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

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ABSTRACT

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

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

1. Introduction

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

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

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

Camels are well-known producers of milk which is used as main food resource for populations in the arid countries and hot regions of the world. Camel milk is a potential source of all the essential nutrients already found in cow milk. Besides, it is rich in iron, lactoferrin and vitamin C (Kappeler et al., 1999). Compared to the bovine whey, camel whey is devoid of the β-lg which has been considered as one of the most dominant bovine milk allergen limiting the use of this milk for the preparation of infant formulae (Uchida et al., 1996). Thus, the α-la is the major protein of camel soluble protein fraction, with a concentration of 2.2 g/L (Omar et al., 2016). As bovine α-la, camel α-la is a calcium-metalloprotein composed of 123 amino acid residues, with a molecular weight of 14.43 kDa (Beg et al., 1985). However, when compared to bovine α-la, camel one presents a different structure justified by a difference in the nature of 39 amino acids between these two different proteins. Thus, the percentage sequence similarity and identity between the sequences are 82.9% and 69.1%, respectively. Camel α-la has a considerably more hydrophobic core than its bovine

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counterpart at positions 25–35. The secondary structure of this protein is more preserved during heat treatment, so, the camel α-la is more thermostable than its bovine equivalent. However, its conformation is more sensitive to calcium loss (Atri et al., 2010).

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

Few studies describe the emulsifying behavior and interfacial properties of camel whey proteins (Laleye et al., 2008) even if for cow proteins, these properties were extensively studied and reported (Fachin and Viotto, 2005; Lam and Nickerson, 2015a; Jiang et al., 2018). Thus, the mechanism of emulsion creation of camel milk proteins has not been widely studied in the literature and the absence of the β-lg is suggested to have a significant impact on the resulting emulsifying properties at different pH values.

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

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

2. Materials and methods

2.1. Whey separation

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

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

Sweet wheys of bovine and camel milks were extracted from skimmed milks after an enzymatic coagulation at 37 °C for 1–2 h in the presence of microbial rennet enzyme (0.35 and 1.4 mL per liter of skimmed bovine and camel milks, respectively) (Parachimic, Laboratories Arrazi, Sfax, Tunisia, strength = 1:10,000). Whereas, acid wheys were isolated after the acidification of skimmed milks using a solution of HCl (1 M) until pH = 4.3 and 4.6 for camel and bovine milks, respectively (Felfoul et al., 2015).

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

2.2. Whey solution preparation

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

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

2.3. Emulsifying properties

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

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

The created emulsions were kept undisturbed for 10 min and then 100 mL aliquots were taken and dispersed into 900 μL of 0.1% SDS solution. The absorbance of the emulsion-SDS mixture was also measured at 500 nm as described above. Emulsifying activity index (EAI, m^2/g) and emulsion stability index (ESI, %) of whey solutions were calculated using Eq. (1) and Eq. (2) suggested by Pearce and Kinsella (1978):

$$EAI\ (m^2/g) = \frac{2 \times 2.303 \times A_{500} \times d}{C \times (1 - \Phi) \times 10^4} \quad (1)$$

$$ESI\ (\%) = \frac{A_{10}}{A_0} \times 100 \quad (2)$$

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

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

2.4. SDS-PAGE electrophoresis

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

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

2.5. Thiol groups content and denaturation rate

Free thiol groups were quantified as described by Ellman (1959). 300 μL of the extracted wheys at a concentration of 1 g/L were mixed with 50 μL of DTNB (5,5′-dithio-bis(2-nitrobenzoic acid) solution (2 mM DTNB, 50 mM sodium acetate (NaAc) in H_2O), 100 μL of Tris solution (1 M Tris, pH 8.0) and 550 μL of distilled water.

Mixture was incubated for 5 min at 37 °C using water bath. Then, the optical density (DO) was measured at λ = 412 nm.

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

$$CSH\ (M) = (DO_{412nm}/\varepsilon 412) \times (1000/300) \quad (3)$$

where DO_{412nm} is the absorbance at λ = 412 nm; ε_{412} is the DTNB

extinction coefficient (13,600 M^{-1}/cm) at λ = 412 nm 1000 μL is the cuvette volume and 300 μL is the protein sample volume.

