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Protocols

The effect of pH and heat treatments on the foaming properties of purified α-lactalbumin from camel milk

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ABSTRACT

The effect of pH (4.3 or 6.5) and heat treatment (70 °C or 90 °C for 30 min) on the foaming and interfacial properties of α-lactalbumin extracted from camel milk were studied. The increased temperature treatment changed the foaming properties of camel α-lactalbumin solution and its ability to unfold at the air–water interface. At neutral pH, heat treatment was found to improve foamability, whereas at acid pH (4.3) this property decreased. Foams were more stable after a heat treatment at pH 4.3 than at 6.5, due to higher levels of protein aggregation at low pH.

Heat treatment at 90 °C for 30 min affected the physicochemical properties of the camel α-lactalbumin by increasing free thiol group concentration at pH 6.5. Heat treatment also caused changes in α-lactalbumin's surface charge. These results also confirm the pronounced aggregation of heated camel α-lactalbumin solution at acid pH.

1. Introduction

Camel milk has an important role in human nutrition in the arid and semi-arid regions in the world. This milk contains all the nutritious components already found in bovine milk and is even richer in iron, lactoferrin and vitamin C [1]. Even if the total camel milk proteins and whey proteins concentration are approximately the same as bovine milk, camel whey protein composition is different as β-lactoglobulin is absent in camel serum [2]. Camel α-lactalbumin is the main whey protein in camel milk, with an average concentration of 2.2 g/l, which is close to the concentration in human milk concentration (2.45 g/l) and significantly higher than that of bovine milk (0.5 g/l) [3]. The main biological function of this protein is to regulate lactose synthesis [4].

As the bovine α-lactalbumin, camel α-lactalbumin is a calcium metalloprotein of 123 amino acid residues, with a molecular weight around 14.43 kDa and an isoelectric point between 4.1–4.8 [5]. But this protein has greater antioxidant activity, is richer in essential amino acids and is more digestible than the equivalent bovine protein [6]. The primary sequences of bovine and camel α-lactalbumin show that the percentage sequence similarity and identity are 82.9% and 69.1% respectively, justified by a difference in the nature of 39 residues between these two different proteins. Camel α-lactalbumin has a considerably more hydrophobic core than the bovine protein at positions 25–35 [5,7]. Both proteins have the same number of cysteines at the same positions and the same number of disulfide bonds (Cys^{6}/Cys^{120}, Cys^{28}/Cys^{111}, Cys^{61}/Cys^{77}, and Cys^{73}/Cys^{91}). However, the conformation of camel α-lactalbumin is more sensitive to calcium loss than its bovine equivalent [7]. Furthermore, bovine α-lactalbumin is more flexibile in the unfolded state than it is when folded.

The α-lactalbumin can be denatured in several ways, such as (i) extremes of pH, (ii) heating to 90 °C, (iii) removal of bound Ca^{2+}, (iv) adding denaturant agents, or (v) cleavage of disulphide bridges [8,9]. A variety of physicochemical conditions (including (i)–(iii)) cause α-lactalbumin to assume a molten globule conformation. This is described as a compact state that keeps its secondary structure, but has a poorly defined tertiary structure. This molten globule state increases the accessibility of α-lactalbumin's hydrophobic regions [6,7].

Previous studies reported that holo (calcium-loaded) and apo (calcium-unloaded) camel α-lactalbumins are denatured at 71.7 °C and 39.6 °C respectively. Atri et al. [7] showed that, camel α-lactalbumin is more stable than the bovine protein in both the holo and apo states; the camel protein has more hydrophobic interactions that contribute to its stability.

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Bovine α-lactalbumin is also known to have good emulsifying and foaming properties and has been thoroughly studied [9,10]. The foaming and interfacial properties of camel α-lactalbumin are less well characterized [11,12].

We therefore set out in this study to examine the impact of pH and heat treatments on the foaming and interfacial properties of camel α-lactalbumin. A better understanding of the interfacial properties of this key protein in camel milk will enhance its foaming properties and its exploitation in food applications.

2. Material and methods

2.1. Materials

Fresh raw camel milk was collected manually from 20 different healthy camels (*Camelus dromedarius*) having had between 2 and 12 months of lactation, from a farm in the Medenine region of Tunisia.

The samples were immediately cooled to 4 °C and transported to the laboratory within 24 h. pH values were systematically measured after collecting milk and before storing. The fat was removed by centrifugation (centrifuge Beckman CO-LE80K) at 1000*g* for 15 min at 4 °C [1] and the skimmed milk was stored at −18 °C before experiments.

