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**Foaming and air-water interfacial properties of camel milk proteins  
compared to bovine milk proteins**

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## 22 Abstract

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

46

47 **Keywords:** Camel and bovine proteins, foaming properties, surface tension, viscoelastic  
48 modulus, **intrinsic fluorescence**  
49

## 50        **1. Introduction**

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

71        Caseins and whey proteins are characterized by different surface rheological properties a  
72        flexible and disordered structure allowing changes of their conformation more rapidly and  
73        easily once adsorbed at the interface, whereas, globular whey proteins, stabilized by intra  
74        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 ( $13.01 \pm 0.12$  % for Bo-M and  $12.95 \pm 0.17$  % for Ca-M) a  
84 lipid content around  $34.1 \pm 0.5$  g L<sup>-1</sup> and  $35.4 \pm 0.6$  g L<sup>-1</sup> for both Bo-M and Ca-M, respectively,  
85 and a lactose concentration of  $46.1 \pm 2.2$  g L<sup>-1</sup> and  $43.5 \pm 1.1$  g L<sup>-1</sup> in Bo-M and Ca-M,  
86 respectively. On the other hand, the total mineral content ranges between 6 and 9 g L<sup>-1</sup> of milk  
87 with an average value of 7 g L<sup>-1</sup> and 7.9 g L<sup>-1</sup> 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<sup>-1</sup>) were significantly  
90 lower than those in Bo-M ( $28.36$  g L<sup>-1</sup>) (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  $\beta$ -casein (65% of the total camel caseins compared to 36% (w/w) for  
93 Bo-M) with a concentration of  $15.6$  g L<sup>-1</sup> (Table 1) instead of  $9.5$  g L<sup>-1</sup> 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  $\kappa$ -casein (3.3% of the total camel caseins instead of 13% of  
96 the total casein in Bo-M (Table 1). Camel  $\beta$ -casein is composed of 217 amino-acid residues,  
97 which are more numerous than those of bovine  $\beta$ -casein residues (209 amino-acid residues).  
98 Therefore, the similarity and identity between camel and bovine  $\beta$ -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

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

107 Whey proteins are the second main protein component in Bo-M and Ca-M representing  
108 20% and 24.5% of the total cow and camel proteins, respectively (Ereifej et al., 2011; Madureira  
109 et al., 2010). In bovine whey proteins, the average  $\beta$ -lactoglobulin fraction accounted for 53.6%  
110 of total whey with a concentration of 3.1 g L<sup>-1</sup> (Table 1), followed by the  $\alpha$ -lactalbumin (20.1%)  
111 (Hailu et al., 2016). However, the  $\beta$ -lactoglobulin is absent in camel whey (Merin et al., 2001;  
112 Omar et al., 2016; Lajnaf et al., 2018, Lajnaf, Trigui, Samet-Bali, Attia & Ayadi, 2019; Lajnaf  
113 et al., 2020 a) and the  $\alpha$ -lactalbumin is the major protein in the soluble fraction of Ca-M with  
114 an average concentration of 3.5 g L<sup>-1</sup> which is significantly higher when compared to the  $\alpha$ -  
115 lactalbumin content in Bo-M (1.1 g L<sup>-1</sup>) (Table 1). Camel  $\alpha$ -lactalbumin has 123 amino acids,  
116 a MW of 14.43 kDa and a pI of 4.87. This protein shares some main molecular characteristics  
117 with its bovine counterpart in terms of number of amino-acid residues with a MW of 14.18 kDa  
118 and a pI of 4.65 (El-Agamy, 2009). Thus, camel and bovine  $\alpha$ -lactalbumin show 82.9%  
119 similarity and 69.1% identity (Atri et al., 2010).

120 Various authors reported that the dynamic surface tension parameters are the main  
121 determining factors which are directly associated with the foamability of proteins (Marinova et  
122 al., 2009). For instance, a rapid decrease in surface tension indicates a fast adsorption of proteins  
123 at the interface and thus greater foaming capacity and stabilization of the integrated air bubbles  
124 to avoid coalescence (Tamm, Sauer, Scampicchio & Drisch, 2012). In different studies on  
125 foaming and emulsifying properties of cow proteins (caseins and whey proteins), the surface  
126 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  $\beta$ -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  $\alpha$ -lactalbumin and  $\beta$ -  
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 ( $\beta$ -casein,  $\beta$ -lactoglobulin and  $\alpha$ -  
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  $\beta$ -casein,  $\beta$ -lactoglobulin, and  $\alpha$ -lactalbumin were chosen as proteins pure systems.  
149 For Bo-M, pure  $\beta$ -casein,  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin were used as they represent the  
150 major constituents of the air-water interface in Bo-M foams (Brooker, Anderson & Andrews,  
151 1986; Borcharding et al., 2008). For Ca-M, purified camel  $\alpha$ -lactalbumin and  $\beta$ -casein were  
152 chosen as they represent the main proteins in both soluble and micellar fractions of Ca-M



153 respectively, and as  $\beta$ -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 dromedarius*) 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).

179 Sweet bovine whey was extracted from skim Bo-M after an enzymatic coagulation at  
180 37°C for 1 h in the presence of 0.35 mL microbial rennet enzyme per liter of skim Bo-M  
181 (Parachimic, Laboratories Arrazi, Sfax, Tunisia, strength = 1:10,000) (Lajnaf et al., 2019).  
182 Afterwards, sweet bovine whey was separated from casein fraction by a centrifugation at 5000g  
183 for 20 min at 20 °C.

184 Pure bovine proteins were provided from Sigma-Aldrich and used without further  
185 purification. The purity of  $\beta$ -casein (Product #: C6905, Lot #: SLBH6096V);  $\beta$ -lactoglobulin  
186 (Product #: L2506, Lot #: SLBB4325V) and  $\alpha$ -lactalbumin (Product #: L5385, Lot #:  
187 SLBJ2493V) are  $\geq 98\%$ ,  $\geq 85\%$  and  $\geq 85\%$ , respectively.

### 188 ***2.3. Camel protein fractions***

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

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

196 After milk defatting, the casein fraction of Ca-M was separated from the soluble fraction  
197 by rennet addition (1.4 mL L<sup>-1</sup> of milk) at 37 °C for 1 h (Felfoul, Lopez, Gaucheron, Attia &  
198 Ayadi, 2015; Lajnaf et al., 2018, 2019). The rennet coagulum was centrifuged at 5,000g for 15  
199 min at 20 °C (centrifuge Beckman CO-LE80K, Coulter, Fullerton, CA). Afterwards, the curd  
200 containing the camel caseins was kept for the  $\beta$ -casein purification and the supernatant  
201 representing the soluble fraction of milk identified as sweet camel whey was also retained for  
202  $\alpha$ -lactalbumin purification.

203 Camel  $\alpha$ -lactalbumin was purified from sweet whey by ultrafiltration (UF) as described  
204 by previous works (Salami et al., 2009; Lajnaf, Picart-Palmade, Attia, Marchesseau & Ayadi,  
205 2017; Lajnaf, Gharsallah, Jridi, Attia & Ayadi, 2020 b). Indeed, sweet camel whey was applied  
206 to a UF membrane characterized by a molecular mass cut off of 30 kDa (Amicon-bioseparations  
207 model 8050). The UF system was operated at a pressure of 1 bar and room temperature for 3 h.  
208 The UF permeate containing purified camel  $\alpha$ -lactalbumin was stored at  $-18\text{ }^{\circ}\text{C}$  for further use.

209 Camel  $\beta$ -casein was isolated from rennet camel curd obtained previously using the cold-  
210 extraction method described by Huppertz et al. (2006). A volume of heated demineralized water  
211 ( $80\text{ }^{\circ}\text{C}$ ) equal to that of the removed sweet whey was added to the curd and the mixture was  
212 kept at  $80\text{ }^{\circ}\text{C}$  for 5 min using a water bath to disable the action of the added rennet enzyme and  
213 then centrifuged at 5,000 g for 15 min at  $20\text{ }^{\circ}\text{C}$  using a Beckman centrifuge CO-LE80K  
214 (Coulter, Fullerton, CA). The curd was stored, macerated and suspended in deionized water ( $5$   
215  $^{\circ}\text{C}$ ) at a volume equal to that of the discarded whey. Finally, the protein suspension was kept at  
216  $5\text{ }^{\circ}\text{C}$  for up to 24 h and centrifuged at 5,000 g at  $5\text{ }^{\circ}\text{C}$  for 15 min. The supernatant obtained  
217 containing the isolated camel  $\beta$ -caseins and camel  $\alpha$ -lactalbumin and was also stored at  $-18\text{ }^{\circ}\text{C}$   
218 for further analysis.

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

226 The pH value of camel caseins solution was adjusted to that of milk using 1M NaOH  
227 yielding camel Na-cas. The purity of extracted camel  $\alpha$ -lactalbumin (~ 91.2%) and  $\beta$ -casein (~

228 81.5%) was verified by the reversed-phase high-performance liquid chromatography (RP-  
229 HPLC) (Yüksel & Erdem, 2010) (Fig. 3B).

#### 230 **2.4. Protein solution preparation**

231 The different protein solutions were prepared by dissolving them in 20 mM Tris-HCl  
232 buffer, pH 7 (Atri et al., 2010). The pH value was chosen to approximately correspond to milk  
233 conditions, furthermore the adopted buffer is commonly used to control the pH in similar  
234 studies (Ibanoglu & Ibanoglu, 1999; Lajnaf, Picart-Palmade, Attia, Marchesseau & Ayadi,  
235 2016).

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

240 The proteins fractions studied were:

- 241 - For Bo-M: skim milk, Na-cas, sweet whey,  $\alpha$ -lactalbumin,  $\beta$ -casein and  $\beta$ -  
242 lactoglobulin
- 243 - For Ca-M: skim milk, Na-cas, sweet whey,  $\alpha$ -lactalbumin and  $\beta$ -casein as Ca-M is  
244 devoid of  $\beta$ -lactoglobulin.

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

#### 250 **2.5. RP-HPLC analysis**

251 RP-HPLC (Agilent 1260 Infinity quaternary LC, Germany) was used to separate and  
252 identify the main proteins from camel and bovine milk-derived proteins (Yüksel & Erdem,  
253 2010; Lajnaf et al., 2020 a). A C18 column (Zorbax Eclipse Plus C18, 250 mm length  $\times$  4.6

254 mm, particle size 5  $\mu\text{m}$ , Packing Lot #: B14292) was used for protein separation. The analysis  
255 was performed using a Shimadzu SPD6A-UV detector measuring the optical density. All  
256 solutions were filtered through a nylon filter (47 mm, 0.45  $\mu\text{m}$ , EG0492-1).

257 The chromatographic conditions were as follows: Solvent A: Acetonitrile, water and  
258 trifluoroacetic acid in a ratio of 100:900:1 (v/v/v); Solvent B: Acetonitrile, water and  
259 trifluoroacetic acid in a ratio of 900:100:1 (v/v/v). Total run time: 35 min; Column temperature:  
260 25  $^{\circ}\text{C}$ . Flow rate: 1.0 mL  $\text{min}^{-1}$ . Detection wavelength: 220 nm. Injection volume of the final  
261 diluted sample: 20  $\mu\text{L}$ . For the sample preparation, 500  $\mu\text{L}$  of protein sample (skim milk, Na-  
262 cas, whey and purified camel proteins) were added to 3.7 mL of a solution consisting of solvents  
263 A and B in a 70:30 ratio (v/v). The sample-solvents mixture was vortexed for 10 s and then  
264 filtered through nylon filter (0.45  $\mu\text{m}$ ) before injection into the column. Once, the sample was  
265 injected, a gradient was generated immediately by increasing the proportion of solvent B from  
266 20% at the beginning of the analysis to 46% at the end of the run.

267 Standard individual bovine proteins ( $\beta$ -casein,  $\alpha_{\text{S}}$ -casein,  $\kappa$ -casein,  $\beta$ -lactoglobulin and  $\alpha$ -  
268 lactalbumin) were provided from Sigma Aldrich. Individual pure standards were prepared by  
269 diluting proteins in solvent A and solvent B mixture (70:30, v/v), separately as camel and  
270 bovine milk proteins. Quantitative estimation of the main camel and bovine milk protein  
271 mixtures (skim milk, Na-cas and whey) was performed by calculating the peak area of each  
272 protein.

## 273 **2.6. Foaming properties**

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

277 The protein solution was mixed using the Ultra-Turrax mixer (IKA Labortechnik, Staufen  
278 Germany) at a speed of 13,500 rpm for 2 min at room temperature ( $\sim 20$   $^{\circ}\text{C}$ ) (Lajnaf et al., 2020  
279 a).

