

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

Roua Lajnaf, Laetitia Picart-Palmade, Hamadi Attia, Sylvie Marchesseau, M.A. Ayadi

# ▶ To cite this version:

Roua Lajnaf, Laetitia Picart-Palmade, Hamadi Attia, Sylvie Marchesseau, M.A. Ayadi. Foaming and air-water interfacial properties of camel milk proteins compared to bovine milk proteins. Food Hydrocolloids, 2022, 126, 10.1016/j.foodhyd.2021.107470 . hal-03559081

# $\begin{array}{c} {\rm HAL~Id:~hal\text{-}03559081} \\ {\rm https://hal.inrae.fr/hal\text{-}03559081v1} \end{array}$

Submitted on 8 Jan 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



1 Foaming and air-water interfacial properties of camel milk proteins 2 compared to bovine milk proteins 3 4 Roua Lajnaf<sup>a,b\*</sup>, Laetitia Picart-Palmade<sup>b</sup>, Hamadi Attia<sup>a</sup>, Sylvie Marchesseau<sup>b</sup>, M.A. 5 6 Ayadi<sup>a</sup> 7 8 9 <sup>a</sup>Alimentary Analysis Unit, National Engineering School of Sfax, BPW 3038, Sfax, Tunisia 10 <sup>b</sup>IATE, Univ Montpellier, INRAE, Institut Agro, Montpellier, France **Journal:** Food Hydrocolloids 11 **Shortened Title:** Foaming properties of camel and bovine milk proteins 12 13 14 \* Corresponding author: Roua Lajnaf 15 16 National Engineering School of Sfax, Sfax Tunisia 17 University of Montpellier Tel: +216 74 675761 18 E-mail address: roua\_lajnaf@yahoo.fr; roua.lajnef@enis.tn 19 20 21

## 22 Abstract

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

43

44

45

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

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

### 1. Introduction

50

51

52

53

54

55

56

57

58

59

60

61

62

63

64

65

66

67

68

69

70

71

72

73

74

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

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

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

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

The physico-chemical composition of camel and cow milk (fat, lactose and total solids content) was relatively close to that of Bo-M (Al haj & Al Kanhal, 2010; Lajnaf et al., 2019) with a similar total solids content (13.01  $\pm$  0.12 % for Bo-M and 12.95  $\pm$  0.17 % for Ca-M) a 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, 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, respectively. On the other hand, the total mineral content ranges between 6 and 9 g L<sup>-1</sup> of milk 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 Kanhal, 2010).

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

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

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

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

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

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

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

### 2. Material and methods

### 2.1. Materials

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

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

## 2.2. Bovine protein fractions

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

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

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

## 2.3. Camel protein fractions

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

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

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

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

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

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

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

81.5%) was verified by the reversed-phase high-performance liquid chromatography (RP-

229 HPLC) (Yüksel & Erdem, 2010) (Fig. 3B).

## 2.4. Protein solution preparation

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

235 2016).

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

The proteins fractions studied were:

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

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

## 2.5. RP-HPLC analysis

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

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

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

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

## 2.6. Foaming properties

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

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

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

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

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

- where  $V_{foam}$  is the volume of the created foam at t=0 and  $V_0$  the volume in the initial solution before whipping.
- Foam Stability (FS) is defined as the foam half-time which is the time for drainage of the half of the foam created ( $t_{foam1/2}$ ) (Marinova et al., 2009).

## 2.5. Interfacial properties

- Dynamic surface tension measurements were performed using a pendant-drop tensiometer (IT Concept, Longessaigne, France). An axisymmetric air drop was created at the tip of the needle of a specific syringe dipped into the cuvette that contained the protein solution and driven by a computer. The images of the drop were taken by a camera and digitized.
- The surface tension was calculated according to Laplace's equation (Eq. 2) and calculated by analyzing the drop's profile:

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

296 where:

288

289

290

291

292

- 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
- 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 densities of the two phases, g is the acceleration of gravity, and  $\gamma$  is the surface tension).
- The sinusoidal changes of  $\gamma$  as a function of time are recorded and plotted by the control unit in order to measure the surface viscoelastic modulus ( $\epsilon$ ) which is defined by the equation 3 (Eq. 3):

$$|\varepsilon| = d\gamma / d \ln A, \tag{3}$$

## where A is the surface area of the air drop.

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

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

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

## 2.6. Fluorescence spectroscopy

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

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

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

### 2.7. Statistics

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

## 3. Results and discussion

# 3.1. Protein analysis

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

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

hydrophobic protein in camel milk with a higher hydrophobicity level compared to bovine β-

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

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

## 3.2. Foaming properties

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

with  $\beta$ -casein (FC= 126.9  $\pm$  5.4% and 134.6  $\pm$  3.8% for camel and bovine  $\beta$ -caseins, respectively) followed by milk and Na-cas regardless of milk origin.

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

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

skim Ca-M (up to 165%) when compared to Bo-M (115%) due to the difference in the protein composition between both Ca-M and Bo-M and the highest  $\beta$ -casein content in Ca-M.

Furthermore, bovine whey showed higher foamability compared to camel whey at a protein concentration of 0.5 g L<sup>-1</sup> (FC =  $50 \pm 5.4$  % and  $70.0 \pm 5.4$  % for camel and bovine whey proteins, respectively) with foaming behavior intermediate between those of  $\beta$ -lactoglobulin and bovine  $\alpha$ -lactalbumin alone. No significant difference was observed between camel whey and camel  $\alpha$ -lactalbumin (FC=  $51.9 \pm 1.9$  % and  $56.4 \pm 4.4$ %) suggesting that foaming proteins of camel whey are mostly maintained by the  $\alpha$ -lactalbumin due to its highest content in Ca-M in agreement with Lajnaf et al. (2018) and RP-HPLC results (section 3.1).

