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The foaming properties of camel and bovine whey: The impact of pH and heat treatment

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ABSTRACT

The effect of heat treatment (70 °C or 90 °C for 30 min) on the foaming and interfacial properties of acid and sweet whey obtained from bovine and camel fresh milk was examined. The maximum foamability and foam stability were observed for acid whey when compared to sweet whey for both milks, with higher values for the camel whey. This behavior for acid whey was explained by the proximity of the pI of whey protein (4.9–5.2), where proteins were found to carry the lowest negative charge as confirmed by the zeta potential measurements. Interfacial properties of acid camel whey and acid bovine whey were preserved at air water interface even after a heat treatment at 90 °C. These results confirmed the pronounced foaming and interfacial properties of acid camel whey, even if acid and sweet bovine whey exhibited the highest viscoelastic modulus after heating.

1. Introduction

Camel milk plays an important role in human nutrition in the arid and semi-arid countries and its production has increased on a large commercial scale from modern camel farms (Alhaj et al., 2013). Its consumption is becoming more popular in many countries in Asia, Africa and Europe for its nutritive and therapeutic properties due to the presence of essential nutrients (protein, fat, lactose, minerals) and bioactive substances, such as immunoglobulins, lactoferrin, lysozyme, peptidoglycan recognition protein (PGRP) (El-Agamy, 2009; El-Agamy, Abou-Shloue, & Abdel-Kader, 1998; Kappeler, Heuberger, Farah, & Puhan, 2004; Merin et al., 2001). Another interesting specificity of the camel milk is the β-lactoglobulin deficiency in its whey, the soluble fraction of milk protein. Indeed, in bovine whey, the main components are the β-lactoglobulin (β-Lg), α-lactalbumin (α-La), serum albumin (SA) and the immunoglobulin, representing 55% (w/w), 24% (w/w), 15% (w/w) and 5% (w/w) respectively. In camel whey, the protein concentration is approximately the same as bovine's whey, around 6.3–8 g L^{-1} (Farag & Kebary, 1992), but the composition is different. α-La is the main component of camel whey and represents about 70% (w/w) of the proteins as observed for human milk and β-Lg is absent (Laleye, Jobe, & Wasesa, 2008; Merin et al., 2001; Omar, Harbourne, & Oruna-Concha, 2016).

The whey proteins play a key role in milk foam creation and stabilization, which is due to their interfacial properties closely dependent on their structure. Their structure greatly depends on both the chemical environment and applied heat treatment. Indeed, after a thermal treatment close to 60 °C, at neutral pH and low ionic strength, the β-Lg dissociates from its native dimer structure to native monomers. Its thermal denaturation occurred at a temperature of 78 °C enabling thiol/disulfide exchange reactions and thus, aggregates formation (Kazmierski & Corredig, 2003). For the α-La, a different heat denaturation behavior was observed as this monomeric metalloprotein has the lowest temperature of denaturation of the whey proteins (~64 °C) at neutral pH. At this temperature, it does not aggregate because of the lack of free thiol groups although it contains four buried disulfide bridges. The initiation of thiol/disulfur interchange starts at a temperature of 85 °C, mainly by the C_6–C_{120} thiol-disulfide exchange bond formation which is considered as the most reactive one (Doi, Tokuyama, Fong-Huang, Ibuki, & Kanamori, 1983; Livney, Verespej, & Dalgleish, 2003). Later, free thiol groups form intermolecular disulfide bonded aggregates (Chaplin & Lyster, 1986).

Thereby, denatured whey proteins at neutral pH may polymerize to form aggregates, depending on both the heating temperature and the ionic force. The resulting interfacial and foaming

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properties will therefore be a complex competitive adsorption between native proteins, aggregated and non-aggregated denatured proteins (Schmitt, Bovay, Rouvet, Shojaei-Rami, & Kolodziejczyk, 2007).

Foam properties of whey proteins were found to depend also on the pH value. Protein molecules change their surface charge and hence their flexibility with a decrease of pH. Consequently, both foaming and emulsifying properties of whey proteins are also altered. Many studies describe the foaming and emulsifying properties of bovine whey proteins. Tcholakova, Denkov, Ivanov, and Campbell (2006) have found that the β-Lg had better emulsifying and adsorption properties at acid pH than at pH 6.2, whereas, Lam and Nickerson (2015) have determined that the α-La achieved a better emulsion stability at pH 7 due to the further negative charge.

Few information exists on the foaming and interfacial properties of camel whey proteins even if foaming properties of bovine whey proteins have been intensively studied and reported (Lajnaf, Picart-Palmade, Attia, Marchesseau, & Ayadi, 2016; Laleye et al., 2008). Their interfacial properties have not been widely described in the literature but different behaviors were suggested for the camel whey, as the lack of the β-Lg would impact the resulting foaming properties after a thermal treatment.

Therefore, the aim of this study is to investigate the impact of heat treatments at different pH (acid and sweet camel whey) on the foaming and interfacial properties of camel whey, in comparison with bovine whey. The heat treatments chosen (70 °C and 90 °C for 30 min) were based on the work of Laleye et al. (2008) and Felfoul, Lopez, Gaucheron, Attia, and Ayadi (2015). These parameters correspond to different stages of denaturation of camel α-lactalbumin in order to increase our knowledge on its foaming properties. This would allow the valorization of the camel whey, by-product of the cheese industry, as an ingredient in food industry.

2. Material and methods

2.1. Bovine and camel whey separation

Fresh raw camel milk (*Camelus dromedarius*) was collected from 20 different healthy camels ranging between 2 and 12 months in lactation in local cattle located in the south of Tunisia (region of Medenine). Fresh bovine milk was a bulked milk supplied in a local farmer in the region of Sfax. Once arrived to the laboratory at 4 °C, pH values were measured (Metrohm pH meter). Then, both milks were skimmed by centrifugation at 1000*g* for 15 min at 4 °C (Kappeler, Ackermann, Farah, & Puhan, 1999).

