 2016).

In all samples the total amount of protein was 0.5 g L^{-1} for foaming properties and 11 mg L^{-1} for interfacial properties in agreement with previous works (Ibanoglu & Ibanoglu, 1999; Lajnaf et al., 2016). The same protein concentration was chosen in order to compare the foaming and interfacial properties of all camel and bovine samples studied.

The proteins fractions studied were:

- For Bo-M: skim milk, Na-cas, sweet whey, α-lactalbumin, β-casein and β-lactoglobulin
- For Ca-M: skim milk, Na-cas, sweet whey, α-lactalbumin and β-casein as Ca-M is devoid of β-lactoglobulin.

Skim Ca-M and Bo-M were used during foaming tests and interfacial studies with a pendant drop tensiometer. Indeed, previous studies reported that the milk fat had an adverse effect on foaming and interfacial properties of milk caused by its competitive adsorption with proteins on the interfacial regions as well as an inability to stabilize the air bubbles (Ho, Dhungana, Bhandari & Bansal, 2021; Nylander, Arnebrant, Cárdenas, Bos & Wilde, 2019).

2.5. RP-HPLC analysis

RP-HPLC (Agilent 1260 Infinity quaternary LC, Germany) was used to separate and identify the main proteins from camel and bovine milk-derived proteins (Yüksel & Erdem, 2010; Lajnaf et al., 2020 a). A C18 column (Zorbax Eclipse Plus C18, 250 mm length × 4.6

mm, particle size 5 µm, Packing Lot #: B14292) was used for protein separation. The analysis was performed using a Shimadzu SPD6A-UV detector measuring the optical density. All solutions were filtered through a nylon filter (47 mm, 0.45 µm, EG0492-1).

The chromatographic conditions were as follows: Solvent A: Acetonitrile, water and trifluoroacetic acid in a ratio of 100:900:1 (v/v/v); Solvent B: Acetonitrile, water and trifluoroacetic acid in a ratio of 900:100:1 (v/v/v). Total run time: 35 min; Column temperature: 25 °C. Flow rate: 1.0 mL min^{-1}. Detection wavelength: 220 nm. Injection volume of the final diluted sample: 20 µL. For the sample preparation, 500 µL of protein sample (skim milk, Na-cas, whey and purified camel proteins) were added to 3.7 mL of a solution consisting of solvents A and B in a 70:30 ratio (v/v). The sample-solvents mixture was vortexed for 10 s and then filtered through nylon filter (0.45 µm) before injection into the column. Once, the sample was injected, a gradient was generated immediately by increasing the proportion of solvent B from 20% at the beginning of the analysis to 46% at the end of the run.

Standard individual bovine proteins (β-casein, α_S-casein, κ-casein, β-lactoglobulin and α-lactalbumin) were provided from Sigma Aldrich. Individual pure standards were prepared by diluting proteins in solvent A and solvent B mixture (70:30, v/v), separately as camel and bovine milk proteins. Quantitative estimation of the main camel and bovine milk protein mixtures (skim milk, Na-cas and whey) was performed by calculating the peak area of each protein.

2.6. Foaming properties

Ten milliliters of camel or bovine protein solution (skim milk, Na-cas, whey and pure proteins β-casein, α-lactalbumin and β-lactoglobulin) at concentration of 0.5 g L^{-1} were poured into a measuring cylinder (length 8.5 cm and radius 2 cm) (Ibanoglu & Ibanoglu, 1999).

The protein solution was mixed using the Ultra-Turrax mixer (IKA Labortechnik, Staufen Germany) at a speed of 13,500 rpm for 2 min at room temperature (~20 °C) (Lajnaf et al., 2020 a).

After mixing, the volume of the foam was immediately read in the measuring cylinder and two parameters, foam capacity and stability, were calculated.

Foam Capacity (FC) is defined by Equation 1 (Eq. 1) as:

$$FC = (V_{foam}/V_0) \times 100; (\%) \quad (1)$$

where V_{foam} is the volume of the created foam at t=0 and V_0 the volume in the initial solution before whipping.

Foam Stability (FS) is defined as the foam half-time which is the time for drainage of the half of the foam created ($t_{foam1/2}$) (Marinova et al., 2009).

2.5. Interfacial properties

Dynamic surface tension measurements were performed using a pendant-drop tensiometer (IT Concept, Longessaigne, France). An axisymmetric air drop was created at the tip of the needle of a specific syringe dipped into the cuvette that contained the protein solution and driven by a computer. The images of the drop were taken by a camera and digitized.

The surface tension was calculated according to Laplace's equation (Eq. 2) and calculated by analyzing the drop's profile:

$$(1/x)[d(x \sin \theta)/dx] = (2/b) - cz \quad (2)$$

where :

- x and z : the cartesian coordinates at any point of the created drop profile,
- θ : the angle of the tangent to the drop profile
- b : the radius of curvature of the drop apex,
- c : is the capillary constant (equal to $g \Delta\rho/\gamma$, where $\Delta\rho$ is the difference between the densities of the two phases, g is the acceleration of gravity, and γ is the surface tension).

The sinusoidal changes of γ as a function of time are recorded and plotted by the control unit in order to measure the surface viscoelastic modulus (ε) which is defined by the equation 3 (Eq. 3):

$$|\varepsilon| = d\gamma / d \ln A, \quad (3)$$

where A is the surface area of the air drop.

The temperature-controlled chamber of the apparatus was adjusted to 20 ± 1 °C using water circulation from a thermostat.

The measurement of the surface tension (γ) and viscoelastic moduli (ε) were carried out at a concentration of 11 mg L^{-1} of proteins (for skim milk, Na-cas, whey and pure proteins β-casein, α-lactalbumin and β-lactoglobulin) after dilution using deionized water and for 3000 s (Cases et al., 2005; Ibanoglu & Ibanoglu, 1999). At this concentration (11 mg L^{-1}), the air-water interface was fully covered by the tested proteins and only a very small amount of protein remained in the bulk phase which is necessary for the clarity of the medium (Cases et al., 2005).

From the curves, the adsorption kinetic parameters were determined (Li et al., 2021): The adsorption rate of the protein at the air drop surface which is defined as the initial slope value of the surface tension curve ($AR = -d\gamma(t)/dt|_{t=0}$) (Lajnaf et al., 2017; Marinova et al., 2009). The measurements were done in triplicates.

2.6. Fluorescence spectroscopy

The surface characteristics for bovine and camel pure proteins (β-casein, α-lactalbumin and β-lactoglobulin) was determined by intrinsic fluorescence in order to compare proteins homology of both milk and to explain their interfacial behavior at the air-water interface under native conditions (pH 7.5, temperature 25°C) without any denaturing effect.

Intrinsic fluorescence was measured according to the method of Lam and Nickerson (2015b) at a constant excitation wavelength of 275 nm as a function of emission wavelength between 285 to 450 nm using spectrofluorometer (Aminco Bowman, Foster City, CA) and a 1 nm slit width. Intrinsic fluorescence experiments were performed for 5µM protein solutions for camel and bovine β-caseins in agreement with the work of Esmaili et al. (2011) carried out with camel β-casein and for 30 µM proteins solutions for pure whey proteins (α-lactalbumin and β-lactoglobulin) in agreement with the work of Zhang et al. (2014) which have been made with pure α-lactalbumin and β-lactoglobulin. By this technique, the fluorescence of the aromatic

amino acids was measured especially tyrosine, tryptophan and phenylalanine (Lam & Nickerson, 2015b). All intensity data was expressed as function of emission wavelength in arbitrary units (A.U).

2.7. Statistics

The significance of the main effects of the protein type (camel and bovine β-casein and α-lactalbumin; β-lactoglobulin) on RP-HPLC, foaming properties (FC and FS indices), interfacial properties (surface tension (γ) and viscoelastic modulus (ε)) and conformational state (intrinsic fluorescence) was tested by three-way analysis of variance (ANOVA). Statistical analyses were performed with IBM-SPSS software (Version 19). All experiments were carried out at least in triplicate and results were reported as mean ± one standard deviation.

3. Results and discussion

3.1. Protein analysis

Protein fractions derived from Ca-M and Bo-M were characterized by RP-HPLC (Fig. 2A and B). For bovine milk, five major peaks (with retention time (RT): 20 min, 24.9 min, 26.4 min, 27.5 min and 30.3 min) were detected and identified as κ-casein (~ 7.1%), α-casein (~ 24.6%), β-casein (~ 37.5%), α-lactalbumin (~ 4.7%) and β-lactoglobulin (~ 26.2%). Meanwhile, only four major protein peaks were identified in Ca-M (Fig. 2B). These peaks corresponded to α-casein (~ 28.5%), α-lactalbumin (~ 19.7%), protein fraction (F) (~ 1.4%) and β-casein (~ 50.4%) with RT of 20.4 min, 22.6 min, 26.1 min and 27.9 min, respectively.

Chromatograms showed that β-casein is the main protein of the colloidal fraction of Bo-M and Ca-M representing 48.7% and 55.8% of total bovine and camel Na-casein respectively, in agreement with the results of Davies and Law (1980) and Kappeler et al. (2003). In addition, camel β-casein exhibited the highest RT (~27.9 min) compared to other milk proteins and its bovine counterpart (RT~26.4 min). Thus, camel β-casein is suggested to be the most hydrophobic protein in camel milk with a higher hydrophobicity level compared to bovine β-

357 casein in agreement with Salami et al. (2011) and Lajnaf, Gharsallah, Attia and Ayadi (2021).
358 On the contrary, camel α-lactalbumin showed a lower RT (22.6 min) compared with its bovine
359 counterpart (27.5 min), suggesting a different molecular structure that has a lower surface
360 hydrophobicity. Bovine κ-casein represented 7% of total bovine Na-cas, while camel milk and
361 Na-cas chromatograms also showed that no peak was detected for κ-casein, probably due to its
362 very low concentration in Ca-M, making it masked by β- and α-caseins, in agreement with
363 Farah, Rettenmaier and Atkins (1992) and Lajnaf et al. (2020 a).

364 As expected, no peak corresponding to β-lactoglobulin was detected in Ca-M in
365 agreement with previous authors (Ereifej et al., 2011; Omar et al., 2016; Lajnaf et al., 2018).
366 On the other hand, β-lactoglobulin is the major protein of bovine whey followed by α-
367 lactalbumin representing ~60% and ~28% of the total whey proteins, respectively. Meanwhile,
368 camel α-lactalbumin was found to be the main protein in the camel whey accounting for 84%
369 of the total Ca-M and whey in agreement with Ereifej et al. (2011) and Lajnaf et al. (2018). In
370 Fig. 2A, it is possible to observe a peak of protein fraction in bovine whey with an RT of 20.7
371 min, which is identified as caseinomacropeptide (CMP) in agreement with the work of
372 Svanborg, Johansen, Abrahamsen, Schüller and Skeie (2016). Camel whey (Fig. 2B) also
373 contains a protein fraction (F) with an RT of 26.1 min which represents 10% of the total whey
374 proteins amounts. This protein is suggested to be identified as the CSA (Camel Serum
375 Albumin), PGRP (peptidoglycan recognition protein) or Lactoferrin in agreement with El-
376 Hatmi, Girardet, Gaillard, Yahyaoui and Attia (2007), Ereifej et al. (2011), Felfoul et al. (2015)
377 and Lajnaf et al. (2018).