Similar results were also reported by Lajnaf et al. (2018) for camel and bovine sweet wheys at a higher protein concentration (5 g L<sup>-1</sup>). This previous work showed that sweet bovine whey exhibited higher foaming capacity than sweet camel whey under native conditions. However, this difference in the foaming behavior between camel and bovine wheys was no longer observed after acidifying or heating the whey proteins at 70 °C and 90 °C for 30 min (Lajnaf et al., 2018). For instance, in acidic conditions, the foamability was higher in camel whey than its bovine counterpart because of the lack of the  $\beta$ -lactoglobulin and the dominance of the  $\alpha$ -lactalbumin in camel whey which is in the molten globular state with more active surface than its native state. In acid bovine whey, the  $\beta$ -lactoglobulin forms aggregates with  $\alpha$ -lactalbumin leading to an antifoaming effect (Lainaf et al., 2018).

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

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

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

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

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

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

## 3.3. Surface tension

460

461

462

463

464

465

466

467

468

469

470

471

472

473

474

475

476

477

478

479

480

481

482

483

484

Surface tension for pure bovine and camel protein fractions at the air-water interface are shown in Fig. 4A and B, respectively. Surface tension measurements were carried out at the same protein concentration (11 mg  $L^{-1}$ ) and at pH 7. Surface tension ( $\gamma(t)$ ) is a key parameter in bubble formation. Hence, the foaming properties of milk proteins are determined by their rate of diffusion and adsorption to the interface (Borcherding et al., 2008).

First, Fig. 4 shows that changes in  $\gamma(t)$  developed by proteins adsorption at the air-water could be divided into two main different stages as reported by Cases et al. (2005): a rapid decrease of the surface tension value during the first 500 s followed by its stabilization (up to 3000 s). Furthermore, all surface tension curves start from the initial value of  $72.8 \pm 0.5$  mN m<sup>-1</sup>, which is estimated to be the surface tension of pure water in agreement with Tamm et al. (2012).

At t = 3000 s, Fig. 4A shows that the order of effectiveness for bovine proteins was: 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 ( $\gamma$  = 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  $\pm$  1.1 mN m<sup>-1</sup>) > bovine  $\alpha$ -lactalbumin ( $\gamma$  = 58.2  $\pm$  0.6) (p <0.05). Furthermore, as shown in Fig. 4B, the order of effectiveness for camel proteins fractions at t = 3000 s was: camel  $\beta$ -casein (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 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).

Table 2 illustrates the rate of adsorption (AR) corresponding to the initial slopes of the surface tension curves, (AR =  $-d\gamma(t)/dt|_{t=0}$ , see Fig. 4) in order to characterize the decrease of the surface tension rate when a new air-water surface is created during the foaming process. Very good correlation is observed between FC and R values: camel and bovine  $\beta$ -caseins carried the highest AR values regardless of milk origin (AR=  $0.275 \pm 0.003$  mN m<sup>-1</sup> s<sup>-1</sup> and  $0.362 \pm 0.003$  mN m<sup>-1</sup> s<sup>-1</sup>

0.023 mN m<sup>-1</sup> s<sup>-1</sup> for bovine and camel  $\beta$ -caseins respectively). Besides, Table 2 shows that skim milk and Na-cas exhibited higher AR values than those of whey and its derived proteins ( $\beta$ -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  $\pm$  0.012 mN m<sup>-1</sup> s<sup>-1</sup> for bovine and camel Na-cas, respectively in agreement with previous foaming results (Foam studies, Section 3.2). Besides,  $\beta$ -lactoglobulin and bovine  $\alpha$ -lactalbumin 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>, 0.185  $\pm$  0.01 mN m<sup>-1</sup> s<sup>-1</sup> and  $\pm$ 0.178  $\pm$  0.005 mN m<sup>-1</sup> s<sup>-1</sup> for bovine whey,  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin, respectively). However, no significant difference was found between camel whey and  $\alpha$ -lactalbumin (AR  $\sim$ 0.170 mN m<sup>-1</sup> s<sup>-1</sup>).

Thus, globular whey proteins (camel and bovine  $\alpha$ -lactalbumin, whey and  $\beta$ -lactoglobulin) were characterized by a lower efficiency in reducing surface tension at the airwater interface and lower AR values compared with skim milk and the casein fraction (Na-cas and  $\beta$ -casein), regardless of milk origin. These findings are consistent with those of Mellema and Isenbart (2004) who reported that skim milk proteins give lower final surface tension values when compared with whey proteins in the concentration range 1.4-2.8% (w/w) due to the coadsorption of casein micelles and whey proteins in skim milk leading to lower surface tension values. Molecular structure also plays a key role in the adsorption of milk proteins at the airwater interface.  $\beta$ -casein is more efficient in reducing the surface tension at concentrations of 1 g L<sup>-1</sup> compared with  $\beta$ -lactoglobulin. This behavior was explained by the flexible molecular structure of  $\beta$ -casein in solution which allows easy and rapid reduction of the surface tension over the first minutes compared to  $\beta$ -lactoglobulin (Seta et al., 2014).

The  $\beta$ -lactoglobulin is characterized by an ordered secondary structure as well as a compact tertiary structure. At the pH studied, this protein exists in a dimer linked by non-covalent interactions, and each  $\beta$ -lactoglobulin monomer contains two intramolecular disulfide bridges and a hidden free thiol group. Hence,  $\beta$ -lactoglobulin was not fully unfolded at the air-

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

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

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

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

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

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

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

## 3.4. Viscoelastic modulus

The viscoelastic modulus was also used to determine the rheological properties of camel and bovine proteins fractions at air-water interface (Fig. 5). As the surface tension  $\gamma(t)$  reflects the surface activity and flexibility of the protein molecule, the viscoelastic modulus reflects the rigidity of the film protein created at the interface (Cases et al., 2005).