Camel and bovine wheys were extracted after rennet coagulation at 37 °C for 1 h of fresh skimmed milks in the presence of 1.4 mL rennet enzyme per liter of milk (*M. miehei*, strength = 1:10,000, Laboratories Arrazi, Parachimic, Sfax, Tunisia). Sweet wheys of camel and cow milks were separated from rennet gels by centrifugation at 5000*g* for 20 min at 20 °C (centrifuge Beckman CO-LE80K) (Felfoul et al., 2015).

Acid wheys were obtained after acidification of fresh bovine and camel milks until pH 4.6 and 4.3 respectively using 1 M HCl, followed by centrifugation at 5000*g* for 20 min at 20 °C (centrifuge Beckman CO-LE80K) (Felfoul et al., 2015). Henceforth, the different wheys will be referred to as ABW (acid bovine whey), SBW (sweet bovine whey), ACW (acid camel whey) and SCW (sweet camel whey) respectively.

2.2. Quantitative and qualitative analyzes of wheys

The concentration of proteins from various wheys obtained from camel and bovine milks respectively (ABW; SBW; ACW; SCW) was determined by the Micro BCA™ Protein Assay Kit (G-Biosciences, GenoTechnology, USA). The BCA reagent, 2,2′-biquinoline-4,4′-dicarboxylic acid also known as bicinchoninic acid (BCA) assay Kit was used according to the manufacturer's guiding instructions: 50 μL of diluted whey sample were added to one milliliter BCA working reagent, the mixture was held 120 min in the dark and the optical density was then measured at λ = 562 nm.

A bovine serum albumin (BSA) calibration curve was realized using 8 points of known BSA concentration up to 2 mg mL^{-1}.

Major whey proteins were separated and quantified by SDS-PAGE technique (with a concentration of acrylamide gel of 15%) as described by Laemmli (1970) using a Bio-Rad apparatus (Mini Protean Tetra Cell, BioRad laboratories, USA). Quantitative determination of the protein content was performed using the Gel-Quant.NET software provided by biochemlabsolutions.com.

2.3. Heat treatment

Camel and bovine whey solutions were heated in bottles at 70 °C and 90 °C for 30 min using water bath.

Heat treatment temperatures were chosen according to Laleye et al. (2008) and Felfoul et al. (2015) as mentioned previously. Heating up time was also chosen according to previous works (Lam & Nickerson, 2015). Thermal denaturation was stopped by a rapid decrease in the temperature after ice incubation during 15 min to make sure there is no further denaturation of whey proteins. The control was at 20 °C, corresponding to the native conditions.

2.4. Foam studies

Ten milliliters of whey solution at a protein concentration of 5 g L^{-1} were introduced in a measuring cylinder (radius 2 cm and length 8.5 cm). The content was mixed using the Ultra Turrax T25 mixer (IKA Labortechnik, Staufen Germany) at a speed of 13,500 rpm for 2 min at room temperature. The volume of the foam was directly read in the measuring cylinder as function of time (Lajnaf et al., 2016).

The foamability of whey was determined by foam capacity equation (FC) in %, which is defined as Eq. (1)

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

where V_{foam} is the volume of the created foam at t_0 and V_0 the initial whey volume before whipping (10 mL).

Foam stability (FS, min) can be defined as the foam half time or the time required for draining the half initially created foam ($t_{foam1/2}$) (Marinova et al., 2009).

2.5. Interfacial properties

Dynamic surface tension and viscoelastic modulus measurements were performed with a drop tensiometer (IT Concept, TECLIS, Longessaigne, France) as described by Cases, Rampini, and Cayot (2005). An air drop was formed with an extreme microsyringe into an optical glass curve containing protein solution. The axially symmetric shape of the drop was taken by a suitable camera, connected to a microcomputer. The computer calculated the surface tension according to the Laplace equation (Eq. (2)) applied to the profile of the drop.

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

where:

- x and z are the Cartesian coordinates at any point of the drop profile,
- θ is the angle of the tangent to the drop profile,
- b is the radius of curvature of the drop apex,

- c is the capillary constant, equal to g $\Delta\rho/\gamma$, where $\Delta\rho$ is the difference between the densities of the two phases, γ the surface tension and g the acceleration of gravity.

Besides, the control unit can record and plot the sinusoidal changes of γ as a function of time and can measure the surface viscoelastic modulus (ε) as defined by the Eq. (3):

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

where A is the area of the air drop.

The room temperature of the apparatus was adjusted to 20 ± 1 °C.

The measurement of the interfacial tension (γ) and the viscoelastic modules (ε) were realized for the diluted camel and bovine whey at a concentration of proteins of 11 mg L^{-1} using deionized water (Cases et al., 2005). Thus, at this concentration, all the area of the air bubble formed by the drop tensiometer was covered with proteins content in the glass curve.

2.6. Thiol groups concentration

To quantify free thiol groups, 50 μL of the extracted camel and bovine wheys was respectively mixed with 50 μL of DTNB (5,5′-dithio-bis (2-nitrobenzoic acid) 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 as described by Ellman (1959). The mixture was then incubated for 5 min at 37 °C and the optical density was measured at λ = 412 nm by UVmini-1240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

The free SH groups concentration was calculated by Eq. (4):

$$C_{SH}(M) = (DO_{412nm}/\varepsilon_{412}) \times (1000/50) \quad (4)$$

where DO_{412nm} is the absorbance at λ = 412 nm; ε_{412} is the DTNB extinction coefficient (13,600 M^{-1} cm^{-1}) at λ = 412 nm. 1000 μL is the total volume of the cuvette and 50 μL is the protein sample volume.

2.7. The ζ-potential measurements

The ζ-potential of the whey proteins was determined in triplicate at 20 ± 1 °C using the Zetasizer Nano-ZS90 (Malvern Instruments, Westborough, MA) at a concentration of 1 g L^{-1} (Magnusson & Nilsson, 2011). The ζ-potential (mV) was calculated from the electrophoretic mobility (U_E) using Henry's equation (Eq. (5)):

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

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

2.8. Statistics

All experiments and measurements were performed at least in triplicate and mentioned as the mean ± one standard deviation. A three-way analysis of variance (ANOVA) was used to test for significance in the main effects of the whey samples (ABW, SBW, ACW and SCW), and heat treatment conditions (70 and 90 °C for 30 min), along with their associated interactions on the foaming properties, tension measurements, viscoelastic modulus, thiol groups concentration and ζ-potential of whey proteins. Statistical analyses were performed with the software: IBM SPSS statistics (Version 19, IBM SPSS, USA).