2.2. Purification of camel α-lactalbumin

After defatting, the casein fraction of the skimmed camel milk was coagulated for 2 h at 37 °C by rennet addition (1.4 ml/l of milk) [13]. The remaining liquid whey, known as the sweet whey, was then retrieved by centrifugation at 5000*g* for 20 min at 20 °C (centrifuge Beckman CO-LE80K). The curd containing the casein fraction was discarded and the supernatant containing soluble whey proteins was ultrafiltered at 20 °C on an ultrafiltration (UF) membrane with a 30 kDa molecular mass cut-off (Amicon bioseperations model 8050) [6]. The UF system was operated at a pressure of 1 bar for 3 h at 20 ± 1 °C. The UF retentate was discarded and the permeate containing camel α-lactalbumin was kept at −18 °C for further analysis. The rejection rate (R) of the camel α-lactalbumin by the membrane was determined as Eq. (1):

$$R = \left(1 - \frac{C_p}{C_s}\right) \times 100 \quad (1)$$

where Cp is the camel α-lactalbumin concentration of permeate and Cs is the concentration in the supernatant before ultrafiltration.

Camel α-lactalbumin concentration was determined by the Micro BCA™ Protein Assay Kit (G-Biosciences, GenoTechnology, USA), used according to the manufacturer's instructions (50 μl sample/ml BCA working reagent, 120 min in the dark and read at 562 nm). A BSA (bovine serum albumin) dilution curve consisting of 8 points up to 2 mg/ml BSA was realized to plot the standard curve.

The purity of the camel α-lactalbumin was estimated by 15% acrylamide SDS-PAGE using the technique described by Laemmli [14]. Proportions of the different camel proteins were determined using the GelQuant.NET software provided by biochemlabsolutions.com and expressed as percentage of total protein.

2.3. Camel α-lactalbumin solution preparation

Camel α-lactalbumin solutions were prepared by adjusting pH, from the initial 6.5, to 4.3, using 1 M HCl. All α-lactalbumin solutions were adjusted, before the experiments, to have the same concentration of 1.7 g/l as measured by the BCA Protein Kit. Camel α-lactalbumin solutions (pH 6.5 and 4.3) were heated at 70 °C or 90 °C in a water bath for 30 min. Beakers were then put on ice to decrease the temperature and stop the protein denaturation. All further dilutions were carried out using deionized water from Milli-Q system (Millipore, USA).

2.4. Foaming properties

Ten milliliters of permeate containing the camel α-lactalbumin whey were introduced in a measuring cylinder (radius 2 cm and length 8.5 cm) and mixed using the Ultra Turrax T25 (IKA Labortechnik, Staufen Germany) at 13500 rpm for 2 min at room temperature [12].

Thefoamcapacity(FC)in%, isdefinedthroughEq.(2)

$$FC = (V_{foam}/V_0) \times 100 \quad (2)$$

where V_{foam} is the volume of the formed foam (ml) and V_0 the initial volume before whipping (ml).

Foam stability (FS) is defined as the foam half time or the time for drainage of half of the initially created foam ($t_{foam1/2}$) [15].

2.5. Interfacial properties

Dynamic surface tension measurements were performed with a drop tensiometer (IT Concept, Longessaigne, France). An axisymmetric air drop was formed at the tip of a needle diving into the cuvette containing the purified camel α-lactalbumin solution. Air drop formation was driven by the device software. The interfacial tension was computed by analyzing the profile of the drop according to Laplace Eq. (3):

$$(1/x)[d(x\sin\theta)/dx] = (2/b) - cz, \quad (3)$$

where:

- x and z are the cartesian coordinates at any point of the drop profile,
- b is the radius of curvature of the drop apex,
- θ is the angle of the tangent to the drop profile,
- c is called the capillary constant.

The measurement of the interfacial tension (γ) was carried out for the permeate containing camel α-lactalbumin at a concentration of 0.011 g/l, after dilution using deionized water. At this concentration, all the interface of the air bubble created by the tensiometer was covered with proteins and only a small amount of protein remained in the bulk phase [16].

The adsorption rate of the protein at the air drop surface is defined as the initial slope value of the interfacial tension curve as function of time at t = 0 ($A = -d\gamma/dt|_{t=0}$) and this was also determined in order to characterize the rate of the surface tension decrease [15].

