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Roua Lajnaf, Ines Trigui, Olfa Samet-Bali, Hamadi Attia, M. A. Ayadi. Comparative study on emulsifying and physico-chemical properties of bovine and camel acid and sweet wheys. *Journal of Food Engineering*, 2020, 268, 10.1016/j.jfoodeng.2019.109741 . hal-02330242

**HAL Id: hal-02330242**

**<https://hal.science/hal-02330242>**

Submitted on 20 Jul 2022

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**Comparative study on emulsifying and physico-chemical properties of  
bovine and camel acid and sweet wheys**

Running title:

**Emulsifying properties of bovine and camel wheys**

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**Journal:** Journal of Food Engineering

**Running title:** Emulsifying properties of bovine and camel wheys

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**Abstract**

Main goal of this research was to examine the emulsifying properties of acid and sweet wheys extracted from bovine and camel fresh milks after heating at 70 and 90 °C for 30 min at laboratory scale. Specifically, emulsifying properties (emulsification stability (ESI) and activity (EAI) indexes) and the physico-chemical characteristics (surface hydrophobicity,  $\zeta$ -potential, interfacial tension and denaturation rate) of wheys were assessed. Maximum EAI and ESI were found for sweet wheys (EAI~ 2 m<sup>2</sup>/g; ESI~ 65%), with higher EAI values for the camel whey. This behavior was explained by the strongest electrostatic-repulsive forces between oil droplets under conditions away from the isoelectric-point of proteins in agreement with the  $\zeta$ -potential measurements.

Findings indicate that heating affected the physico-chemical properties of camel and bovine whey proteins in acidic conditions by increasing surface hydrophobicity and the ability to reduce the interfacial tension. These results confirmed the protein-protein aggregation of heated acid wheys as proved by electrophoreses.

**Key words:** Milk protein, techno-functional properties, aggregation, heat treatment, pH.

42        **1. Introduction**

43        Whey is the main by-product of caseins or cheese manufacture. It is of great importance  
44 in the dairy industry. Its demand is increasing for whey proteins preparation due to the high  
45 functional and nutritional values with application in food ingredients industry (Baldasso et al.,  
46 2011). Indeed, whey proteins have become the most employed proteins in food formulations  
47 due to their excellent functional characteristics such as emulsification (Nishanthi et al., 2017;  
48 Tosi et al., 2007).

49        Techno-functional properties such as emulsifying properties of bovine whey proteins  
50 have been intensively studied and reported with special interest in the effect of the physico-  
51 chemical factors such as the applied heat treatment, the chemical environment and the pH  
52 value (Lam and Nickerson, 2015a, 2015b; Mellema and Isenbart, 2004; Slack et al., 1986).  
53 Indeed, the  $\beta$ -lactoglobulin ( $\beta$ -lg), which is the main protein of soluble cow milk fraction, was  
54 found to have a lower emulsion stability at acid pH values when compared to that at pH 6.2,  
55 in spite of the greater adsorption rate near the isoelectric-point (pI) of this protein  
56 (Tcholakova et al., 2006). Furthermore, the  $\alpha$ -lactalbumin ( $\alpha$ -la) protein was found to coat the  
57 emulsion droplets better at neutral pH than that in acid conditions (Lam and Nickerson,  
58 2015b).

59        After a heat treatment, denatured milk proteins may polymerize to create aggregates,  
60 depending on both pH level and the heating temperature value (de la Fuente et al., 2002).  
61 Thus, the resulting emulsifying and foaming properties of proteins will be a competitive  
62 adsorption phenomenon between aggregates and non-aggregated denatured milk proteins  
63 (Schmitt et al., 2007; Lajnaf et al., 2018).

64        Camels are well-known producers of milk which is used as main food resource for  
65 populations in the arid countries and hot regions of the world. Camel milk is a potential  
66 source of all the essential nutrients already found in cow milk. Besides, it is rich in iron,

67 lactoferrin and vitamin C (Kappeler et al., 1999). Compared to the bovine whey, camel whey  
68 is devoid of the  $\beta$ -lg which has been considered as one of the most dominant bovine milk  
69 allergen limiting the use of this milk for the preparation of infant formulae (Uchida et al.,  
70 1996). Thus, the  $\alpha$ -la is the major protein of camel soluble protein fraction, with a  
71 concentration of 2.2 g/L (Omar et al., 2016). As bovine  $\alpha$ -la, camel  $\alpha$ -la is a calcium-  
72 metalloprotein composed of 123 amino acid residues, with a molecular weight of 14.43 kDa  
73 (Beg et al., 1985). However, when compared to bovine  $\alpha$ -la, camel one presents a different  
74 structure justified by a difference in the nature of 39 aminoacids between these two different  
75 proteins. Thus, the percentage sequence similarity and identity between the sequences are  
76 82.9 % and 69.1 %, respectively. Camel  $\alpha$ -la has a considerably more hydrophobic core than  
77 its bovine counterpart at positions 25–35. The secondary structure of this protein is more  
78 preserved during heat treatment, so, the camel  $\alpha$ -la is more thermostable than its bovine  
79 equivalent. However, its conformation is more sensitive to calcium loss (Atri et al., 2010).

80 The  $\alpha$ -la can be denatured in several ways, such as pH and heating temperature. For  
81 instance, at pH < 5, the  $\alpha$ -la has been found to lose its bound calcium ions and assumes the  
82 molten globular state (Matsumura et al., 1994). Furthermore, the  $\alpha$ -la denatures at relatively  
83 low temperatures (~64 °C) but does not aggregate at this temperature value because of its lack  
84 of free thiol groups. However, when held at temperatures > 85 °C, the  $\alpha$ -la evolves free -SH  
85 groups that form intermolecular disulfide-bonded aggregates (Doi et al., 1983; McGuffey et  
86 al., 2005).

87 Few studies describe the emulsifying behavior and interfacial properties of camel whey  
88 proteins (Laleye et al., 2008) even if for cow proteins, these properties were extensively  
89 studied and reported (Fachin and Viotto, 2005; Lam and Nickerson, 2015a; Jiang et al., 2018).  
90 Thus, the mechanism of emulsion creation of camel milk proteins has not been widely studied

91 in the literature and the absence of the  $\beta$ -lg is suggested to have a significant impact on the  
92 resulting emulsifying properties at different pH values.

93 Therefore, the goal of this work is to examine the effect of different heat treatments (70  
94 and 90 °C for 30 min) on the emulsifying properties of the extracted camel and bovine sweet  
95 and acid wheys at laboratory scale. The temperature values of the heat treatment (70 and  
96 90 °C) were chosen according to previous works as Laleye et al. (2008) and Felfoul et al.  
97 (2015). These authors demonstrated that at 70 °C, the  $\beta$ -lg molecules are reduced from dimers  
98 to monomers and begin to unfold. Besides, the denaturation temperature values of both bovine  
99 and camel  $\alpha$ -la are near 70 °C. Felfoul et al. (2015) reported also that 90 °C is the temperature  
100 of the total denaturation and aggregation of whey proteins.

101 This work would allow the valorization of the camel and bovine whey by-product of the  
102 cheese industry, as an ingredient in food industry.

103

## 104 **2. Materials and methods**

### 105 **2.1. Whey separation**

106 Fresh raw camel milk (*Camelus dromedarius*) was obtained from a cattle belonging to a  
107 farm which is located in the region of Medenine in Tunisia. Fresh bovine milk was derived  
108 from a local breed in the region of Sfax (Tunisia).

109 When arriving to the laboratory (at 4 °C), the physico-chemical composition of camel and  
110 bovine milk was systematically determined according to AOAC Official Method (AOAC,  
111 1984). Both milk samples were skimmed by centrifugation at 3000g for 20 min at 4 °C using  
112 a Thermo Scientific Heraeus Megafuge Centrifuge (Germany) and pH values of milk were  
113 measured using the pH meter “Metrohm” (Felfoul et al., 2015).