Fig. 5A and B show the variation of the viscoelastic modulus  $\epsilon(t)$  of the protein film developed by the bovine and camel protein systems studied (skim milk, Na-cas, whey,  $\beta$ -casein,  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin) as a function of time during 3000 s and at 20 °C.

The magnitude of  $\epsilon(t)$  values varied significantly with protein type and milk origin. For Bo-M and Ca-M, Fig. 5A and B show that using skim milk, Na-cas and  $\beta$ -casein led immediately to the final and lowest  $\epsilon$  value ( $\epsilon$ ~13 mN m<sup>-1</sup>) from t=500 s compared with the other protein fractions (whey,  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin). For instance, the order of effectiveness in the creation of the most rigid surface film by Bo-M proteins at t=3000 s was (Fig. 5A):  $\beta$ -lactoglobulin ( $\epsilon=50.3\pm1.7$  mN m<sup>-1</sup>) > bovine whey ( $\epsilon=45.6\pm0.5$  mN m<sup>-1</sup>) > bovine  $\alpha$ -lactalbumin ( $\epsilon=37.3\pm2.1$  mN m<sup>-1</sup>) > bovine  $\beta$ -casein ( $\epsilon=13.3\pm1.7$  mN m<sup>-1</sup>) = skim Bo-M ( $\epsilon=12.7\pm1.5$  mN m<sup>-1</sup>) = bovine Na-cas ( $\epsilon=12.5\pm1.1$  mN m<sup>-1</sup>). While for Ca-M proteins, Fig. 5B shows that the order of efficiency t=3000 s was camel  $\alpha$ -lactalbumin ( $\epsilon=23.2\pm1.5$  mN m<sup>-1</sup>) = camel whey ( $\epsilon=20.5\pm1.5$  mN m<sup>-1</sup>) > camel  $\beta$ -casein ( $\epsilon=13.7\pm1.1$  mN m<sup>-1</sup>) = camel Na-cas ( $\epsilon=12.9\pm1.1$  mN m<sup>-1</sup>) = skim Ca-M ( $\epsilon=12.8\pm1.5$  mN m<sup>-1</sup>).

Thus, findings indicated that skim milk, Na-cas and  $\beta$ -casein have the lowest viscoelastic modulus values compared with globular proteins (whey,  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin), regardless of the origin of the milk (Fig. 5). Dilatational rheology plays an important role in the stability of foams and emulsions and it is a very sensitive technique to monitor the interfacial behavior and the competitive adsorption of proteins. Chen et al. (1993) and Bos and Van Vliet

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

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

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

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

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

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

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

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

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

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

# 3.5. Protein conformational state

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

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

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

camel  $\beta$ -casein is totally deficient in tryptophan with a higher aminoacid residues of tyrosine. Salmen, Abu-Tarboush, Al-Saleh and Metwalli (2012) reported that camel  $\beta$ -casein contains greater tyrosine residues whose percentage (4.77%) is significantly higher when compared to bovine  $\beta$ -casein (2.92%). Hence, the excitation of camel  $\beta$ -casein at 275 nm caused the emission of tyrosine residues whose  $\lambda_{max}$  is close to 303.1 nm with a higher intensity than that of the bovine  $\beta$ -casein (fluorescent intensity 159.82 A.U and 215.57 A.U for bovine and camel  $\beta$ -caseins, respectively) suggesting a higher hydrophobicity of camel  $\beta$ -casein in agreement with the findings of Ellouze, Vial, Attia and Ayadi (2021), Esmaili et al. (2011) and Lam and Nickerson (2015b). Indeed, Esmaili et al. (2011) and Ellouze et al. (2021) reported that fluorescence intensity of a protein is mainly due to tryptophan residues.

The *in silico* prediction of surface hydrophobicity index of camel β-casein is -0.339 is obtained from on ExPASy SIB Bioinformatics Resources Portal (Gasteiger et al., 2005), which is the highest hydrophobicity among camel caseins (Salami et al., 2011). Therefore, the hydrophobicity index of bovine β-casein is -0.355 (Gasteiger et al., 2005), which explain a higher hydrophobicity level compared to camel β-casein and according to the hydropathy scale of Kyte and Doolittle (1982). The difference in hydrophobicity index is mainly due to the difference in the amino-acid composition of the two β-caseins (identity level of 67.2%). Camel β-casein contains 5 tyrosine and 10 phenylalanine residues, which are mainly located in the hydrophobic part of its primary structure. It is devoid of tryptophan comparing to bovine βcasein which contains 1 tryptophan but lacks of tyrosine and phenylalanine residues leading to a higher emulsifying properties of camel β-casein compared to its bovine counterpart especially at pH 9 and 3 (Ellouze et al., 2021). These findings are in agreement with the interfacial tension results (Section 3.4) as follows: the different structural characteristics as well as a highest hydrophobicity could explain the highest tensioactive properties of camel  $\beta$ -casein when compared to bovine β-casein and its ability to lower the interfacial tension at the air-water and to stabilize milk foams.

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

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

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

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

## 4. Conclusion

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

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

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

**Acknowledgements** This work was funded by the Ministry of Scientific Research and Technology of Tunisia. We are greatly indebted to UMR-IATE for the drop-tensiometry measurements and to Professor Ammar Ben Ali for editing the manuscript.