2.6. Determination of the thiol content

The concentration of free thiols was determined using 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB) as described by Ellman [17]. Aliquots (50 μl) of the reaction samples were mixed with 50 μl of DTNB solution (50 mM sodium acetate (NaAc), 2 mM DTNB in H_2O), 100 μl of Tris solution (1 M Tris, pH 8.0 and 800 μl of distilled water). The mixture was incubated for 5 min at room temperature, the absorbance was read at 412 nm using a UVmini-1240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) and the concentration of free SH groups (C_{SH} (mM)) was calculated by the Eq. (4):

$$C_{SH}(mM) = (DO_{412nm}/\varepsilon_{412}) \times (1000/200) \quad (4)$$

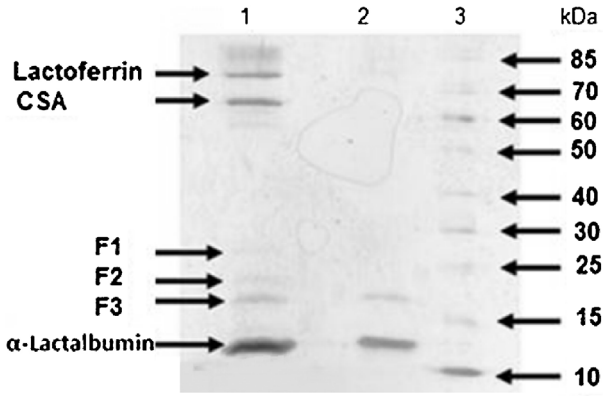

Fig. 1. SDS-PAGE profiles of different steps for purification of camel α-lactalbumin. Sweet whey and purified sample of camel α-lactalbumin (after treatment with UF membrane) are shown in Lanes 1 and 2, respectively and represents an amount of 35 μg of protein per lane. Lane 3 represents molecular mass markers; CSA = camel serum albumin, F = fraction.

where DO_{412nm} is the absorbance at 412 nm; ε_{412} is the extinction coefficient (13600 M^{-1} cm^{-1}); 1000 μl is the total volume of the cuvette and 200 μl is the sample volume.

2.7. Electrical charge (ζ-potential measurements)

The ζ-potential of the camel α-lactalbumin solution was determined at 20 ± 1 °C using a Zetasizer Nano-ZS90 (Malvern Instruments, Westborough, MA). All protein samples were measured at a concentration of 1.7 ± 0.1 g/l.

The ζ-potential (ζ, mV) was determined from the electrophoretic mobility (U_E) using Henry's equation [9] (5):

$$U_E = \frac{2\varepsilon\zeta f(k\alpha)}{3\eta} \quad (5)$$

where ε is the permittivity (Farad/m); $f(k.\alpha)$ the function related to the ratio of particle radius (α, nm); k, the Debye length (nm^{-1}) and η is the dispersion viscosity (mPa s).

2.8. Statistics

The significance of the main effects of pH (4.3, 6.5) and heat treatment conditions (70 °C, 90 °C) on camel α-lactalbumin foaming properties, ζ-potential and surface tension measurements was tested by three-way analysis of variance (ANOVA). Statistical analyses were performed with the IBM SPSS statistics software, Version 19. All experiments were performed at least in triplicate and results were reported as the mean ± one standard deviation.

3. Results and discussion

3.1. Purification of α-lactalbumin

The purification of the camel α-lactalbumin from camel milk represents an important step of this work. This purification needs a milk centrifugation and an ultrafiltration of the camel serum obtained as stated in Materials and methods. The isolated camel α-lactalbumin purity was systematically checked by SDS-PAGE. Fig. 1 shows the SDS-PAGE profiles before and after UF purification of camel α-lactalbumin. Camel whey (lane 1, Fig. 1) is composed of different proteins: lactoferrin (19%), camel serum albumin (CSA) (19%), fraction 1 (2%), fraction 2 (3%), fraction 3 (7%) and the camel α-lactalbumin (49%). After UF purification (line 2, Fig. 1), the isolated camel α-lactalbumin proteins were found in the permeate whereas proteins with high molecular weight were kept in the retentate. The α-lactalbumin percentage rose from 49% before UF to 90% after UF. These percentages are in accordance with the work of Salami et al. [6] and Lajnaf et al. [12]. As also seen in Fig. 1, lane 2, the fraction protein F3 was still present after UF purification at a percentage of 10%. The molecular mass of this fraction was estimated at ~20 kDa and it could correspond to the peptidoglycan recognition protein (PGRP). Indeed, the molecular mass of this protein is 19.1 kDa [18].

The concentration of total protein amount was 6.65 ± 0.25 g/l in the camel serum and 1.7 ± 0.1 g/l after UF purification, but the purity of α-lactalbumin was higher after this step. The extraction yield of camel α-lactalbumin, i.e., grams of protein extracted per grams of protein in the initial whey, represented 47.3% of total camel α-lactalbumin. Rejection rate (R) of the camel α-lactalbumin by the membrane during the UF purification reached 52.7% as a consequence of membrane fouling and solution chemistry [19].