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

$$DR(\%) = \frac{CSH(heated\ whey) - CSH(native\ whey)}{CSH(native\ whey)} \times 100 \quad (4)$$

2.6. ζ-potential measurements

The ζ-potential values of the extracted whey proteins were determined at 25 ± 1 °C using the Zetasizer Nano-ZS90 apparatus (Malvern Instruments, Westborough, MA) at a protein concentration of 0.5 g/L as suggested by Lam and Nickerson (2015b).

The ζ-potential value (mV) was measured using Henry's equation (Eq. (5)):

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

where: U_E is the electrophoretic mobility, ε the permittivity (Farad/m); k, the Debye length; f(kα) the function of the radius of particle (α, nm); η the viscosity of the dispersion (mPa s).

2.7. Determination of hydrophobicity

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

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

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

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

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

2.8. Interfacial tension

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

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

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

The interfacial tension was calculated by Eq. (7)

$$\gamma = \frac{Fmax}{4\pi R\beta} \quad (7)$$

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

2.9. Statistics

All experimental analyses and measurements in this work were performed in triplicate and mentioned as the mean value ± standard deviation.

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

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

3. Results and discussion

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

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

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

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

3.2. Emulsifying properties

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

Thus, it was found that sweet wheys, regardless of milk origin and heating temperature (70 and 90 °C for 30 min) coated the oil droplets better than acid wheys with higher EAI values of the sweet camel whey (2.2 and 1.9 m^2/g for sweet camel and bovine wheys, respectively under native conditions). Whereas, in acidic conditions, a better emulsification activity was achieved with the bovine whey when compared to its camel counterpart (1.7 and 1.3 m^2/g for both acid bovine and camel wheys, respectively). Statistical analyses showed that heating reduced significantly the EAI values ($p < 0.05$) for acid wheys probably due to the

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

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

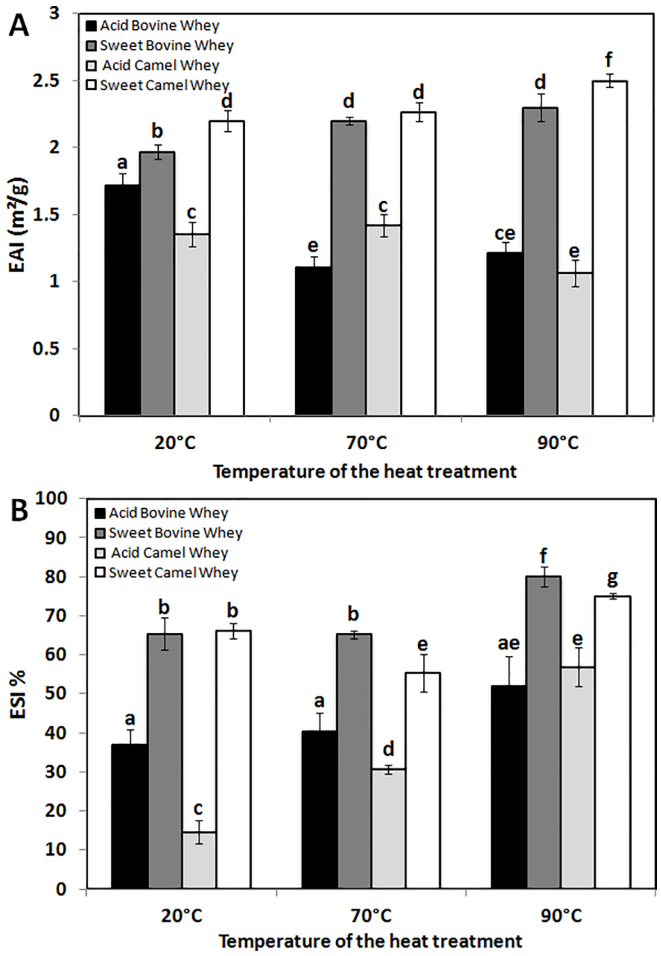

Fig. 1. Emulsifying Activity Index (EAI) (A) and Emulsion Stability Index (ESI) (B) of camel and bovine whey protein solutions, at a protein concentration of 5 g/L and as function temperature of the heat-treatment temperature (70 and 90 °C for 30 min). [a-g] Samples represented with different letters are significantly different from each other ($p < 0.05$). Error bars show the standard deviations of mean values of EAI and ESI.

extensive aggregation and denaturation of preheated whey proteins at acid pH values (Lam and Nickerson, 2015a).