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

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

$$283 \quad FC = (V_{\text{foam}}/V_0) \times 100; (\%) \quad (1)$$

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

286 Foam Stability (FS) is defined as the foam half-time which is the time for drainage of the  
287 half of the foam created ( $t_{\text{foam}1/2}$ ) (Marinova et al., 2009).

## 288 ***2.5. Interfacial properties***

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

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

$$295 \quad (1/x)[d(x \sin \theta)/dx] = (2/b) - cz \quad (2)$$

296 where :

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

302 The sinusoidal changes of  $\gamma$  as a function of time are recorded and plotted by the control  
303 unit in order to measure the surface viscoelastic modulus ( $\epsilon$ ) which is defined by the equation  
304 3 (Eq. 3):

$$305 \quad |\epsilon| = d\gamma / d \ln A, \quad (3)$$

306 where  $A$  is the surface area of the air drop.

307 The temperature-controlled chamber of the apparatus was adjusted to  $20 \pm 1$  °C using water  
308 circulation from a thermostat.

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

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

## 319 ***2.6. Fluorescence spectroscopy***

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

324 Intrinsic fluorescence was measured according to the method of Lam and Nickerson  
325 (2015b) at a constant excitation wavelength of 275 nm as a function of emission wavelength  
326 between 285 to 450 nm using spectrofluorometer (Aminco Bowman, Foster City, CA) and a 1  
327 nm slit width. Intrinsic fluorescence experiments were performed for  $5 \mu\text{M}$  protein solutions  
328 for camel and bovine  $\beta$ -caseins in agreement with the work of Esmaili et al. (2011) carried out  
329 with camel  $\beta$ -casein and for  $30 \mu\text{M}$  proteins solutions for pure whey proteins ( $\alpha$ -lactalbumin  
330 and  $\beta$ -lactoglobulin) in agreement with the work of Zhang et al. (2014) which have been made  
331 with pure  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin. By this technique, the fluorescence of the aromatic

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

## 335 **2.7. Statistics**

336 The significance of the main effects of the protein type (camel and bovine  $\beta$ -casein and  $\alpha$ -  
337 lactalbumin;  $\beta$ -lactoglobulin) on RP-HPLC, foaming properties (FC and FS indices), interfacial  
338 properties (surface tension ( $\gamma$ ) and viscoelastic modulus ( $\epsilon$ )) and conformational state (intrinsic  
339 fluorescence) was tested by three-way analysis of variance (ANOVA). Statistical analyses were  
340 performed with IBM-SPSS software (Version 19). All experiments were carried out at least in  
341 triplicate and results were reported as mean  $\pm$  one standard deviation.

## 342 **3. Results and discussion**

### 343 **3.1. Protein analysis**

344 Protein fractions derived from Ca-M and Bo-M were characterized by RP-HPLC (Fig.  
345 2A and B). For bovine milk, five major peaks (with retention time (RT): 20 min, 24.9 min, 26.4  
346 min, 27.5 min and 30.3 min) were detected and identified as  $\kappa$ -casein (~ 7.1%),  $\alpha$ -casein (~  
347 24.6%),  $\beta$ -casein (~ 37.5%),  $\alpha$ -lactalbumin (~ 4.7%) and  $\beta$ -lactoglobulin (~ 26.2%).  
348 Meanwhile, only four major protein peaks were identified in Ca-M (Fig. 2B). These peaks  
349 corresponded to  $\alpha$ -casein (~ 28.5%),  $\alpha$ -lactalbumin (~ 19.7%), protein fraction (F) (~ 1.4%) and  
350  $\beta$ -casein (~ 50.4%) with RT of 20.4 min, 22.6 min, 26.1 min and 27.9 min, respectively.

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



357 casein in agreement with Salami et al. (2011) and Lajnaf, Gharsallah, Attia and Ayadi (2021).  
358 On the contrary, camel  $\alpha$ -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  $\kappa$ -casein represented 7% of total bovine Na-cas, while camel milk and  
361 Na-cas chromatograms also showed that no peak was detected for  $\kappa$ -casein, probably due to its  
362 very low concentration in Ca-M, making it masked by  $\beta$ - and  $\alpha$ -caseins, in agreement with  
363 Farah, Rettenmaier and Atkins (1992) and Lajnaf et al. (2020 a).

364 As expected, no peak corresponding to  $\beta$ -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,  $\beta$ -lactoglobulin is the major protein of bovine whey followed by  $\alpha$ -  
367 lactalbumin representing ~60% and ~28% of the total whey proteins, respectively. Meanwhile,  
368 camel  $\alpha$ -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 caseinomacropptide (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  $\beta$ -casein yielded better foam than whey protein  
380 fractions (whey,  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin), with higher FC values of camel proteins  
381 reaching  $111.5 \pm 5.4\%$  and  $103.8 \pm 5.4\%$  for camel milk and Na-cas, respectively and  $96.2 \pm$   
382  $5.5\%$  and  $80.7 \pm 4.9\%$  for Bo-M and Na-cas, respectively. Maximum foamability was obtained

383 with  $\beta$ -casein (FC=  $126.9 \pm 5.4\%$  and  $134.6 \pm 3.8 \%$  for camel and bovine  $\beta$ -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  $\beta$ -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 ( $\beta$ -lactoglobulin and  $\alpha$ -  
390 lactalbumin), especially in  $\beta$ -casein with a concentration of  $1.44 \pm 0.06 \text{ mg mL}^{-1}$  in the foam  
391 phase leading to the highest foam Enrichment Ratio among all milk proteins ( $\sim 2.80$ ). While  
392 whey proteins were less competitive at adsorbing to foam than caseins with Enrichment Ratios  
393 of  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin of 1.67 and 1.77, respectively (Zhang et al., 2004). Thus,  
394  $\beta$ -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  $\alpha$ -helix,  $\beta$ -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  $\alpha_{S1}$  and  $\beta$  caseins leading to the formation of a complex with a  
405 higher ratio of  $\alpha_{S1}$ -casein to  $\beta$ -casein. This complex was characterized by a greater stability than  
406 that of  $\alpha_{S1}$ - $\alpha_{S1}$  and  $\beta$ - $\beta$  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  $\beta$ -casein content in Ca-M.

411 Furthermore, bovine whey showed higher foamability compared to camel whey at a  
412 protein concentration of  $0.5 \text{ g L}^{-1}$  (FC =  $50 \pm 5.4 \%$  and  $70.0 \pm 5.4 \%$  for camel and bovine  
413 whey proteins, respectively) with foaming behavior intermediate between those of  $\beta$ -  
414 lactoglobulin and bovine  $\alpha$ -lactalbumin alone. No significant difference was observed between  
415 camel whey and camel  $\alpha$ -lactalbumin (FC=  $51.9 \pm 1.9 \%$  and  $56.4 \pm 4.4\%$ ) suggesting that  
416 foaming proteins of camel whey are mostly maintained by the  $\alpha$ -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 ( $5 \text{ g L}^{-1}$ ). 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  $70 \text{ }^\circ\text{C}$  and  $90 \text{ }^\circ\text{C}$  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  $\beta$ -lactoglobulin and the dominance  
425 of the  $\alpha$ -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  $\beta$ -lactoglobulin forms aggregates with  $\alpha$ -  
427 lactalbumin leading to an antifoaming effect (Lajnaf et al., 2018).

428 The foaming stability (FS) values of pure proteins ( $\beta$ -lactoglobulin,  $\beta$ -casein and  $\alpha$ -  
429 lactalbumin) and naturally mixed proteins systems (skim milk, Na-cas and whey) at a  
430 concentration of  $0.5 \text{ g L}^{-1}$  are given in Fig. 3B. The bovine proteins fractions (skim milk, Na-  
431 cas and  $\beta$ -casein) gave the highest foams stability among all samples studied, reaching  
432 approximately  $\sim 1000 \text{ s}$  for bovine proteins and  $\sim 600 \text{ s}$  for their camel counterparts.

433 The difference of FS between the bovine and camel  $\beta$ -casein can be mainly explained by  
434 the different physico-chemical characteristics of the two counterparts. First, it is obvious that

435 camel  $\beta$ -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  $\beta$ -casein compared to camel  $\beta$ -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  $\beta$ -casein to stabilize foams compared with camel  $\beta$ -casein.  
442 Furthermore, Bo-M contains higher amounts of  $\kappa$ -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  $\kappa$ -casein due to its  
445 structured form compared with  $\alpha$ -casein and  $\beta$ -casein.

446 Fig. 3B showed that FS values of caseinates in both Ca-M and Bo-M were significantly  
447 higher than that of  $\beta$ -lactoglobulin (FS =  $480 \pm 45$  s), whey (FS =  $82.5 \pm 10.6$  s and  $70 \pm 35$  s  
448 for bovine and camel whey, respectively) and  $\alpha$ -lactalbumin (FS =  $31.6 \pm 12.5$  s and  $27.0 \pm 1.41$   
449 s for bovine and camel  $\alpha$ -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,  $\alpha$ -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  $\alpha$ -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<sup>-1</sup>) 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  $\alpha$ -lactalbumin  
477 and cold solubilization for camel  $\beta$ -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<sup>-1</sup>) 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  $72.8 \pm 0.5$  mN m<sup>-1</sup>  
494 <sup>1</sup>, 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 ( $\gamma = 47.1 \pm 0.1$  mN m<sup>-1</sup>) > bovine  $\beta$ -casein ( $\gamma = 48.8 \pm 0.5$  mN m<sup>-1</sup>) = Bo-M ( $\gamma$   
498  $= 48.9 \pm 0.1$  mN m<sup>-1</sup>) >  $\beta$ -lactoglobulin ( $\gamma = 52.9 \pm 0.1$  mN m<sup>-1</sup>) > bovine whey ( $\gamma = 55.8 \pm$   
499  $1.1$  mN m<sup>-1</sup>) > bovine  $\alpha$ -lactalbumin ( $\gamma = 58.2 \pm 0.6$ ) ( $p < 0.05$ ). Furthermore, as shown in Fig.  
500 4B, the order of effectiveness for camel proteins fractions at  $t = 3000$  s was: camel  $\beta$ -casein  
501 ( $44.9 \pm 0.5$  mN m<sup>-1</sup>) > camel Na-cas ( $47.6 \pm 0.5$  mN m<sup>-1</sup>) = Ca-M ( $48.1 \pm 0.2$  mN m<sup>-1</sup>) > camel  
502 whey ( $50.9 \pm 0.1$  mN m<sup>-1</sup>) = camel  $\alpha$ -lactalbumin ( $49.8 \pm 0.6$  mN m<sup>-1</sup>) ( $p < 0.05$ ).

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  $\beta$ -caseins carried  
507 the highest AR values regardless of milk origin ( $AR = 0.275 \pm 0.003$  mN m<sup>-1</sup> s<sup>-1</sup> and  $0.362 \pm$

508 0.023 mN m<sup>-1</sup> s<sup>-1</sup> for bovine and camel  $\beta$ -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 ( $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin) reaching AR values of  $0.252 \pm 0.005$  mN m<sup>-1</sup> s<sup>-1</sup> and  $0.300$   
511  $\pm 0.012$  mN m<sup>-1</sup> s<sup>-1</sup> for bovine and camel Na-cas, respectively in agreement with previous  
512 foaming results (Foam studies, Section 3.2). Besides,  $\beta$ -lactoglobulin and bovine  $\alpha$ -lactalbumin  
513 were better adsorbed at the air drop interface than bovine whey (AR=  $0.153 \pm 0.003$  mN m<sup>-1</sup> s<sup>-1</sup>  
514 <sup>1</sup>,  $0.185 \pm 0.01$  mN m<sup>-1</sup> s<sup>-1</sup> and  $0.178 \pm 0.005$  mN m<sup>-1</sup> s<sup>-1</sup> for bovine whey,  $\beta$ -lactoglobulin and  
515  $\alpha$ -lactalbumin, respectively). However, no significant difference was found between camel  
516 whey and  $\alpha$ -lactalbumin (AR  $\sim 0.170$  mN m<sup>-1</sup> s<sup>-1</sup>).

517 Thus, globular whey proteins (camel and bovine  $\alpha$ -lactalbumin, whey and  $\beta$ -  
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  $\beta$ -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.  $\beta$ -casein is more efficient in reducing the surface tension at concentrations of 1  
526 g L<sup>-1</sup> compared with  $\beta$ -lactoglobulin. This behavior was explained by the flexible molecular  
527 structure of  $\beta$ -casein in solution which allows easy and rapid reduction of the surface tension  
528 over the first minutes compared to  $\beta$ -lactoglobulin (Seta et al., 2014).