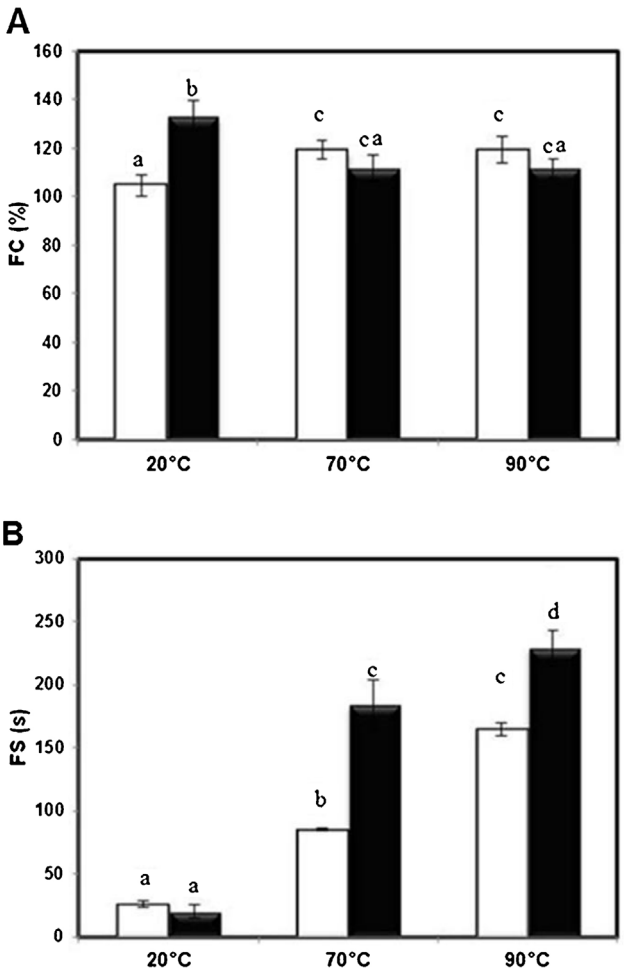

Fig. 2. Foam capacity: FC (A) and foam stability: FS (B) of camel α-lactalbumin solution (at a concentration of 1.7 g/l) (pH 4.3 ■ and 6.5 □) as function of the temperature of the heat treatment (70 °C and 90 °C for 30 min).
[a–d]Samples represented with different letters are significantly different from each other ($p < 0.05$). Error bars are standard deviations of mean values of FC and FS.

3.2. Foam properties

The average values of the foam capacity (FC) of denatured camel α-lactalbumin solutions (1.7 g/l) at different pH (4.3 and 6.5) and after different heat treatments (70 °C or 90 °C for 30 min) are shown in Fig. 2A and compared to native α-lactalbumin solutions (20 °C, pH 6.5).

In its native form i.e. at 20 °C and pH 6.5, the foam capacity of camel α-lactalbumin was 105%. A decrease of pH value to 4.3 increased foaming by 36%, suggesting that in these acid conditions camel α-lactalbumin coats the air bubbles better than at pH 6.5. Indeed, camel α-lactalbumin is probably more flexible at pH 4.3 due to the reduced electrostatic repulsions of proteins at a pH close to its isoelectric point. The same trends were reported for bovine α-lactalbumin by Zhang et al. [20] who observed that at pH < 5, α-lactalbumin loses its bound calcium ions and assumes the molten globular state, thus becoming more surface active.

Fig. 2A also shows the impact of heat treatments at native (6.5) or acid pH (4.3) on the FC values of camel α-lactalbumin. At pH 6.5, a thermal treatment for 30 min at 70 °C or 90 °C induced a significant increase ($p < 0.05$) of the FC from 105% to 120%. No significant change was found on the evolution of FC between 70 °C and 90 °C. The opposite results were found for the acidified α-lactalbumin solution, since in this case thermal treatments significantly decreased ($p < 0.05$) the FC from 136% to 110%. At pH 4.3, FC stayed constant when the temperature increased from 70 °C to 90 °C. In agreement with previous work [9] carried out with bovine α-lactalbumin, our results show a decrease of the interfacial properties of the α-lactalbumin protein after a thermal treatment at acid pH, compared to native pH. Lam and Nickerson [9] explained that this behavior was due to heat-induced aggregation, which occurs less at neutral pH than at acid pH due to charge repulsion forces. Indeed, electrostatic repulsive forces at neutral pH preserve α-lactalbumin proteins from thermal aggregation. These repulsive forces lead to better adsorption of α-lactalbumin at the air-water interface.