The emulsification ability significantly increased for both sweet wheys after heating at 90 °C for 30 min ($p < 0.05$) and achieved maximum EAI values in these conditions (EAI ~ 2.5 min). These results are in agreement with those of Dissanayake and Vasiljevic (2009) who reported that the EAI values of whey proteins increased with thermal treatment and proteins denaturation. On the other hand, Fachin and Viotto (2005) found that the emulsifying properties were considerably improved after heating at pH 7. This improvement in emulsifying properties was justified by the greater denaturation of whey proteins in these conditions. Mellema and Isenbart (2004) reported that the heat treatment (85 °C for 20 min) of whey protein solutions at pH 6.7 improved the ability of proteins to adsorb at oil-water interface resulting in lower interfacial tension values, and clearly elevated values for the viscoelastic modulus as compared to the native whey solutions (without heating). On the other hand, Lajnaf et al. (2017) found that, the foamability and the interfacial properties of the purified camel α-la solution at neutral pH are significantly improved by heat treatment (i.e. 70 and 90 °C for 30 min).

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

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

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

Indeed, the magnitude of the electrical charge on the oil droplets surface decreased at pH values close to the pI of proteins. Consequently, the negative charge of acid wheys proteins is insufficient to generate electrostatic-repulsive forces between the created oil droplets leading to various attractive droplet-droplet interactions as van der Waals and the hydrophobic forces and then, droplets aggregate forming large flocculates (McClements, 2005).

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

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

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

A comparison between camel and bovine wheys-based emulsifier agents and commercial dairy emulsifiers which are whey protein isolate (WPI) and sodium caseinates (Na-cas) reveled that, for both dairy emulsifiers, EAI values were higher than those of camel and bovine

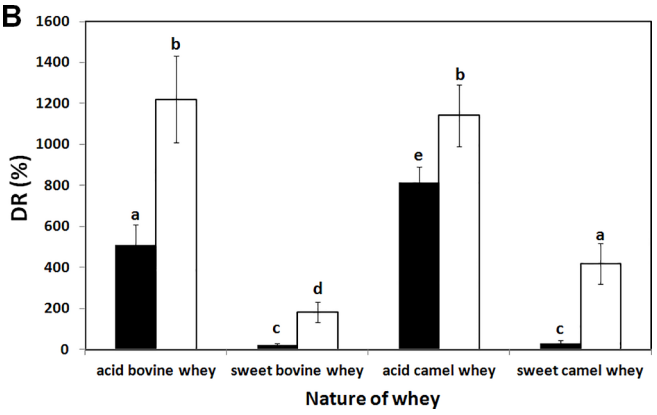

Fig. 2. Effect of temperature (70 (black bars) and 90 °C (white bars) for 30 min) on camel and bovine whey denaturation rate (DR). [a-d] Samples represented with different letters are significantly different from each other ($p < 0.05$). Error bars show the standard deviations of mean values of DR.

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

3.3. Protein denaturation and aggregation

3.3.1. Denaturation rate

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

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

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

Acid wheys carried higher DR values compared to their sweet counterparts regardless of heating temperature value. This result confirmed that acid wheys are characterized by a higher thermal sensitivity than the sweet wheys. Lam and Nickerson (2015a) reported that the size of whey proteins' aggregates is the greatest in acidic conditions due to the minimized electrostatic repulsion between neighboring proteins molecules leading them to interact and aggregate. Whereas, the high electronegative charge of sweet whey proteins may electrostatically keep them from aggregation after heating.