812

#### References

- Al haj, O. A., & Al Kanhal, H. A. (2010). Compositional, technological and nutritional aspects
- of dromedary camel milk. *International Dairy Journal*, 20(12), 811–821.
- 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  $\alpha$ -
- lactalbumin. *Journal of Dairy Research*. 77,43–49.
- Bahri, A., Henriquet, C., Pugnière, M., Marchesseau, S., & Chevalier-Lucia, D. (2019). Binding
- analysis between monomeric  $\beta$ -casein and hydrophobic bioactive compounds investigated
- by surface plasmon resonance and fluorescence spectroscopy. Food Chemistry, 286, 289–
- 821 **296**.
- Barzegar, A., Yousefi, R., Sharifzadeh, A., Dalgalarrondo, M., Chobert, J. M., Ganjali, M. R.,
- Norouzi, P., Ehsani, M. R., Niasari-Naslaji, A., Saboury, A. A., Haertlé, T., & Moosavi-
- Movahedi, A. A. (2008). Chaperone activities of bovine and camel  $\beta$ -caseins: Importance
- of their surface hydrophobicity in protection against alcohol dehydrogenase aggregation.
- 826 International Journal of Biological Macromolecules, 42(4), 392–399
- Borcherding, K., Lorenzen, P. C., Hoffmann, W., & Schrader, K. (2008). Effect of foaming
- temperature and varying time/temperature-conditions of pre-heating on the foaming
- properties of skimmed milk. *International Dairy Journal*, 18(4), 349–358.
- Bos, M. A., & Van Vliet, T. (2001). Interfacial rheological properties of adsorbed protein layers
- and surfactants: a review. Advances in Colloid and Interface Science, 91(3), 437–471.
- Brooker, B. E., Anderson, M., & Andrews, A. T. (1986). The development of structure in
- whipped cream. *Food Structure*, 5(2), 12.
- 834 Cao, Y., Xiong, Y. L., Cao, Y., & True, A. D. (2018). Interfacial properties of whey protein
- foams as influenced by preheating and phenolic binding at neutral pH. Food

- 836 *Hydrocolloids*, 82, 379–387.
- Cases, E., Rampini, C., & Cayot, P. (2005). Interfacial properties of acidified skim milk.
- *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  $\beta$ -case in 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 β-
- lactoglobulin and α-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
- and emulsion stability. *Food Structure*, 12(2), 1.
- 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
- β-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
- 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 α-lactalbumin and β-
- lactoglobulin at air-water interfaces. Journal of Colloid and Interface Science, 214(2),
- 856 129–142.
- Davies, D. T., & Law, A. J. R. (1980). The content and composition of protein in creamery
- milks in south-west Scotland. *Journal of Dairy Research*, 47(01), 83–90.

- De Kruif C.G., & Holt C. (2003). Casein Micelle Structure, Functions and Interactions. In: Fox
- P.F., McSweeney P.L.H. (eds) Advanced Dairy Chemistry—1 Proteins. Springer, Boston,
- 861 MA.
- Diao, M., Liang, Y., Zhao, J., Zhao, C., Zhang, J., & Zhang, T. (2021). Enhanced cytotoxicity
- and antioxidant capacity of kaempferol complexed with  $\alpha$ -lactal bumin. Food and
- 864 *Chemical Toxicology*, *153*, 112265.
- Dickinson, E, Horne, D. S., Phipps, J. S., & Richardson, R. M. (1993). A neutron reflectivity
- study of the adsorption of .beta.-casein at fluid interfaces. *Langmuir*, 9(1), 242–248.
- Dickinson, E. (1989). Surface and emulsifying properties of caseins. Journal of Dairy
- 868 Research, 56(03), 471–477.
- Dickinson, E. (2001). Milk protein interfacial layers and the relationship to emulsion stability
- and rheology. *Colloids and Surfaces B: Biointerfaces*, 20(3), 197–210.
- Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of
- dispersed systems. *Food Hydrocolloids*, 17(1), 25–39.
- 873 Dickinson, E, & Hong, S.T. (1994). Surface Coverage of β-lactoglobulin at the oil-water
- interface: influence of protein heat treatment and various emulsifiers. Journal of
- 875 Agricultural and Food Chemistry, 42(8), 1602–1606.
- Dombrowski, J., Dechau, J., & Kulozik, U. (2016). Multiscale approach to characterize bulk,
- surface and foaming behavior of casein micelles as a function of alkalinisation. *Food*
- 878 *Hydrocolloids*, *57*, 92–102.
- 879 Eigel, W. N., Butler, J. E., Ernstrom, C. A., Farrell, H. M., Harwalkar, V. R., Jenness, R., &
- 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).
- 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 α-
- lactalbumin at the oil-water interface: Effect of protein concentration and pH change on
- 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 α-lactalbumin at the oil-water
- interface: Effect of pH and heat treatment on the structure, surface characteristics and
- 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
- 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
- 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
- application. LWT Food Science and Technology, 44(10), 2166–2172.