378 ***3.2. Foaming properties***

379 Fig. 3A shows that skim milk, Na-cas and β-casein yielded better foam than whey protein
380 fractions (whey, β-lactoglobulin and α-lactalbumin), with higher FC values of camel proteins
381 reaching 111.5 ± 5.4% and 103.8 ± 5.4% for camel milk and Na-cas, respectively and 96.2 ±
382 5.5% and 80.7 ± 4.9% for Bo-M and Na-cas, respectively. Maximum foamability was obtained

383 with β-casein (FC= 126.9 ± 5.4% and 134.6 ± 3.8 % for camel and bovine β-caseins,
384 respectively) followed by milk and Na-cas regardless of milk origin.

385 Foaming results of bovine protein fractions are in agreement with those of Zhang,
386 Dalgleish and Goff (2004) who reported that β-casein is the most competitive protein among
387 all milk proteins as it is highly present in the foam phase. Capillary electrophoresis results
388 obtained by Zhang et al. (2004) showed that caseins were more enriched in the skim milk foam
389 phase, corresponding to foam floating on the top, than whey proteins (β-lactoglobulin and α-
390 lactalbumin), especially in β-casein with a concentration of 1.44 ± 0.06 mg mL^{-1} in the foam
391 phase leading to the highest foam Enrichment Ratio among all milk proteins (~2.80). While
392 whey proteins were less competitive at adsorbing to foam than caseins with Enrichment Ratios
393 of β-lactoglobulin and α-lactalbumin of 1.67 and 1.77, respectively (Zhang et al., 2004). Thus,
394 β-casein is considered as the most surface-active protein due to its relatively high
395 hydrophobicity and its unordered structure compared to other milk proteins. On the other hand,
396 whey proteins contain high amount of α-helix, β-sheet and intramolecular disulfide bonds.
397 Hence, more energy and longer time are needed to unfold the native structure of globular
398 proteins and to fully spread at the air-water interface compared with flexible proteins (Fox,
399 McSweeney & Paul, 1998; Zhang et al., 2004).

400 Lorient, Closs and Courthaudon (1991) noted that purified proteins (caseins or whey
401 proteins) are usually more surface active than mixture (whole casein or whey) from which they
402 were isolated. This behavior was explained by the competitive adsorption of different proteins
403 in the same mixture. Furthermore, Cayot, Courthaudon and Lorient (1991) observed an
404 heterogeneous association of α_{S1} and β caseins leading to the formation of a complex with a
405 higher ratio of α_{S1}-casein to β-casein. This complex was characterized by a greater stability than
406 that of α_{S1}-α_{S1} and β-β complexes and a lower number of remaining monomers leading to lower
407 efficiency in reducing the interfacial tension at the oil-water interface (Cayot et al., 1991;
408 Lorient et al., 1991). For camel proteins, Lajnaf et al. (2020 a) found greater foamability for

409 skim Ca-M (up to 165%) when compared to Bo-M (115 %) due to the difference in the protein
410 composition between both Ca-M and Bo-M and the highest β-casein content in Ca-M.
411 Furthermore, bovine whey showed higher foamability compared to camel whey at a
412 protein concentration of 0.5 g L^{-1} (FC = 50 ± 5.4 % and 70.0 ± 5.4 % for camel and bovine
413 whey proteins, respectively) with foaming behavior intermediate between those of β-
414 lactoglobulin and bovine α-lactalbumin alone. No significant difference was observed between
415 camel whey and camel α-lactalbumin (FC= 51.9 ± 1.9 % and 56.4 ± 4.4%) suggesting that
416 foaming proteins of camel whey are mostly maintained by the α-lactalbumin due to its highest
417 content in Ca-M in agreement with Lajnaf et al. (2018) and RP-HPLC results (section 3.1).
418 Similar results were also reported by Lajnaf et al. (2018) for camel and bovine sweet
419 wheys at a higher protein concentration (5 g L^{-1}). This previous work showed that sweet bovine
420 whey exhibited higher foaming capacity than sweet camel whey under native conditions.
421 However, this difference in the foaming behavior between camel and bovine wheys was no
422 longer observed after acidifying or heating the whey proteins at 70 °C and 90 °C for 30 min
423 (Lajnaf et al., 2018). For instance, in acidic conditions, the foamability was higher in camel
424 whey than its bovine counterpart because of the lack of the β-lactoglobulin and the dominance
425 of the α-lactalbumin in camel whey which is in the molten globular state with more active
426 surface than its native state. In acid bovine whey, the β-lactoglobulin forms aggregates with α-
427 lactalbumin leading to an antifoaming effect (Lajnaf et al., 2018).
428 The foaming stability (FS) values of pure proteins (β-lactoglobulin, β-casein and α-
429 lactalbumin) and naturally mixed proteins systems (skim milk, Na-cas and whey) at a
430 concentration of 0.5 g L^{-1} are given in Fig. 3B. The bovine proteins fractions (skim milk, Na-
431 cas and β-casein) gave the highest foams stability among all samples studied, reaching
432 approximately ~1000 s for bovine proteins and ~ 600 s for their camel counterparts.
433 The difference of FS between the bovine and camel β-casein can be mainly explained by
434 the different physico-chemical characteristics of the two counterparts. First, it is obvious that

435 camel β-casein is slightly larger than its bovine counterpart. It contains a higher number of
436 amino-acid residues with an identity of 67.2% with its bovine counterpart (Kappeler, 1998;
437 Barzegar et al., 2008) and a higher pI value. On the other hand, better emulsion stability of
438 bovine β-casein compared to camel β-casein was previously observed by Lajnaf et al. (2021)
439 despite its lower efficiency in reducing surface tension at oil-water interface.

440 Hence, the higher foam stability of bovine milk and Na-cas may be associated to the
441 greater ability of bovine β-casein to stabilize foams compared with camel β-casein.
442 Furthermore, Bo-M contains higher amounts of κ-casein (7.1%, RP-HPLC results) compared
443 to Ca-M, which could also explain the highest FS values of Bo-M. Indeed, Closs, Courthaudon
444 and Lorient (1990) noted that the stability of milk foams is maintained by κ-casein due to its
445 structured form compared with α-casein and β-casein.

446 Fig. 3B showed that FS values of caseinates in both Ca-M and Bo-M were significantly
447 higher than that of β-lactoglobulin (FS = 480 ± 45 s), whey (FS = 82.5 ± 10.6 s and 70 ± 35 s
448 for bovine and camel whey, respectively) and α-lactalbumin (FS = 31.6 ±12.5 s and 27.0 ± 1.41
449 s for bovine and camel α-lactalbumin, respectively). These results are consistent with Marinova
450 et al. (2009) who reported that casein adsorption layers are thicker and denser and can ensure
451 better foam stabilization. However, globular whey molecules cannot compact well to provide
452 the necessary stabilization of proteins films and foams away from their pI, even after adding
453 electrolytes or increasing the protein concentration. For example, α-lactalbumin is known as a
454 small protein with good foaming properties but with a relatively poor ability to stabilize the
455 created foam. This protein can migrate easily at the air-water interface due to its low MW (~14
456 kDa), while it is unable to ensure film protein consistency (Slack, Amundson & Hill, 1986).
457 The results showed that camel and bovine α-lactalbumin presented similar foaming and
458 stabilizing properties (Fig.3 A and B). This behavior can be explained by similar physico-
459 chemical characteristics and the same number of amino-acid residues (123 residues).

460 Competitive adsorption of proteins to the air-interface in an aqueous foam is affected not
461 only by the physicochemical properties of proteins, but also by other factors such the viscosity,
462 the solubility, the presence of lactose, fat and minerals and by the processing history. For
463 instance, the presence of lactose in camel and bovine skim milk and sweet whey could present
464 a great water holding capacity which contributes to an increase in the viscosity of the protein
465 solution leading to a higher foam stability (Gamboa & Barraquio, 2012). Furthermore, the
466 presence of minerals in camel and bovine proteins fractions has an indirect effect on the foaming
467 properties of proteins as it has significant impact on their conformations, their stability, and
468 their state of distribution between the colloidal and serum phases of milk. The presence of
469 calcium (10-20 mmol L^{-1}) in reconstituted skim milk proteins was found to improve their
470 foaming capacity whereas it reduced the ability of proteins to stabilize foams (Ho, Bhandari &
471 Bansal, 2021; Zayas, 1997). This could explain the lower foaming stability which is observed
472 for camel and bovine wheys (Fig. 3B). The destructive effects of milk fat on the foaming
473 properties of milks have been reported even at low amounts. Indeed, the foamability of milk
474 was found to significantly decrease with an increase in the fat contents from 0 to 1.5% (w/w).
475 The processing history also affects the foaming properties of camel and bovine derived protein
476 fractions. For camel proteins, the purification methods (ultrafiltration for camel α-lactalbumin
477 and cold solubilization for camel β-casein) led to obtain camel proteins in their native form
478 without denaturation (Huppertz et al., 2006; Salami et al., 2009). However, Na-cas proteins
479 were extracted using caseins acid precipitation at pH below pI and solubilization at neutral pH
480 (6.7 and 6.5 for bovine and camel Na-cas, respectively). Zhang et al. (2004) noted that caseins
481 re-solubilization after their precipitation leads to the recovery of their foamability reaching
482 maximal values. This behavior may explain the difference in foaming properties between
483 extracted Na-cas and skim milk in Bo-M and Ca-M (Fig. 3A and B).