529 The  $\beta$ -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  $\beta$ -lactoglobulin monomer contains two intramolecular disulfide  
532 bridges and a hidden free thiol group. Hence,  $\beta$ -lactoglobulin was not fully unfolded at the air-

533 water interface and the rate of the surface tension decrease was lower than that of  $\beta$ -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:  $\beta$ -lactoglobulin and bovine  $\alpha$ -lactalbumin alone. On the other hand,  
537 the evolution of the surface tension curves of camel whey and camel  $\alpha$ -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  $\beta$ -  
541 lactoglobulin is preferentially adsorbed over  $\alpha$ -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  $\beta$ -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<sup>-1</sup>) when compared to that  
547 of bovine skim milk and  $\beta$ -casein ( $\sim 48.8$  mN m<sup>-1</sup>). 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  $\beta$ -casein,  
550 followed by the surface tension value stabilization state where the effect of  $\beta$ -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  $\gamma(t)$  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  $\alpha$ -lactalbumin  
562 exhibited similar interfacial behavior, suggesting that the interfacial and foaming behavior of  
563 camel whey is maintained by camel  $\alpha$ -lactalbumin, representing 84% of the total camel whey  
564 proteins (RP-HPLC results, section 3.1). Hence, the absence of  $\beta$ -lactoglobulin in camel whey  
565 allows camel  $\alpha$ -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  $\beta$ -casein. These results lead to the finding that  $\beta$ -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  $\alpha$ -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  $\beta$ - and  $\alpha_{s1}$ -caseins,  $\beta$ -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  $\beta$ -casein over other caseins ( $\alpha$ - and  $\kappa$ -caseins)  
576 and caseins over whey proteins.

577 For Ca-M, Lajnaf et al. (2016) found that mixtures with a higher camel  $\beta$ -casein amount  
578 are more efficient in reducing the surface tension at the air-water interface. Camel  $\beta$ -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  $\beta$ -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  $\beta$ -casein than bovine  $\beta$ -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  $\gamma(t)$  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  $\epsilon(t)$  of the protein film  
590 developed by the bovine and camel protein systems studied (skim milk, Na-cas, whey,  $\beta$ -casein,  
591  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin) as a function of time during 3000 s and at 20 °C.

592 The magnitude of  $\epsilon(t)$  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  $\beta$ -casein led  
594 immediately to the final and lowest  $\epsilon$  value ( $\epsilon \sim 13 \text{ mN m}^{-1}$ ) from  $t = 500 \text{ s}$  compared with the  
595 other protein fractions (whey,  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin). For instance, the order of  
596 effectiveness in the creation of the most rigid surface film by Bo-M proteins at  $t = 3000 \text{ s}$  was  
597 (Fig. 5A) :  $\beta$ -lactoglobulin ( $\epsilon = 50.3 \pm 1.7 \text{ mN m}^{-1}$ ) > bovine whey ( $\epsilon = 45.6 \pm 0.5 \text{ mN m}^{-1}$ ) >  
598 bovine  $\alpha$ -lactalbumin ( $\epsilon = 37.3 \pm 2.1 \text{ mN m}^{-1}$ ) > bovine  $\beta$ -casein ( $\epsilon = 13.3 \pm 1.7 \text{ mN m}^{-1}$ ) =  
599 skim Bo-M ( $\epsilon = 12.7 \pm 1.5 \text{ mN m}^{-1}$ ) = bovine Na-cas ( $\epsilon = 12.5 \pm 1.1 \text{ mN m}^{-1}$ ). While for Ca-  
600 M proteins, Fig. 5B shows that the order of efficiency  $t = 3000 \text{ s}$  was camel  $\alpha$ -lactalbumin ( $\epsilon =$   
601  $23.2 \pm 1.5 \text{ mN m}^{-1}$ ) = camel whey ( $\epsilon = 20.5 \pm 1.5 \text{ mN m}^{-1}$ ) > camel  $\beta$ -casein ( $\epsilon = 13.7 \pm 1.1$   
602  $\text{mN m}^{-1}$ ) = camel Na-cas ( $\epsilon = 12.9 \pm 1.1 \text{ mN m}^{-1}$ ) = skim Ca-M ( $\epsilon = 12.8 \pm 1.5 \text{ mN m}^{-1}$ ).

603 Thus, findings indicated that skim milk, Na-cas and  $\beta$ -casein have the lowest viscoelastic  
604 modulus values compared with globular proteins (whey,  $\alpha$ -lactalbumin and  $\beta$ -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  $\beta$ -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  $\beta$ -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  $\beta$ -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  $\beta$ -lactoglobulin at the interface is  
620 attributed to the high packing density and strong protein-protein connections. Once adsorbed,  
621 the  $\beta$ -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  $\beta$ -  
625 lactoglobulin and  $\alpha$ -lactalbumin, suggesting that the adsorbed film protein of bovine whey at  
626 the air-water interface consist of both of  $\beta$ -lactoglobulin and  $\alpha$ -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  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin. On the other hand, Zhang et al. (2004) have shown that there  
631 was preferential adsorption of  $\beta$ -lactoglobulin over  $\alpha$ -lactalbumin in the foam phase made with  
632 whey protein isolate at neutral pH values in contrast to acidic pH levels where  $\alpha$ -lactalbumin is  
633 more dominant at the interface than the  $\beta$ -lactoglobulin. Hence, this led to the conclusion that

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

637 Findings also indicated that bovine whey and  $\alpha$ -lactalbumin show significantly higher  
638 viscoelastic modulus values compared to those of camel whey and  $\alpha$ -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  $\alpha$ -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  $\alpha$ -lactalbumin displayed a  
647 compact globular structure with a more disordered structure for camel  $\alpha$ -lactalbumin  
648 (Redington, Breydo, Almehdar, Redwan & Uversky, 2016). Therefore, the lower viscoelastic  
649 values of camel  $\alpha$ -lactalbumin can be attributed to a less rigid and cohesive interfacial film  
650 compared with that of bovine  $\alpha$ -lactalbumin, despite the similar shape of  $\gamma(t)$  curve of both  
651 proteins.

652 **The** comparison between the  $\epsilon(t)$  curves of whey and  $\alpha$ -lactalbumin from camel milk  
653 fractions (Fig. 5B) revealed that camel whey exhibited similar rheological properties suggesting  
654 the creation of an  $\alpha$ -lactalbumin interfacial film. According to Cases et al. (2005), the increase  
655 in the first stage of  $\epsilon(t)$  is attributed to the protein rearrangement which occurs rapidly when the  
656 surfactant used is  $\beta$ -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  $\alpha$ -lactalbumin, while the viscoelastic modulus values of skim camel

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

661 Therefore, the adsorption layers of Bo-M proteins can be modeled as follows :  $\beta$ -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  $\beta$ -lactoglobulin dimers and  $\alpha$ -lactalbumin  
665 monomers with preferential adsorption of the  $\beta$ -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  $\beta$ -lactoglobulin is totally absent. First, camel  $\beta$ -casein is adsorbed as train–loop–tail model  
669 as observed for bovine  $\beta$ -casein. Afterwards, camel  $\alpha$ -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  $\beta$ -  
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  $\alpha$ -  
674 lactalbumin leading to higher pseudo-equilibrium surface pressure (Paulsson & Dejmek, 1992).  
675 Jara, Carrera Sánchez, Patino and Pilosof (2014) reported that  $\beta$ -lactoglobulin shows a higher  
676 degree of denaturation once adsorbed at the interface with irreversible conformational changes,  
677 while  $\alpha$ -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,  $\alpha$ -lactalbumin and  $\beta$ -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

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

### 690 **3.5. Protein conformational state**

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

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

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

710 camel  $\beta$ -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  $\beta$ -casein contains  
712 greater tyrosine residues whose percentage (4.77%) is significantly higher when compared to  
713 bovine  $\beta$ -casein (2.92%). Hence, the excitation of camel  $\beta$ -casein at 275 nm caused the emission  
714 of tyrosine residues whose  $\lambda_{\text{max}}$  is close to 303.1 nm with a higher intensity than that of the  
715 bovine  $\beta$ -casein (fluorescent intensity 159.82 A.U and 215.57 A.U for bovine and camel  $\beta$ -  
716 caseins, respectively) suggesting a higher hydrophobicity of camel  $\beta$ -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  $\beta$ -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  $\beta$ -casein is -0.355 (Gasteiger et al., 2005), which explain a  
724 higher hydrophobicity level compared to camel  $\beta$ -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  $\beta$ -caseins (identity level of 67.2%). Camel  
727  $\beta$ -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  $\beta$ -  
729 casein which contains 1 tryptophan but lacks of tyrosine and phenylalanine residues leading to  
730 a higher emulsifying properties of camel  $\beta$ -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  $\beta$ -casein when  
734 compared to bovine  $\beta$ -casein and its ability to lower the interfacial tension at the air-water and  
735 to stabilize milk foams.

736 Fig. 6B shows the fluorescence emission spectra of the studied whey proteins including  
737 bovine  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin as well as camel  $\alpha$ -lactalbumin. First, fluorescence  
738 emission spectra of  $\beta$ -lactoglobulin display a peak at 334.80 nm as shown in Fig. 6B. These  
739 findings are in agreement with those of Kong et al. (2020). These authors noted that the  $\lambda_{\max}$  of  
740 the pure bovine  $\beta$ -lactoglobulin is of 334 nm after an excitation of tryptophan residues at 280  
741 nm (Kong et al., 2020). These authors have attributed the intrinsic fluorescence of  $\beta$ -  
742 lactoglobulin to the residues tryptophan-19 which is located in a hydrophobic pocket and well-  
743 protected as well as tryptophan-61 which is completely exposed to the solvent (Kong et al.,  
744 2020).

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

756 Fig. 6B shows that fluorescence emission spectra of camel  $\alpha$ -lactalbumin were different  
757 when compared to those of its bovine counterpart with a lower  $\lambda_{\max}$  value of 304.25 nm and a  
758 lower fluorescence intensity suggesting different molecular structure of both proteins and lower  
759 hydrophobicity of the camel  $\alpha$ -lactalbumin. These results are in great consistence with those of  
760 Ellouze et al. (2019) and Ellouze et al. (2020). These authors reported that camel  $\alpha$ -lactalbumin  
761 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  $\alpha$ -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  $\alpha$ -  
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  $\alpha$ -lactalbumin and the higher viscoelastic modulus of bovine  $\alpha$ -  
769 lactalbumin compared to its camel counterpart.

#### 770 **4. Conclusion**

771 The results obtained in this work indicate that skim milk, sodium caseinates and  $\beta$ -casein  
772 exhibited the highest foaming and stabilizing properties when compared to globular whey  
773 proteins (whey,  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin fractions). The maximum foamability was  
774 observed with camel milk proteins fractions especially camel Na-cas and  $\beta$ -casein while bovine  
775 proteins gave the highest foam stability with higher FS values for bovine  $\beta$ -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  $\beta$ -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 reformation more difficult but the resulting surface viscoelasticity higher contrary to  
785 caseins.

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

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

807  
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811

812 **References**

813 Al haj, O. A., & Al Kanhal, H. A. (2010). Compositional, technological and nutritional aspects  
814 of dromedary camel milk. *International Dairy Journal*, 20(12), 811–821.

815 Atri, M. S., Saboury, A. A., Yousefi, R., Chobert, J., Haertle, T., & Moosavi-Movahedi, A. A.  
816 (2010). Comparative study on heat stability of camel and bovine apo and holo  $\alpha$ -  
817 lactalbumin. *Journal of Dairy Research*. 77,43–49.

818 Bahri, A., Henriquet, C., Pugnère, M., Marchesseau, S., & Chevalier-Lucia, D. (2019). Binding  
819 analysis between monomeric  $\beta$ -casein and hydrophobic bioactive compounds investigated  
820 by surface plasmon resonance and fluorescence spectroscopy. *Food Chemistry*, 286, 289–  
821 296.