- 906 Farah, Z., Rettenmaier, R., & Atkins, D. (1992). Vitamin content of camel milk. *International*
- *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
- 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.
- Gasteiger, E., Hoogland, C., Gattiker, A., Wilkins, M. R., Appel, R. D., & Bairoch, A. (2005).
- 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
- 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
- other factors in foaming properties of milk: a review. Critical Reviews in Food Science
- 923 *and Nutrition*, 1–21.
- Ho, T. M., Dhungana, P., Bhandari, B., & Bansal, N. (2021). Effect of the native fat globule
- 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 β-casein. *Food Chemistry*, 99(1), 45–50.
- 929 Ibanoglu, E., & Ibanoglu, Ş. (1999). Foaming behaviour of EDTA-treated α-lactalbumin. *Food*
- 930 *Chemistry*, 66(4), 477–481.
- Jara, F. L., Sánchez, C. C., Patino, R. J. M., & Pilosof, A. M. R. (2014). Competitive adsorption
- behavior of β-lactoglobulin, α-lactalbumin, bovin serum albumin in presence of
- hydroxypropylmethylcellulose. Influence of pH. *Food Hydrocolloids*, *35*, 189–197.
- Kanduc, D. (2012). Homology, similarity, and identity in peptide epitope immunodefinition.
- 935 *Journal of Peptide Science*, 18(8), 487–494.
- 836 Kappeler, S, Farah, Z., & Puhan, Z. (1998). Sequence analysis of *Camelus dromedarius* milk
- 937 caseins. The Journal of Dairy Research, 65(2), 209–222.
- 838 Kappeler, S., Farah, Z., & Puhan, Z. (2003). 5'-Flanking regions of camel milk genes are highly
- similar to homologue regions of other species and can be divided into two Distinct Groups.
- 940 *Journal of Dairy Science*, 86(2), 498–508.
- 841 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.:
- 243 Zdenko Puhan; Korref.: Zakaria Farah; Korref: Gerald Stranzinger.
- 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.
- 846 Kong, F., Tian, J., Yang, M., Zheng, Y., Cao, X., & Yue, X. (2020). Characteristics of the
- interaction mechanisms of xylitol with  $\beta$ -lactoglobulin and  $\beta$ -casein: Amulti-spectral
- 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
- of a protein. *Journal of Molecular Biology*, 157(1), 105–132.

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

- functionality of camel and bovine milk whey proteins. *Journal of Dairy Science*, 91(12),
- 976 4527–4534.
- 277 Lam, R. S. H., & Nickerson, M. T. (2015 a). The effect of pH and temperature pre-treatments
- on the physicochemical and emulsifying properties of whey protein isolate. *LWT Food*
- 979 *Science and Technology*, 60(1), 427–434.
- Lam, R. S. H., & Nickerson, M. T. (2015 b). The effect of pH and temperature pre-treatments
- on the structure, surface characteristics and emulsifying properties of alpha-lactalbumin.
- 982 Food Chemistry, 173, 163–170.
- Li, M., Blecker, C., & Karboune, S. (2021). Molecular and air-water interfacial properties of
- potato protein upon modification via laccase-catalyzed cross-linking and conjugation with
- 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).
- Protein profile of whole camel milk resulting from commercial thermal treatment. *LWT*-
- 988 Food Science & Technology, 134, 110256.
- 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.
- 294 Lorient, D., Closs, B., & Courthaudon, J. L. (1991). Connaissances nouvelles sur les propriétés
- 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
- omponents: 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).
- Invited review: physiological properties of bioactive peptides obtained from whey
- 1001 proteins. *Journal of Dairy Science*, *93*(2), 437–455.
- Marinova, K. G., Basheva, E. S., Nenova, B., Temelska, M., Mirarefi, A. Y., Campbell, B., &
- Ivanov, I. B. (2009). Physico-chemical factors controlling the foamability and foam
- stability of milk proteins: Sodium caseinate and whey protein concentrates. Food
- 1005 *Hydrocolloids*, 23(7), 1864–1876.
- Mellema, M., & Isenbart, J. G. (2004). Effect of acidification and heating on the rheological
- properties of oil-water interfaces with adsorbed milk proteins. *Journal of Dairy Science*,
- 1008 87(9), 2769–2778.
- Merin, U., Bernstein, S., Yagil, R., Creveld, C. Van, Lindner, P., & Gollop, N. (2001). A
- comparative study of milk serum proteins in camel (Camelus dromedarius) and bovine
- 1011 colostrum. *Livestock Production Science*, 67, 297–301.
- Murray, B. S. (2020). Recent Developments in Food Foams. Current Opinion in Colloid &
- 1013 Interface Science, 101394.
- Nicorescu, I., Vial, C., Talansier, E., Lechevalier, V., Loisel, C., Valle, D. Della, Riaublanc, A.,
- Djelveh, G., & Legrand, J. (2011). Comparative effect of thermal treatment on the
- physicochemical properties of whey and egg white protein foams. Food Hydrocolloids,
- 1017 *25*(4), 797–808.
- Nylander, T., Arnebrant, T., Cárdenas, M., Bos, M., & Wilde, P. (2019). Protein/emulsifier
- interactions. In *Food emulsifiers and their applications* (pp. 101–192). Springer.
- Omar, A., Harbourne, N., & Oruna-Concha, M. J. (2016). Quantification of major camel milk
- proteins by capillary electrophoresis. *International Dairy Journal*, 58, 31–35.
- Paulsson, M., & Dejmek, P. (1992). Surface film pressure of β-lactoglobulin, α-lactalbumin and