3. Results and discussion

3.1. Quantitative and qualitative analyzes of camel and bovine wheys

The analysis of the protein concentration of the different extracted wheys indicated that the protein content in ABW, SBW, ACW and SCW samples were 8.08 ± 0.19; 9.74 ± 0.89; 6.03 ± 0.06 and 7.01 ± 0.06 g L^{-1} of whey respectively. The protein content in sweet whey is higher than that in acid whey probably due to the presence of caseino/glycomacropeptide in the sweet whey after rennet coagulation. Camel and bovine whey protein fractions were characterized by SDS-PAGE (Fig. 1). Densitometry analysis of the gel showed that, in lanes 1 and 2, corresponding to ABW and SBW respectively, four major protein bands (150 kDa, 66 kDa, 18 kDa and 14 kDa) were identified as Immunoglobulins (1%), BSA (3%), β-Lg (72%) and α-La (24%).

For the camel whey (lanes 3 and 4 corresponding to ACW and SCW), three major proteins bands with molecular weight (MW) of 80 kDa, 66 kDa and 14 kDa were identified, corresponding to lactoferrin (4.1%), SA (7.8%) and α-La (72.8%) respectively. No band

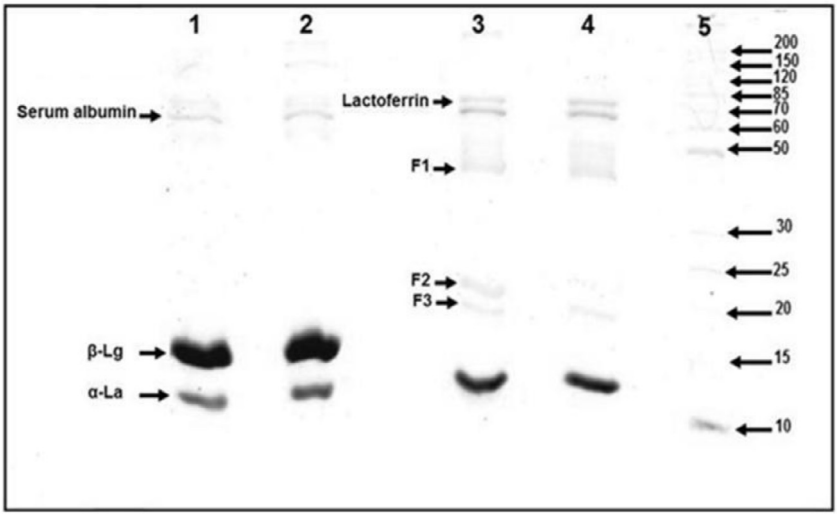

Fig. 1. SDS-PAGE patterns of bovine and camel whey proteins: lane 1, ABW; lane 2 SBW; lane 3, ACW; lane 4, SCW; lane 5, standard milk proteins MW in kDa; β-Lg = β-lactoglobulin, α-La = α-lactoglobulin, F = fraction.

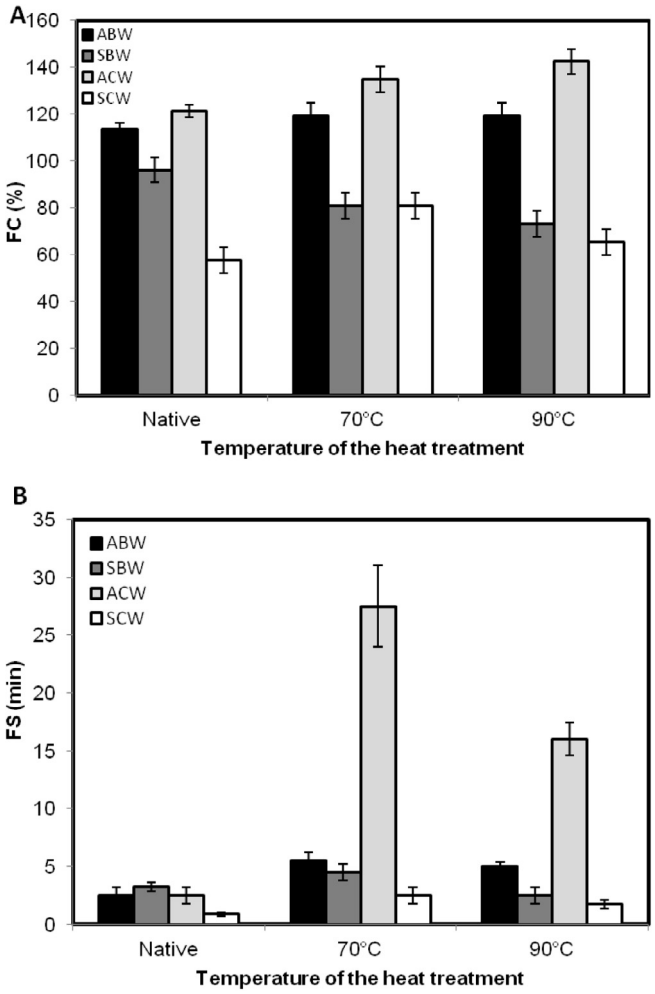

Fig. 2. Foam capacity: FC (A) and foam stability: FS (B) of camel and bovine wheys; at a concentration of 5 g L^{-1} as function of the temperature of the heat treatment (70 °C and 90 °C) for 30 min.

corresponding to β-Lg was detected, as already described by previous authors (El-Hatmi, Girardet, Gaillard, Yahyaoui, & Attia, 2007; Ereifej, Alu'datt, Alkhalidy, Alli, & Rababah, 2011; Omar et al., 2016).