114 Sweet wheys of bovine and camel milks were extracted from skimmed milks after an  
115 enzymatic coagulation at 37 °C for 1-2 h in the presence of microbial rennet enzyme (0.35

116 and 1.4 mL per liter of skimmed bovine and camel milks, respectively) (Parachimic,  
117 Laboratories Arrazi, Sfax, Tunisia, strength = 1:10,000). Whereas, acid wheys were isolated  
118 after the acidification of skimmed milks using a solution of HCl (1 M) until pH = 4.3 and 4.6  
119 for camel and bovine milks, respectively (Felfoul et al., 2015).

120 Afterwards, wheys were separated from casein fraction by a centrifugation at 3000g for  
121 20 min at 20 °C (Thermo Scientific Heraeus Megafuge Centrifuge, Germany).

## 122 **2.2. Whey solution preparation**

123 The protein content of the isolated wheys was determined using the Kjeldahl method  
124 (AOAC, 1984).

125 Camel and bovine heated wheys were obtained after a heat treatment using water-bath at  
126 70 and 90 °C for 30 min followed by an ice incubation to stop the proteins denaturation. The  
127 control whey sample was at 20 °C. It corresponds to the native conditions. The selected  
128 parameters of the thermal treatment temperatures are chosen according to previous studies  
129 (Felfoul et al., 2015; Laleye et al., 2008).

## 130 **2.3. Emulsifying properties**

131 Oil-in-water emulsions were prepared by mixing 15% (v/v) corn oil (3.75 mL) and 85%  
132 (v/v) (21.25 mL) whey protein solutions at a concentration of 5 g/L using the Ultra-Turrax  
133 T25 high-speed mixer (IKA Labortechnik, Staufen, Germany) at a speed of 21,500 rpm for 30  
134 seconds.

135 A 100 µL aliquot of freshly created emulsion was taken from the bottom of the beaker  
136 and dispersed into 900 µL of 0.1% sodium dodecyl-sulfate (SDS) solution (w/v). The mixture  
137 was vortexed for 10 seconds and the absorbance was measured at  $\lambda = 500$  nm using a UV  
138 mini-1240 PC spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

139 The created emulsions were kept undisturbed for 10 min and then 100 µL aliquots were  
140 taken and dispersed into 900 µL of 0.1% SDS solution. The absorbance of the emulsion-SDS

141 mixture was also measured at 500 nm as described above. Emulsifying activity index (EAI,  
142 m<sup>2</sup>/g) and emulsion stability index (ESI, %) of whey solutions were calculated using Eq. (1)  
143 and Eq. (2) suggested by Pearce and Kinsella (1978):

144

$$145 \quad \text{EAI (m}^2\text{/g)} = [2 \times 2.303 \times A_{500} \times \text{dilution}] / [C \times (1 - \Phi) \times 10^4] \quad (1)$$

$$146 \quad \text{ESI (\%)} = [A_{10} / A_0] \times 100 \quad (2)$$

147

148 where  $A_{500}$  represents the absorbance of analysis whey sample at 500 nm, C is the protein  
149 concentration (g/mL),  $\Phi$  is the volume of the oil fraction (v/v) of the created emulsion ( $\Phi =$   
150 0.15), 100 is the dilution,  $A_0$  and  $A_{10}$  represent the absorbance at 500 nm at time zero and after  
151 10 min, respectively.

152 **Emulsifying activity index (EAI) and emulsion stability index (ESI) of Whey Protein**  
153 **Isolate (WPI, Lactalis Ingredient, Laval, France) and Sodium caseinates (C8654, Lot#**  
154 **BCBC3986V, Product of New Zealand) were also determined (at a protein concentration of 5**  
155 **g/L) in order to compare emulsifying properties of camel and bovine wheys-based emulsifier**  
156 **agents to commercial emulsifiers.**

157

#### 158 **2.4. SDS-PAGE electrophoresis**

159 SDS-PAGE electrophoresis was carried out on gels (12% acrylamide gel) using the  
160 technique described by Erefej et al. (2011) and Laemmli (1970). Electrophoresis was  
161 performed at a constant current 120V for 1.5-2 h (Mini Protean Tetra Cell, BioRad  
162 laboratories, USA).

163 Quantitative estimation of each whey protein amount was carried out using an  
164 appropriate software (Gel-Quant.NET; biochemlabsolutions.com).

#### 165 **2.5. Thiol groups content and denaturation rate**



166 Free thiol groups were quantified as described by Ellman (1959). 300  $\mu\text{L}$  of the extracted  
167 wheys at a concentration of 1 g/L were mixed with 50  $\mu\text{L}$  of DTNB (5,5'-dithio-bis(2-  
168 nitrobenzoic acid) solution (2 mM DTNB, 50 mM sodium acetate (NaAc) in  $\text{H}_2\text{O}$ ), 100  $\mu\text{L}$  of  
169 Tris solution (1 M Tris, pH 8.0) and 550  $\mu\text{L}$  of distilled water.

170 Mixture was incubated for 5 min at 37  $^\circ\text{C}$  using water bath. Then, the optical density  
171 (DO) was measured at  $\lambda = 412\text{nm}$ .

172

173 The free SH groups concentration ( $C_{\text{SH}}$ ) was calculated by Eq. (3):

174

$$175 \quad C_{\text{SH}} (\text{M}) = (DO_{412\text{nm}} / \varepsilon_{412}) \times (1000/300) \quad (3)$$

176

177 where  $DO_{412\text{nm}}$  is the absorbance at  $\lambda = 412 \text{ nm}$ ;  $\varepsilon_{412}$  is the DTNB extinction coefficient  
178 ( $13,600 \text{ M}^{-1}/\text{cm}$ ) at  $\lambda = 412\text{nm}$ . 1000  $\mu\text{L}$  is the cuvette volume and 300  $\mu\text{L}$  is the protein  
179 sample volume.

180 In this assay, the whey proteins' denaturation rate (DR) was calculated by the Eq. (4):

$$181 \quad \text{Denaturation rate (\%)} = \frac{C_{\text{SH}}(\text{heated whey}) - C_{\text{SH}}(\text{native whey})}{C_{\text{SH}}(\text{native whey})} \times 100 \quad (4)$$

## 182 **2.6. $\zeta$ -potential measurements**

183 The  $\zeta$ -potential values of the extracted whey proteins were determined at  $25 \pm 1 \text{ }^\circ\text{C}$   
184 using the Zetasizer Nano-ZS90 apparatus (Malvern Instruments, Westborough, MA) at a  
185 protein concentration of 0.5 g/L as suggested by Lam and Nickerson (2015b) .

186 The  $\zeta$ -potential value (mV) was measured using Henry's equation (Eq. (5)):

$$187 \quad U_{\text{E}} = \frac{2\varepsilon\zeta f(k\alpha)}{3\eta} \quad (5)$$

188 where:  $U_{\text{E}}$  is the electrophoretic mobility,  $\varepsilon$  the permittivity (Farad/m);  $k$ , the Debye  
189 length;  $f(k\alpha)$  the function of the radius of particle ( $\alpha$ , nm);  $\eta$  the viscosity of the dispersion  
190 (mPa s).

191 **2.7. Determination of hydrophobicity**

192 The surface hydrophobicity of the whey proteins was measured using the method  
193 described by Chelh et al. (2006). Briefly, 1 mL of whey sample (at a protein concentration of  
194 1 mg/mL) and 200  $\mu$ L of 1 mg/mL bromophenol blue (BPB) were added and mixed well.

195 A control was prepared using Tris-HCl buffer (20 mM, pH 8.0) instead of sample.  
196 Whey samples were kept under agitation for 10 min at room temperature and then centrifuged  
197 at 2,000g for 15 min at 25  $^{\circ}$ C.

198 Supernatants were diluted 1:10 and the DO was then measured at  $\lambda = 595$  nm against a  
199 blank Tris-HCl buffer.

200 In this assay, a higher amount of bound-BPB indicates a higher proteins'  
201 hydrophobicity which was calculated by Eq. (6):

$$202 \quad \text{Bound-BPB } (\mu\text{g}) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 200 \mu\text{g} \quad (6)$$

203

204 **2.8. Interfacial tension**

205 Interfacial tension for each whey protein solution was measured using a TSD-971  
206 Tensiometry System Digital (Gibertini Elettronica, Italia) via the "Du Noüy methodology" as  
207 described by Lam and Nickerson (2015b).