- bovine serum albumin at the air/water interface studied by Wilhelmy plate and drop
- volume. *Journal of Colloid and Interface Science*, 150(2), 394–403.
- Razumovsky, L., & Damodaran, S. (1999). Surface activity– compressibility relationship of
- proteins at the air– water interface. *Langmuir*, 15(4), 1392–1399.
- Redington, J., Breydo, L., Almehdar, H., Redwan, E., & Uversky, V. (2016). α-Lactalbumin:
- of camels and cows. *Protein & Peptide Letters*, 23(12), 1072–1080.
- Rouimi, S., Schorsch, C., Valentini, C., & Vaslin, S. (2005). Foam stability and interfacial
- properties of milk protein-surfactant systems. *Food Hydrocolloids*, 19(3), 467–478.
- Salami, M., Moosavi-Movahedi, A. A., Moosavi-Movahedi, F., Ehsani, M. R., Yousefi, R.,
- Farhadi, M., Niasari-Naslaji, A., Saboury, A. A., Chobert, J.-M., & Haertlé, T. (2011).
- Biological activity of camel milk casein following enzymatic digestion. *Journal of Dairy*
- 1034 *Research*, 78(04), 471–478.
- Salami, M., Yousefi, R., Ehsani, M. R., Razavi, S. H., Chobert, J.M., Haertlé, T., Saboury, A.
- A., Atri, M. S., Niasari-Naslaji, A., & Ahmad, F. (2009). Enzymatic digestion and
- antioxidant activity of the native and molten globule states of camel  $\alpha$ -lactal bumin:
- Possible significance for use in infant formula. *International Dairy Journal*, 19(9), 518–
- 1039 523.
- Salmen, S. H., Abu-Tarboush, H. M., Al-Saleh, A. A., & Metwalli, A. A. (2012). Amino acids
- content and electrophoretic profile of camel milk casein from different camel breeds in
- Saudi Arabia. Saudi Journal of Biological Sciences, 19(2), 177–183.
- Seta, L., Baldino, N., Gabriele, D., Lupi, F. R., & De Cindio, B. (2014). Rheology and
- adsorption behaviour of  $\beta$ -casein and  $\beta$ -lactoglobulin mixed layers at the sunflower
- oil/water interface. Colloids and Surfaces A: Physicochemical and Engineering Aspects,
- 1046 *441*, 669–677.

- 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
- Foundation of Science. 285–306.
- Slack, A. W., Amundson, C. H., & Hill, C. G. (1986). Foaming and emulsifying characteristics
- of fractionated whey protein. *Journal of Food Processing and Preservation*, 10(2), 81–88.
- Svanborg, S., Johansen, A.-G., Abrahamsen, R. K., Schüller, R. B., & Skeie, S. B. (2016).
- 1053 Caseinomacropeptide influences the functional properties of a whey protein concentrate.
- 1054 International Dairy Journal, 60, 14–23.
- Tamm, F., Sauer, G., Scampicchio, M., & Drusch, S. (2012). Pendant drop tensiometry for the
- evaluation of the foaming properties of milk-derived proteins. *Food Hydrocolloids*, 27(2),
- 1057 371–377.
- Thompson, A., Boland, M. J., & Singh, H. (2009). Milk proteins, from expression to food, SL
- 1059 *Taylor*. Elsevier, Academic Press, Amsterdam.
- Wangoh, J., Farah, Z., & Puhan, Z. (1998). Iso-electric focusing of camel milk proteins.
- 1061 *International Dairy Journal*, 8(7), 617–621.
- Williams, A., & Prins, A. (1996). Comparison of the dilational behaviour of adsorbed milk
- proteins at the air-water and oil-water interfaces. Colloids and Surfaces A:
- 1064 *Physicochemical and Engineering Aspects*, 114, 267–275.
- Xiong, X., Ho, M. T., Bhandari, B., & Bansal, N. (2020). Foaming properties of milk protein
- dispersions at different protein content and casein to whey protein ratios. *International*
- 1067 Dairy Journal, 109, 104758.
- Yang, H., Xiao, X., Zhao, X., & Wu, Y. (2017). Intrinsic fluorescence spectra of tryptophan,
- tyrosine and phenyloalanine. Selected Papers of the Chinese Society for Optical
- Engineering Conferences Held October and November 2016, 10255, 102554M.

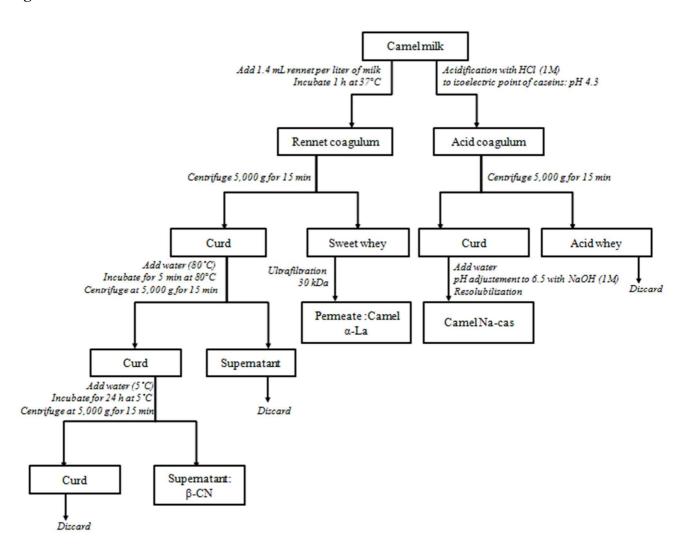
Yin, Z., Qie, X., Zeng, M., Wang, Z., Qin, F., Chen, J., Li, W., & He, Z. (2022). Effect of 1071 1072 thermal treatment on the molecular-level interactions and antioxidant activities in β-casein and chlorogenic acid complexes. Food Hydrocolloids, 123, 107177. 1073 1074 Yüksel, Z., & Erdem, Y. K. (2010). Detection of the milk proteins by RP-HPLC. GIDA-Journal of Food, 35(1), 5–11. 1075 1076 Zayas, J. F. (1997). Foaming properties of proteins. In Functionality of proteins in food (pp. 260–309). Springer. 1077 Zhang, H., Yu, D., Sun, J., Guo, H., Ding, Q., Liu, R., & Ren, F. (2014). Interaction of milk 1078 whey protein with common phenolic acids. Journal of Molecular Structure, 1058, 228-1079 233. 1080 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 milk proteins. Colloids and Surfaces B: Biointerfaces, 34(2), 113–121. 1083 1084 Zhou, X., Sala, G., & Sagis, L. M. C. (2020 a). Bulk and interfacial properties of milk fat emulsions stabilized by whey protein isolate and whey protein aggregates. Food 1085 Hydrocolloids, 109, 106100. 1086 Zhou, B., Tobin, J. T., Drusch, S., & Hogan, S. A. (2020 b). Interfacial properties of milk 1087 proteins: A review. Advances in Colloid and Interface Science, 102347. 1088 1089