The foaming stability (FS) values of camel α-lactalbumin foams in response to pH and/or thermal treatments are given in Fig. 2B. The camel α-lactalbumin FS values were highly pH and temperature dependent. At 20 °C, foams made with camel α-lactalbumin solutions were found to be unstable regardless of pH value (FS < 30 s). Foam stability increased after heating the protein solution to 70 °C or 90 °C and it was significantly higher at pH 4.3 than at pH 6.5 (Fig. 2B). For camel α-lactalbumin at pH 4.3, FS values increased greatly from 20 s at 20 °C to 184 s or 229 s after a heat treatment at 70 °C or 90 °C, respectively. Likewise for camel α-lactalbumin at pH 6.5, FS values substantially increased from 26 s, for the native form, to 85 s at 70 °C, and then to 164 s at 90 °C ($p < 0.05$). Dickinson [21] has reported that the increase in foam stability of milk proteins after heat treatment is due to an increase in the diffusion and adsorption velocity of milk proteins at the interface. Mellema and Isenbart [22] have found that for bovine whey protein, the combination of heating (at 85 °C for 20 min) and acidification (pH 4.6) leads to extensive aggregation, which results in stable emulsions. Thus, the open structure of the acidified α-lactalbumin, the thermal denaturation and the reduced electrostatic repulsion near the isoelectric point, are all factors that promote the creation of large aggregates.

Murray and Ettelaie [23] reported that protein aggregation could improve foam stability. They suggested that proteins are adsorbed rapidly at the air water interface and that this contributes to the formation of foam, while aggregates are adsorbed slower, which stabilizes the interfacial film of protein already formed. Furthermore, a better foam stability was achieved at pH values close to the isoelectric point of α-lactalbumin, due to the further reduction of electrostatic repulsions between proteins [20].

3.3. Interfacial properties

The influence of pH and/or thermal treatments on the air/water interfacial behavior of the camel α-lactalbumin at low concentration (0.011 g/l) is presented in Fig. 3A and B. The corresponding initial rates of protein adsorption at the air/water interface are shown in Fig. 3C. The evolution of the surface tension as a function of time γ (t), induced by the different camel α-lactalbumin solutions was characterized by a rapid decrease of γ (t) in the first 500 s followed by a more progressive decrease until 3000 s (Fig. 3A and B). In all cases, the initial value of the surface tension measured was around 75 mN/m and was estimated to be close to the surface tension of pure water. At pH 6.5, thermal treatments at 70 or 90 °C