These results are in agreement with Omar et al. (2016) who found that α-La had the greatest concentration in camel whey. For the lactoferrin, identified with a MW of 80 kDa, Ereifej, Alu'datt, Alkhalidy, Alli, and Rababah (2011) have found concentration in different camel milks that ranged between non-detectable amount and amount of 4.3% (w/w) of total camel protein. Camel whey also contains three protein fractions with MW of 48 kDa (Fraction 1), 22 kDa (Fraction 2) and 19 kDa (Fraction 3), which represent 3.3%, 5.7% and 2.2% of the total amount of whey proteins, respectively. These whey fractions are specific components of camel milk and not comparable to any bovine protein. Therefore, the protein fraction F3 is suggested to be identified as the PGRP (peptidoglycan recognition protein) whose MW is 19.1 kDa (Kappeler et al., 2004; Alhaj & Al Kanhal, 2010; Ereifej, Alu'datt, Alkhalidy, Alli, & Rababah, 2011).

3.2. Foam studies

Fig. 2A and B shows respectively the average values of the foam capacity (FC) and foam stability (FS) of camel and bovine wheys studied at a protein concentration of 5 g L^{-1}. A same whey protein concentration was chosen for the four samples in order to compare the protein properties under native conditions and after different heat treatments (70 °C or 90 °C for 30 min). Fig. 2A shows that acid whey gives better foam than sweet whey (whatever camel or bovine milk) and the maximum foam volume was achieved with ACW followed by ABW regardless of heating temperature. Indeed, after a heat treatment of 90 °C for 30 min, FC value of ACW and ABW reached 142% and 119% respectively (Fig. 2A).

For the sweet whey, statistical analysis showed that heating reduced significantly the foamability ($p < 0.05$) in comparison to native whey with a better foaming achieved with the SBW, but no significant differences in the FC could be found between SBW and SCW after heat treatments applied at 70 °C and 90 °C.

These results are in agreement with those supported by Zhang, Dalgleish, and Goff (2004) carried out with whey protein isolate. Thus, the high foam of whey proteins at acid pH is due to the reduced electrostatic repulsion of proteins at a pH value close to their isoelectric point (pI) ($pI_{\alpha\text{-La}} = 4.1–4.8$; $pI_{\beta\text{-Lg}} = 5.2$). The overall foamability was found to be higher in ACW than the bovine counterpart (ABW) in spite of the proximity isoelectric point of both whey proteins. This is partly caused by the lack of the β-Lg and the dominance of the α-La in camel whey. Thus, in bovine whey for acid pH between 3 and 5, β-Lg forms aggregates with α-La which may have antifoaming effect (Marinova et al., 2009; Zhang et al., 2004).

Besides, ACW has better foamed properties due to the molten globular state of α-La. Indeed, at pH <5, α-La becomes more surface active by losing its bound calcium ions and taking the molten globular state (Zhang et al., 2004). When the α-La carried less negative charges, its conformation allows better adsorption at the interface and higher protein-protein interactions to form a viscoelastic film (Lam & Nickerson, 2015). The high proportion of α-La (>70%) in acid camel whey can explain the high foamability of ACW compared to ABW.

The foaming stability (FS) values of camel and bovine wheys in response to thermal treatment are given in Fig. 2B. Under native conditions, there is no significant difference for the stability of foams made with camel and bovine wheys (FS <5 min). Stability of foams greatly increased ($p < 0.05$) for ACW after heating at 70 °C for 30 min and achieved maximum FS values in these conditions (FS ~28 min). At 90 °C, acid bovine and camel whey proteins improved their ability to adsorb at air/water interface and stabilized air bubbles better than in sweet one.

These results are in agreement with those of Zhang et al. (2004) who reported that better foam stability was achieved with both α-La and β-Lg at pH close to pI due to the further reduction of electrostatic repulsion between proteins. Whey proteins remain soluble at low pH (acidic) despite the lowest negative charge (Anandharamakrishnan, Rielly, & Stapley, 2008). Thus, Marinova et al. (2009) have found that whey proteins adsorbed layers at air water interface are denser with minimal electrostatic repulsion between molecules at pH value between 4 and 4.5. Consequently, foams created by whey proteins at acid pH are more stable than that at neutral pH.

Mellema and Isenbart (2004) reported that the heat treatment (85 °C for 20 min) of pre-acidified whey protein solutions improved the emulsion stability due to the creation of extensive aggregation. The denaturation temperature of the major three bovine proteins in their pure forms have been reported as 81 °C for β-Lg, 74 °C for serum albumin SA and 61 °C for α-La respectively (Bernal & Jelen, 1985). For camel whey, the camel α-La was found to denature at 71.7 °C (Atri et al., 2010). The denaturation temperature values of the individual camel and bovine proteins can explain the increase in foam stability when whey was preheated at 70 °C for 30 min. Moreover, Tosi, Canna, Lucero, and Ré (2007) have found that heat treatment could greatly improve

the foam stability (2–4 time more stable) of heated whey proteins when compared to the reference untreated whey. However, temperature must not be higher than 85 °C to avoid an excessive denaturation effect which can reduce foaming properties of whey.

3.3. Interfacial studies

Many authors have reported that the dynamic surface tension is the main determining factor which is directly associated to the foaming properties of proteins. Overall, a rapid decrease in the surface tension indicates a fast protein adsorption and stabilization of air bubbles against coalescence (Marinova et al., 2009).

The impact of heat treatment (70 °C and 90 °C for 30 min) on bovine and camel wheys respectively has been studied through the evolution of the surface tension γ (t) as function of time (Fig. 3). Concentration of 11 mg L^{-1} has been chosen as recommended by previous work (Cases et al., 2005). All surface tension curves start from the initial value of 75 mN m^{-1}, which is estimated to be the surface tension of pure water. In native conditions, the decrease in the surface tension observed for ABW to achieve the final value of $\gamma = 51.1 \pm 0.6$ mN m^{-1} after 3000 s, was significantly more rapid and effective if compared to SBW (final value of $\gamma = 57.1 \pm 0.5$ mN m^{-1}). This result confirmed that bovine acid wheys are more efficient to reduce the tension at air water interface than the sweet one in agreement with our previous results of foaming properties. For camel wheys, ACW was found to be more efficient to reduce the surface tension for the first 800 s, followed by a non significant difference between ACW and SCW samples to achieve the final surface tension value of $\gamma \sim 51.5$ mN m^{-1}.