208 Thus, for the determination of the surface tension, the whey solution was placed in an  
209 oil-water system. Indeed, within a 40 mm diameter glass sample beaker, 20 mL of whey  
210 solution at a protein concentration of 1 g/L were added, followed by the immersion of the Du  
211 Nüoy ring (20 mm diameter) and then the addition of upper corn oil layer (20 mL).

212 Finally, the ring was pulled upwards to stretch the oil-water interface to determine the  
213 maximum force and then to calculate the interfacial tension value (mN/m).

214 The interfacial tension was calculated by the Eq. (7)

$$215 \quad \gamma = \frac{F_{max}}{4\pi R\beta} \quad (7)$$

216 where  $\gamma$  is the interfacial tension (mN/m),  $F_{max}$  is the maximum force (mN), R is the radius of  
217 the used ring (20 mm),  $\beta$  is a correction factor which depends on two factors : the density of  
218 the liquid and the dimensions of the ring. All interfacial tension analyses were realized at  
219 25 °C.

## 220 **2.9. Statistics**

221 All experimental analyses and measurements in this work were performed in triplicate  
222 and mentioned as the mean value  $\pm$  standard deviation.

223 The ANOVA test was used to test for significance in the main effects of the extracted  
224 whey samples and heat treatment conditions, along with their associated interactions on the  
225 hydrophobicity, surface tension, thiol groups' content,  $\zeta$ -potential measurements and  
226 emulsifying properties indexes of proteins.

227 Statistical analyses were determined using SPSS-statistics (Version 19, IBM, USA).

## 228 **3. Results and discussion**

### 229 **3.1. Chemical composition of camel and bovine milk and whey proteins content**

230 The physico-chemical composition of camel and bovine milk was determined in this  
231 study as shown in the Table 1. Table 1 shows that the main values of protein concentration in  
232 camel milk ( $22.06 \pm 0.73$  g/L) were significantly lower than that in bovine milk ( $28.36 \pm 0.27$   
233 g/L) ( $p < 0.05$ ). Moreover, the main components of camel milk were relatively close to that of  
234 bovine milk including fat, lactose, ash and total solids.

235 Analysis of the protein content of the different extracted wheys indicated that the  
236 protein concentration in acid and sweet bovine whey samples were  $10.79 \pm 0.10$  and  $11.55 \pm$   
237  $0.12$  g/L of whey, respectively. The protein content in sweet bovine whey was significantly  
238 higher ( $p < 0.05$ ) than that in its acid counterpart probably due to the presence of  
239 caseinomacropeptide in the sweet whey after rennet coagulation.

240 Whereas, no significant differences in the proteins concentration were observed  
241 between acid and sweet camel wheys ( $6.44\pm 0.18$ ;  $6.52\pm 0.01$  g/L for acid and sweet camel  
242 wheys, respectively). This behavior could be explained by the lack of the caseinomacropptide  
243 in camel whey which is mainly related to the reduced  $\kappa$ -casein content in camel milk  
244 (Ekstrand et al., 1980; Al haj and Al Kanhal, 2010).

245

### 246 ***3.2.Emulsifying properties***

247 The EAI measures how well proteins can coat an oil droplet surface within a dilute  
248 emulsion, while the ESI gives an estimate of the emulsion's stability as function of time  
249 (Pearce and Kinsella, 1978). EAI values of camel and bovine wheys' solutions at a protein  
250 concentration of 5 g/L as function of the temperature of the thermal treatment (70 and 90 °C)  
251 are shown in Figure 1A.

252 Thus, it was found that sweet wheys, regardless of milk origin and heating temperature  
253 (70 and 90 °C for 30 min) coated the oil droplets better than acid wheys with higher EAI  
254 values of the sweet camel whey (2.2 and 1.9 m<sup>2</sup>/g for sweet camel and bovine wheys,  
255 respectively under native conditions). Whereas, in acidic conditions, a better emulsification  
256 activity was achieved with the bovine whey when compared to its camel counterpart (1.7 and  
257 1.3 m<sup>2</sup>/g for both acid bovine and camel wheys, respectively). Statistical analyses showed that  
258 heating reduced significantly the EAI values ( $p < 0.05$ ) for acid wheys probably due to the  
259 extensive aggregation and denaturation of preheated whey proteins at acid pH values (Lam  
260 and Nickerson, 2015a).

261 The emulsification ability significantly increased for both sweet wheys after heating at  
262 90 °C for 30 min ( $p < 0.05$ ) and achieved maximum EAI values in these conditions (EAI ~  
263 2.5 min). These results are in agreement with those of Dissanayake and Vasiljevic (2009) who  
264 reported that the EAI values of whey proteins increased with thermal treatment and proteins

265 denaturation. On the other hand, Fachin and Viotto (2005) found that the emulsifying  
266 properties were considerably improved after heating at pH 7. This improvement in  
267 emulsifying properties was justified by the greater denaturation of whey proteins in these  
268 conditions. Mellema and Isenbart (2004) reported that the heat treatment (85 °C for 20 min)  
269 of whey protein solutions at pH 6.7 improved the ability of proteins to adsorb at oil-water  
270 interface resulting in lower interfacial tension values, and clearly elevated values for the  
271 viscoelastic modulus as compared to the native whey solutions (without heating). On the  
272 other hand, Lajnaf et al. (2017) found that, the foamability and the interfacial properties of the  
273 purified camel  $\alpha$ -lactalbumin solution at neutral pH are significantly improved by heat  
274 treatment (i.e. 70 and 90 °C for 30 min).

275         Difference between camel and bovine wheys can be attributed to the difference in  
276 protein composition of both wheys and their conformational change at neutral and acidic pH  
277 values. Suttiprasit et al. (1992) noted that at neutral pH, the  $\alpha$ -la is more efficient to reduce the  
278 interfacial tension than the  $\beta$ -lg, as it is more flexible and smaller. Whereas, Laleye et al.  
279 (2008) reported that the lower emulsifying properties of pre-acidified camel whey are  
280 believed to be due to the pronounced aggregation of camel whey protein molecules. These  
281 authors noted that the aggregation behavior of camel milk proteins at lower pH levels is  
282 explained by the high content of the  $\alpha$ -la. Thus, the high proportion of the  $\alpha$ -la can explain the  
283 low EAI values of acid camel whey compared to acid bovine whey.

284         The ESI values of camel and bovine whey solutions as a function of temperature pre-  
285 treatment are shown in Figure 1B. ESI values of sweet wheys were higher than those of acid  
286 wheys regardless of the heating temperature value in agreement with the findings of Lam and  
287 Nickerson (2015a, 2015b).

288 Yamauchi et al. (1980) and Leman et al. (1988) reported that the stability of whey  
289 protein emulsions increases when pH level is increased from 5 to 7, which is probably due to  
290 an increase in repulsion by the electrostatic charge of the proteins

291 Indeed, the magnitude of the electrical charge on the oil droplets surface decreased at  
292 pH values close to the pI of proteins. Consequently, the negative charge of acid wheys  
293 proteins is insufficient to generate electrostatic-repulsive forces between the created oil  
294 droplets leading to various attractive droplet-droplet interactions as Van der Waals and the  
295 hydrophobic forces and then, droplets aggregate forming large flocculates (McClements,  
296 2004).

297 For sweet bovine whey, ESI data were similar under native conditions and at 70 °C  
298 (~65%), and then increased to 80% min at 90 °C (Figure 2B). For sweet camel whey, ESI  
299 values increased significantly from 54% at 70 °C, to 75% at 90 °C (p<0.05).

300 ESI of acid wheys rose significantly (p<0.05) as function of temperature. Thus, ESI  
301 reached their maximum at 90 °C with values of 54% and 60% for acid bovine and camel  
302 wheys, respectively.

303 This behavior could be attributed to the increase in the adsorption velocity and the  
304 diffusion of heated milk proteins at the oil-water interface as reported by Dickinson (2003).  
305 Jiang et al. (2018) have found that heated whey proteins (at 85 °C for 30 min) give more  
306 stable emulsions than unheated proteins due to the creation of large protein aggregates and the  
307 further negative charge after heating. For the camel whey, Lajnaf et al. (2017) noted that the  
308 open structure of the camel  $\alpha$ -la molecule and the reduced electrostatic repulsion of this  
309 protein near its pI are all factors that could promote the creation of large aggregates resulting  
310 in a greater ability to stabilize foam and emulsions.