822 Barzegar, A., Yousefi, R., Sharifzadeh, A., Dalgalarondo, M., Chobert, J. M., Ganjali, M. R.,  
823 Norouzi, P., Ehsani, M. R., Niasari-Naslaji, A., Saboury, A. A., Haertlé, T., & Moosavi-  
824 Movahedi, A. A. (2008). Chaperone activities of bovine and camel  $\beta$ -caseins: Importance  
825 of their surface hydrophobicity in protection against alcohol dehydrogenase aggregation.  
826 *International Journal of Biological Macromolecules*, 42(4), 392–399

827 Borcharding, K., Lorenzen, P. C., Hoffmann, W., & Schrader, K. (2008). Effect of foaming  
828 temperature and varying time/temperature-conditions of pre-heating on the foaming  
829 properties of skimmed milk. *International Dairy Journal*, 18(4), 349–358.

830 Bos, M. A., & Van Vliet, T. (2001). Interfacial rheological properties of adsorbed protein layers  
831 and surfactants: a review. *Advances in Colloid and Interface Science*, 91(3), 437–471.

832 Brooker, B. E., Anderson, M., & Andrews, A. T. (1986). The development of structure in  
833 whipped cream. *Food Structure*, 5(2), 12.

834 Cao, Y., Xiong, Y. L., Cao, Y., & True, A. D. (2018). Interfacial properties of whey protein  
835 foams as influenced by preheating and phenolic binding at neutral pH. *Food*

836 *Hydrocolloids*, 82, 379–387.

837 Cases, E., Rampini, C., & Cayot, P. (2005). Interfacial properties of acidified skim milk.  
838 *Journal of Colloid and Interface Science*, 282(1), 133–141.

839 Cayot, P., Courthaudon, J. L., & Lorient, D. (1991). Emulsifying properties of pure and mixed.  
840  $\alpha_{S1}$ - and  $\beta$ -casein fractions: effects of chemical glycosylation. *Journal of Agricultural and*  
841 *Food Chemistry*, 39(8), 1369–1373.

842 Chatterton, D. E. W., Smithers, G., Roupas, P., & Brodkorb, A. (2006). Bioactivity of  $\beta$ -  
843 lactoglobulin and  $\alpha$ -lactalbumin-Technological implications for processing. *International*  
844 *Dairy Journal*, 16(11), 1229–1240.

845 Chen, J., Dickinson, E., & Iveson, G. (1993). Interfacial interactions, competitive adsorption  
846 and emulsion stability. *Food Structure*, 12(2), 1.

847 Clark, D. C., Husband, F., Wilde, P. J., Cornec, M., Miller, R., Krägel, J., & Wüstneck, R.  
848 (1995). Evidence of extraneous surfactant adsorption altering adsorbed layer properties of  
849  $\beta$ -lactoglobulin. *Journal of the Chemical Society, Faraday Transactions*, 91(13), 1991–  
850 1996.

851 Closs, B., Courthaudon, J., & Lorient, D. (1990). Effect of chemical glycosylation on the  
852 surface properties of the soluble fraction of casein. *Journal of Food Science*, 55(2), 437–  
853 439.

854 Cornec, M., Cho, D., & Narsimhan, G. (1999). Adsorption dynamics of  $\alpha$ -lactalbumin and  $\beta$ -  
855 lactoglobulin at air–water interfaces. *Journal of Colloid and Interface Science*, 214(2),  
856 129–142.

857 Davies, D. T., & Law, A. J. R. (1980). The content and composition of protein in creamery  
858 milks in south-west Scotland. *Journal of Dairy Research*, 47(01), 83–90.

859 De Kruijff C.G., & Holt C. (2003). Casein Micelle Structure, Functions and Interactions. In: Fox  
860 P.F., McSweeney P.L.H. (eds) *Advanced Dairy Chemistry—1 Proteins*. Springer, Boston,  
861 MA.

862 Diao, M., Liang, Y., Zhao, J., Zhao, C., Zhang, J., & Zhang, T. (2021). Enhanced cytotoxicity  
863 and antioxidant capacity of kaempferol complexed with  $\alpha$ -lactalbumin. *Food and*  
864 *Chemical Toxicology*, *153*, 112265.

865 Dickinson, E., Horne, D. S., Phipps, J. S., & Richardson, R. M. (1993). A neutron reflectivity  
866 study of the adsorption of  $\beta$ -casein at fluid interfaces. *Langmuir*, *9*(1), 242–248.

867 Dickinson, E. (1989). Surface and emulsifying properties of caseins. *Journal of Dairy*  
868 *Research*, *56*(03), 471–477.

869 Dickinson, E. (2001). Milk protein interfacial layers and the relationship to emulsion stability  
870 and rheology. *Colloids and Surfaces B: Biointerfaces*, *20*(3), 197–210.

871 Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of  
872 dispersed systems. *Food Hydrocolloids*, *17*(1), 25–39.

873 Dickinson, E., & Hong, S.T. (1994). Surface Coverage of  $\beta$ -lactoglobulin at the oil-water  
874 interface: influence of protein heat treatment and various emulsifiers. *Journal of*  
875 *Agricultural and Food Chemistry*, *42*(8), 1602–1606.

876 Dombrowski, J., Dechau, J., & Kulozik, U. (2016). Multiscale approach to characterize bulk,  
877 surface and foaming behavior of casein micelles as a function of alkalisation. *Food*  
878 *Hydrocolloids*, *57*, 92–102.

879 Eigel, W. N., Butler, J. E., Ernstrom, C. A., Farrell, H. M., Harwalkar, V. R., Jenness, R., &  
880 Whitney, R. M. (1984). Nomenclature of proteins of cow's milk: fifth revision. *Journal of*  
881 *Dairy Science*, *67*(8), 1599–1631.

- 882 El-Agamy, E I. (2006). Camel milk. *Handbook of Milk of Non-Bovine Mammals*, 297–344.
- 883 El-Agamy, E. I. (2009). Bioactive components in camel milk. *Bioactive Components in Milk*  
884 *and Dairy Products*, 107, 159.
- 885 El-Hatmi, H., Girardet, J. M., Gaillard, J. L., Yahyaoui, M. H., & Attia, H. (2007).  
886 Characterisation of whey proteins of camel (*Camelus dromedarius*) milk and colostrum.  
887 *Small Ruminant Research*, 70(2–3), 267–271.
- 888 Ellouze, M., Lajnaf, R., Zouari, A., Attia, H., Ayadi, M. A., & Vial, C. (2020). Camel  $\alpha$ -  
889 lactalbumin at the oil-water interface: Effect of protein concentration and pH change on  
890 surface characteristics and emulsifying properties. *Colloids and Surfaces B: Biointerfaces*,  
891 189, 110654.
- 892 Ellouze, M., Vial, C., Attia, H., & Ayadi, M. A. (2019). Camel  $\alpha$ -lactalbumin at the oil-water  
893 interface: Effect of pH and heat treatment on the structure, surface characteristics and  
894 emulsifying properties. *LWT - Food Science & Technology*, 116, 108550.
- 895 Ellouze, M., Vial, C., Attia, H., & Ayadi, M. A. (2021). Effect of pH and heat treatment on  
896 structure, surface characteristics and emulsifying properties of purified camel  $\beta$ -casein.  
897 *Food Chemistry*, 365, 130421.
- 898 Ereifej, K. I., Alu'datt, M. H., Alkhalidy, H. A., Alli, I., & Rababah, T. (2011). Comparison  
899 and characterisation of fat and protein composition for camel milk from eight Jordanian  
900 locations. *Food Chemistry*, 127(1), 282–289.
- 901 Esmaili, M., Ghaffari, S. M., Moosavi-Movahedi, Z., Atri, M. S., Sharifizadeh, A., Farhadi,  
902 M., Yousefi, R., Chobert, J. M., Haertlé, T., & Moosavi-Movahedi, A. A. (2011). Beta  
903 casein-micelle as a nano vehicle for solubility enhancement of curcumin; food industry  
904 application. *LWT - Food Science and Technology*, 44(10), 2166–2172.

905

906 Farah, Z., Rettenmaier, R., & Atkins, D. (1992). Vitamin content of camel milk. *International*  
907 *Journal for Vitamin and Nutrition Research*, 62(1), 30–33.

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

911 Fox, P. F., McSweeney, P. L. H., & Paul, L. H. (1998). *Dairy chemistry and biochemistry*.  
912 Springer.

913 Gamboa, G. V., & Barraquio, V. L. (2012). Foaming properties at different fat levels and age of  
914 milk. *Philippine Agricultural Scientist*, 95(4), 416–421.

915 Gasteiger, E., Hoogland, C., Gattiker, A., Wilkins, M. R., Appel, R. D., & Bairoch, A. (2005).  
916 Protein identification and analysis tools on the ExPASy server. *The Proteomics Protocols*  
917 *Handbook*, 571–607.

918 Hailu, Y., Hansen, E. B., Seifu, E., Eshetu, M., Ipsen, R., & Kappeler, S. (2016). Functional  
919 and technological properties of camel milk proteins: a review. *Journal of Dairy Research*,  
920 83(4), 422–429.

921 Ho, T. M., Bhandari, B. R., & Bansal, N. (2021). Functionality of bovine milk proteins and  
922 other factors in foaming properties of milk: a review. *Critical Reviews in Food Science*  
923 *and Nutrition*, 1–21.

924 Ho, T. M., Dhungana, P., Bhandari, B., & Bansal, N. (2021). Effect of the native fat globule  
925 size on foaming properties and foam structure of milk. *Journal of Food Engineering*, 291,  
926 110227.

927 Huppertz, T., Hennebel, J. B., Considine, T., Shakeel-Ur-Rehman, Kelly, A. L., & Fox, P. F.

928 (2006). A method for the large-scale isolation of  $\beta$ -casein. *Food Chemistry*, 99(1), 45–50.

929 Ibanoglu, E., & Ibanoglu, Ş. (1999). Foaming behaviour of EDTA-treated  $\alpha$ -lactalbumin. *Food*  
930 *Chemistry*, 66(4), 477–481.

931 Jara, F. L., Sánchez, C. C., Patino, R. J. M., & Pilosof, A. M. R. (2014). Competitive adsorption  
932 behavior of  $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin, bovin serum albumin in presence of  
933 hydroxypropylmethylcellulose. Influence of pH. *Food Hydrocolloids*, 35, 189–197.

934 Kanduc, D. (2012). Homology, similarity, and identity in peptide epitope immunodefinition.  
935 *Journal of Peptide Science*, 18(8), 487–494.

936 Kappeler, S, Farah, Z., & Puhan, Z. (1998). Sequence analysis of *Camelus dromedarius* milk  
937 caseins. *The Journal of Dairy Research*, 65(2), 209–222.

938 Kappeler, S., Farah, Z., & Puhan, Z. (2003). 5'-Flanking regions of camel milk genes are highly  
939 similar to homologue regions of other species and can be divided into two Distinct Groups.  
940 *Journal of Dairy Science*, 86(2), 498–508.

941 Kappeler, S. (1998). *Compositional and structural analysis of camel milk proteins with*  
942 *emphasis on protective proteins*. Diss. Techn. Wiss. ETH-Zürich, Nr. 12947, 1998. Ref.:  
943 Zdenko Puhan; Korref.: Zakaria Farah; Korref: Gerald Stranzinger.

944 Kappeler, S. R., Ackermann, M., Farah, Z., & Puhan, Z. (1999). Sequence analysis of camel  
945 (*Camelus dromedarius*) lactoferrin. *International Dairy Journal*, 9(7), 481-486.

946 Kong, F., Tian, J., Yang, M., Zheng, Y., Cao, X., & Yue, X. (2020). Characteristics of the  
947 interaction mechanisms of xylitol with  $\beta$ -lactoglobulin and  $\beta$ -casein: A multi-spectral  
948 method and docking study. *Spectrochimica Acta Part A: Molecular and Biomolecular*  
949 *Spectroscopy*, 243, 118824.

950 Kyte, J., & Doolittle, R. F. (1982). A simple method for displaying the hydropathic character  
951 of a protein. *Journal of Molecular Biology*, 157(1), 105–132.