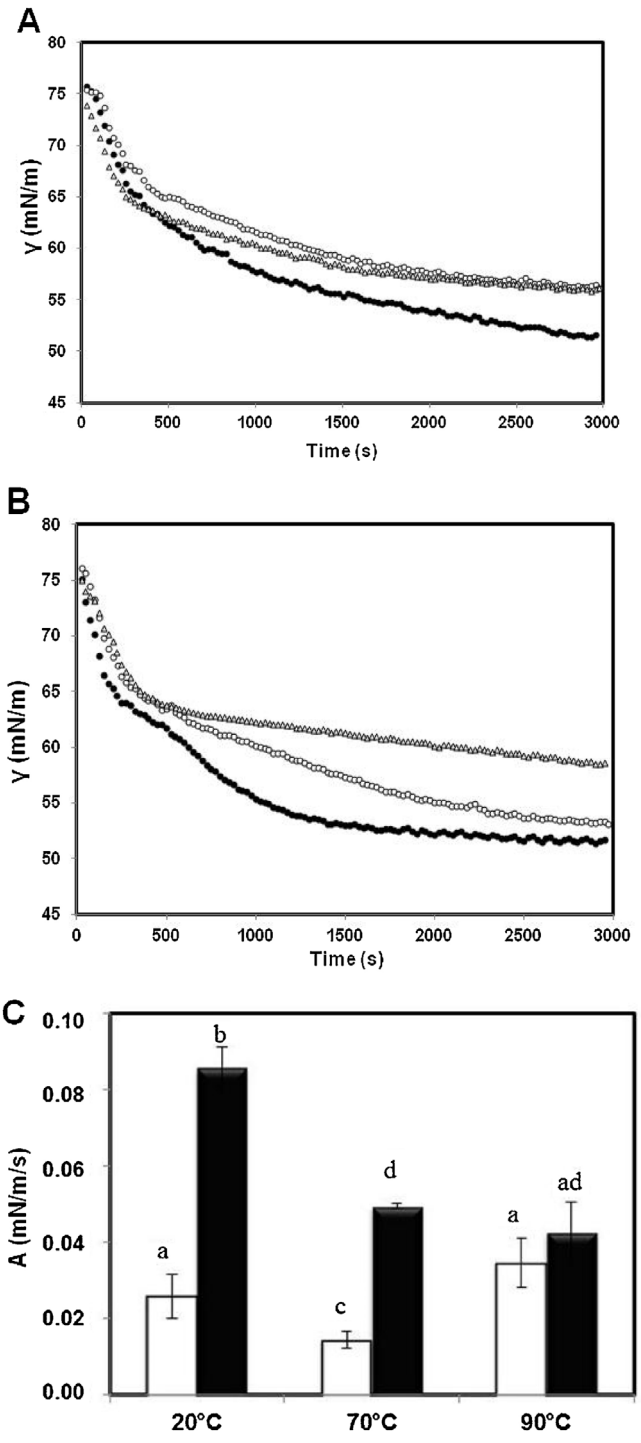

Fig. 3. Time-dependent changes in interfacial tension γ at air/water interface for 0.011 g/l native (●) and heated camel α-lactalbumin solution at 70 °C (○) and 90 °C (Δ) for 30 min at pH 6.5 (A) and pH 4.3 (B). (C) The adsorption rate of the 0.011 g/l camel α-lactalbumin (rate of initial decrease of the dynamic surface tension, $A = -d\gamma/dt|_{t=0}$) is presented at pH 6.5 (□) and pH 4.3 (■) for two different heat-treatments (70 °C and 90 °C for 30 min). The control was measured at 20 °C.

for 30 min have a small effect on the initial rate of absorption of the camel α-lactalbumin (Fig. 3C), but significantly decreased its capacity to lower the surface tension (Fig. 3A). Indeed, after 3000 s, the order of effectiveness was: native ($\gamma = 51.6 \pm 0.1$ mN/m) > heated at 70 °C ($\gamma = 56.2 \pm 0.2$ mN/m) = heated at 90 °C ($\gamma = 56.1 \pm 0.1$ mN/m) (Fig. 3A), although there was no significant difference between the camel α-lactalbumin heated at 70 or 90 °C.

The surface tension at the air/water interface of camel α-lactalbumin solution in previous studies (at a concentration of 0.01 g/l and pH 7) was found to be similar to that of bovine α-lactalbumin. It achieved a value of 58.8 mN/m within an hour starting from initial surface tension value around 68.1 mN/m [12]. We found that decreasing the pH of the native camel α-lactalbumin from 6.5 to 4.3 induced a significant increase of the initial rate of adsorption from 0.025 to 0.085 mN/m/s, respectively (Fig. 3C) but has no effect on the final interfacial activity since after 3000 s $\gamma = 51.6 \pm 0.1$ mN/m (Fig. 3A and B). Furthermore, at pH 4.3, thermal treatment induced a strong decrease of the initial adsorption rate from 0.085 mN/m/s for the unheated α-lactalbumin to 0.05 mN/m/s or 0.04 mN/m/s for 70 or 90 °C for 30 min respectively. The order of effectiveness at 3000 s was: native α-lactalbumin ($\gamma = 51.68 \pm 0.05$ mN/m) > heated at 70 °C ($\gamma = 53.3 \pm 0.2$ mN/m) > heated at 90 °C ($\gamma = 59.3 \pm 0.9$ mN/m) (Fig. 3B). Thus, at pH 4.3, the effect of heating temperature on the interfacial behavior of camel α-lactalbumin was more pronounced than at pH 6.5 (Fig. 3A and C). Furthermore, comparing all the different conditions, unheated camel α-lactalbumin at pH 4.3 had the best interfacial properties with the fastest lowering of surface tension. These findings are in agreement with the highest foam capacity observed with the same solution of α-lactalbumin (Fig. 2A) and with the results of Lam and Nickerson [9]. Indeed, these results reported that, regardless of treatment temperature, the surface tension for bovine α-lactalbumin was lower near its isoelectric point. The bovine protein had a lower net charge at the isoelectric point than at neutral pH. The lower charge allowed for better protein adsorption at the interface and it also enabled protein interactions to form a viscoelastic film.