As it was reported in previous works, the interfacial behavior of bovine whey at neutral pH is due to the presence of the both proteins: β-Lg and α-La (Zhang et al., 2004).

The serum albumin (SA) has lower interfacial properties due to its low concentration in whey and its high MW (66 kDa) (Suttiprasit, Krisdhasima, & McGuire, 1992). For the camel whey, the lack of β-Lg suggested that α-La is the key protein involved in the protective protein layer created at the air water interface (Laleye et al., 2008; Marinova et al., 2009). Thus, the other proteins (SA, lactoferrin and fractions F) have been suggested to have a minor role in the creation and stabilization of foams.

For both camel and bovine wheys, the behavior of the proteins at the air water interface is a consequence of the change of their conformation, tertiary and quaternary structure, to states with less regular structures (Cases et al., 2005). Indeed, proteins have the best foam properties at pH levels where their are less compact and more flexible (Zhang et al., 2004).

The difference of interfacial properties between the sweet bovine and camel whey (SBW and SCW), and acid and sweet whey for both mammal milks (SCW and ACW or SBW and ABW) can be explained by the difference in protein composition and their different conformations at acidic pH. Suttiprasit et al. (1992) have found that at neutral pH, α-La is more efficient to reduce the surface tension than the β-Lg molecule, as it is smaller and more flexible. However, at acidic pH, Zhang et al. (2004) observed that β-Lg is more rigid and less competitive at the interface than α-La present in its molten globular state at low pH. Consequently, acid whey reduces the surface tension at the air water interface better than sweet whey. However in both ACW and SCW, the entire interface is suggested to be mainly covered by the α-La regardless of pH value. The efficiency of the camel whey to reduce the surface tension depends on the tensioactivity of the main protein, the camel α-La, greatly dependent of the pH value and the presence/absence of calcium fixation.

Fig. 3B and C shows that thermal treatments at 70 °C or 90 °C for 30 min have a considerable effect on the surface tension for both acid and sweet wheys. After a heat treatment of 70 °C for

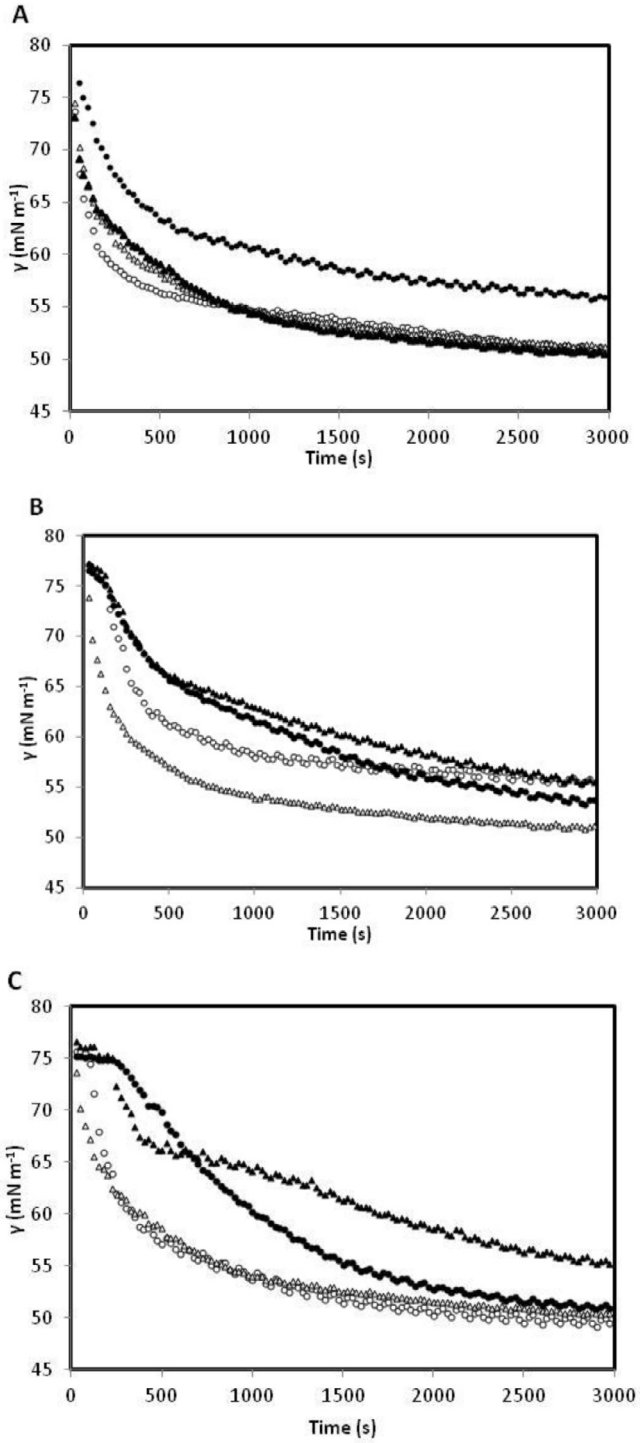

Fig. 3. Time-dependent changes in interfacial tension γ (mN m^{-1}) at air/water interface for 0.011 g L^{-1} of camel and bovine whey solution in native conditions (A), after a heat treatment of 70 °C (B) and 90 °C (C) for 30 min (ABW ○ SBW ● ACW Δ and SCW ▲).