311 A comparison between camel and bovine wheys-based emulsifier agents and  
312 commercial dairy emulsifiers which are whey protein isolate (WPI) and sodium caseinates

313 (Na-cas) revealed that, for both dairy emulsifiers, EAI values were higher than those of camel  
314 and bovine wheys-based emulsifiers' agents (EAI~ 4.33 and 4.87 m<sup>2</sup>/g for WPI and Na-cas,  
315 respectively). On the other hand, emulsions created by camel and bovine wheys were more  
316 stable than those of WPI solutions (ESI~ 38.65%). Na-cas had the best stabilizing properties  
317 (ESI~ 88.8%). Furthermore, the heat treated WPI and Na-cas solutions (i.e. 70 and 90 °C for  
318 30 min) were found to coat the emulsion droplets better than both heated camel and bovine  
319 wheys reaching EAI values ~ 5.57 and 4.91 m<sup>2</sup>/g for heated WPI and Na-cas, respectively.  
320 No significant difference was observed between ESI values of heated camel and bovine  
321 wheys and WPI solutions (ESI~ 66.66%). Heat treated Na-cas exhibited the highest emulsion  
322 stabilizing abilities when compared to the extracted wheys (ESI> 90%). These results are in  
323 agreement with the findings of Casper et al. (1999). Indeed, these authors reported that  
324 Bovine cheese whey showed lower emulsifying capability at pH 8 when compared to WPI.  
325 Therefore, this behavior depended on protein composition and physico-chemical conditions.

### 326 ***3.3. Protein denaturation and aggregation***

#### 327 ***3.3.1. Denaturation rate***

328 Figure 2 compares the DR values of wheys at a protein concentration of 1g/L as a  
329 function of pH level and/or thermal treatment temperature (70 and 90 °C for 30 min).

330 As expected, the free thiol groups' concentration of both wheys raised significantly after  
331 heating as function of temperature. Consequently, the DR values reached their maximum at  
332 90 °C with values of 1220±211% and 183±50% for acid and sweet bovine wheys,  
333 respectively and 1143±150% and 419±100% for acid and sweet camel wheys, respectively.

334 It could be explained by the milk proteins denaturation which happened during 30 min  
335 of heating at 90 °C regardless of the milk proteins origin as reported by Felfoul et al. (2015).

336 Acid wheys carried higher DR values compared to their sweet counterparts regardless of  
337 heating temperature value. This result confirmed that acid wheys are characterized by a higher

338 thermal sensitivity than the sweet wheys. Lam and Nickerson (2015a) reported that the size of  
339 whey proteins' aggregates is the greatest in acidic conditions due to the minimized  
340 electrostatic repulsion between neighboring proteins molecules leading them to interact and  
341 aggregate. Whereas, the high electronegative charge of sweet whey proteins may  
342 electrostatically keep them from aggregation after heating.

### 343 **3.3.2. Electrophoresis patterns**

344 We characterized the aggregation and the denaturation behavior of camel and bovine  
345 wheys after heating (at 70 and 90 °C for 30 min) by the gel electrophoretic patterns (Figure 3).  
346 The densitometry analysis of the gel corresponding to native acid and sweet bovine wheys  
347 (Figure 3A L1 and Figure 3B L1, respectively) showed that five major protein bands (150  
348 kDa, 70 kDa, 30 kDa, 18 kDa and 14 kDa) were identified as immunoglobulins (~3%), BSA  
349 (~8%), protein fraction F (~4%),  $\beta$ -Ig (~65%) and  $\alpha$ -la (~20%).

350 For native acid and sweet camel wheys (Figure 3C L1 and Figure 3D L1, respectively),  
351 five major protein bands with molecular weight of 80 kDa, 66 kDa, 22 kDa, 19 kDa and 14  
352 kDa were identified, corresponding to lactoferrin (~2%), camel serum albumin (CSA) (~21%),  
353 protein fractions F1 (~10%) and F2 (~15%) and  $\alpha$ -la (~52%), respectively. As expected, no  
354 band corresponding to  $\beta$ -Ig was detected as already reported by previous authors (Ereifej et al.,  
355 2011; Omar et al., 2016).

356 Figure 3A shows that when acid bovine whey was heated, the serum albumin and the  $\alpha$ -  
357 la appeared to decrease in intensity with temperature value (L2 and L3); whereas, for sweet  
358 bovine whey (Figure 3B), no effect was recorded on  $\beta$ -Ig and  $\alpha$ -la protein bands heated at 70  
359 and 90 °C for 30 min. Heated bovine whey samples showed the appearance of a protein band  
360 (42 kDa) which is probably formed during heating.

361 When acid camel whey was heated at (70 or 90 °C),  $\alpha$ -la and protein fractions (F1 and  
362 F2) bands appeared to increase in intensity (Figure 3C). For raw sweet camel whey (Figure



363 3D), the thermal treatment at 70 °C (L2) and 90 °C (L3) for 30 min induced an immediate  
364 disappearance of the  $\alpha$ -la and the appearance of several faint distinct bands, which were  
365 probably intermediate protein species (e.g.,  $\alpha$ -la dimers, trimers) that were formed during  
366 heating.

367 Similar results were obtained by Felfoul et al. (2015). These authors noticed that the  
368 disappearance of the CSA and  $\alpha$ -la electrophoretic bands could be the consequence of camel  
369 proteins denaturation and/or aggregation. For bovine wheys, the denaturation is maintained by  
370 the presence of the  $\beta$ -lg as reported by de la Fuente et al. (2002). Thus, these authors noted  
371 that the  $\beta$ -lg dominates the behavior of the milk protein aggregation under different thermal  
372 treatments due to its particular molecular characteristics. Thus, this protein reacts more easily  
373 with itself than with the  $\alpha$ -la leading to the creation of heat induced complexes rich in  $\beta$ -lg.  
374 However, the rate of disappearance of the  $\alpha$ -la was greater when compared to the  $\beta$ -lg because  
375 of the higher number of intramolecular disulphide bonds and lower thermal transition  
376 temperature of the  $\alpha$ -la (Schokker et al., 2000).

377 In order to understand the aggregation phenomena in camel whey, Felfoul et al. (2015)  
378 showed that the  $\alpha$ -la in acid camel whey has a lower denaturation temperature (60.5 °C) than  
379 that in sweet camel whey (73.8 °C). Besides, Atri et al. (2010) found that the camel  $\alpha$ -la apo  
380 state is more sensitive to heat treatment than the holo state.

### 381 ***3.4. Surface characteristics***

#### 382 ***3.4.1. Surface hydrophobicity***

383 Hydrophobicity of bovine and camel isolated wheys at a protein concentration of 1g/L  
384 as a function of heating temperature value (70 and 90 °C for 30 min) is shown in Figure 4.

385 The BPB-bound amounts were  $58.4 \pm 7.1$   $\mu\text{g/mL}$  and  $5.5 \pm 1.1$   $\mu\text{g/mL}$  for acid and sweet  
386 bovine wheys, respectively, and  $98.9 \pm 7$   $\mu\text{g/mL}$  and  $11.5 \pm 4$   $\mu\text{g/mL}$  for acid and sweet camel  
387 wheys, respectively.

388 Acid wheys carried higher BPB-bound amounts compared to sweet wheys and the  
389 maximum hydrophobicity values were achieved with the acid wheys regardless of the  
390 temperature of the thermal treatment. Indeed, after heating at 90 °C for 30 min, BPB-bound  
391 amounts of acid camel and bovine wheys reached 174 and 147 µg/mL of whey, respectively.

392 For both sweet wheys, statistical analysis showed that heating has no significant effect  
393 on the protein's hydrophobicity with the lowest BPB-bound amounts (~15 µg/mL) after  
394 heating at 90 °C for 30 min.