The overall results presented in Fig. 3 also show that thermal treatment at pH 6.5 or 4.3 induces a modification of the interfacial properties of camel α-lactalbumin. Heat treatment at low pH also decreases the initial adsorption rate and/or increases the final surface tension. We also demonstrate that foams produced from heated camel α-lactalbumin solutions, at pH 6.5 or 4.3, are more stable than foams from unheated solutions (Fig. 2B). In the case of bovine milk, Mellema and Isenbart [22] reported that the combination of heating and acidification of whey proteins led to extensive aggregation. The resulting aggregates were less surface active and less competitive at the interface but could form a more stable interfacial film due to higher interactions between proteins [22].

3.4. Determination of ζ-potential and free thiol groups

Fig. 4 shows the changes in ζ-potential of camel α-lactalbumin as a function of pH and/or of thermal treatment temperature. The ζ-potential of native camel α-lactalbumin was approximately −16.8 mV at pH 6.5 and −11 mV at pH 4.3. Steinhauer et al. [24] have reported that the ζ-potential of bovine α-lactalbumin at pH > 5.3 was ∼−17 mV and this increased to 0 mV when the pH was reduced to 4.3, which is its isoelectric point. We found the camel α-lactalbumin had a negative charge at pH 4.3 (∼−11 mV) unlike the results of Steinhauer et al. [24]. This difference could be explained by a difference in the camel α-lactalbumin mineral environment and/or by the presence of charged impurities (presence of fraction F3).

At any given pH, the net charge of camel α-lactalbumin was not significantly modified after treatment at 70 °C for 30 min while

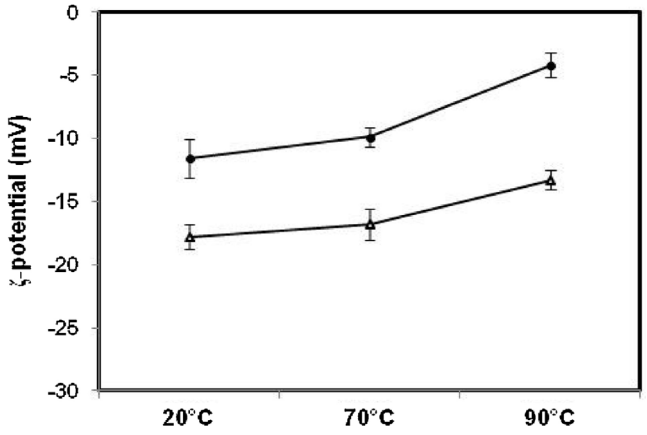

Fig. 4. ζ-potential of camel α-lactalbumin solution at a concentration of 1.7 g/l as a function of pH (pH 4.3 (●) and 6.5 (Δ)) and temperature treatments (70 °C and 90 °C for 30 min).

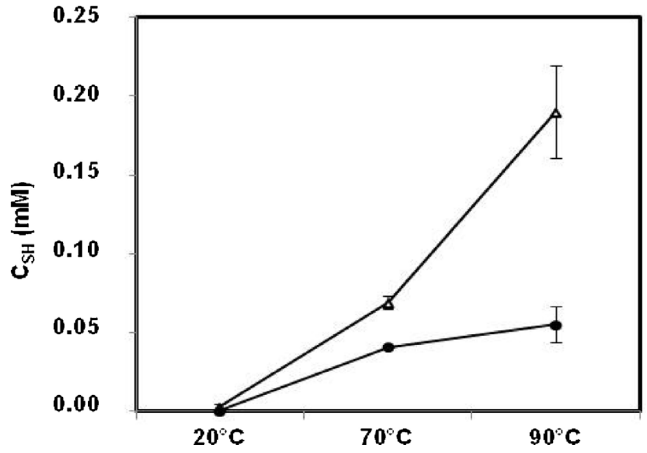

Fig. 5. Free thiol groups concentration (C_{SH}) of camel α-lactalbumin solution at a concentration of 1.7 g/l as a function of pH (pH 4.3 (●) and 6.5 (Δ)) and temperature treatments (70 °C and 90 °C for 30 min).

after a treatment at 90 °C it significantly increased up to ∼−13.3 mV or ∼−4.2 mV at pH 6.5 or 4.3, respectively. This could be explained by protein denaturation after a heat treatment, amplified by aggregation phenomenon at pH 4.3. Felfoul et al. [13] reported that the aggregation phenomena of camel proteins started after 30 min of heating at 70 °C and 90 °C.