30 min, only ACW has preserved its tensioactive properties at air water interface, with a final tension value of $\gamma = 51.05 \pm 0.12$ mN m^{-1} in agreement with previous foaming results (Section 3.2 Foam studies). At 90 °C, acid whey has retained the best interfacial properties compared to the sweet one especially during the first 1000 s. The order of effectiveness presented in Fig. 3C was: ACW ($\gamma = 54.3 \pm 0.4$ mN m^{-1}) and ABW ($\gamma = 55.2 \pm 0.3$ mN m^{-1}) > SBW ($\gamma = 58.3 \pm 0.3$ mN m^{-1}) > SCW

(γ = 64.6 ± 0.5 mN m^{-1}). It can also be concluded that acid whey preserved the flexibility and the interfacial properties even at 90 °C. However for sweet whey, the tensioactive properties of proteins were reduced after heat treatment at 70 °C and 90 °C for 30 min. The difference in behavior of wheys protein at air water interface is not only due to the pH of the whey but also to the denaturation ability of the soluble proteins after the heat treatment.

For the case of bovine whey, regardless of its pH value, heat treatment induced aggregation between the β-Lg and α-La. In acid whey, according to Havea, Singh, and Creamer (2002), aggregates were found to be soluble and stabilized by disulfide bridges. Whereas, for sweet whey, heat treatment induced the creation of large aggregates associated by non-covalent bonds such as hydrophobic and ionic interactions.

Felfoul et al. (2015) have also shown by SDS PAGE gels that when SCW has been heated at 90 °C, CSA and α-La bands disappeared. Therefore, for ACW, two other bands were created whose MW are 20 and 30 kDa respectively, but in very low concentration. These bands could correspond to specific camel whey proteins or caseins traces. Different behavior of bovine wheys were observed after heating at 90 °C for 30 min at acid pH as both β-Lg and α-La bands disappeared, suggesting proteins denaturation and aggregation. So, in acid wheys, protein aggregation under heat treatment was promoted but aggregates were still surface active and achieve lower surface tension value.

The viscoelastic modulus was also used to examine the impact of the heating treatment (70 and 90 °C for 30 min) on the rheological properties of camel and bovine whey at air water interface (Fig. 4). As the surface tension γ (t) reflects the flexibility and the tensioactivity of the molecule, the viscoelastic modulus is found to reflect the rigidity of the film created by proteins located at the interface (Cases et al., 2005). Fig. 4A showed the variation of the viscoelastic modulus of bovine and camel wheys as a function of time in native conditions, respectively. Depending on the nature of the protein at the interface, their structure (denatured or not) and the pH value, the magnitude of ε(t) values varied significantly. The camel whey, regardless of pH value, led rapidly (t = 300 s) to the final ε value (ε = 20 ± 0.1 mN m^{-1}). This result was in accordance with Laleye et al. (2008) which reported that in camel whey, the predominant protein at the interface at acid or neutral pH value was the α-La. However for bovine whey, the viscoelastic modulus depended on the pH value of the whey. After t = 3000 s, the order of effectiveness was SBW (ε = 45 ± 0.5 mN m^{-1}) > ABW (ε = 23 ± 1.2 mN m^{-1}). Zhang et al. (2004) reported that there is preferential adsorption of α-La over β-Lg in the foam phase at acid pH values. Hence α-La is dominant at the air water interface for acid pH whereas β-Lg covers rapidly the interface at neutral pH. These differences in the protein film at the interface explain the difference of the viscoelastic modulus.

Fig. 4B and C show that the magnitude and the general shape of ε(t) curves of wheys samples were affected by the temperature of heating treatment, depending on the pH value and the whey composition. For bovine whey, no significant effect was observed for the sweet whey (SBW). It exhibited at all temperature values the highest viscoelastic modulus whereas the heat treatment has affected the rigidity of the film created in acid condition (ABW): the viscoelastic modulus of ACW rose significantly after t = 3000 s, from ε = 30 ± 5.1 mN m^{-1} at 70 °C to ε = 43 ± 2.1 mN m^{-1} at 90 °C. In support of these results, Mellema and Isenbart (2004) have found that the heat treatment (85 °C/20 min) of a pre-acidified bovine whey protein concentrate solution (pH 4.6) resulted in high modulus value (ε = 60 mN m^{-1}). This high modulus is caused by the high sensibility of whey proteins due to extensive aggregation and denaturation.