3.3.2. Electrophoresis patterns

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

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

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

When acid camel whey was heated at (70 or 90 °C), α-la and protein fractions (F1 and F2) bands appeared to increase in intensity (Fig. 3C).

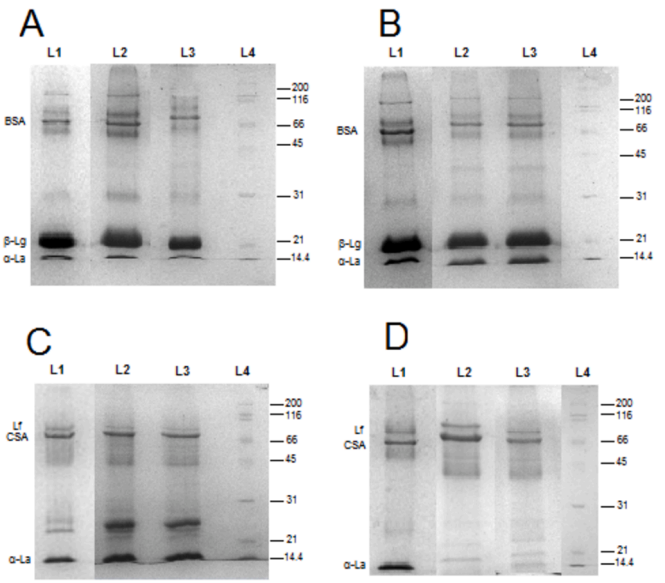

Fig. 3. SDS-PAGE electrophoresis patterns of acid bovine whey (A), sweet bovine whey (B) acid camel whey (C) and sweet camel whey (D) proteins heated at 70 and 90 °C for 30 min. L1: native whey, L2: heated whey (70 °C for 30 min), and L3: heated whey (90 °C for 30 min). L4 represents molecular mass markers; CSA = camel serum albumins, BSA = bovine serum albumin, Lf = lactoferrin, F = fraction, α-La = α-lactalbumin, β-Lg = β-lactoglobulin.

For raw sweet camel whey (Fig. 3D), the thermal treatment at 70 °C (L2) and 90 °C (L3) for 30 min induced an immediate disappearance of the α-la and the appearance of several faint distinct bands, which were probably intermediate protein species (e.g., α-la dimers, trimers) that were formed during heating.

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

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

3.4. Surface characteristics

3.4.1. Surface hydrophobicity

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

The BPB-bound amounts were 58.4 ± 7.1 μg/mL and 5.5 ± 1.1 μg/mL for acid and sweet bovine wheys, respectively, and 98.9 ± 7 μg/mL and 11.5 ± 4 μg/mL for acid and sweet camel wheys, respectively.

Acid wheys carried higher BPB-bound amounts compared to sweet wheys and the maximum hydrophobicity values were achieved with the acid wheys regardless of the temperature of the thermal treatment. Indeed, after heating at 90 °C for 30 min, BPB-bound amounts of acid

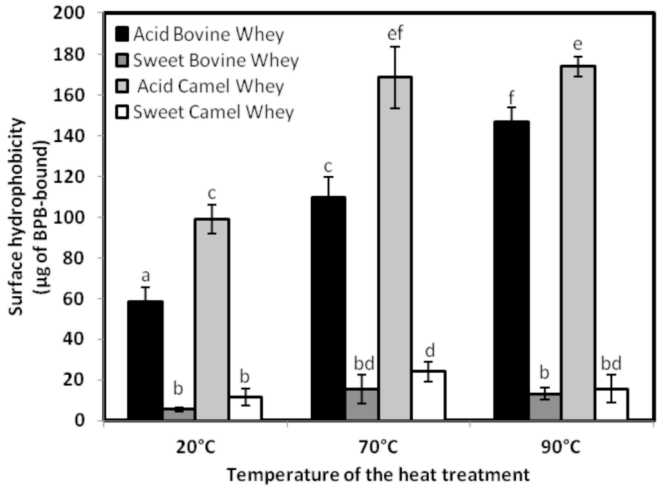

Fig. 4. Surface hydrophobicity (µg of BPB-bound) of camel and bovine whey protein solutions, at a protein concentration of 1 g/L and as function temperature of the heat-treatment temperature (70 and 90 °C for 30 min). [a-f] Samples represented with different letters are significantly different from each other ($p < 0.05$). Error bars show the standard deviations of mean values of Surface hydrophobicity.