#### Figure captions

- 1093 Fig. 1. Flow diagram of the camel milk protein isolation procedure. Abbreviations are: Na-cas,
- 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; β-CN,
- 1097 β-casein; α-La, α-lactalbumin; β-Lg, β-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
- caseinates (Na-cas),  $\beta$ -casein ( $\beta$ -CN), whey,  $\beta$ -lactoglobulin ( $\beta$ -Lg) and  $\alpha$ -lactalbumin ( $\alpha$ -La).
- The experiments were performed in 20 mM Tris-HCl buffer, pH 7.0, at 25 °C at a protein
- 1102 concentration of  $0.5 \text{ g L}^{-1}$ .
- 1103 a-f Samples represented with different letters are significantly different from each other (p<0.05). Error bars show
- the standard deviations of mean values of foam capacity and stability.
- 1105 Fig. 4. Time-dependent changes in surface tension  $\gamma(t)$  (mN m<sup>-1</sup>) at air-water interface of
- bovine(A) and camel (B) proteins systems: Skim bovine milk (skim Bo-M), skim camel milk
- (skim Ca-M), sodium caseinates (Na-cas), whey,  $\alpha$ -lactalbumin ( $\alpha$ -La),  $\beta$ -casein ( $\beta$ -CN) and  $\beta$ -
- lactoglobulin ( $\beta$ -Lg), at a concentration of 11 mg L<sup>-1</sup>, pH 7 and temperature 20 °C.
- **Fig. 5.** Time-dependent changes in viscoelastic modulus ε(t) (mN m<sup>-1</sup>) at air-water interface of
- bovine (A) and camel (B) proteins systems: Skim bovine milk (skim Bo-M), skim camel milk
- (skim Ca-M), sodium caseinates (Na-cas), whey,  $\alpha$ -lactalbumin ( $\alpha$ -La),  $\beta$ -casein ( $\beta$ -CN) and  $\beta$ -
- lactoglobulin ( $\beta$ -Lg), at a concentration of 11 mg L<sup>-1</sup>, pH 7 and temperature 20 °C.

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

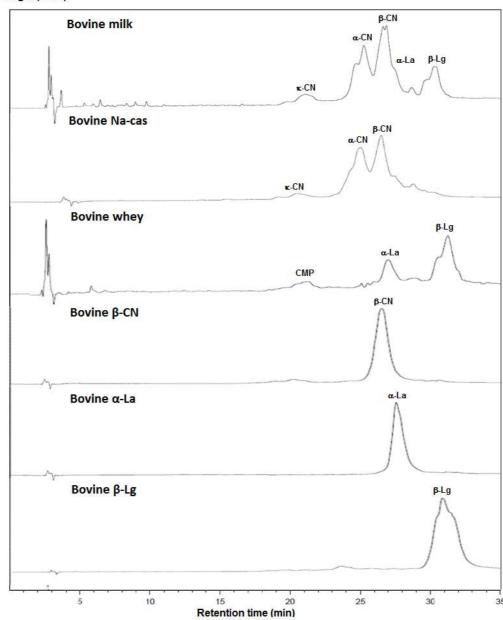
### 1120 Figures:



1122 Fig. 1

## Manuscript R2 (2022)

# A Height (mAU)



## Manuscript R2 (2022)

B

Height (mAU)

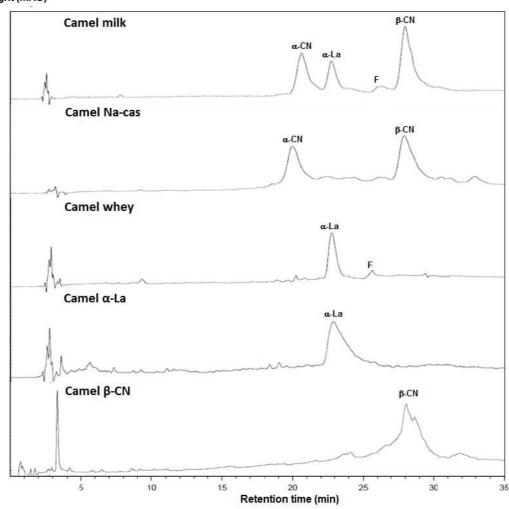
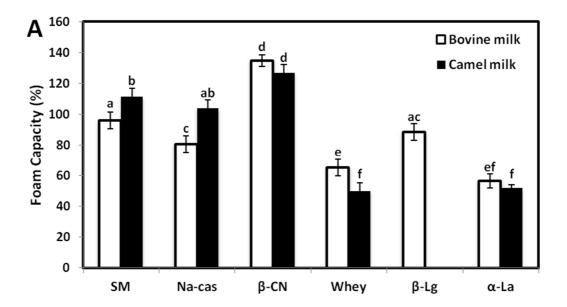
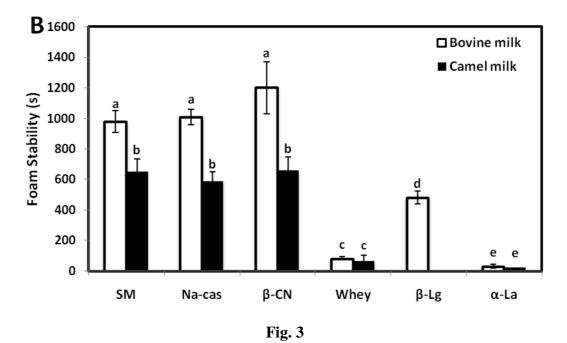