484 ***3.3. Surface tension***

485 Surface tension for pure bovine and camel protein fractions at the air-water interface are
486 shown in Fig. 4A and B, respectively. Surface tension measurements were carried out at the
487 same protein concentration (11 mg L^{-1}) and at pH 7. Surface tension ($\gamma(t)$) is a key parameter in
488 bubble formation. Hence, the foaming properties of milk proteins are determined by their rate
489 of diffusion and adsorption to the interface (Borcherding et al., 2008).
490 First, Fig. 4 shows that changes in $\gamma(t)$ developed by proteins adsorption at the air-water
491 could be divided into two main different stages as reported by Cases et al. (2005): a rapid
492 decrease of the surface tension value during the first 500 s followed by its stabilization (up to
493 3000 s). Furthermore, all surface tension curves start from the initial value of 72.8 ± 0.5 mN m^{-1},
494 which is estimated to be the surface tension of pure water in agreement with Tamm et al.
495 (2012).
496 At t = 3000 s, Fig. 4A shows that the order of effectiveness for bovine proteins was:
497 bovine Na-cas (γ = 47.1 ± 0.1 mN m^{-1}) > bovine β-casein (γ = 48.8 ± 0.5 mN m^{-1}) = Bo-M (γ
498 = 48.9 ± 0.1 mN m^{-1}) > β-lactoglobulin (γ = 52.9 ± 0.1 mN m^{-1}) > bovine whey (γ = 55.8 ±
499 1.1 mN m^{-1}) > bovine α-lactalbumin (γ = 58.2 ± 0.6) ($p < 0.05$). Furthermore, as shown in Fig.
500 4B, the order of effectiveness for camel proteins fractions at t = 3000 s was: camel β-casein
501 (44.9 ± 0.5 mN m^{-1}) > camel Na-cas (47.6 ± 0.5 mN m^{-1}) = Ca-M (48.1 ± 0.2 mN m^{-1}) > camel
502 whey (50.9 ± 0.1 mN m^{-1}) = camel α-lactalbumin (49.8 ± 0.6 mN m^{-1}) ($p < 0.05$).
503 Table 2 illustrates the rate of adsorption (AR) corresponding to the initial slopes of the
504 surface tension curves, ($AR = -d\gamma(t)/dt|_{t=0}$, see Fig. 4) in order to characterize the decrease of
505 the surface tension rate when a new air-water surface is created during the foaming process.
506 Very good correlation is observed between FC and R values: camel and bovine β-caseins carried
507 the highest AR values regardless of milk origin (AR= 0.275 ± 0.003 mN m^{-1} s^{-1} and 0.362 ±

508 0.023 mN m^{-1} s^{-1} for bovine and camel β-caseins respectively). Besides, Table 2 shows that
509 skim milk and Na-cas exhibited higher AR values than those of whey and its derived proteins
510 (β-lactoglobulin and α-lactalbumin) reaching AR values of 0.252 ± 0.005 mN m^{-1} s^{-1} and 0.300
511 ± 0.012 mN m^{-1} s^{-1} for bovine and camel Na-cas, respectively in agreement with previous
512 foaming results (Foam studies, Section 3.2). Besides, β-lactoglobulin and bovine α-lactalbumin
513 were better adsorbed at the air drop interface than bovine whey (AR= 0.153 ± 0.003 mN m^{-1} s^{-1}, 0.185 ± 0.01 mN m^{-1} s^{-1} and ~~1~~0.178 ± 0.005 mN m^{-1} s^{-1} for bovine whey, β-lactoglobulin and
514 α-lactalbumin, respectively). However, no significant difference was found between camel
515 whey and α-lactalbumin (AR ~0.170 mN m^{-1} s^{-1}).
516
517 Thus, globular whey proteins (camel and bovine α-lactalbumin, whey and β-
518 lactoglobulin) were characterized by a lower efficiency in reducing surface tension at the air-
519 water interface and lower AR values compared with skim milk and the casein fraction (Na-cas
520 and β-casein), regardless of milk origin. These findings are consistent with those of Mellema
521 and Isenbart (2004) who reported that skim milk proteins give lower final surface tension values
522 when compared with whey proteins in the concentration range 1.4-2.8% (w/w) due to the co-
523 adsorption of casein micelles and whey proteins in skim milk leading to lower surface tension
524 values. Molecular structure also plays a key role in the adsorption of milk proteins at the air-
525 water interface. β-casein is more efficient in reducing the surface tension at concentrations of 1
526 g L^{-1} compared with β-lactoglobulin. This behavior was explained by the flexible molecular
527 structure of β-casein in solution which allows easy and rapid reduction of the surface tension
528 over the first minutes compared to β-lactoglobulin (Seta et al., 2014).
529 The β-lactoglobulin is characterized by an ordered secondary structure as well as a
530 compact tertiary structure. At the pH studied, this protein exists in a dimer linked by non-
531 covalent interactions, and each β-lactoglobulin monomer contains two intramolecular disulfide
532 bridges and a hidden free thiol group. Hence, β-lactoglobulin was not fully unfolded at the air-

533 water interface and the rate of the surface tension decrease was lower than that of β-casein,
534 which is considered as a mobile disordered milk protein (Cases et al., 2005).

535 Bovine whey was characterized by a surface tension behavior intermediate between those
536 of the main whey proteins: β-lactoglobulin and bovine α-lactalbumin alone. On the other hand,
537 the evolution of the surface tension curves of camel whey and camel α-lactalbumin are very
538 similar, in agreement with previous foaming results (Section 3.2). Many studies describe the
539 interfacial properties of bovine whey proteins (Lam & Nickerson, 2015; Mellema & Isenbart,
540 2004; Zhou et al., 2020 a; Zhou et al., 2020 b). Similarly, Zhang et al. (2004) noted that β-
541 lactoglobulin is preferentially adsorbed over α-lactalbumin, in the foam phase at pH values
542 above neutrality, due to the changes in conformation and quaternary structure of whey proteins
543 with pH.

544 Overall, the comparison between bovine protein fractions revealed that skim Bo-M, Na-
545 cas and β-casein exhibited a similar interfacial behavior. However, bovine Na-cas presented the
546 lowest final surface tension value at t = 3000 s ($\gamma = 47.1 \pm 0.1$ mN m^{-1}) when compared to that
547 of bovine skim milk and β-casein (~ 48.8 mN m^{-1}). It is then suggested that the interfacial
548 behavior of Bo-M is divided into two main stages: an initial adsorption state occurring during
549 the first 30 min where the surface tension values are mainly dominated by the presence β-casein,
550 followed by the surface tension value stabilization state where the effect of β-casein on lowering
551 the surface tension is amplified by ageing of the protein layer and relaxation processes at the
552 interface, protein-protein interactions and re-arrangements of protein species leading to lower
553 energy states. In addition, Cases et al. (2005) reported a further increase in rate of interfacial
554 tension γ(t) of milk proteins at the oil-water interface and interpreted this behavior as greater
555 exchangeability between the adsorbed casein molecules due to enhanced flexibility and
556 exposure of their hydrophobic residues. On the other hand, Zhou et al. (2020 b) noted that the
557 final stage of the surface stabilization involves continued reorientation of adsorbed protein layer
558 to get a more energetically favorable conformation. Thus, the rate of interfacial pressure

559 increases slowly during this phase, reaching an equilibrium state when the monolayer was
560 saturated.

561 For Ca-M, different mechanisms can be suggested: camel sweet whey and α-lactalbumin
562 exhibited similar interfacial behavior, suggesting that the interfacial and foaming behavior of
563 camel whey is maintained by camel α-lactalbumin, representing 84% of the total camel whey
564 proteins (RP-HPLC results, section 3.1). Hence, the absence of β-lactoglobulin in camel whey
565 allows camel α-lactalbumin to adsorb more easily and rapidly at the interface (Laleye, Jobe &
566 Wasesa, 2008).

567 On the other hand, camel Na-cas milk has an intermediate interfacial behavior between
568 those of skim milk and camel β-casein. These results lead to the finding that β-casein has the
569 main role in the creation of Ca-M foams at neutral pH. This protein was more surface-active
570 compared to globular proteins (whey and camel α-lactalbumin) and whole caseins. This is
571 consistent with the results of Lorient et al. (1991) who reported that purified caseins are often
572 more surface-active than casein mixture systems. Dickinson (1989) noted that an equimolar
573 mixture of β- and α_{S1}-caseins, β-casein is the first protein adsorbed; it diffuses rapidly to the
574 interface and remains predominant. A similar behavior was observed by Lorient et al. (1989)
575 who demonstrated the preferential adsorption of β-casein over other caseins (α- and κ-caseins)
576 and caseins over whey proteins.

577 For Ca-M, Lajnaf et al. (2016) found that mixtures with a higher camel β-casein amount
578 are more efficient in reducing the surface tension at the air-water interface. Camel β-casein was
579 characterized by higher tensioactive properties at the air-water interface compared to its bovine
580 counterpart due to its different amino-acid residue composition and higher hydrophobicity in
581 agreement with the different RT between camel and bovine β-caseins (section 3.1). Lajnaf et
582 al. (2021) showed higher surface hydrophobicity and efficiency in reducing interfacial tension
583 at oil-water interface of the camel β-casein than bovine β-casein at both pH levels 7 and 9.

584 ***3.4. Viscoelastic modulus***

585 The viscoelastic modulus was also used to determine the rheological properties of camel
586 and bovine proteins fractions at air-water interface (Fig. 5). As the surface tension γ(t) reflects
587 the surface activity and flexibility of the protein molecule, the viscoelastic modulus reflects the
588 rigidity of the film protein created at the interface (Cases et al., 2005).
589 Fig. 5A and B show the variation of the viscoelastic modulus ε(t) of the protein film
590 developed by the bovine and camel protein systems studied (skim milk, Na-cas, whey, β-casein,
591 α-lactalbumin and β-lactoglobulin) as a function of time during 3000 s and at 20 °C.
592 The magnitude of ε(t) values varied significantly with protein type and milk origin. For
593 Bo-M and Ca-M, Fig. 5A and B show that using skim milk, Na-cas and β-casein led
594 immediately to the final and lowest ε value (ε~13 mN m^{-1}) from t = 500 s compared with the
595 other protein fractions (whey, α-lactalbumin and β-lactoglobulin). For instance, the order of
596 effectiveness in the creation of the most rigid surface film by Bo-M proteins at t = 3000 s was
597 (Fig. 5A) : β-lactoglobulin (ε = 50.3 ± 1.7 mN m^{-1}) > bovine whey (ε = 45.6 ± 0.5 mN m^{-1}) >
598 bovine α-lactalbumin (ε = 37.3 ± 2.1 mN m^{-1}) > bovine β-casein (ε = 13.3 ± 1.7 mN m^{-1}) =
599 skim Bo-M (ε = 12.7 ± 1.5 mN m^{-1}) = bovine Na-cas (ε = 12.5 ± 1.1 mN m^{-1}). While for Ca-
600 M proteins, Fig. 5B shows that the order of efficiency t = 3000 s was camel α-lactalbumin (ε =
601 23.2 ± 1.5 mN m^{-1}) = camel whey (ε = 20.5 ± 1.5 mN m^{-1}) > camel β-casein (ε = 13.7 ± 1.1
602 mN m^{-1}) = camel Na-cas (ε = 12.9 ± 1.1 mN m^{-1}) = skim Ca-M (ε = 12.8 ± 1.5 mN m^{-1}).
603 Thus, findings indicated that skim milk, Na-cas and β-casein have the lowest viscoelastic
604 modulus values compared with globular proteins (whey, α-lactalbumin and β-lactoglobulin),
605 regardless of the origin of the milk (Fig. 5). Dilatational rheology plays an important role in the
606 stability of foams and emulsions and it is a very sensitive technique to monitor the interfacial
607 behavior and the competitive adsorption of proteins. Chen et al. (1993) and Bos and Van Vliet

608 (2001) reported a direct relationship between the surface rheology of β-lactoglobulin at the oil-
609 water interface and the stability of the oil-in-water emulsions created by this proteins. On the
610 other hand, it has been separately reported that the heating of β-lactoglobulin at the oil-water
611 interface significantly enhanced the surface viscoelasticity of the adsorbed layer leading to a
612 higher interfacial shear viscosity. Meanwhile, good correlation was observed with the
613 adsorption and the rheological properties of Tween 20 and β-lactoglobulin mixtures at the air-
614 water. Indeed, a reduction in the dilatational modulus and an increase in foam stability were
615 simultaneously observed at a constant protein concentration of 0.2% (w/w) (Clark et al., 1995;
616 Dickinson & Hong, 1994). Whey proteins are adsorbed in two steps, the first representing
617 protein adsorption and the second representing rearrangement and unfolding of whey proteins
618 (Mellema & Isenbart, 2004; Cases et al., 2005; Seta et al., 2014). Cases et al. (2005) noted that
619 the highly viscoelastic character of globular proteins such as β-lactoglobulin at the interface is
620 attributed to the high packing density and strong protein-protein connections. Once adsorbed,
621 the β-lactoglobulin is partially unfolded which allows the exposure of the sulfhydryl group
622 leading to polymerization of the protein through the exchange between sulfhydryl and disulfide
623 groups in the adsorbed protein layer (Cases et al., 2005).