395 In support of these results, Voutsinas et al. (1983) found that the surface hydrophobicity  
396 of whey proteins increases with heating (80 °C for 4 min). Lam and Nickerson (2015a) have  
397 found that the heat-treatment (85 °C for 30 min) of a pre-acidified whey protein isolate (WPI)  
398 solution (pH 5) resulted in high hydrophobicity rate values. These authors reported that the  
399 high hydrophobicity is mainly attributed to the combined effect of reduced net charge and  
400 protein denaturation of whey. Indeed, the combination of acidification and heating of whey  
401 proteins led to the exposure of the buried hydrophobic moieties to the surface of the unfolded  
402 protein. But, the reduced hydrophobicity of whey proteins at neutral pH after heating may  
403 reflect the greater contribution of surface negative charge which could restrict complete whey  
404 protein unfolding (Kato and Nakai, 1980; Lam and Nickerson, 2015a).

405 The different behavior of both acid wheys could be explained by their protein  
406 composition. For the bovine whey, Zhang et al. (2004) reported that the β-Ig is  
407 thermodynamically stable in acidic conditions and more rigid than the α-la.

408 The highest surface hydrophobicity of acid camel whey proteins can be attributed to the  
409 lack of the β-Ig in this serum and also to the greatest hydrophobicity of camel α-la. Thus,  
410 fluorescence results obtained by Atri et al. (2010) reported that camel α-la shows a greater  
411 surface hydrophobicity than its bovine counterpart. Indeed, primary structure of camel α-la  
412 contains more hydrophobic residues such as Tryptophan (Trp). Thus, after calcium removal,

413 the camel  $\alpha$ -la showed a greater surface hydrophobicity due to the greater hydrophobicity of  
414 the N-terminal part of its  $\alpha$ -helical domain.

#### 415 **3.4.2. Determination of $\zeta$ -potential**

416 Surface charge values (or  $\zeta$ -potential) for bovine and camel wheys (acid and sweet) at a  
417 protein concentration of 0.5g/L after heating at 70 and 90 °C for 30 min were measured and  
418 given in Figure 5.

419 Overall, the  $\zeta$ -potential values of acid wheys were significantly lower than that of their  
420 sweet counterparts ( $p < 0.05$ ). Under native conditions, the  $\zeta$ -potential values were  $\sim -2.5 \pm 0.4$   
421 and  $\sim -18.12 \pm 0.6$  mV for acid and sweet camel wheys, respectively and  $\sim -3.85 \pm 0.5$  and  $\sim$   
422  $20.79 \pm 1.2$  mV for acid and sweet bovine wheys, respectively. These findings are in agreement  
423 with the  $\zeta$ -potential results of Momen et al. (2018). These authors confirmed that whey  
424 proteins of camel milk carried lower negative charge than cow whey proteins. They suggested  
425 that this difference can be explained by the variance in the protein composition, the pI of both  
426  $\alpha$ -la (4.87 and 4.65 for camel and bovine  $\alpha$ -la, respectively) and the presence of the lactoferrin  
427 in camel whey which is a highly basic protein (pI = 8.8).

428 Figure 5 shows also that, the electronegative charge of both sweet wheys was not  
429 significantly modified after heating, while after a heat-treatment at 70 °C, it significantly  
430 decreased to  $\sim -1.16$  and  $\sim -1.04$  mV for acid bovine and camel wheys, respectively.

431 These results are in agreement and with the findings of Lam and Nickerson (2015a) and  
432 with the highest surface hydrophobicity observed with the acid wheys (Figure 4). Indeed, at  
433 neutral pH, whey proteins molecules were strongly negatively charged which may  
434 electrostatically repel BPB from binding to the protein. In contrast, near whey protein's pI, the  
435 reduction of the electrostatic repulsion and the open structure could promote protein–BPB  
436 interactions.

#### 437 **3.4.3. Interfacial properties**

438 The interfacial tension between corn oil and whey proteins' solutions (at a protein  
439 concentration of 1 g/L) in response to temperature pre-treatments (70 and 90 °C for 30 min)  
440 and pH values (acid and sweet wheys) are shown in Figure 6. Overall, both camel and bovine  
441 wheys have significantly reduced the interfacial tension at oil-water interface from 29.1mN/m  
442 ( $p < 0.05$ ).

443 Thus, in native conditions, the order of effectiveness to reduce the surface tension at the  
444 oil-water interface was: acid camel whey ( $\gamma = 19.6 \pm 1.9$  mN/m) > acid bovine whey ( $\gamma =$   
445  $23.4 \pm 0.8$  mN/m) > sweet camel whey ( $\gamma = 25.1 \pm 0.7$  mN/m) > sweet bovine whey ( $\gamma =$   
446  $26.9 \pm 0.5$  mN/m).

447 The heat-treatment improved the tensioactivity of whey proteins and their ability to  
448 reduce the interfacial tension at oil-water interface depending on the pH value.

449 Indeed, when heating sweet wheys at 70 °C for 30 min, the surface tension declined  
450 significantly ( $p < 0.05$ ) from 26.9 to 24.3 mN/m and from 25.1 to 22.7 mN/m for sweet bovine  
451 and camel wheys, respectively. No significant change was found on the evolution of surface  
452 tension values of sweet wheys between 70 and 90 °C.

453 After a heat-treatment of 90 °C for 30 min, acid wheys have retained the best interfacial  
454 properties when compared to the sweet wheys. Thus, the order of effectiveness at 90 °C was:  
455 acid camel whey ( $\gamma = 9.35 \pm 1.34$  mN/m) > acid bovine whey ( $\gamma = 17.5 \pm 1.0$  mN/m) > sweet  
456 camel whey ( $\gamma = 21.45 \pm 1.5$  mN/m) and sweet bovine whey ( $\gamma = 23.05 \pm 0.8$  mN/m).

457 These results are in agreement with those of Lam and Nickerson (2015a) who noted that  
458 pH plays a key role in the adsorption of whey proteins at the oil-water interface. Indeed,  
459 electrostatic repulsion between whey proteins molecules leads to greater difficulties in  
460 aligning at the interface to create a viscoelastic film. Whereas, in acidic conditions, whey  
461 proteins carried less negative charge near their pI, allowing for higher proteins interactions  
462 and better adsorption at the interface.

463 Previous studies (Shimizu et al., 1985; Hunt and Dalgleish, 1994) noted that at low pH  
464 values the  $\alpha$ -la is more dominant at the interface than the  $\beta$ -lg, which could explain the lower  
465 surface tension values of the acid wheys.

466 Mellema and Isenbart (2004) have shown by surface tension measurements that the  
467 heat-treatment of pre-acidified bovine whey proteins (85 °C for 20 min) are not stable in  
468 solution and will have a high tendency to aggregate or adsorb leading to surface active  
469 aggregates and giving high viscoelastic modulus values. These authors reported that, for whey  
470 proteins isolates, pre-acidification dominates the interfacial behavior at the oil-water interface  
471 over pre-heating. Thus, the combination of pre-acidification and pre-heating of these proteins  
472 was found to give results similar to pre-acidification without heating.

473 Lajnaf et al. (2017) observed that the  $\alpha$ -la isolated from camel milk was more flexible at  
474 acid pH, regardless of heating temperature, due to the reduced negative charge of this proteins  
475 and its molten globular state at low pH values.

476 Thus, it can be suggested that the interfacial behavior of camel whey, at the oil-water  
477 interface, is maintained by the major protein which is the camel  $\alpha$ -la regardless of the applied  
478 heat-treatment. Thus, a greater tensioactivity of the acid camel whey can be attributed to the  
479 highest hydrophobicity rate of camel  $\alpha$ -la (section 3.3.1).

480 Despite the highest tensioactivity of acid wheys, greater droplet coverage and higher  
481 emulsion stability were found for sweet wheys regardless of the milk origin. These findings  
482 suggest that better oil droplet coverage could not be maintained by neutrally charged proteins  
483 even if they exhibited the highest effectiveness to reduce the surface tension at the oil-water  
484 interface in agreement with of Lam and Nickerson (2015a). Whereas, for sweet wheys,  
485 electrostatic repulsion may help proteins to spread out at the oil droplet surface leading to  
486 droplet coverage by the negatively charged proteins.