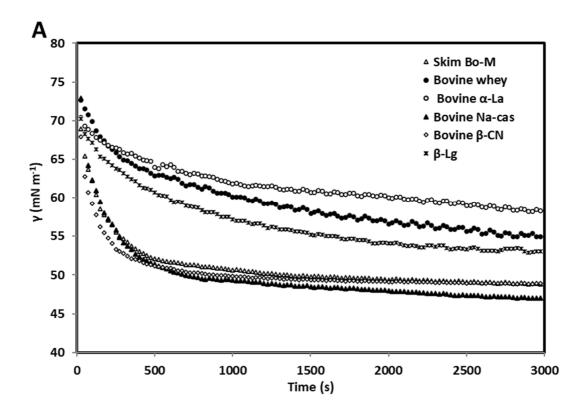
Fig. 2

1142

1143







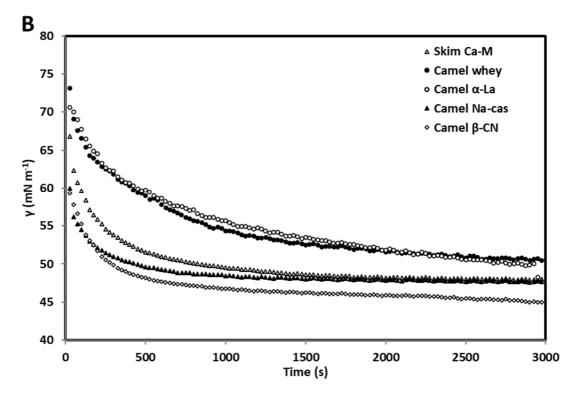
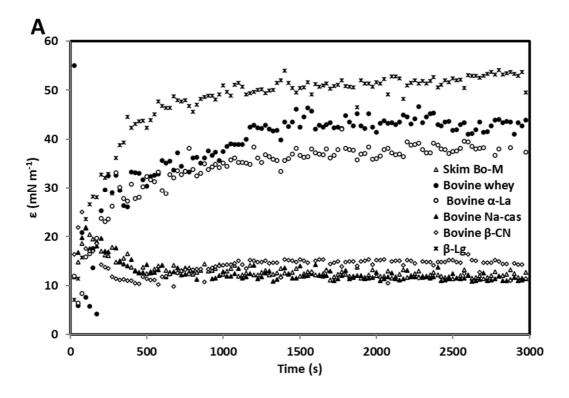
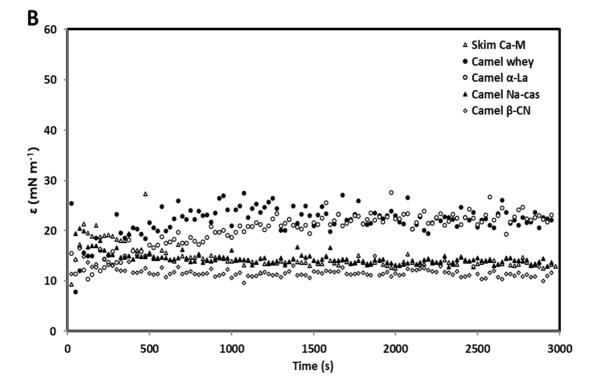
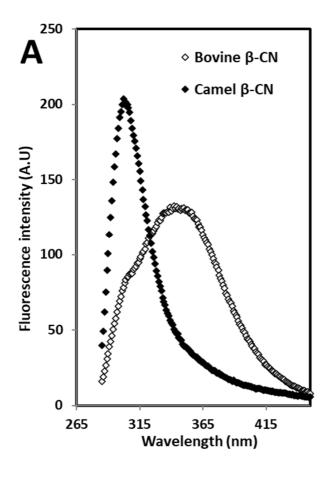


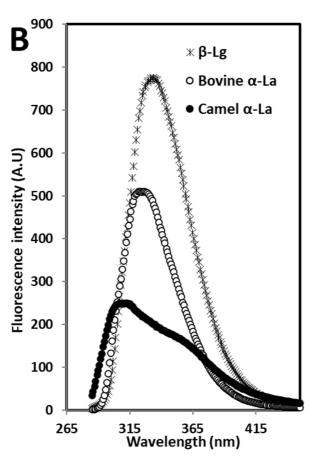
Fig. 4





1161 Fig. 5





1165 Fig. 6

### Table caption

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

1177 Tables

**Table 1.** 

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

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

<sup>&</sup>lt;sup>b</sup>: Percentage of the protein in the serum fraction of milk

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

<sup>&</sup>lt;sup>d</sup> Protein sequence similarity: similarities between aminoacid sequences including residues with similar biochemical properties

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

a-h Samples represented with different letters are significantly different from each other (p<0.05). Error bars show

**Table 2.** 

the standard deviations of mean values of adsorption rate (AR).

Bo-M	Ca-M	
$0.238 \pm 0.016$ de	$0.218 \pm 0.025$ e	
$0.252 \pm 0.005$ d	$0.300 \pm 0.012$ b	
$0.153 \pm 0.003$ h	$0.165 \pm 0.002$ g	
$0.275 \pm 0.003$ °	$0.362 \pm 0.023$ a	
$0.185 \pm 0.011$ f		
$0.178 \pm 0.005$ f	$0.170 \pm 0.013$ fgh	
	$0.238 \pm 0.016^{\text{ de}}$ $0.252 \pm 0.005^{\text{ d}}$ $0.153 \pm 0.003^{\text{ h}}$ $0.275 \pm 0.003^{\text{ c}}$ $0.185 \pm 0.011^{\text{ f}}$	