624 Sweet bovine whey exhibited a viscoelastic modulus intermediate between that of β-
625 lactoglobulin and α-lactalbumin, suggesting that the adsorbed film protein of bovine whey at
626 the air-water interface consist of both of β-lactoglobulin and α-lactalbumin. These findings are
627 highly consistent with Marinova et al. (2009) who noted that the adsorbed layer of whey protein
628 isolate at the air-water interface cannot be modeled with a single protein. These authors
629 suggested that the film protein created is composed of an average of whey proteins, including
630 β-lactoglobulin and α-lactalbumin. On the other hand, Zhang et al. (2004) have shown that there
631 was preferential adsorption of β-lactoglobulin over α-lactalbumin in the foam phase made with
632 whey protein isolate at neutral pH values in contrast to acidic pH levels where α-lactalbumin is
633 more dominant at the interface than the β-lactoglobulin. Hence, this led to the conclusion that

634 the adsorbed proteins from bovine sweet whey are composed of β-lactoglobulin dimers and α-
635 lactalbumin monomers with preferential adsorption of the β-lactoglobulin dimers which
636 probably interacts with the interface.

637 Findings also indicated that bovine whey and α-lactalbumin show significantly higher
638 viscoelastic modulus values compared to those of camel whey and α-lactalbumin. This behavior
639 can be explained by a different amino-acid composition (identity level of 69.1%) as well as by
640 a different adsorption behavior of bovine and camel α-lactalbumin, despite their similar
641 efficiency in reducing the surface tension at the air-water interface, as confirmed by the
642 different RT value in RP-HPLC chromatograms (section 3.1). Overall, the increase in
643 viscoelastic modulus values is observed with the decrease in protein flexibility (Seta et al.,
644 2014). Williams and Prins (1996) noted that the proteins which can adsorb and rearrange
645 quickly at the interfaces are expected to yield lower dilatational moduli. On the other hand, the
646 structural characterization revealed that both camel and bovine α-lactalbumin displayed a
647 compact globular structure with a more disordered structure for camel α-lactalbumin
648 (Redington, Breydo, Almehdar, Redwan & Uversky, 2016). Therefore, the lower viscoelastic
649 values of camel α-lactalbumin can be attributed to a less rigid and cohesive interfacial film
650 compared with that of bovine α-lactalbumin, despite the similar shape of γ(t) curve of both
651 proteins.

652 The comparison between the ε(t) curves of whey and α-lactalbumin from camel milk
653 fractions (Fig. 5B) revealed that camel whey exhibited similar rheological properties suggesting
654 the creation of an α-lactalbumin interfacial film. According to Cases et al. (2005), the increase
655 in the first stage of ε(t) is attributed to the protein rearrangement which occurs rapidly when the
656 surfactant used is β-casein leading directly to the final viscoelastic modulus value. From these
657 results, it was concluded that the viscoelastic modulus values of camel whey system are mainly
658 dominated by the presence α-lactalbumin, while the viscoelastic modulus values of skim camel

659 milk system is dominated by caseins, especially β-casein which remains mainly at the air-water
660 interface.

661 Therefore, the adsorption layers of Bo-M proteins can be modeled as follows : β-casein
662 polypeptide is the first adsorbed as inner adjacent layer at the air-water interface in a "train"
663 and outer layer extending into the aqueous phase as a "tail" or "loop" (Dickinson, Horne, Phipps
664 & Richardson, 1993) following by adsorption of β-lactoglobulin dimers and α-lactalbumin
665 monomers with preferential adsorption of the β-lactoglobulin dimers resulting in an increased
666 rigidity of the surface film (Marinova et al., 2009).

667 The modeling of the protein adsorption layers of Ca-M proteins shows some differences
668 since β-lactoglobulin is totally absent. First, camel β-casein is adsorbed as train–loop–tail model
669 as observed for bovine β-casein. Afterwards, camel α-lactalbumin monomers are adsorbed
670 leading to an increased the stiffness of the film created, but ultimately leading to a less rigid
671 film compared to that of Bo-M (low viscoelastic modulus). Indeed, the adsorbed β-
672 lactoglobulin molecules exert a greater effect on the surface pressure, which is the difference
673 between the surface tensions of the protein solution and the pure solvent, than the adsorbed α-
674 lactalbumin leading to higher pseudo-equilibrium surface pressure (Paulsson & Dejmek, 1992).
675 Jara, Carrera Sánchez, Patino and Pilosof (2014) reported that β-lactoglobulin shows a higher
676 degree of denaturation once adsorbed at the interface with irreversible conformational changes,
677 while α-lactalbumin is characterized by reversible denaturation upon adsorption at the air-water
678 interface without breaking buried disulfide bonds (Razumovsky & Damodaran, 1999).

679 Finally, the results of the interfacial rheology revealed a relationship between dilatational
680 rheological parameters and foaming properties of camel and bovine protein fractions. For both
681 milk samples : globular whey proteins (whey, α-lactalbumin and β-lactoglobulin) exhibited the
682 highest interfacial viscoelastic modulus values and the lowest ability to stabilize foams (section
683 3.2) leading to suggest that the extent of protein rigidity made the molecular re-conformation
684 more difficult but the resulting surface viscoelasticity was higher in agreement with the findings

of Cases et al. (2005). On the contrary, skim milk and flexible caseins (Na-cas and β-casein) had the lowest viscoelastic modulus values and the highest rate of adsorption. Based on this result, it can be concluded that the extent of protein flexibility is higher (skim milk, Na-cas and β-casein) the molecular re-conformation at the air-water interface easier, leading to weaker surface viscoelasticity.

3.5. Protein conformational state

The protein conformation state of bovine and camel pure proteins (β-casein, α-lactalbumin and β-lactoglobulin) was determined by measuring the intrinsic fluorescence of exposed hydrophobic amino acid residues which are tryptophan, tyrosine and phenylalanine without adding extra reagent to the protein solution. The fluorescence emission spectra of the pure β-caseins (camel and bovine β-casein) and whey proteins (camel and bovine α-lactalbumin and β-lactoglobulin) are shown in Fig. 6 A and B, respectively.

Fig. 6A shows that the fluorescence emission spectra of the bovine β-casein display a peak at 345,13 nm due to the presence of a single tryptophan of individual β-casein which is located in its hydrophobic fragment in agreement with the findings of Bahri, Henriquet, Pugnière, Marchesseau and Chevalier-Lucia (2019) and Yin et al. (2022). Overall, the protein excitation at 280 nm caused the emission of mainly tryptophan residues and, in aqueous solution, the emission maximum wavelength of free tryptophan is close to 350 nm. On the other hand, the excitation at 275 nm and 260 nm caused the emulsion of tyrosine and phenylalanine residues, reaching emission maximum wavelengths (λ_{max}) of 303 nm and 280 nm respectively (Yang et al., 2017). Bahri et al. (2019) noted that the lower λ_{max} of the pure β-casein compared to that of the free tryptophan is attributed to the apolar environment location of this residue.

A different fluorescence emission spectrum was observed for the pure camel β-casein when compared to its bovine counterpart with a peak at 303.1 nm suggesting a different molecular structure between these homologous proteins. Indeed, the aminoacid sequence of

710 camel β-casein is totally deficient in tryptophan with a higher aminoacid residues of tyrosine.
711 Salmen, Abu-Tarboush, Al-Saleh and Metwalli (2012) reported that camel β-casein contains
712 greater tyrosine residues whose percentage (4.77%) is significantly higher when compared to
713 bovine β-casein (2.92%). Hence, the excitation of camel β-casein at 275 nm caused the emission
714 of tyrosine residues whose λ_{max} is close to 303.1 nm with a higher intensity than that of the
715 bovine β-casein (fluorescent intensity 159.82 A.U and 215.57 A.U for bovine and camel β-
716 caseins, respectively) suggesting a higher hydrophobicity of camel β-casein in agreement with
717 the findings of Ellouze, Vial, Attia and Ayadi (2021), Esmaili et al. (2011) and Lam and
718 Nickerson (2015b). Indeed, Esmaili et al. (2011) and Ellouze et al. (2021) reported that
719 fluorescence intensity of a protein is mainly due to tryptophan residues.
720 The *in silico* prediction of surface hydrophobicity index of camel β-casein is -0.339 is
721 obtained from on ExPASy SIB Bioinformatics Resources Portal (Gasteiger et al., 2005), which
722 is the highest hydrophobicity among camel caseins (Salami et al., 2011). Therefore, the
723 hydrophobicity index of bovine β-casein is -0.355 (Gasteiger et al., 2005), which explain a
724 higher hydrophobicity level compared to camel β-casein and according to the hydropathy scale
725 of Kyte and Doolittle (1982). The difference in hydrophobicity index is mainly due to the
726 difference in the amino-acid composition of the two β-caseins (identity level of 67.2%). Camel
727 β-casein contains 5 tyrosine and 10 phenylalanine residues, which are mainly located in the
728 hydrophobic part of its primary structure. It is devoid of tryptophan comparing to bovine β-
729 casein which contains 1 tryptophan but lacks of tyrosine and phenylalanine residues leading to
730 a higher emulsifying properties of camel β-casein compared to its bovine counterpart especially
731 at pH 9 and 3 (Ellouze et al., 2021). These findings are in agreement with the interfacial tension
732 results (Section 3.4) as follows: the different structural characteristics as well as a highest
733 hydrophobicity could explain the highest tensioactive properties of camel β-casein when
734 compared to bovine β-casein and its ability to lower the interfacial tension at the air-water and
735 to stabilize milk foams.