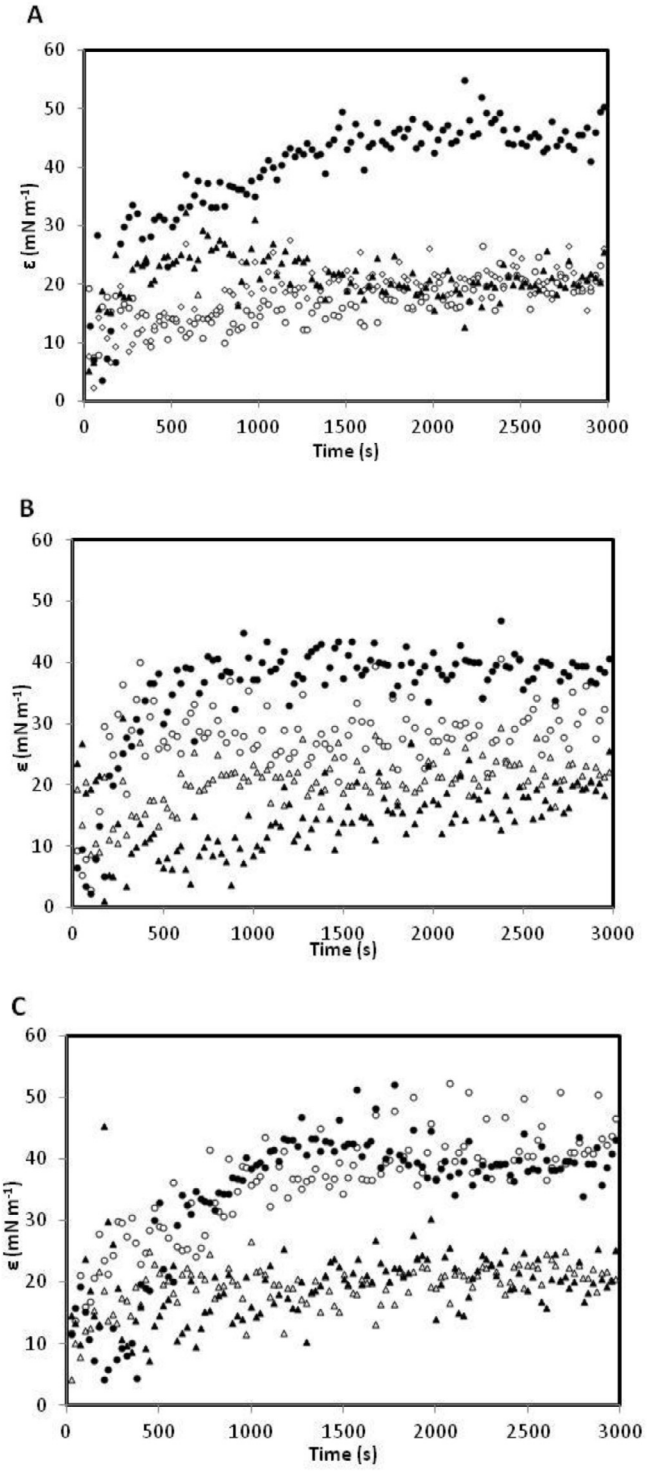

Fig. 4. Time-dependent changes in viscoelastic modulus ε (mN m^{-1}) at air/water interface for 0.011 g L^{-1} of camel and bovine whey solution in native conditions (A), after a heat treatment of 70 °C (B) and 90 °C (C) for 30 min (ABW ○ SBW ● ACW △ and SCW ▲).

Overall, as compared to camel whey, bovine whey exhibited a high viscoelastic modulus values especially after heat treatment suggesting that the protein adsorbed layer in mainly formed by β-Lg. In agreement with Cases et al. (2005), denatured β-Lg exhibits a high viscoelastic character in protein adsorbed layers which can be attributed to a high packing density and strong protein–protein connections. The unfolding of the β-Lg allows an exposure of free

thiol groups. Consequently, this protein polymerizes in the adsorbed layer due to the occurring interchange between thiol and disulfide groups.

A different behavior was observed for acid and sweet camel wheys (ACW and SCW). Both of them were found to give low modulus values at t = 3000 s, regardless of heating temperature ($\varepsilon = 21 \pm 1.3$ mN m^{-1}). Seta, Baldino, Gabriele, Lupi, and Cindio (2014) have reported that lower viscoelastic modulus at interface are given by proteins characterized by a lower MW and a greater flexibility due to a rapid interface recovery at short and long times. So, it can be suggested that the camel α-La is characterized not only by a high flexibility but also a greater thermostability in accordance with Atri et al. (2010). The absence of β-Lg in this serum reduces the rigidity properties of the protein film created at the air water interface.

3.4. Determination of ζ-potential

Fig. 5 shows the changes in ζ potential of bovine and camel wheys (acid and sweet) as a function of thermal treatment temperature (70 °C and 90 °C for 30 min). In the native conditions, the ζ potential values of acid wheys (pH 4.6 and 4.3 for ABW and ACW respectively) were significantly lower ($p < 0.05$) than that of sweet wheys (pH 6.7 and 6.5 for SBW and SCW respectively). The ζ-potential were ∼−11.4 ± 0.2 mV; −23.3 ± 0.4 mV; −9.9 ± 0.4 mV and −21.3 ± 0.9 mV for ABW, SBW, ACW and SCW respectively. Camel wheys carried lower negative charge compared to their bovine counterparts in agreement with the results of Hinz, O'Connor, Huppertz, Ross, and Kelly (2012). The difference in protein composition and the presence of highly basic protein as lactoferrin (pI = 8.8) in camel whey explain the less negatively charge of the camel whey proteins (Teo et al., 2016). The temperature has not a great impact on the charge except on the sweet bovine whey proteins that became greatly negatively charged (−30.2 mV) after a heat treatment of 90 °C for 30 min. The denaturation of the different whey proteins may play a role in this change of negative charge. In acid conditions, ACW appeared less negatively charged after a heating at 90 °C for 30 min (ζ-potential ∼−6.5 ± 0.7 mV), mainly due to the behavior of the camel α-La in agreement with results of Lam and Nickerson (2015).

3.5. Determination of free thiol groups

The evolution of free thiol concentrations for camel and bovine whey proteins, in sweet and acid wheys under different temperatures for heat treatment (70 °C and 90 °C for 30 min) is shown in Fig. 6. Under native conditions, no significant difference was observed between whey regardless of the milk origin and pH. After heating, the free SH group concentration of bovine sweet and acid wheys raised significantly ($p < 0.05$) as function of temperature. Free SH group concentrations reached their maximum at 90 °C with values of 1.29 ± 0.06 mM and 1.18 ± 0.03 mM for ABW and SBW respectively. It could be explained by the whey proteins denaturation which begins at 70 °C for 30 min. For camel wheys, thiol group concentrations varied depending on both pH and temperature: in sweet whey (SCW), thiol group concentration (0.4 mM under native conditions) increased to 0.73 ± 0.02 mM at 70 °C and 1.63 ± 0.05 mM at 90 °C. In acidic conditions (ACW), no significant evolution of thiol group concentration was observed.

The heat aggregation phenomenon of the bovine whey proteins have been extensively studied and explained (De la Fuente, Singh, & Hemar, 2002; Havea et al., 2002). Firstly, β-Lg dimer dissociates in a monomeric form, which causes the exposure of the β-Lg monomers free –SH groups and the reactivity of the protein towards thiol/disulphide exchange reactions. So, β-Lg reacts with the α-La monomers via thiol/disulphide exchange reactions forming dimers, which can react with other whey molecules leading to the creation of aggregates. A similar behavior was observed for ABW and SBW, suggesting that pH doesn't have a significant impact on the heat denaturation phenomena for bovine whey.

In order to understand the heat denaturation phenomena of the camel whey, Felfoul et al. (2015) compared the thiol groups concentration of both camel and bovine wheys and reported that the whole proteins denaturation happened during 30 min of heating at 90 °C regardless of the milk origin with a higher thermal sensitivity for camel whey.