- 952 Lajnaf, R., Picart-Palmade, L., Attia, H., Marchesseau, S., & Ayadi, M. A. (2017). The effect  
953 of pH and heat treatments on the foaming properties of purified  $\alpha$ -lactalbumin from camel  
954 milk. *Colloids and Surfaces B: Biointerfaces*, 156, 55–61.
- 955 Lajnaf, R., Picart-Palmade, L., Cases, E., Attia, H., Marchesseau, S., & Ayadi, M. A. (2018).  
956 The foaming properties of camel and bovine whey: The impact of pH and heat treatment.  
957 *Food Chemistry*, 240, 295–303.
- 958 Lajnaf, R., Zouari, A., Trigui, I., Attia, H., & Ayadi, M. A. (2020 a). Effect of different heating  
959 temperatures on foaming properties of camel milk proteins: A comparison with bovine  
960 milk proteins. *International Dairy Journal*, 104, 104643.
- 961 Lajnaf, R., Gharsallah, H., Jridi, M., Attia, H., & Ayadi, M. A. (2020 b). Antioxidant and  
962 antibacterial activities, interfacial and emulsifying properties of the apo and holo forms of  
963 purified camel and bovine  $\alpha$ -lactalbumin. *International Journal of Biological*  
964 *Macromolecules*, 165, 205–213.
- 965 Lajnaf, R., Gharsallah, H., Attia, H., & Ayadi, M. A. (2021). Comparative study on antioxidant,  
966 antimicrobial, emulsifying and physico-chemical properties of purified bovine and camel  
967  $\beta$ -casein. *LWT- Food Science & Technology*, 140, 110842.
- 968 Lajnaf, R., Picart-Palmade, L., Attia, H., Marchesseau, S., & Ayadi, M. A. (2016). Foaming  
969 and adsorption behavior of bovine and camel proteins mixed layers at the air/water  
970 interface. *Colloids and Surfaces B: Biointerfaces*, 151, 287–294.
- 971 Lajnaf, R., Trigui, I., Samet-Bali, O., Attia, H., & Ayadi, M. A. (2019). Comparative study on  
972 emulsifying and physico-chemical properties of bovine and camel acid and sweet wheys.  
973 *Journal of Food Engineering*, 109741.
- 974 Laleye, L. C., Jobe, B., & Wasesa, A. A. H. (2008). Comparative study on heat stability and

975 functionality of camel and bovine milk whey proteins. *Journal of Dairy Science*, 91(12),  
976 4527–4534.

977 Lam, R. S. H., & Nickerson, M. T. (2015 a). The effect of pH and temperature pre-treatments  
978 on the physicochemical and emulsifying properties of whey protein isolate. *LWT - Food  
979 Science and Technology*, 60(1), 427–434.

980 Lam, R. S. H., & Nickerson, M. T. (2015 b). The effect of pH and temperature pre-treatments  
981 on the structure, surface characteristics and emulsifying properties of alpha-lactalbumin.  
982 *Food Chemistry*, 173, 163–170.

983 Li, M., Blecker, C., & Karboune, S. (2021). Molecular and air-water interfacial properties of  
984 potato protein upon modification via laccase-catalyzed cross-linking and conjugation with  
985 sugar beet pectin. *Food Hydrocolloids*, 112, 106236.

986 Li, R. R., Yue, H. T., Shi, Z. Y., Shen, T., Yao, H. B., Zhang, J. W., Gao, Y., & Yang, J. (2020).  
987 Protein profile of whole camel milk resulting from commercial thermal treatment. *LWT-  
988 Food Science & Technology*, 134, 110256.

989 Li, X., Murray, B. S., Yang, Y., & Sarkar, A. (2020). Egg white protein microgels as aqueous  
990 Pickering foam stabilizers: Bubble stability and interfacial properties. *Food  
991 Hydrocolloids*, 98, 105292.

992 Liang, L. I., & Luo, Y. (2020). Casein and pectin: Structures, interactions, and applications.  
993 *Trends in Food Science & Technology*, 97, 391–403.

994 Lorient, D., Closs, B., & Courthaudon, J. L. (1991). Connaissances nouvelles sur les propriétés  
995 fonctionnelles des protéines du lait et des dérivés. *Le Lait*, 71(2), 141–171.

996 Lorient, D., Closs, B., & Courthaudon, J. L. (1989). Surface properties of the bovine casein  
997 components: relationships between structure and foaming properties. *Journal of Dairy  
998 Research*, 56(3), 495–502.

- 999 Madureira, A. R., Tavares, T., Gomes, A. M. P., Pintado, M. E., & Malcata, F. X. (2010).  
1000 Invited review: physiological properties of bioactive peptides obtained from whey  
1001 proteins. *Journal of Dairy Science*, *93*(2), 437–455.
- 1002 Marinova, K. G., Basheva, E. S., Nenova, B., Temelska, M., Mirarefi, A. Y., Campbell, B., &  
1003 Ivanov, I. B. (2009). Physico-chemical factors controlling the foamability and foam  
1004 stability of milk proteins: Sodium caseinate and whey protein concentrates. *Food*  
1005 *Hydrocolloids*, *23*(7), 1864–1876.
- 1006 Mellema, M., & Isenbart, J. G. (2004). Effect of acidification and heating on the rheological  
1007 properties of oil-water interfaces with adsorbed milk proteins. *Journal of Dairy Science*,  
1008 *87*(9), 2769–2778.
- 1009 Merin, U., Bernstein, S., Yagil, R., Creveld, C. Van, Lindner, P., & Gollop, N. (2001). A  
1010 comparative study of milk serum proteins in camel (*Camelus dromedarius*) and bovine  
1011 colostrum. *Livestock Production Science*, *67*, 297–301.
- 1012 Murray, B. S. (2020). Recent Developments in Food Foams. *Current Opinion in Colloid &*  
1013 *Interface Science*, 101394.
- 1014 Nicorescu, I., Vial, C., Talansier, E., Lechevalier, V., Loisel, C., Valle, D. Della, Riaublanc, A.,  
1015 Djelveh, G., & Legrand, J. (2011). Comparative effect of thermal treatment on the  
1016 physicochemical properties of whey and egg white protein foams. *Food Hydrocolloids*,  
1017 *25*(4), 797–808.
- 1018 Nylander, T., Arnebrant, T., Cárdenas, M., Bos, M., & Wilde, P. (2019). Protein/emulsifier  
1019 interactions. In *Food emulsifiers and their applications* (pp. 101–192). Springer.
- 1020 Omar, A., Harbourne, N., & Oruna-Concha, M. J. (2016). Quantification of major camel milk  
1021 proteins by capillary electrophoresis. *International Dairy Journal*, *58*, 31–35.
- 1022 Paulsson, M., & Dejmek, P. (1992). Surface film pressure of  $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin and

1023 bovine serum albumin at the air/water interface studied by Wilhelmy plate and drop  
1024 volume. *Journal of Colloid and Interface Science*, 150(2), 394–403.

1025 Razumovsky, L., & Damodaran, S. (1999). Surface activity– compressibility relationship of  
1026 proteins at the air– water interface. *Langmuir*, 15(4), 1392–1399.

1027 Redington, J., Breydo, L., Almehdar, H., Redwan, E., & Uversky, V. (2016).  $\alpha$ -Lactalbumin:  
1028 of camels and cows. *Protein & Peptide Letters*, 23(12), 1072–1080.

1029 Rouimi, S., Schorsch, C., Valentini, C., & Vaslin, S. (2005). Foam stability and interfacial  
1030 properties of milk protein-surfactant systems. *Food Hydrocolloids*, 19(3), 467–478.

1031 Salami, M., Moosavi-Movahedi, A. A., Moosavi-Movahedi, F., Ehsani, M. R., Yousefi, R.,  
1032 Farhadi, M., Niasari-Naslaji, A., Saboury, A. A., Chobert, J.-M., & Haertlé, T. (2011).  
1033 Biological activity of camel milk casein following enzymatic digestion. *Journal of Dairy*  
1034 *Research*, 78(04), 471–478.

1035 Salami, M., Yousefi, R., Ehsani, M. R., Razavi, S. H., Chobert, J.M., Haertlé, T., Saboury, A.  
1036 A., Atri, M. S., Niasari-Naslaji, A., & Ahmad, F. (2009). Enzymatic digestion and  
1037 antioxidant activity of the native and molten globule states of camel  $\alpha$ -lactalbumin:  
1038 Possible significance for use in infant formula. *International Dairy Journal*, 19(9), 518–  
1039 523.

1040 Salmen, S. H., Abu-Tarboush, H. M., Al-Saleh, A. A., & Metwalli, A. A. (2012). Amino acids  
1041 content and electrophoretic profile of camel milk casein from different camel breeds in  
1042 Saudi Arabia. *Saudi Journal of Biological Sciences*, 19(2), 177–183.

1043 Seta, L., Baldino, N., Gabriele, D., Lupi, F. R., & De Cindio, B. (2014). Rheology and  
1044 adsorption behaviour of  $\beta$ -casein and  $\beta$ -lactoglobulin mixed layers at the sunflower  
1045 oil/water interface. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*,  
1046 441, 669–677.

1047 Shalash, M. R. (1979). Utilization of camel meat and milk in human nourishment. Provisional  
1048 Report No. 6 *Workshop on camels, Khartoum, Sudan*. Stockholm, Sweden: International  
1049 Foundation of Science. 285–306.

1050 Slack, A. W., Amundson, C. H., & Hill, C. G. (1986). Foaming and emulsifying characteristics  
1051 of fractionated whey protein. *Journal of Food Processing and Preservation*, 10(2), 81–88.

1052 Svanborg, S., Johansen, A.-G., Abrahamsen, R. K., Schüller, R. B., & Skeie, S. B. (2016).  
1053 Caseinomacropptide influences the functional properties of a whey protein concentrate.  
1054 *International Dairy Journal*, 60, 14–23.

1055 Tamm, F., Sauer, G., Scampicchio, M., & Drusch, S. (2012). Pendant drop tensiometry for the  
1056 evaluation of the foaming properties of milk-derived proteins. *Food Hydrocolloids*, 27(2),  
1057 371–377.

1058 Thompson, A., Boland, M. J., & Singh, H. (2009). *Milk proteins, from expression to food*, SL  
1059 Taylor. Elsevier, Academic Press, Amsterdam.

1060 Wangoh, J., Farah, Z., & Puhan, Z. (1998). Iso-electric focusing of camel milk proteins.  
1061 *International Dairy Journal*, 8(7), 617–621.

1062 Williams, A., & Prins, A. (1996). Comparison of the dilational behaviour of adsorbed milk  
1063 proteins at the air-water and oil-water interfaces. *Colloids and Surfaces A:  
1064 Physicochemical and Engineering Aspects*, 114, 267–275.

1065 Xiong, X., Ho, M. T., Bhandari, B., & Bansal, N. (2020). Foaming properties of milk protein  
1066 dispersions at different protein content and casein to whey protein ratios. *International  
1067 Dairy Journal*, 109, 104758.

1068 Yang, H., Xiao, X., Zhao, X., & Wu, Y. (2017). Intrinsic fluorescence spectra of tryptophan,  
1069 tyrosine and phenylalanine. *Selected Papers of the Chinese Society for Optical  
1070 Engineering Conferences Held October and November 2016*, 10255, 102554M.

1071 Yin, Z., Qie, X., Zeng, M., Wang, Z., Qin, F., Chen, J., Li, W., & He, Z. (2022). Effect of  
1072 thermal treatment on the molecular-level interactions and antioxidant activities in  $\beta$ -casein  
1073 and chlorogenic acid complexes. *Food Hydrocolloids*, 123, 107177.

1074 Yüksel, Z., & Erdem, Y. K. (2010). Detection of the milk proteins by RP-HPLC. *GIDA-Journal*  
1075 *of Food*, 35(1), 5–11.

1076 Zayas, J. F. (1997). Foaming properties of proteins. In *Functionality of proteins in food* (pp.  
1077 260–309). Springer.

1078 Zhang, H., Yu, D., Sun, J., Guo, H., Ding, Q., Liu, R., & Ren, F. (2014). Interaction of milk  
1079 whey protein with common phenolic acids. *Journal of Molecular Structure*, 1058, 228–  
1080 233.

1081 Zhang, Z., Dalgleish, D. G., & Goff, H. D. (2004). Effect of pH and ionic strength on  
1082 competitive protein adsorption to air/water interfaces in aqueous foams made with mixed  
1083 milk proteins. *Colloids and Surfaces B: Biointerfaces*, 34(2), 113–121.

1084 Zhou, X., Sala, G., & Sagis, L. M. C. (2020 a). Bulk and interfacial properties of milk fat  
1085 emulsions stabilized by whey protein isolate and whey protein aggregates. *Food*  
1086 *Hydrocolloids*, 109, 106100.

1087 Zhou, B., Tobin, J. T., Drusch, S., & Hogan, S. A. (2020 b). Interfacial properties of milk  
1088 proteins: A review. *Advances in Colloid and Interface Science*, 102347.

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1092 **Figure captions**

1093 **Fig. 1.** Flow diagram of the camel milk protein isolation procedure. Abbreviations are: Na-cas,  
1094 sodium caseinates;  $\beta$ -CN,  $\beta$ -casein; and  $\alpha$ -La,  $\alpha$ -lactalbumin.