Fig. 6B shows the fluorescence emission spectra of the studied whey proteins including bovine β-lactoglobulin and α-lactalbumin as well as camel α-lactalbumin. First, fluorescence emission spectra of β-lactoglobulin display a peak at 334.80 nm as shown in Fig. 6B. These findings are in agreement with those of Kong et al. (2020). These authors noted that the λ_{max} of the pure bovine β-lactoglobulin is of 334 nm after an excitation of tryptophan residues at 280 nm (Kong et al., 2020). These authors have attributed the intrinsic fluorescence of β-lactoglobulin to the residues tryptophan-19 which is located in a hydrophobic pocket and well-protected as well as tryptophan-61 which is completely exposed to the solvent (Kong et al., 2020).

On the other hand, fluorescence emission spectra of bovine α-lactalbumin display a peak at 320.92 nm as shown in Fig. 6B in agreement with the results of Diao et al. (2021). These authors noted that the emission maximum of the bovine α-lactalbumin was 321 nm (excitation at 280 nm). Thus, this protein fluoresces due to the presence of four tryptophan residues including tryptophan-26, tryptophan-60, tryptophan-104, tryptophan-118 (Diao et al., 2021). Fig.6B shows a higher fluorescent intensity for β-lactalbumin than for bovine α-lactalbumin under the same conditions of pH and protein concentrations despite the presence of four and two tryptophan residues in the protein sequences of α-lactalbumin and β-lactoglobulin, respectively. This behavior indicated that the β-lactoglobulin displayed a greater hydrophobic structure with more exposing hydrophobic residues especially the tryptophan-61 in agreement with Lam and Nickerson (2015a).

Fig. 6B shows that fluorescence emission spectra of camel α-lactalbumin were different when compared to those of its bovine counterpart with a lower λ_{max} value of 304.25 nm and a lower fluorescence intensity suggesting different molecular structure of both proteins and lower hydrophobicity of the camel α-lactalbumin. These results are in great consistance with those of Ellouze et al. (2019) and Ellouze et al. (2020). These authors reported that camel α-lactalbumin exhibits a lower fluorescent intensity compared to its bovine counterpart regardless of the

762 denaturing conditions of pH (3.0, 6.0 and 9.0) and heating temperatures (25 °C, 65 °C and
763 95°C). The primary structure of camel α-lactalbumin contains 5 tryptophan, 4 phenylalanine
764 and 3 tyrosine, while its bovine counterpart contains 4 tryptophan, 4 phenylalanine and 4
765 tyrosine. However, Redington et al. (2016) reported that tryptophan residues in bovine α-
766 lactalbumin are more solvent accessible than those of camel protein leading to different
767 fluorescence spectra. This behavior could explain the different interfacial properties between
768 pure camel and bovine α-lactalbumin and the higher viscoelastic modulus of bovine α-
769 lactalbumin compared to its camel counterpart.

770 **4. Conclusion**

771 The results obtained in this work indicate that skim milk, sodium caseinates and β-casein
772 exhibited the highest foaming and stabilizing properties when compared to globular whey
773 proteins (whey, α-lactalbumin and β-lactoglobulin fractions). The maximum foamability was
774 observed with camel milk proteins fractions especially camel Na-cas and β-casein while bovine
775 proteins gave the highest foam stability with higher FS values for bovine β-casein. Caseins and
776 whey proteins adsorb competitively upon milk foaming in camel and bovine milk. This
777 adsorption is followed by the proteins rearrangements and interactions leading to the creation
778 and the stability of the foam film. The study of the interfacial behavior of skim Bo-M and Ca-
779 M at the air-water interface has revealed that skim milk was almost as surface active as Na-cas
780 and β-casein in terms of the final lowering of the surface tension value, the adsorption rate and
781 the viscoelastic modulus. However, globular whey proteins exhibited the highest interfacial
782 viscoelastic modulus values and the lowest ability to reduce the surface tension and to stabilize
783 foams. This behavior confirms that the extent of protein rigidity made the molecular
784 reconformation more difficult but the resulting surface viscoelasticity higher contrary to
785 caseins.

Camel and bovine β-casein presented similar foamability with a more efficient reduction of surface tension at the air-water interface for the camel β-casein and a better foam stability for the bovine β-casein due to their different molecular structure (identity level of 67.2%) and a higher hydrophobic structure for camel β-casein. Thus, intrinsic fluorescence which was performed in order to measure the fluorescence of the aromatic amino acids including tyrosine, tryptophan and phenylalanine and to reveal the different structural characteristics between pure proteins, showed that camel and bovine β-casein display different fluorescence emission spectra with a higher fluorescence intensity for camel β-casein. It is due the higher tyrosine residues despite the absence of tryptophan in its primary structure comparing to bovine β-casein leading to a higher tensioactive properties of camel β-casein compared to its bovine counterpart.

For whey proteins, camel and bovine α-lactalbumin have an identity level of 69.1% and show similar foaming and stabilizing properties, with the creation of a stiffer surface film at the air-water interface for the bovine α-lactalbumin. Thus, due to the absence of β-lactoglobulin in Ca-M, camel α-lactalbumin increases the stiffness of the protein films in Ca-M foam with lower viscoelastic modulus and weaker rheological properties compared to Bo-M proteins. Fluorescence emission spectra of camel α-lactalbumin were different when compared to those of its bovine counterpart with a lower λ_{max} value and a lower fluorescence intensity suggesting different molecular structure of both proteins and lower hydrophobicity of the camel α-lactalbumin. This may explain the lower viscoelastic modulus of camel α-lactalbumin compared to its bovine counterpart and hence, the lower FS values of Ca-M compared to Bo-M.

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Figure captions

Fig. 1. Flow diagram of the camel milk protein isolation procedure. Abbreviations are: Na-cas, sodium caseinates; β-CN, β-casein; and α-La, α-lactalbumin.

Fig. 2. RP-HPLC chromatograms recorded at 220 nm for bovine and camel protein fractions (chromatograms A and B, respectively). Abbreviations are: Na-cas, sodium caseinates; β-CN, β-casein; α-La, α-lactalbumin; β-Lg, β-lactoglobulin. F, protein fraction; CMP: caseinomacropeptide.

Fig. 3. Foam capacity (A) and Foam Stability (B) of camel and bovine skim milk (SM), sodium caseinates (Na-cas), β-casein (β-CN), whey, β-lactoglobulin (β-Lg) and α-lactalbumin (α-La). The experiments were performed in 20 mM Tris-HCl buffer, pH 7.0, at 25 °C at a protein concentration of 0.5 g L^{-1}.

[a-f] Samples represented with different letters are significantly different from each other ($p<0.05$). Error bars show the standard deviations of mean values of foam capacity and stability.

Fig. 4. Time-dependent changes in surface tension γ(t) (mN m^{-1}) at air-water interface of bovine(A) and camel (B) proteins systems: Skim bovine milk (skim Bo-M), skim camel milk (skim Ca-M), sodium caseinates (Na-cas), whey, α-lactalbumin (α-La), β-casein (β-CN) and β-lactoglobulin (β-Lg), at a concentration of 11 mg L^{-1}, pH 7 and temperature 20 °C.

Fig. 5. Time-dependent changes in viscoelastic modulus ε(t) (mN m^{-1}) at air-water interface of bovine (A) and camel (B) proteins systems: Skim bovine milk (skim Bo-M), skim camel milk (skim Ca-M), sodium caseinates (Na-cas), whey, α-lactalbumin (α-La), β-casein (β-CN) and β-lactoglobulin (β-Lg), at a concentration of 11 mg L^{-1}, pH 7 and temperature 20 °C.

Fig. 6. Intrinsic fluorescence intensity of bovine and camel β-caseins (protein concentration 5μM) (A) and pure whey proteins: β-lactalbumin and camel and bovine α-lactalbumin (protein concentration 30μM) (B) at a constant excitation wavelength of 275 nm as a function of emission wavelength between 285 to 450 nm (temperature 25°C).

Figures:

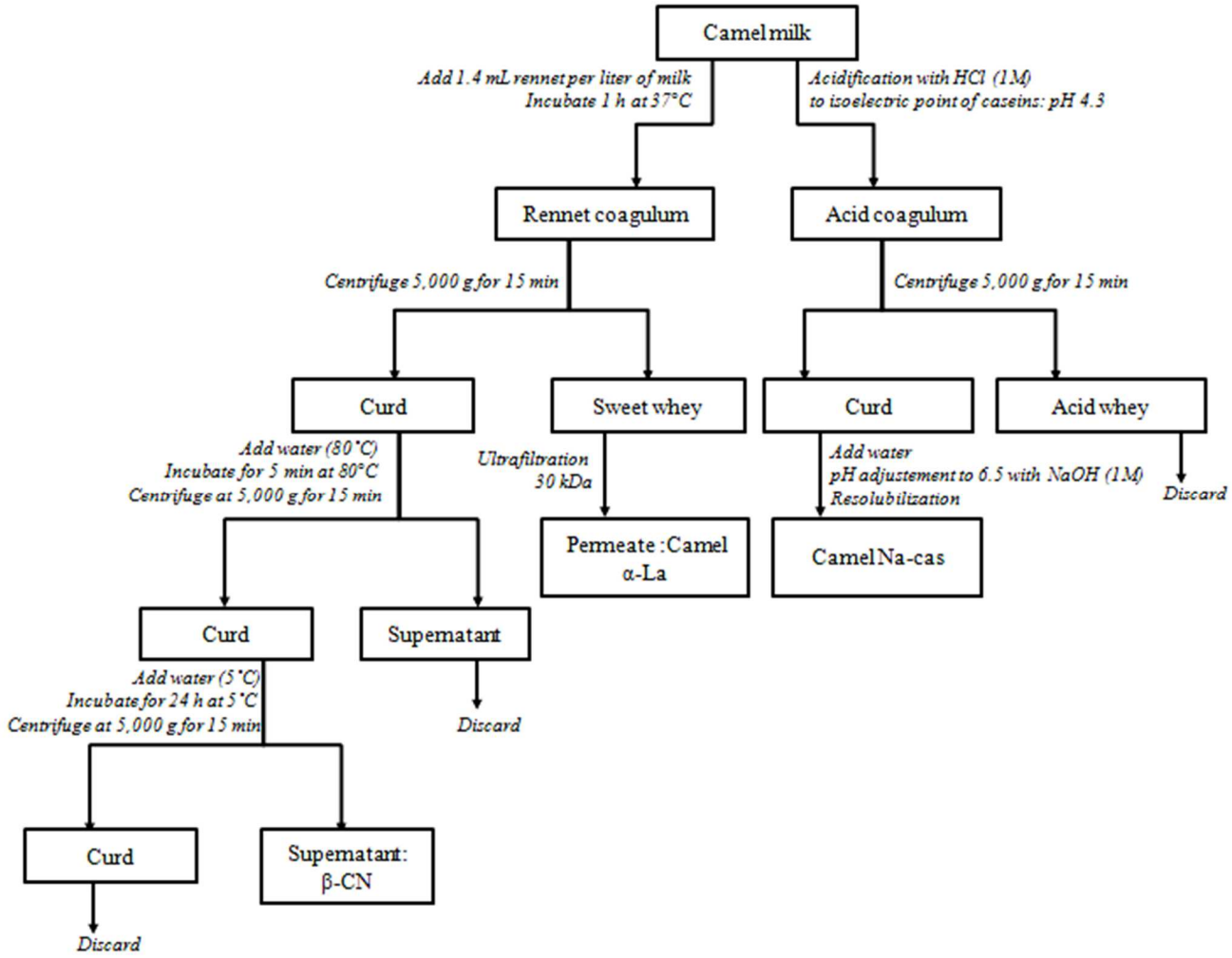
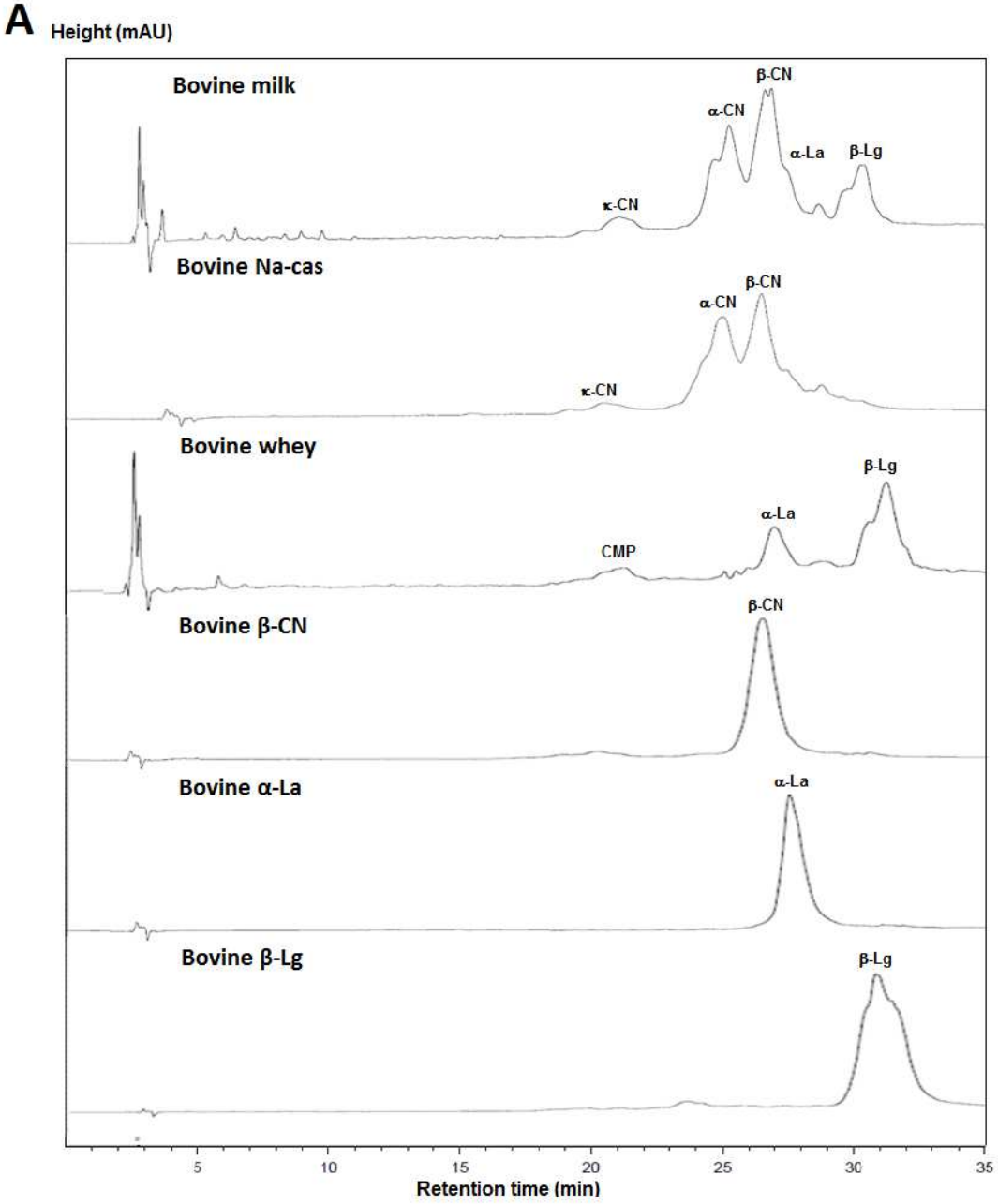


Fig. 1


A
Height (mAU)
Bovine milk
κ-CN
α-CN
β-CN
α-La
β-Lg
Bovine Na-cas
κ-CN
α-CN
β-CN
Bovine whey
CMP
α-La
β-Lg
Bovine β-CN
β-CN
Bovine α-La
α-La
Bovine β-Lg
β-Lg
5
10
15
20
25
30
35
Retention time (min)

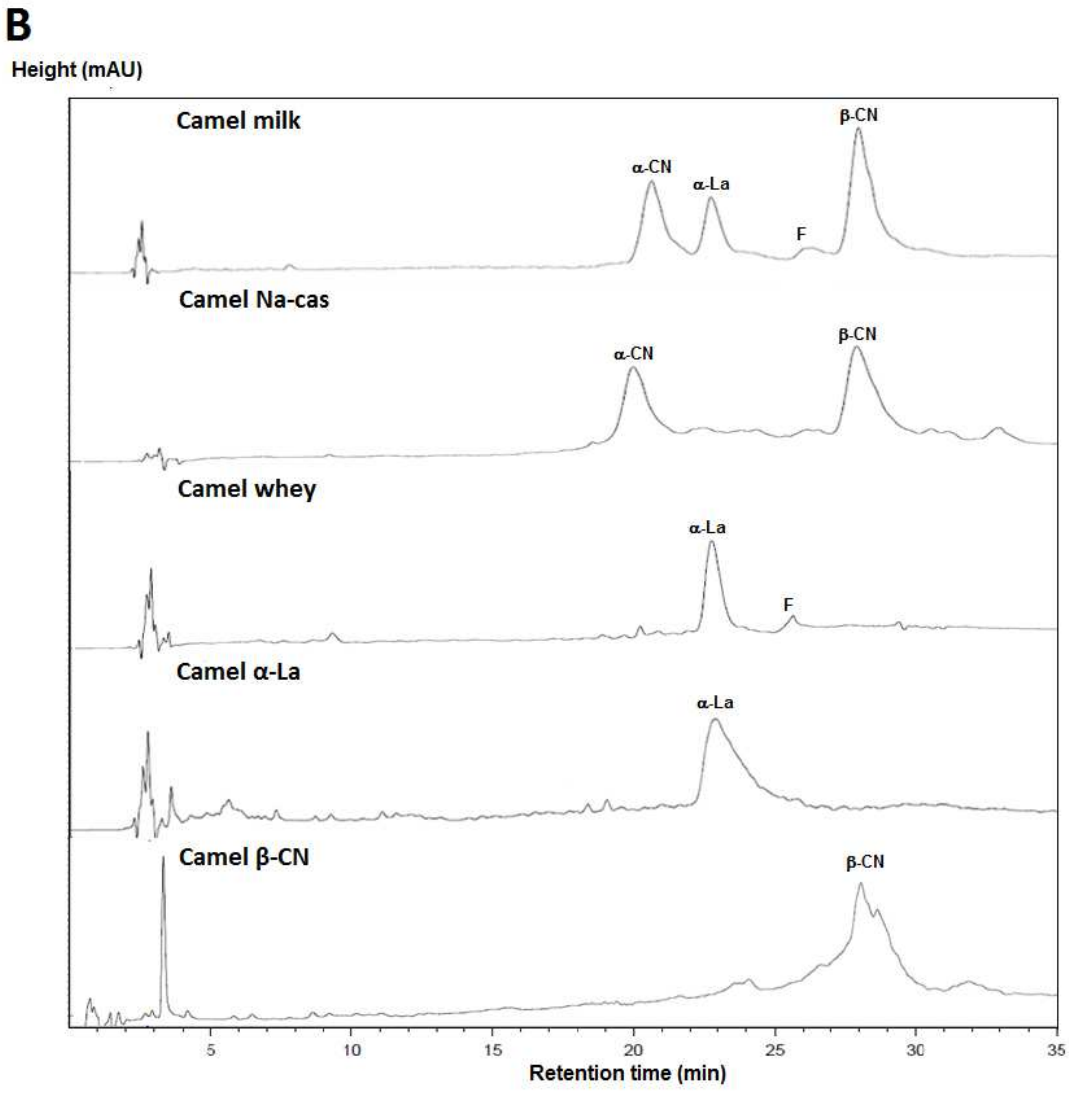
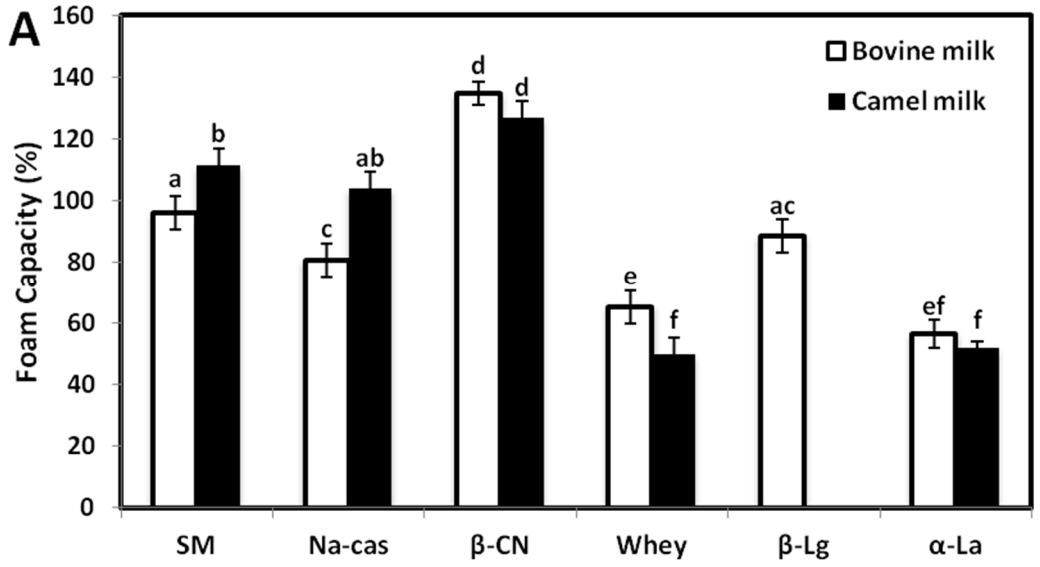

B
Height (mAU)
Camel milk
α-CN
α-La
F
β-CN
Camel Na-cas
α-CN
β-CN
Camel whey
α-La
F
Camel α-La
α-La
Camel β-CN
β-CN
5
10
15
20
25
30
35
Retention time (min)