487

#### 488 **4. Conclusion**

489 Whey emulsifying properties depended on the pH level, the protein composition and the  
490 degree of denaturation of these proteins after a thermal treatment. A Higher oil droplets  
491 surface coverage (EAI) was obtained for sweet wheys especially the sweet camel whey.  
492 Furthermore, stability of emulsions seemed greatest for sweet wheys due to the presence of  
493 electrostatic repulsive forces between proteins as confirmed by the  $\zeta$ -potential measurements.  
494 A heat-treatment at 70 and 90 °C of the acid wheys for 30 min resulted in a significant  
495 increase in ESI values due to the denaturation and aggregaton of proteins. Finally, these  
496 results confirmed the strong potential of camel and bovine wheys as emulsifier agent for  
497 potential applications in industrial emulsion production.

498

499 **Acknowledgements** This work was funded by the Ministry of Scientific Research and  
500 Technology of Tunisia. We are greatly indebted to Professor A. Ben Ali for editing the  
501 manuscript.

502

#### 503 **References**

504 AOAC, 1984. Horowitz, W. (Ed.), Methods of analysis of the association of official analytical  
505 chemists (14th ed.). Washington, DC.

506 Al haj, O. A., Al Kanhal, H. A., 2010. Compositional, technological and nutritional aspects of  
507 dromedary camel milk. *Int. Dairy J.* 20, 811–821.

508 Atri, M.S., Saboury, A.A., Yousefi, R., Chobert, J., Haertle, T., Moosavi-Movahedi, A.A.,  
509 2010. Comparative study on heat stability of camel and bovine apo and holo  $\alpha$ -  
510 lactalbumin. *J. Dairy Res.* 77, 43–49.

511 Baldasso, C., Barros, T.C., Tessaro, I.C., 2011. Concentration and purification of whey  
512 proteins by ultrafiltration. *Desalination* 278, 381–386.

513 Beg, O.U., von Bahr-Lindström, H., Zaidi, Z.H., Jörnvall, H., 1985. The primary structure of  
514 alpha-lactalbumin from camel milk. *Eur. J. Biochem.* 147, 233–239.

515 Casper, J.L., Wendorff, W.L., Thomas, D.L., 1999. Functional properties of whey protein  
516 concentrates from caprine and ovine specialty cheese wheys. *J. Dairy Sci.* 82, 265–271.

517 Chelh, I., Gatellier, P., Santé-Lhoutellier, V., 2006. A simplified procedure for myofibril  
518 hydrophobicity determination. *Meat Sci.* 74, 681–683.

519 de la Fuente, M.A., Singh, H., Hemar, Y., 2002. Recent advances in the characterization of  
520 heat-induced aggregates and intermediates of whey proteins. *Trends Food Sci. Technol.*  
521 13, 262–274.

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

524 Dissanayake, M., Vasiljevic, T., 2009. Functional properties of whey proteins affected by heat  
525 treatment and hydrodynamic high-pressure shearing. *J. Dairy Sci.* 92, 1387–1397.

526 Doi, H., Tokuyama, T., Fong-Huang, K.U.O., Ibuki, F., Kanamori, M., 1983. Heat-induced  
527 complex formation between  $\kappa$ -casein and  $\alpha$ -lactalbumin. *Agric. Biol. Chem.* 47, 2817–  
528 2824.

529 Ekstrand, B., Larsson-Raźnikiewicz, M., Perlmann, C., 1980. Casein micelle size and  
530 composition related to the enzymatic coagulation process. *Biochim. Biophys. Acta*  
531 (BBA)-General Subj. 630, 361–366.

532 Ellman, G.L., 1959. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* 82, 70–77.

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

536 Fachin, L., Viotto, W.H., 2005. Effect of pH and heat treatment of cheese whey on solubility

537 and emulsifying properties of whey protein concentrate produced by ultrafiltration. Int.  
538 Dairy J. 15, 325–332.

539 Felfoul, I., Lopez, C., Gaucheron, F., Attia, H., Ayadi, M. A., 2015. A laboratory  
540 investigation of cow and camel whey proteins deposition under different heat treatments.  
541 Food Bioprod. Process. 96, 256–263.

542 Hunt, J.A., Dalgleish, D.G., 1994. Effect of pH on the stability and surface composition of  
543 emulsions made with whey protein isolate. J. Agric. Food Chem. 42, 2131–2135.

544 Jiang, S., Cheng, J., Jiang, Z., Geng, H., Sun, Y., Sun, C., Hou, J., 2018. Effect of heat  
545 treatment on physicochemical and emulsifying properties of polymerized whey protein  
546 concentrate and polymerized whey protein isolate. LWT - Food Sci. Technol. 98, 134–  
547 140.

548 Kappeler, S.R., Ackermann, M., Farah, Z., Puhan, Z., 1999. Sequence analysis of camel  
549 (*Camelus dromedarius*) lactoferrin Int. Dairy J. 9, 481–486.

550 Kato, A., Nakai, S., 1980. Hydrophobicity determined by a fluorescence probe method and its  
551 correlation with surface properties of proteins. Biochim. Biophys. acta (BBA)-Protein  
552 Struct. 624, 13–20.

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

555 Lajnaf, R., Picart-Palmade, L., Attia, H., Marchesseau, S., Ayadi, M.A., 2017. The effect of  
556 pH and heat treatments on the foaming properties of purified  $\alpha$ -lactalbumin from camel  
557 milk. Colloids Surfaces B Biointerfaces 156, 55–61.

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



561 Laleye, L.C., Jobe, B., Wasesa, A.A.H., 2008. Comparative study on heat stability and  
562 functionality of camel and bovine milk whey proteins. *J. Dairy Sci.* 91, 4527–4534.

563 Lam, R.S.H., Nickerson, M.T., 2015a. The effect of pH and temperature pre-treatments on the  
564 physicochemical and emulsifying properties of whey protein isolate. *LWT - Food Sci.*  
565 *Technol.* 60, 427–434.

566 Lam, R.S.H., Nickerson, M.T., 2015b. The effect of pH and temperature pre-treatments on the  
567 structure, surface characteristics and emulsifying properties of alpha-lactalbumin. *Food*  
568 *Chem.* 173, 163–170.

569 Leman, J., Haque, J., Kinsella, J.E., 1988. Creaming stability of fluid emulsions containing  
570 different milk protein preparations. *Milchwiss.* 43, 286–289.

571 Matsumura, Y., Mitsui, S., Dickinson, E., Mori, T., 1994. Competitive adsorption of  $\alpha$ -  
572 lactalbumin in the molten globule state. *Food Hydrocoll.* 8, 555–566.

573 McClements, D.J. (2005). *Food emulsions: Principles, practice and techniques* (2nd ed.).  
574 BocaRaton, FL: CRC Press Taylor & Francis Group.

575 McGuffey, M.K., Epting, K.L., Kelly, R.M., Foegeding, E.A., 2005. Denaturation and  
576 aggregation of three  $\alpha$ -lactalbumin preparations at neutral pH. *J. Agric. Food Chem.* 53,  
577 3182–3190.

578 Mellema, M., Isenbart, J.G., 2004. Effect of acidification and heating on the rheological  
579 properties of oil-water interfaces with adsorbed milk proteins. *J. Dairy Sci.* 87, 2769–  
580 2778.

581 Momen, S., Salami, M., Emam-Djomeh, Z., Hosseini, E., Sheibani, N., Moosavi-Movahedi,  
582 A.A., 2018. Effect of dry heating on physico-chemical, functional properties and  
583 digestibility of camel whey protein. *Int. Dairy J.* 86, 9–20.

584 Nishanthi, M., Chandrapala, J., Vasiljevic, T., 2017. Properties of whey protein concentrate

585 powders obtained by spray drying of sweet, salty and acid whey under varying storage  
586 conditions. *J. Food Eng.* 214, 137–146.

587 Omar, A., Harbourne, N., Oruna-Concha, M.J., 2016. Quantification of major camel milk  
588 proteins by capillary electrophoresis. *Int. Dairy J.* 58, 31-35.

589 Pearce, K.N., Kinsella, J.E., 1978. Emulsifying properties of proteins: evaluation of a  
590 turbidimetric technique. *J. Agric. Food Chem.* 26, 716–723.