1095 **Fig. 2.** RP-HPLC chromatograms recorded at 220 nm for bovine and camel protein fractions  
1096 (chromatograms A and B, respectively). Abbreviations are: Na-cas, sodium caseinates;  $\beta$ -CN,  
1097  $\beta$ -casein;  $\alpha$ -La,  $\alpha$ -lactalbumin;  $\beta$ -Lg,  $\beta$ -lactoglobulin. F, protein fraction; CMP:  
1098 caseinomacropeptide.

1099 **Fig. 3.** Foam capacity (A) and Foam Stability (B) of camel and bovine skim milk (SM), sodium  
1100 caseinates (Na-cas),  $\beta$ -casein ( $\beta$ -CN), whey,  $\beta$ -lactoglobulin ( $\beta$ -Lg) and  $\alpha$ -lactalbumin ( $\alpha$ -La).  
1101 The experiments were performed in 20 mM Tris-HCl buffer, pH 7.0, at 25 °C at a protein  
1102 concentration of 0.5 g L<sup>-1</sup>.

1103 <sup>a-f</sup> Samples represented with different letters are significantly different from each other (p<0.05). Error bars show  
1104 the standard deviations of mean values of foam capacity and stability.

1105 **Fig. 4.** Time-dependent changes in surface tension  $\gamma(t)$  (mN m<sup>-1</sup>) at air-water interface of  
1106 bovine(A) and camel (B) proteins systems: Skim bovine milk (skim Bo-M), skim camel milk  
1107 (skim Ca-M), sodium caseinates (Na-cas), whey,  $\alpha$ -lactalbumin ( $\alpha$ -La),  $\beta$ -casein ( $\beta$ -CN) and  $\beta$ -  
1108 lactoglobulin ( $\beta$ -Lg), at a concentration of 11 mg L<sup>-1</sup>, pH 7 and temperature 20 °C.

1109 **Fig. 5.** Time-dependent changes in viscoelastic modulus  $\varepsilon(t)$  (mN m<sup>-1</sup>) at air-water interface of  
1110 bovine (A) and camel (B) proteins systems: Skim bovine milk (skim Bo-M), skim camel milk  
1111 (skim Ca-M), sodium caseinates (Na-cas), whey,  $\alpha$ -lactalbumin ( $\alpha$ -La),  $\beta$ -casein ( $\beta$ -CN) and  $\beta$ -  
1112 lactoglobulin ( $\beta$ -Lg), at a concentration of 11 mg L<sup>-1</sup>, pH 7 and temperature 20 °C.

1113 **Fig. 6.** Intrinsic fluorescence intensity of bovine and camel  $\beta$ -caseins (protein concentration  
1114  $5\mu\text{M}$ ) (A) and pure whey proteins:  $\beta$ -lactalbumin and camel and bovine  $\alpha$ -lactalbumin (protein  
1115 concentration  $30\mu\text{M}$ ) (B) at a constant excitation wavelength of 275 nm as a function of  
1116 emission wavelength between 285 to 450 nm (temperature  $25^\circ\text{C}$ ).

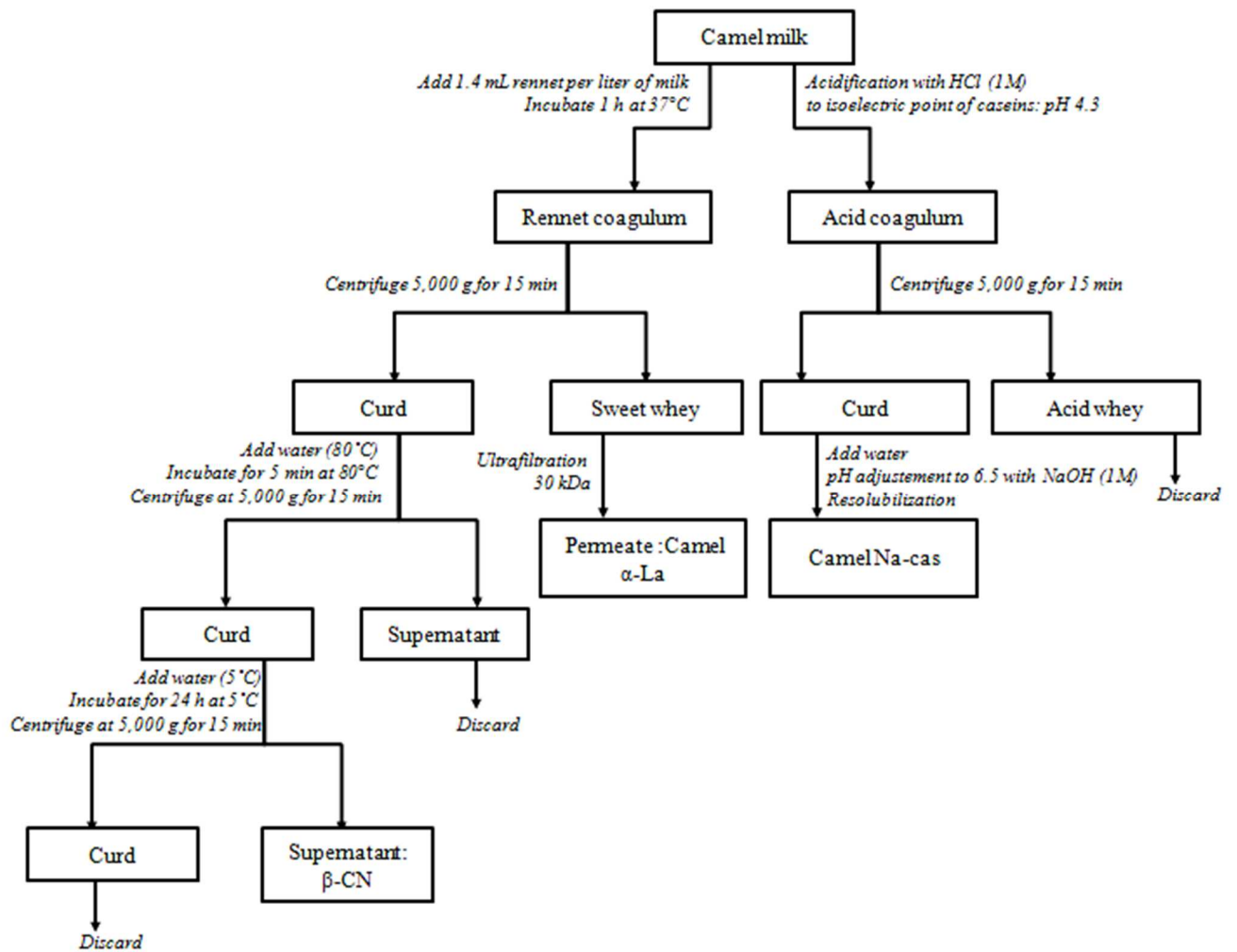
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1120 **Figures:**



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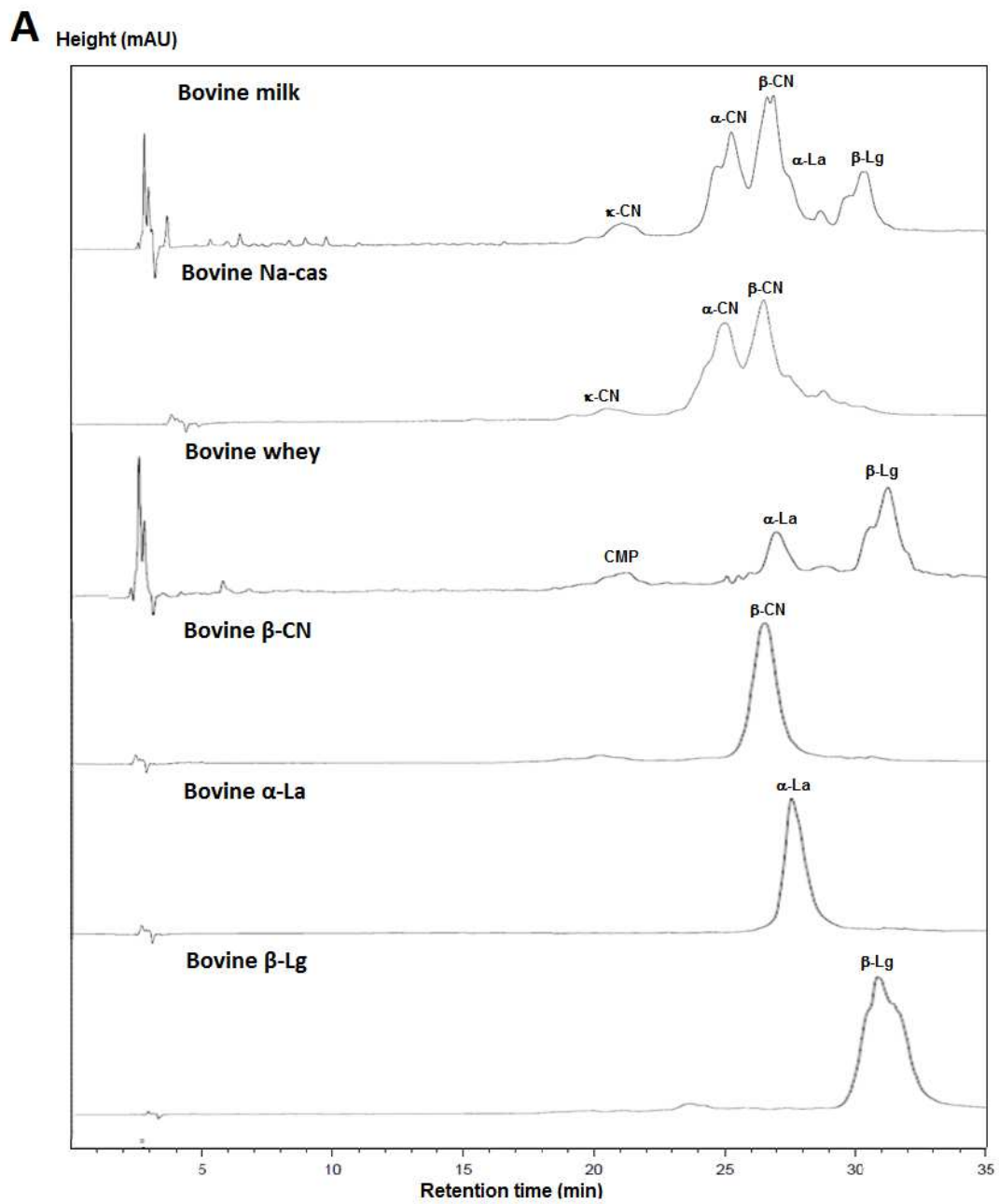
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**Fig. 1**



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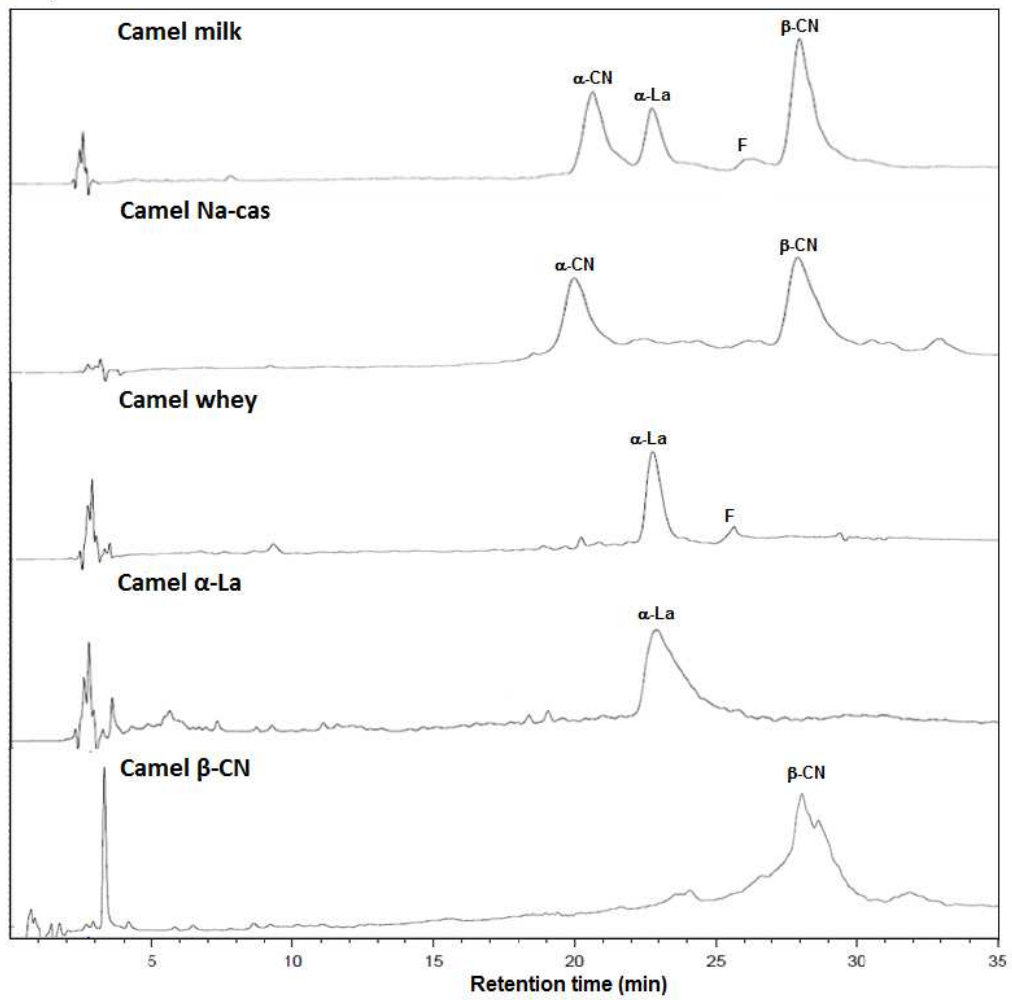
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**B**