Fig. 2



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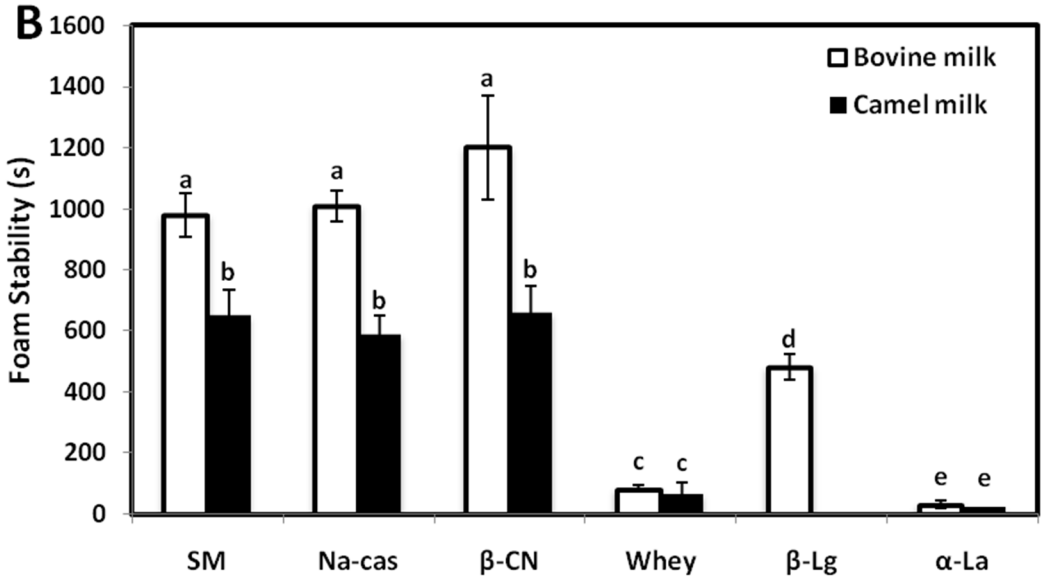

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1147 **Fig. 3**

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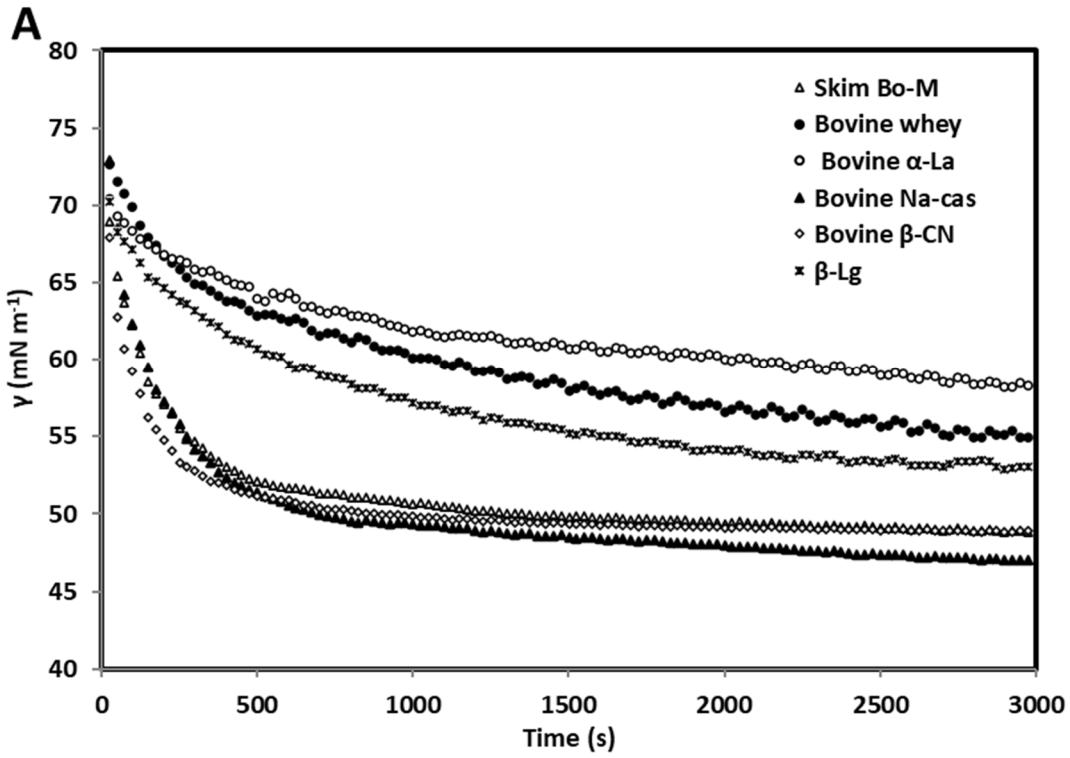

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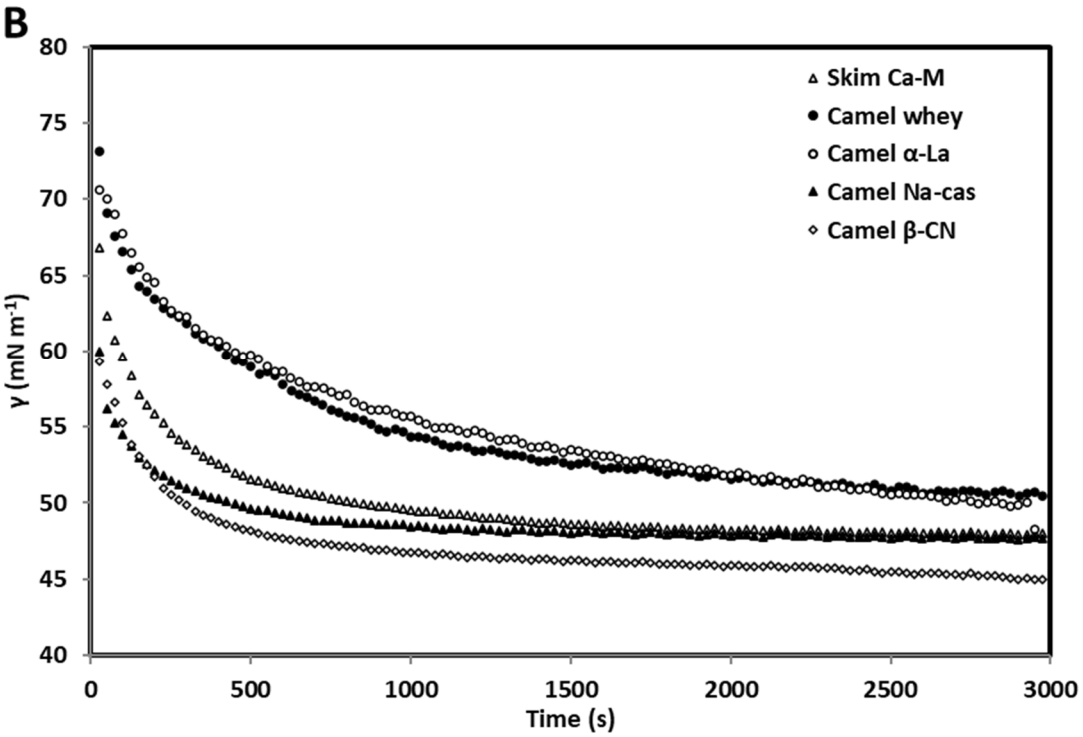

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1151 **Fig. 4**

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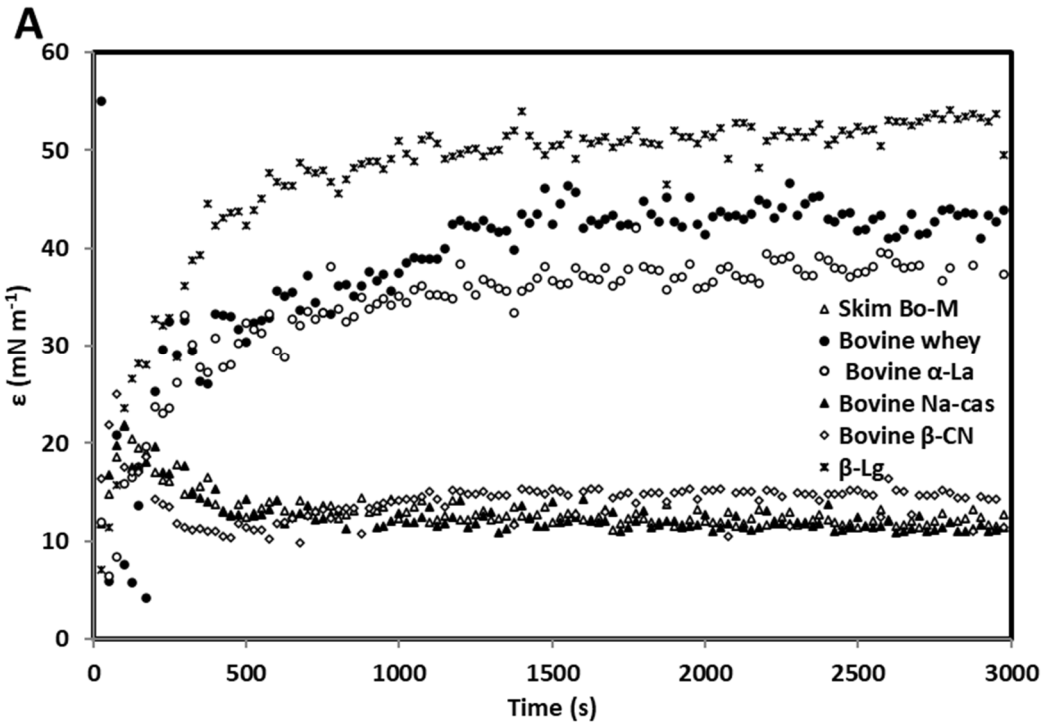

A
ε (mN m-1)
Time (s)
Skim Bo-M
Bovine whey
Bovine α-La
Bovine Na-cas
Bovine β-CN
β-Lg