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

594 Schokker, E.P., Singh, H., Creamer, L.K., 2000. Heat-induced aggregation of  $\beta$ -lactoglobulin  
595 A and B with  $\alpha$ -lactalbumin. *Int. Dairy J.* 10, 843–853.

596 Shimizu, M., Saito, M., Yamauchi, K., 1985. Emulsifying and structural properties of  $\beta$ -  
597 lactoglobulin at different pHs. *Agric. Biol. Chem.* 49, 189–194.

598 Slack, A.W., Amundson, C.H., Hill, C.G., 1986. Foaming and emulsifying characteristics of  
599 fractionated whey protein. *J. Food Process. Preserv.* 10, 81–88.

600 Suttiprasit, P., Krisdhasima, V., McGuire, J., 1992. The surface activity of  $\alpha$ -lactalbumin,  $\beta$ -  
601 lactoglobulin, and bovine serum albumin. *J. Colloid Interface Sci.* 154, 316–326.

602 Tcholakova, S., Denkov, N.D., Ivanov, I.B., Campbell, B., 2006. Coalescence stability of  
603 emulsions containing globular milk proteins. *Adv. Colloid Interface Sci.* 123–126, 259–

604 Tosi, E., Canna, L., Lucero, H., Ré, E., 2007. Foaming properties of sweet whey solutions as  
605 modified by thermal treatment. *Food Chem.* 100, 794–799.

606 Uchida, Y., Shimatani, M., Mitsuhashi, T., Koutake, M., 1996. Process for preparing a  
607 fraction having a high content of  $\alpha$ -lactalbumin from whey and nutritional compositions  
608 containing such fractions. *US Patent*, 5, 503–864.

609 Voutsinas, L.P., Cheung, E., Nakai, S., 1983. Relationships of hydrophobicity to emulsifying  
 610 properties of heat denatured proteins. *J. Food Sci.* 48, 26–32.

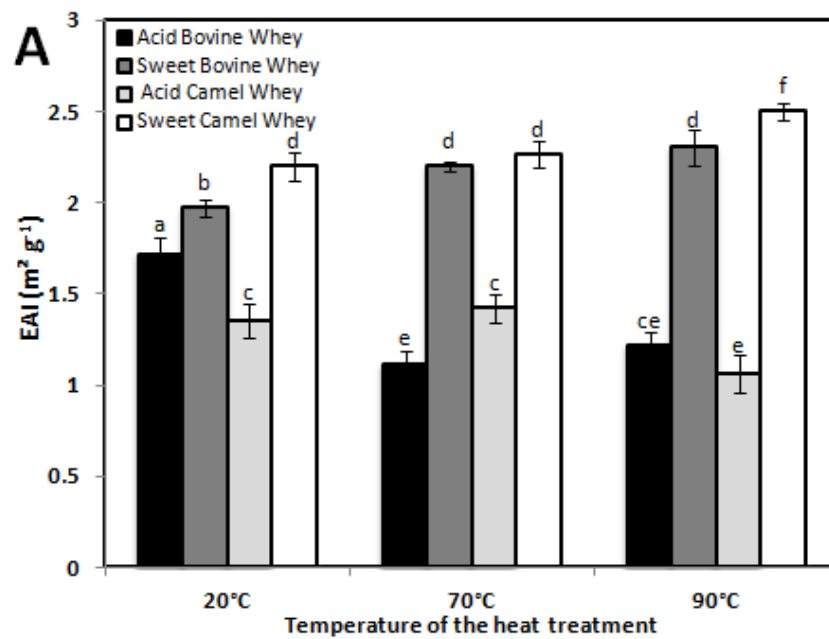
611 Yamauchi, K., Shimizu, M., Kamiya, T., 1980. Emulsifying properties of whey protein. *J.*  
 612 *Food Sci.* 45, 1237–1242.

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

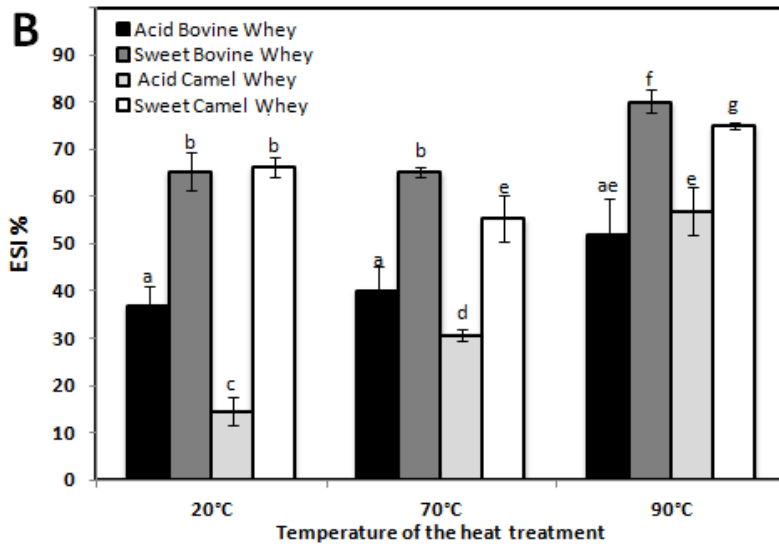
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617 **Figures**

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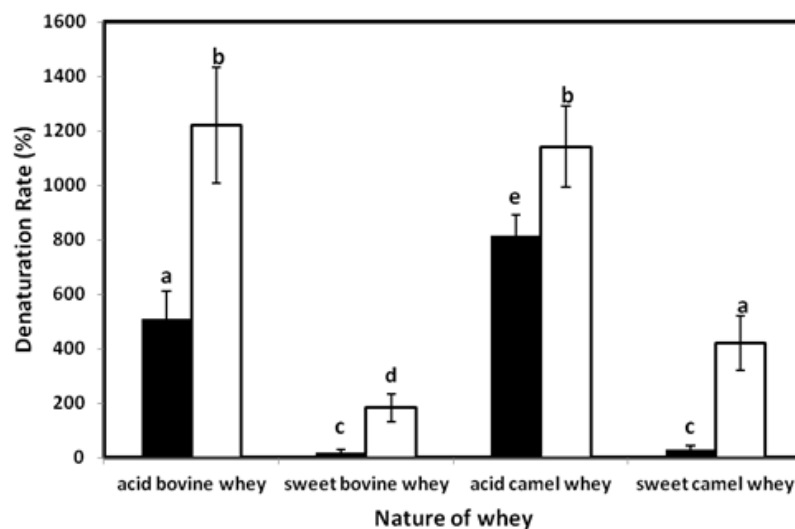
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621 **Figure 1.** Emulsifying Activity Index (EAI) (A) and Emulsion Stability Index (ESI) (B) of  
 622 camel and bovine whey protein solutions, at a protein concentration of 5 g/L and as function  
 623 temperature of the heat-treatment temperature (70 and 90 °C for 30 min).

624 <sup>a-g</sup> Samples represented with different letters are significantly different from each other (p<0.05). Error bars show  
 625 the standard deviations of mean values of EAI and ESI.

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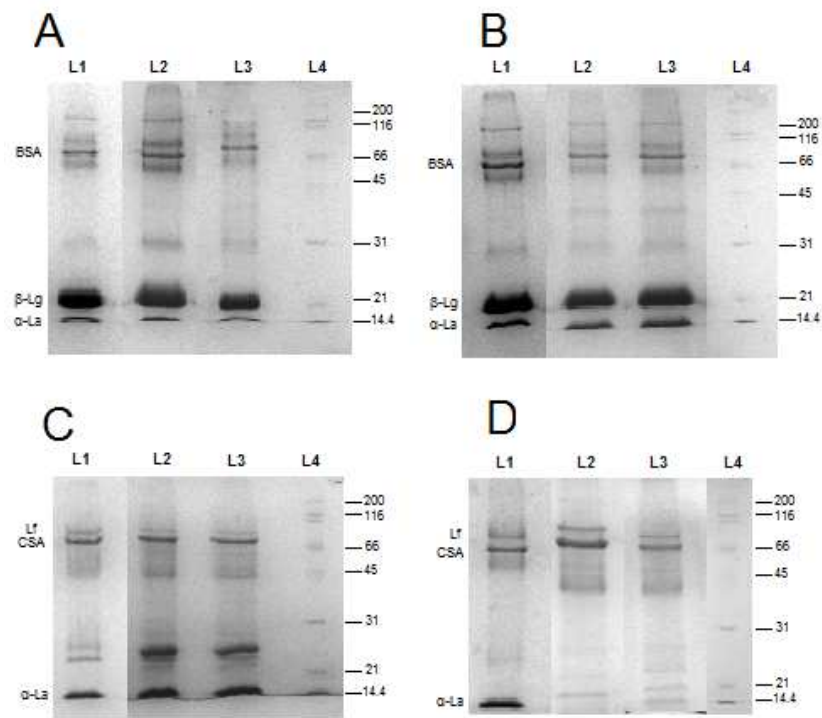
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628

629 **Figure 2.** Effect of temperature (70 (■) and 90 °C (□) for 30 min) on camel and bovine whey  
 630 denaturation rate (DR).