We characterized the denaturation and aggregation rate of camel α-lactalbumin by determining reactive thiol groups concentration with DTNB. Fig. 5 compares the concentration of free thiol groups of camel α-lactalbumin as a function of pH and/or thermal treatment temperature. As expected, native camel α-lactalbumin did not contain any free thiol groups, at either pH (6.5 or 4.3) [25]. At pH 6.5, the concentration of –SH groups of the camel α-lactalbumin increased significantly ($p < 0.05$) after a thermal treatment at 70 or 90 °C for 30 min, to reach 0.06 mM or 0.19 mM, respectively. At pH 4.3, the concentration of free thiol groups also increased after the same thermal treatments, but to a lesser extent. The free thiol groups concentration measurements for camel α-lactalbumin, heated at 90 °C for 30 min at pH 6.5, are in reasonable agreement with the —SH groups concentration of 0.2 mM found by McGuffey et al. [26] after a treatment of the bovine α-lactalbumin at 95 °C. According to McGuffey et al. [26], this behavior is a consequence of the relationship between thiol reactivity of heated α-lactalbumin and the development of hydrophobic interactions between protein molecules. When thiol groups were reduced, hydrophobic inter-

actions induced the formation of large aggregates [25]. Thus, in the present study, large aggregates could be differently created in camel α-lactalbumin solution at pH 4.3 compared to pH 6.5. At pH 6.5 the concentration of thiol groups released increased with temperature. At neutral pH, electrostatic repulsive forces keep whey proteins from aggregating despite there being more hydrophobic parts at the surface at this pH, whereby the smaller aggregates provide enhanced stability in solution [9].

In the same way, Tcholakova et al. [27] reported that electrostatic repulsive forces and the presence of calcium-protein interactions prevented whey protein aggregation during heat treatments. In contrast, at pH value near isoelectric point, different factors such as the reduction of the electrostatic repulsion, the open structure and the loss of calcium ion could promote protein–protein interactions and the formation of large aggregates [9,20]. The phenomenon of denaturation of bovine α-lactalbumin after a heat treatment has been well studied [25,26,28]. At 63.7 °C, it has been shown that the α-lactalbumin was denatured but did not aggregate. However, at temperatures above 85 °C, disulphide bridges were broken and free thiol groups were exposed. Interaction occurred between the free SH groups of Cys^{111} which is the most active one [29]. Chaplin and Lyster [28] confirmed that the thermal denaturation of bovine α-lactalbumin is not irreversible. They found that when α-lactalbumin is heated at 77 °C and then cooled, the protein denaturation was reversible at 90%. However, at a higher temperature value of 95 °C (during 14 min) only 40% of the α-lactalbumin can recover its native state. The aggregation of α-lactalbumin depended mainly on temperature, protein concentration and pH value [28].

According to McGuffey et al. [25], heat treatment of bovine α-lactalbumin at 90 °C and neutral pH induced a significant increase in the aggregation phenomena, which is related to the reactivity of the cysteine residues Cys^{111} and Cys^{120} [29]. On the other hand, Bernal and Jelen [30] found that the calcium-loaded α-lactalbumin greatly enhances the heat stability of the protein in the pH range of 6.5. DSC results obtained by Atri et al. [7] also showed that holo state of the camel α-lactalbumin is more heat stable than the apo state. In their work on camel whey, Felfoul et al. [13] confirmed that the camel α-lactalbumin in the acid whey has a lower temperature of denaturation (60.5 °C) than in the sweet whey (73.8 °C). However, SDS-PAGE gels showed lower aggregates formation in the case of acid camel whey comparing to that of sweet one.

4. Conclusion

pH and temperature impact the foaming and interfacial properties of the camel α-lactalbumin purified from camel milk. We show here that the foamability of camel α-lactalbumin solution was maximal at 20 °C in acid condition, near its effective isoelectric point and this was confirmed by its high adsorption rate at the air-water interface.

At this acid pH, the protonation of the negative groups decreased the electrostatic repulsions of the protein and induced a partially denaturation with the release of its chelated calcium. This apo state enhanced the foaming properties of the protein.

At neutral pH, the foam capacity of the protein solution is significantly improved by heat treatment. An increase of temperature (i.e. either 70 °C or 90 °C for 30 min) at neutral pH affected the physicochemical properties of camel α-lactalbumin with an increase of thiol group concentration and a slight decrease of its negative ζ-potential. Furthermore, the foam created by camel α-lactalbumin was found to be unstable at 20 °C. After heat treatment, the stability of the foam greatly increased due to the presence of aggregated camel α-lactalbumin at the air-liquid interface. Thus, aggregates contributed to improve foam stability but slowed the adsorption of proteins and the creation of foam.

The camel α-lactalbumin is of great interest to create foam. Its aggregated state, obtained after heat treatment and enhanced in acid conditions, is needed to greatly improve the foam stability of camel α-lactalbumin solutions.

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