The different behavior of camel and bovine wheys could be explained by the whey protein composition and the denaturation temperature values of whey proteins. Felfoul et al. (2015) noted that the denaturation of bovine whey is maintained by the presence of the β-Lg whose denaturation temperatures were 79.6 °C and 83.4 °C in the sweet and the acid wheys respectively. On the other hand, these authors found that the denaturation temperatures of camel α-La were 73.5 °C and 60.5 °C in sweet and acid wheys respectively. Thus, for the ACW, the camel α-La is suggested to be already aggregated at 70 °C and 90 °C which explained the lowest thiol groups concentration (Lajnaf, Picart-Palmade, Attia, Marchesseau, & Ayadi, 2017). For SCW, the highest free thiol groups concentration may be due to the denaturing temperature of α-La (73.8 °C) and also to the presence of CSA (Camel Serum albumin) (91.9 °C). Indeed, the molecular structure of the CSA is characterized by the presence of 7 buried disulphide bridges.

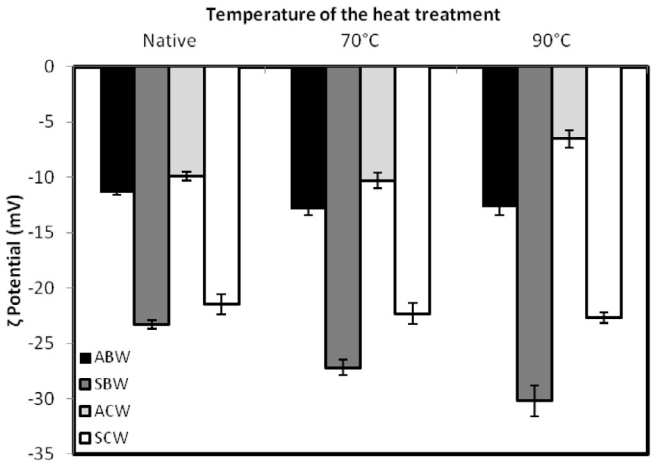

Fig. 5. ζ-potential of camel and bovine whey solution at a concentration of 1 g L^{-1} as a function of temperature treatments (70 °C and 90 °C for 30 min).

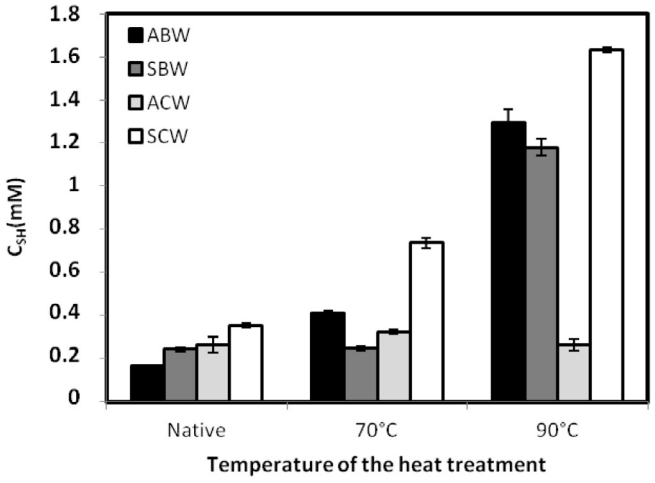

Fig. 6. Free thiol groups concentration (C_{SH}) of whey solution at initial concentrations as function temperature treatments (70 °C and 90 °C for 30 min).

The solubility of camel and bovine wheys samples was measured at a heat treatment of 70 °C and 90 °C for 30 min (data not shown). The obtained results showed that the solubility values decreased significantly with a heat treatment temperature (70 °C and 90 °C for 30 min) with a higher thermal sensitivity to acid wheys. Thus, bovine wheys were found to be more soluble regardless of heating temperature value in agreement with the results of Laleye et al. (2008). These authors found that the solubility of bovine whey proteins is higher than that of camel whey proteins due to the difference in the protein composition between bovine and camel wheys and the lack of the β-Lg in bovine whey. Furthermore, as reported by previous studies, the solubility of the wheys is mainly attributed to the pH value and the electronegative charge associated with whey proteins which may keep them from thermal aggregation (McClements, 2004; Laleye et al., 2008; Lam & Nickerson, 2015). These findings are in agreement with the ζ-potential results (Section 3.4), as sweet wheys carried the highest negative charge when compared to acid wheys. The ACW was found to have the lowest values of solubility, negative charge and thiol groups concentration compared to other wheys which suggests that the aggregation of camel whey proteins at acid pH was promoted after a heat treatment of 70 °C and 90 °C.

Therefore, the highest aggregation rate of ACW explains its behavior in stabilizing foams (Section 3.2). Thus, at the air-water interface, the proteins contributed to the creation of the foam, whereas the aggregates stabilized the interfacial film of proteins already formed.

4. Conclusion

Milk whey foaming properties depended on the pH value, the protein composition and their degree of denaturation after a heat treatment. Whey solutions had the best foamability closed of the isoelectric point of β-Lg and α-La (around pH 4–5), regardless to heating temperature. A heat treatment at 70 °C for 30 min improved the foaming properties of bovine and camel acid wheys, whereas, stability of foam greatly increase only for the acid camel whey. In all results, ACW present the best properties to create foam with the highest stability if compared to other whey, with an increase of these properties after a heat treatment. Drop tensiometer showed that ACW preserved its tensioactive properties at air water interface even after heating at 90 °C during 30 min. The same thiol groups concentration obtained after two heat treatments showed that heating didn't affect the denaturation of the camel α-La in ACW. However thiol group concentration rose significantly for the other whey (SBW, ABW and SBW). Besides, ACW carried out the lowest negative charge at all temperature values. These findings explained the foaming and interfacial behavior of this camel whey.

This behavior is mainly due to the lack of the β-Lg, the dominance of α-La in camel whey and its properties in acid conditions toward heat denaturation (low level of thiol groups). A different behavior was observed for the SCW. It exhibited the lowest foamability and foam stability for all heating temperature values. Interfacial properties of SCW were lost after heating whey at 70 and 90 °C due to protein denaturation.

These experiments confirm the interesting foaming properties of camel whey and highlight the importance of the protein composition and their state of denaturation in acidic or sweet conditions after a heat treatment.

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