631 <sup>a-d</sup> Samples represented with different letters are significantly different from each other ( $p < 0.05$ ). Error bars show  
632 the standard deviations of mean values of DR.

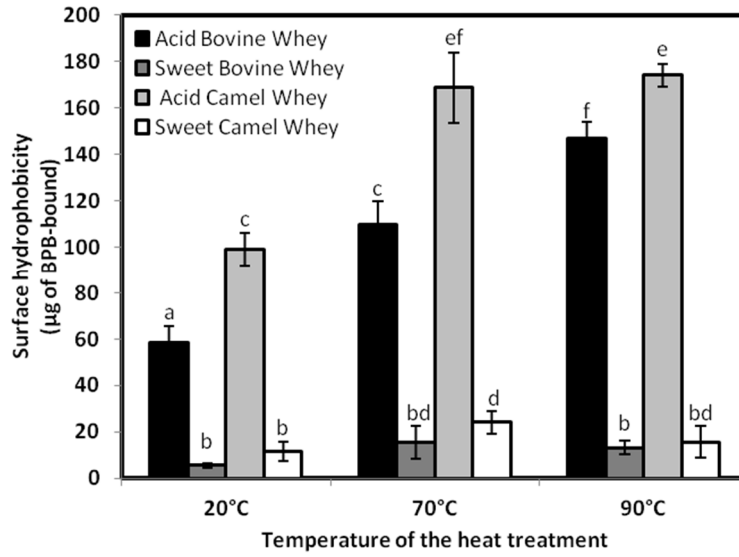


633

634 **Figure 3.** SDS-PAGE electrophoresis patterns of acid bovine whey (A), sweet bovine whey  
635 (B) acid camel whey (C) and sweet camel whey (D) proteins heated at 70 and 90 °C for 30  
636 min.

637 L1: native whey, L2: heated whey (70 °C for 30 min), and L3: heated whey (90 °C for 30  
638 min). L4 represents molecular mass markers; CSA = camel serum albumin, BSA = bovine  
639 serum albumin, Lf = lactoferrin, F = fraction,  $\alpha$ -La =  $\alpha$ -lactalbumin,  $\beta$ -Lg =  $\beta$ -lactoglobulin.

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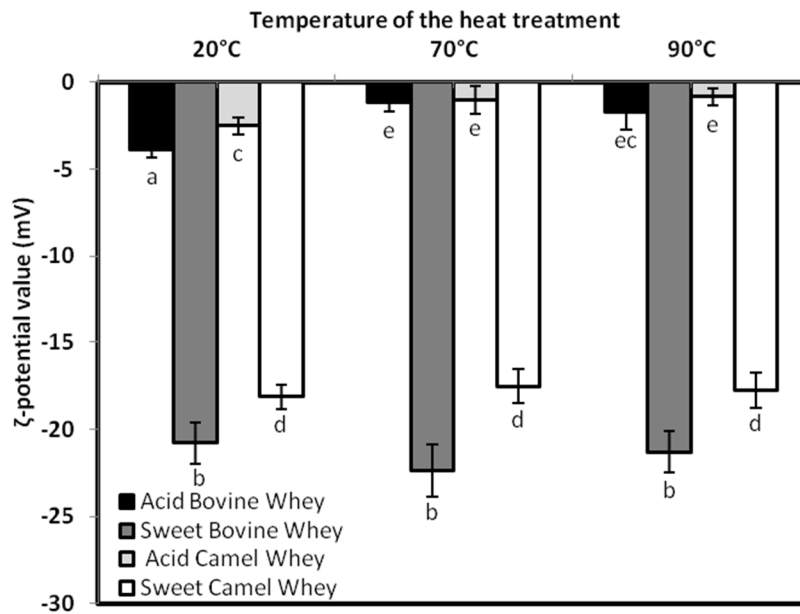


641

642 **Figure 4.** Surface hydrophobicity ( $\mu\text{g}$  of BPB-bound) of camel and bovine whey protein  
 643 solutions, at a protein concentration of 1 g/L and as function temperature of the heat-treatment  
 644 temperature (70 and 90 °C for 30 min).

645 <sup>a-f</sup> Samples represented with different letters are significantly different from each other ( $p < 0.05$ ). Error bars show  
 646 the standard deviations of mean values of Surface hydrophobicity.

647

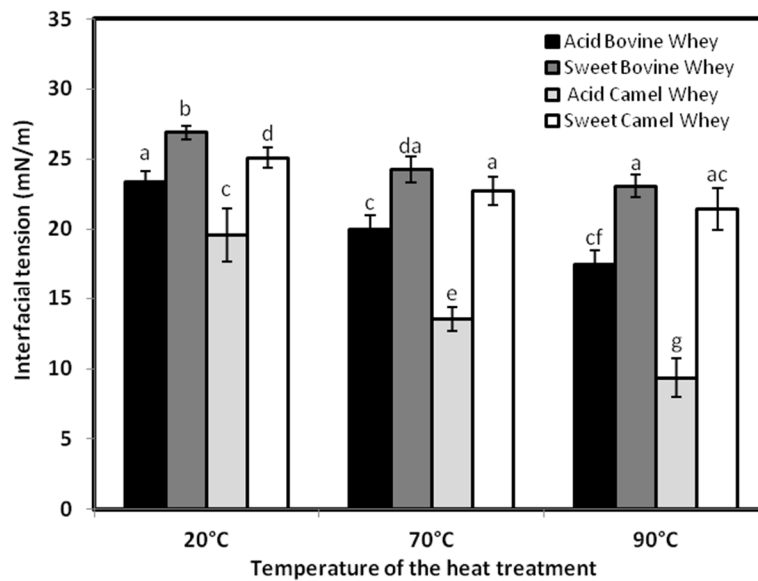


648

649 **Figure 5.**  $\zeta$ -potential measurements (mV) of camel and bovine whey protein solutions, at a  
650 protein concentration of 0.5 g/L and as function temperature of the heat-treatment temperature  
651 (70 and 90 °C for 30 min).

652 <sup>a-c</sup> Samples represented with different letters are significantly different from each other ( $p < 0.05$ ). Error bars show  
653 the standard deviations of mean values of  $\zeta$ -potential.

654



655

656 **Figure 6.** The interfacial tension (mN/m) of camel and bovine whey protein solutions, at a  
657 concentration of 1g/L and as function temperature of the heat-treatment temperature (70 and  
658 90 °C for 30 min).

659 <sup>a-g</sup> Samples represented with different letters are significantly different from each other ( $p < 0.05$ ). Error bars show  
660 the standard deviations of mean values of interfacial tension.

661

662

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664 **Tables**

665 **Table 1.** Chemical composition of camel and bovine milk (AOAC, 1984)

	Bovine milk	Camel milk
Protein (g/L)	28.36±0.27	22.06±0.73
Fat (g/L)	34.1±0.5	35.4 ± 0.6
Lactose (g/L)	46.1±2.2	43.5±1.1
Ash (%)	0.89±0.01	0.69±0.04
NPN (non-protein nitrogen) (g/L)	0.69±0.03	0.43±0.03
Total solids (%)	13.01±0.12	12.95±0.17

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