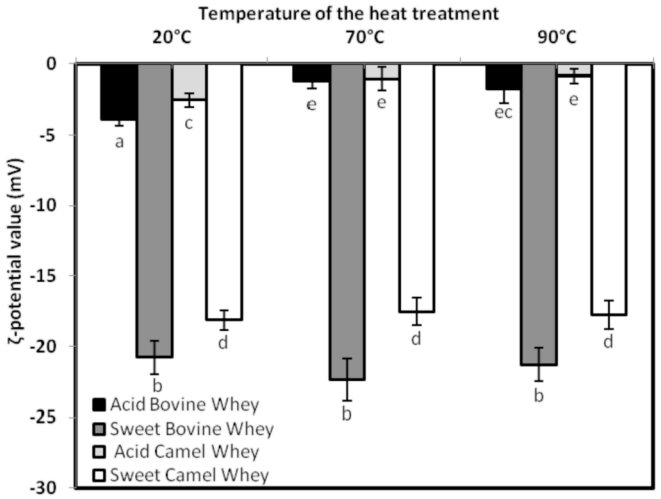

Fig. 5. ζ-Potential measurements (mV) of camel and bovine whey protein solutions, at a protein concentration of 0.5 g/L and as function temperature of the heat-treatment temperature (70 and 90 °C for 30 min). [a-e] Samples represented with different letters are significantly different from each other ($p < 0.05$). Error bars show the standard deviations of mean values of ζ-potential.

camel and bovine wheys reached 174 and 147 µg/mL of whey, respectively.

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

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

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

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

3.4.2. *Determination of ζ-potential*

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

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

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

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

3.4.3. *Interfacial properties*

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

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

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

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

After a heat-treatment of 90 °C for 30 min, acid wheys have retained

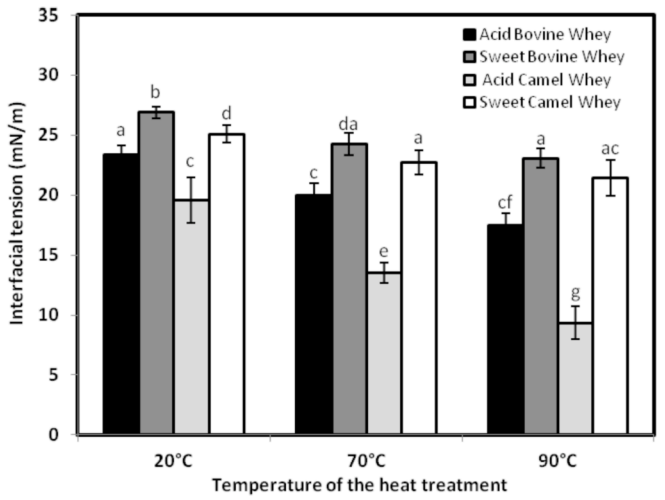

Fig. 6. The interfacial tension (mN/m) of camel and bovine whey protein solutions, at a concentration of 1 g/L and as function temperature of the heat-treatment temperature (70 and 90 °C for 30 min). [a-g] Samples represented with different letters are significantly different from each other ($p < 0.05$). Error bars show the standard deviations of mean values of interfacial tension.

the best interfacial properties when compared to the sweet wheys. Thus, the order of effectiveness at 90 °C was: acid camel whey ($\gamma = 9.35 \pm 1.34$ mN/m) > acid bovine whey ($\gamma = 17.5 \pm 1.0$ mN/m) > sweet camel whey ($\gamma = 21.45 \pm 1.5$ mN/m) and sweet bovine whey ($\gamma = 23.05 \pm 0.8$ mN/m).

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

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

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

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

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

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

4. Conclusion

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

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