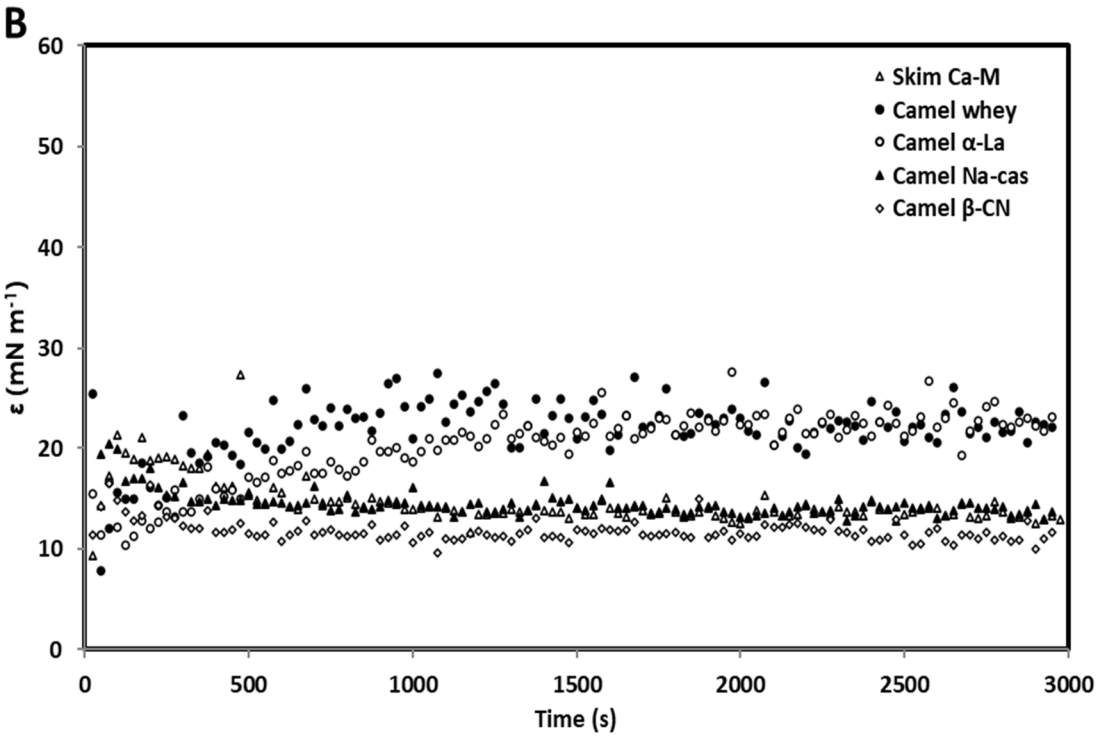
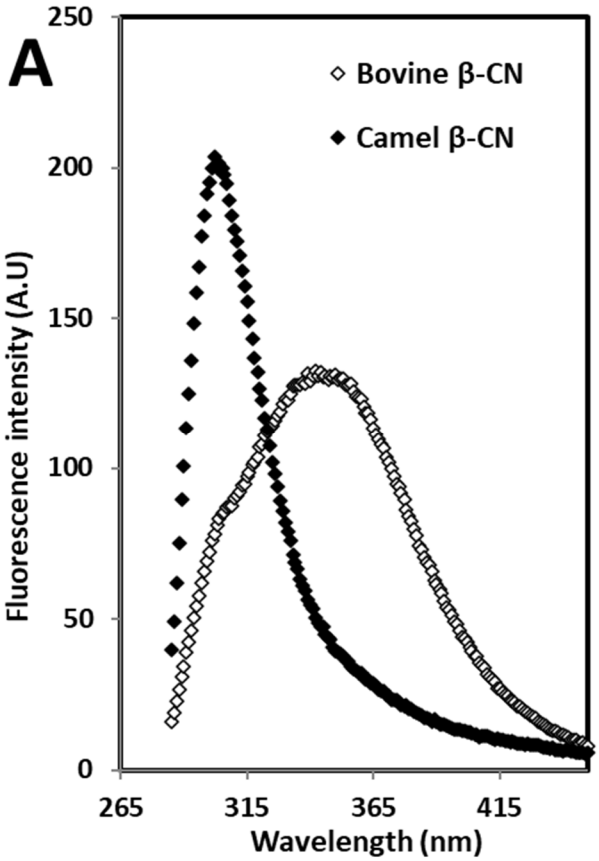

B
ε (mN m-1)
Time (s)
Skim Ca-M
Camel whey
Camel α-La
Camel Na-cas
Camel β-CN

Fig. 5



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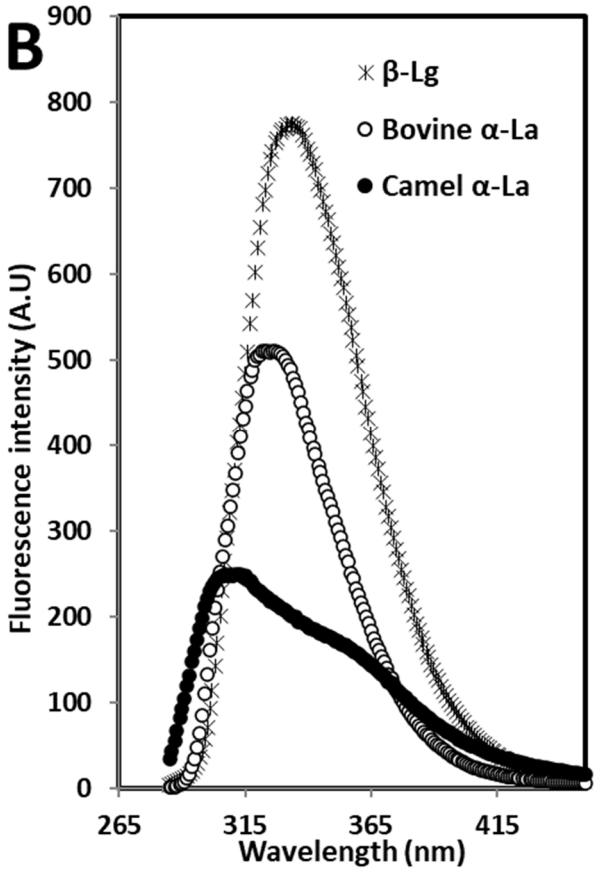

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1165 **Fig. 6**

Table caption

Table 1: Comparison of the proportion of the main proteins of bovine milk (Bo-M) and camel milk (Ca-M) (Atri et al., 2010; Barzegar et al., 2008; Chatterton et al., 2006; El-Agamy, 2006; Hailu et al., 2016; Huang & Miller, 1991; Kappeler et al., 2003; Lajnaf et al., 2017, 2019).

[a] : Proportion of individual protein in the casein fraction of milk.

[b] : Percentage of the protein in the serum fraction of milk

[c] Protein sequence identity: the ratio of the number of identical residues in a pair of aligned protein sequences to the length of the shorter one

[d] Protein sequence similarity: similarities between aminoacid sequences including residues with similar biochemical properties

Tables

Table 1.

Milk fraction	**Proteins**	**Bo-M (g L^{-1})**	**Ca-M (g L^{-1})**	**Identity [c] (%)**	**Similarity [d] (%)**
Caseins	α_{S1}-casein	9.5 (38% [a])	5.3 (22% [a])	44,6	59,7
	α_{S2}-casein	2.5 (10% [a])	2.3 (9.6% [a])	58,3	69,2
	β-casein	9.8 (39% [a])	15.6 (65% [a])	67.2	84.5
	κ-casein	3.3 (13% [a])	0.8 (3.3% [a])	58.4	66,3
Whey proteins	β-lactoglobulin	3.1 (53.6%[b])	-	n.d	n.d
	α-lactalbumin	1.1 (20.1%[b])	3.5 (52% [b])	69.1	82.9
	Serum Albumin	0.35 (6.2%[b])	1.4 (21% [b])	90.4	80.1
	Immunoglobulins	0.20 (3.5 [b])	n.d	n.d	n.d
	Lactoferrine	n.d	0.1 (2% [b])	n.d	n.d

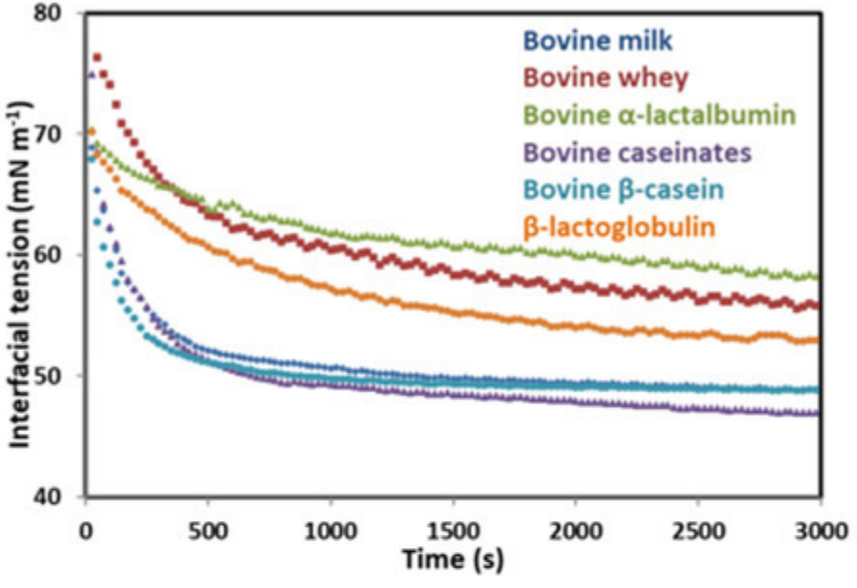
Table 2: Adsorption kinetic parameters of camel and bovine milk proteins fractions at the air-water interface: rate of adsorption (mN m^{-1} s^{-1}). Abbreviations: Bo-M: Bovine milk, Ca-M: Camel milk.

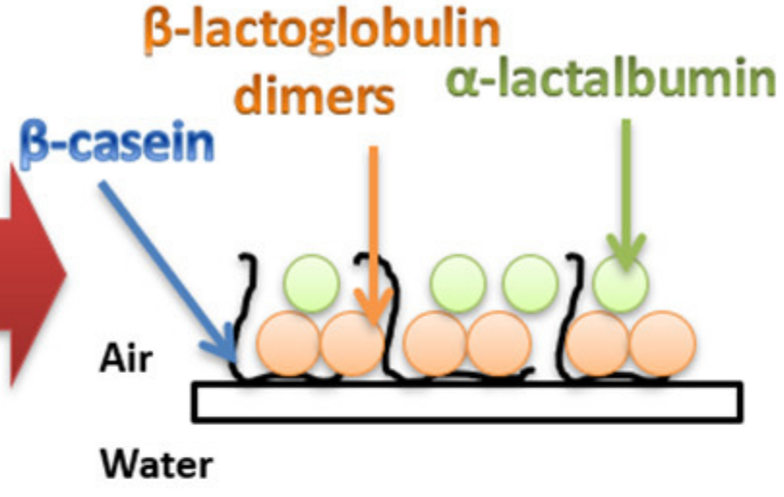
[a-h] Samples represented with different letters are significantly different from each other ($p<0.05$). Error bars show the standard deviations of mean values of adsorption rate (AR).

Table 2.

Protein fraction	Bo-M	Ca-M
Skim milk	0.238 ± 0.016 [de]	0.218 ± 0.025 [e]
Na-cas	0.252 ± 0.005 [d]	0.300 ± 0.012 [b]
Whey	0.153 ± 0.003 [h]	0.165 ± 0.002 [g]
β-casein	0.275 ± 0.003 [c]	0.362 ± 0.023 [a]
β-lactoglobulin	0.185 ± 0.011 [f]	--
α-lactalbumin	0.178 ± 0.005 [f]	0.170 ± 0.013 [fgh]

Bovine milk





Camel milk