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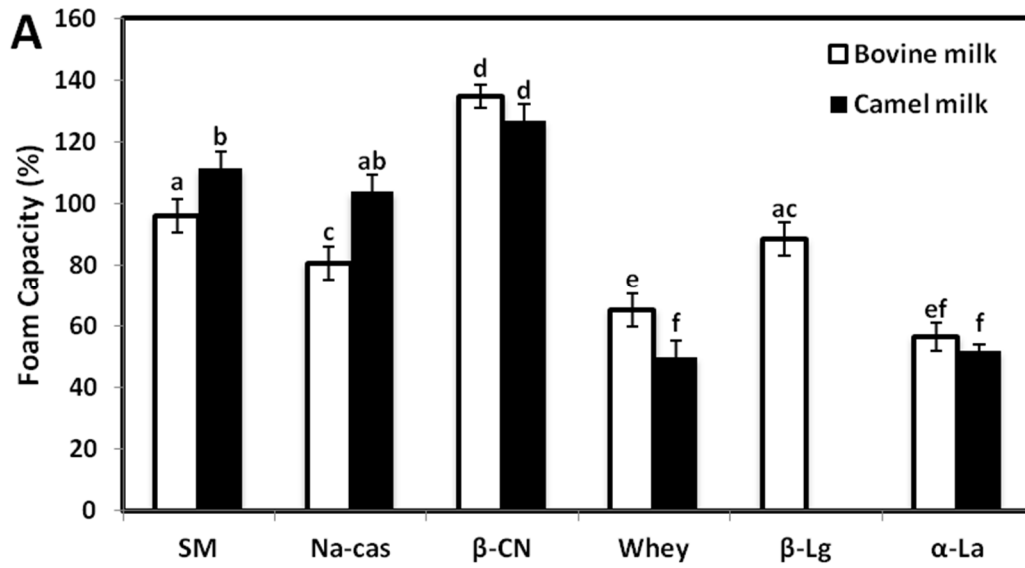


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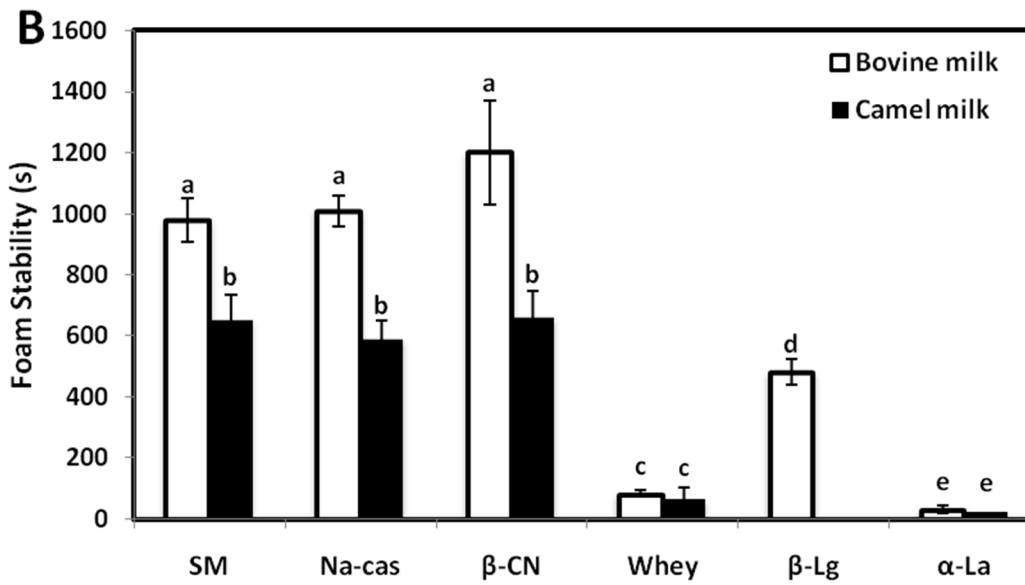
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**Fig. 2**



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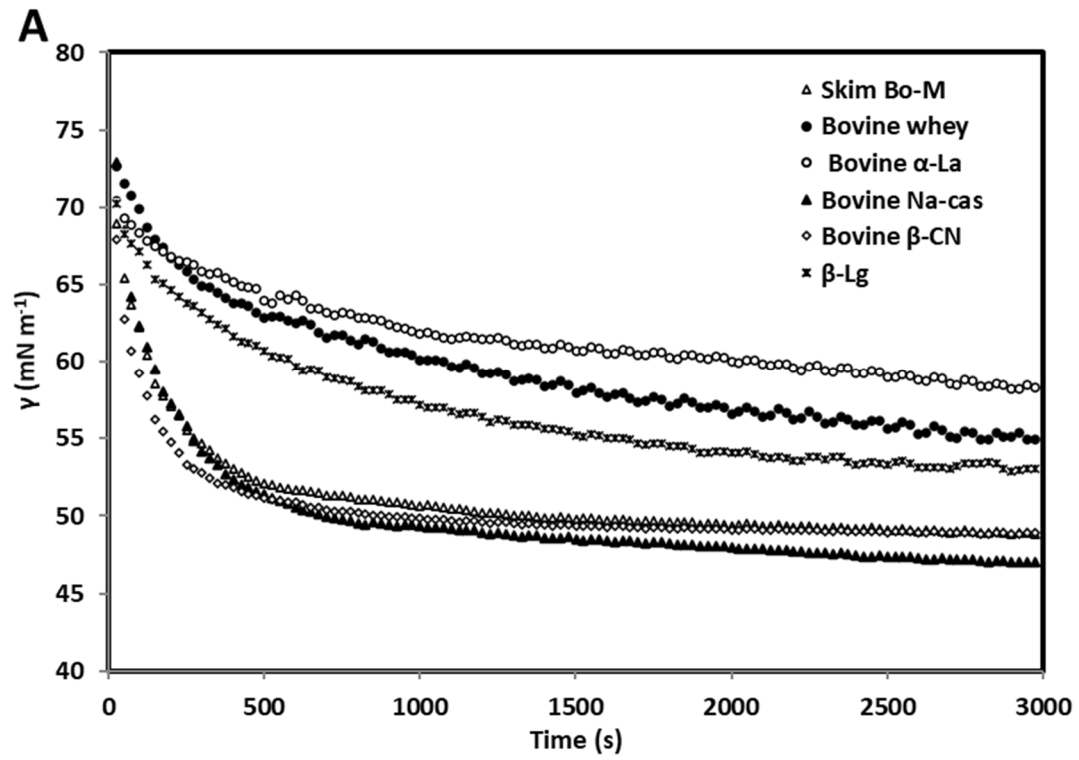


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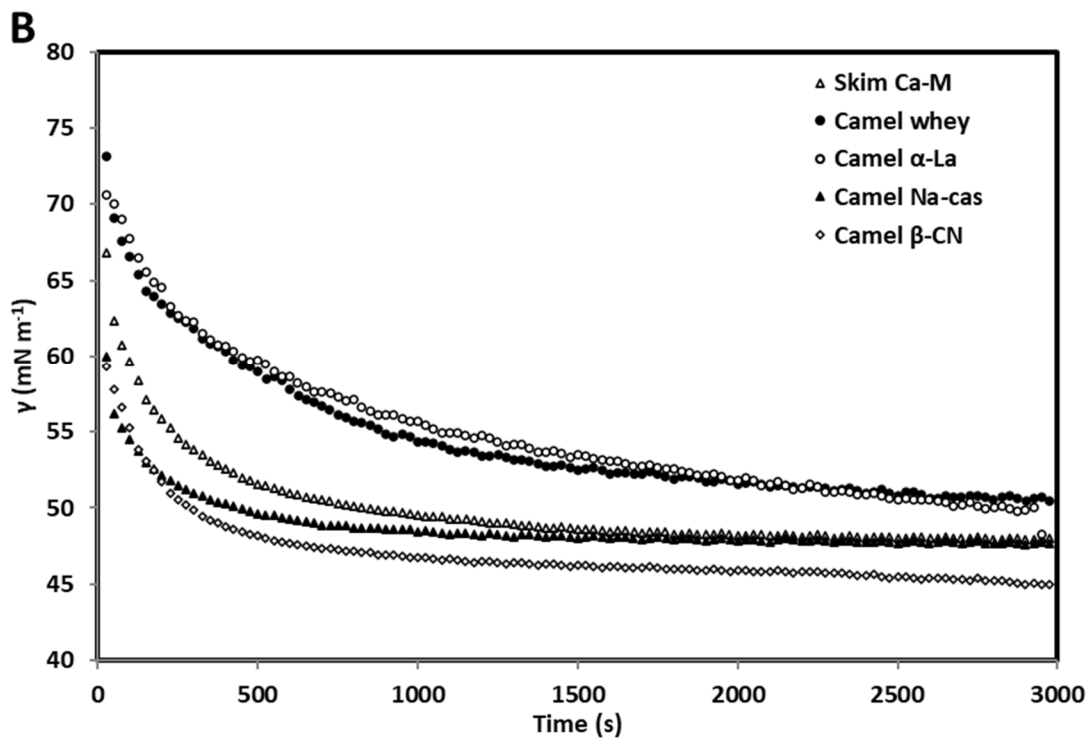
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**Fig. 3**



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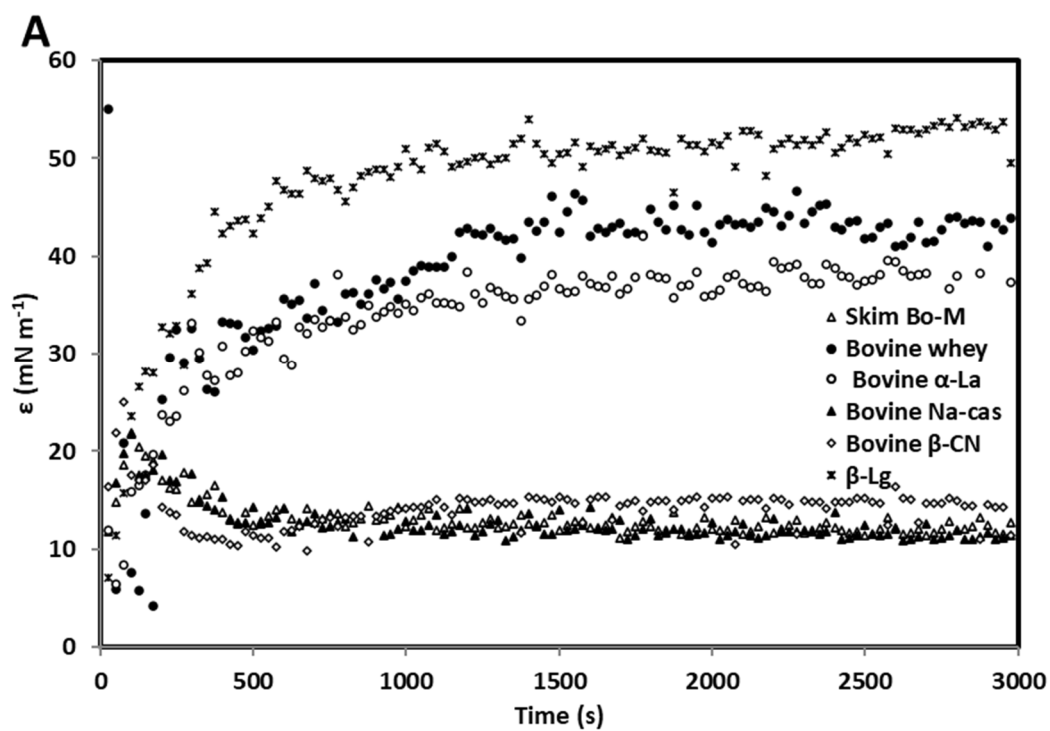
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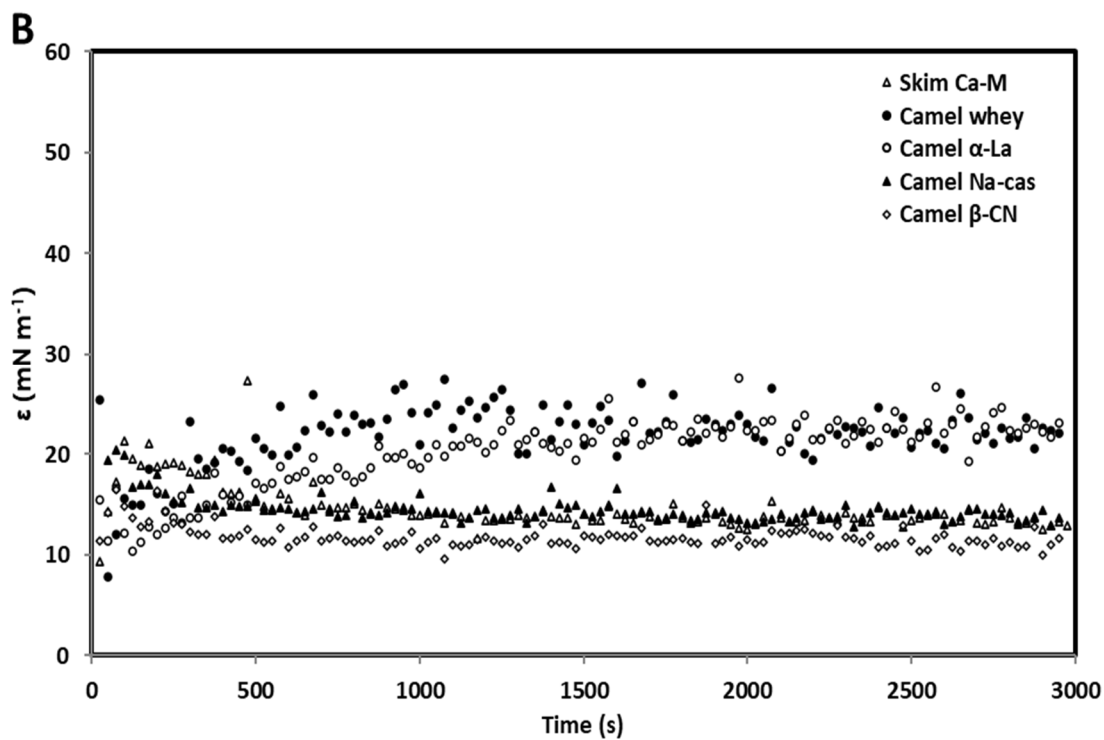
Fig. 4

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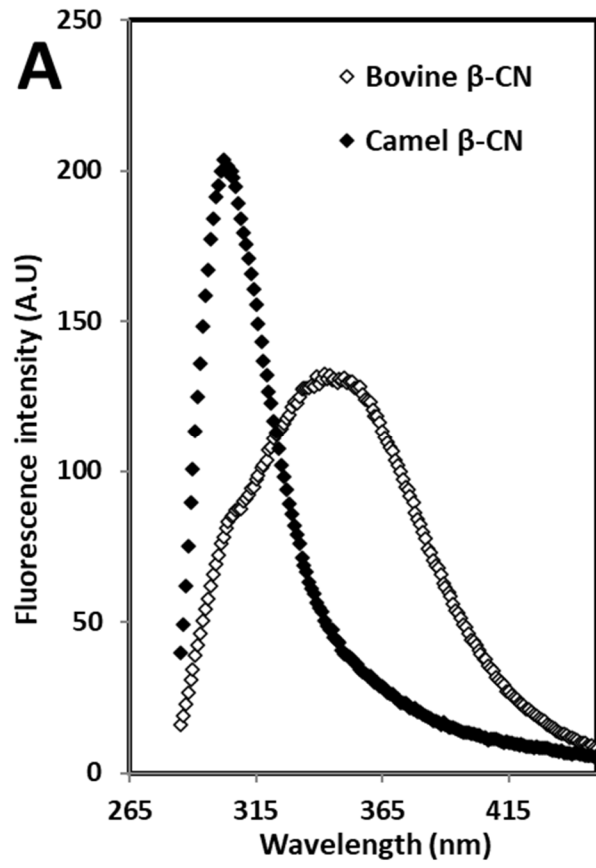
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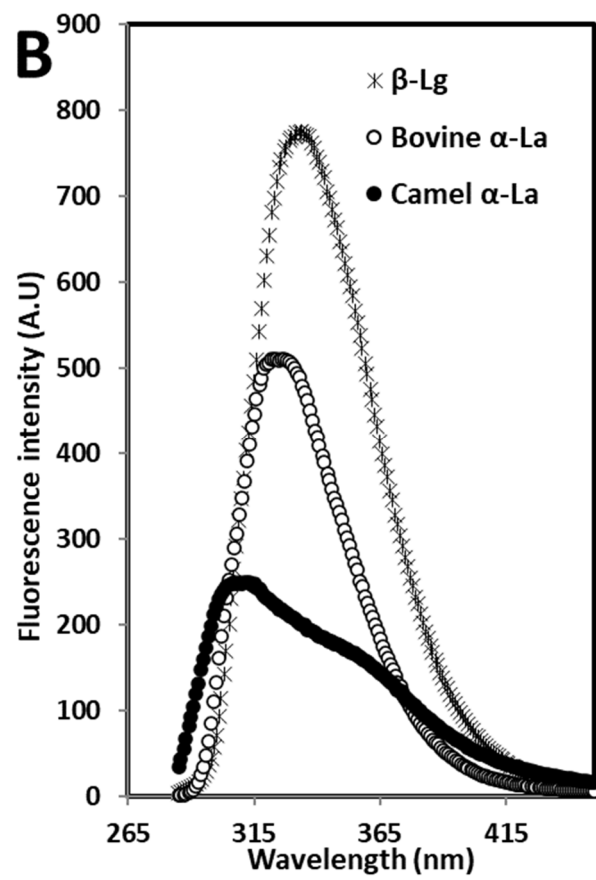
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Fig. 5



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**Fig. 6**

1166 **Table caption**

1167 **Table 1:** Comparison of the proportion of the main proteins of bovine milk (Bo-M) and camel  
 1168 milk (Ca-M) (Atri et al., 2010; Barzegar et al., 2008; Chatterton et al., 2006; El-Agamy, 2006;  
 1169 Hailu et al., 2016; Huang & Miller, 1991; Kappeler et al., 2003; Lajnaf et al., 2017, 2019).

1170 <sup>a</sup> : Proportion of individual protein in the casein fraction of milk.

1171 <sup>b</sup> : Percentage of the protein in the serum fraction of milk

1172 <sup>c</sup> Protein sequence identity: the ratio of the number of identical residues in a pair of aligned  
 1173 protein sequences to the length of the shorter one

1174 <sup>d</sup> Protein sequence similarity: similarities between aminoacid sequences including residues with  
 1175 similar biochemical properties

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1177 **Tables**

1178 **Table 1.**

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



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1180 **Table 2:** Adsorption kinetic parameters of camel and bovine milk proteins fractions at the air-  
1181 water interface: rate of adsorption ( $\text{mN m}^{-1} \text{s}^{-1}$ ). Abbreviations: Bo-M: Bovine milk, Ca-M:  
1182 Camel milk.

1183 <sup>a-h</sup> Samples represented with different letters are significantly different from each other ( $p < 0.05$ ). Error bars show  
1184 the standard deviations of mean values of adsorption rate (AR).

1185

**Table 2.**

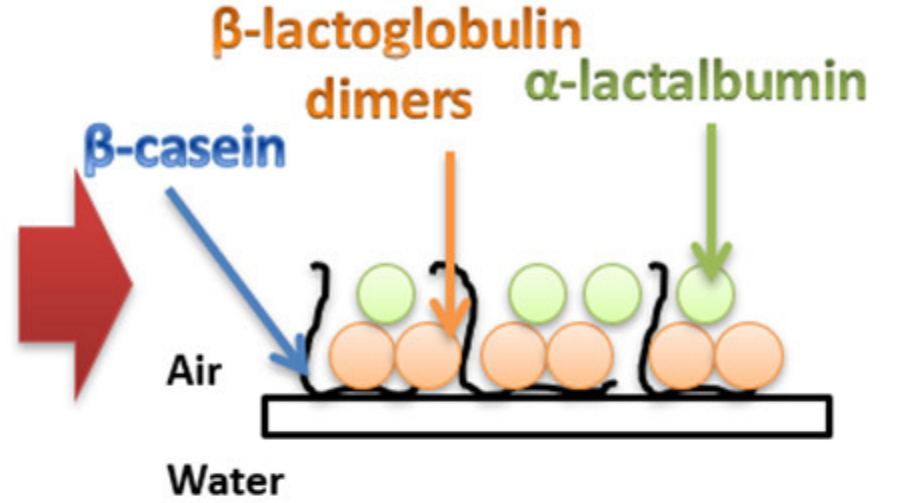
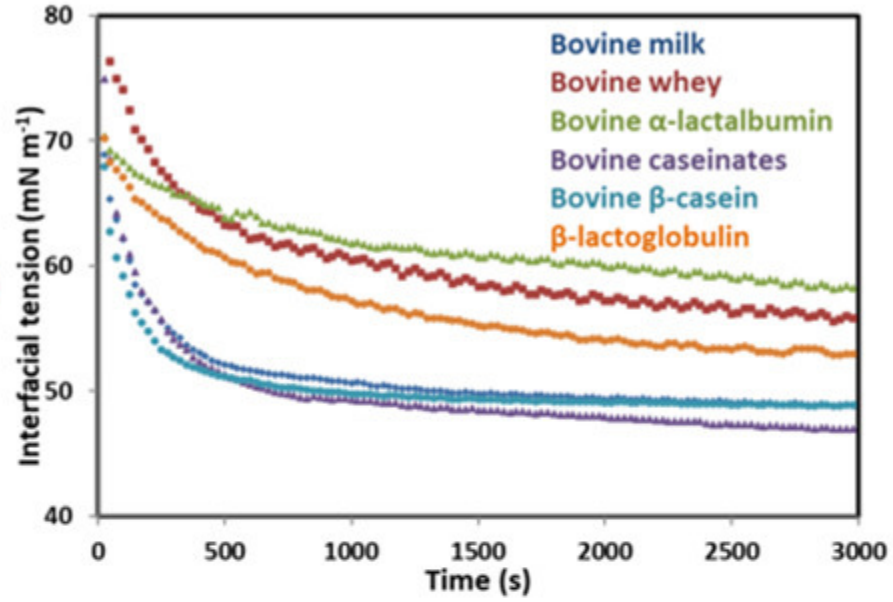
| <b>Protein fraction</b>                 | <b>Bo-M</b>                   | <b>Ca-M</b>                    |
|---|-------------------------------|--------------------------------|
| <b>Skim milk</b>                        | $0.238 \pm 0.016^{\text{de}}$ | $0.218 \pm 0.025^{\text{e}}$   |
| <b>Na-cas</b>                           | $0.252 \pm 0.005^{\text{d}}$  | $0.300 \pm 0.012^{\text{b}}$   |
| <b>Whey</b>                             | $0.153 \pm 0.003^{\text{h}}$  | $0.165 \pm 0.002^{\text{g}}$   |
| <b><math>\beta</math>-casein</b>        | $0.275 \pm 0.003^{\text{c}}$  | $0.362 \pm 0.023^{\text{a}}$   |
| <b><math>\beta</math>-lactoglobulin</b> | $0.185 \pm 0.011^{\text{f}}$  | --                             |
| <b><math>\alpha</math>-lactalbumin</b>  | $0.178 \pm 0.005^{\text{f}}$  | $0.170 \pm 0.013^{\text{fgh}}$ |

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Bovine milk



Camel milk

