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Camel α−lactalbumin at the oil−water interface: Effect of protein concentration and pH change on surface characteristics and emulsifying properties

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ABSTRACT

Camel α−lactalbumin (Ala−C), the main whey protein of camel milk, was purified by membrane filtration. Surface hydrophobicity as well as interfacial tension were examined at different levels of pH (3.0, 6.0, 9.0) and protein concentration (0.1 %, 0.2 %, 0.4 % w/w), and compared to bovine α−lactalbumin (Ala−B). The emulsifying properties (EAI and ESI) of oil-in-water emulsions (20 %/80 %) were investigated for both proteins. The stability of the processed emulsions was characterised by ζ−potential, particle size and viscosity measurements.

The main findings indicate that Ala−C exhibited greater surface hydrophobicity and undergone changes in conformational structure when pH decreased from 9.0–3.0. These changes were enhanced by increasing protein concentration from 0.1 % to 0.4 % (w/w). However, high concentrations showed low emulsifying activity, especially at pH 6.0 where interfacial tension was lower. In comparison with Ala−B, maximum EAI was close, despite the lower surface hydrophobicity of Ala−C under similar conditions.

Overall, emulsions were more viscous at pH 3.0 due to the greater surface coverage than at 9.0 and 6.0. Under the conditions of this study, a protein concentration of 0.2 % resulted in the finest oil droplets and highest viscosity for both types of α−lactalbumin, and Ala−C conferred the highest long-term stability to the emulsions.

1. Introduction

Milk proteins are widely used as ingredients in processed food, cosmetics and pharmaceutical products, in which they perform a wide range of key functions, including thickening, gelling foaming and emulsifying. The latter is a very important functionality of milk proteins, attributed to their ability to facilitate the formation and stabilisation of oil droplets in emulsions. The ability of milk proteins to adsorb at the oil−water interface and to stabilise emulsions has been exploited by many industries of food (nutritional products, specialised medical foods, dietary formulations, cream liqueurs and dairy desserts), cosmetics (cleansers, body lotions, face creams, serum mask) as well as pharmaceutical products (encapsulation of active ingredients).

Camel milk (*Camelus dromedarius*) is recognized for its therapeutic characteristics, and its production has grown on a large commercial scale in modern camel farms, mainly in desert areas of Asia and Africa. Recent studies on camel milk have focused in comparing its composition to cow's milk which is mainly marked by the absence of β−lactoglobulin (β−lg). In addition, camel milk is also reported to have protective proteins with immunologic, bactericidal and viricidal properties more effective than other studied types of milk [1]. More recently, some functional properties of camel milk were explored, in particular their foaming activity [2–4]. A recent study using camel whey proteins in a model beverage emulsion [5] also showed their significant emulsifying properties compared to bovine whey at specific ranges of pH (3.3) and at high protein content (3−8 %). Understanding the mechanism of camel milk protein adsorption at the oil−water interface at larger ranges of pH and protein content is, therefore, of great interest, especially for the food and the pharmaceutical industries.

The α−lactalbumin is thus known to be the most abundant whey protein of camel milk, exceeding 40 % of total whey protein content [6]. Camel α−lactalbumin (Ala−C) is a globular protein that has a

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molecular mass of 14.6 kDa and an isoelectric point (pI) about 5.1 − 5.3 [7]. The primary sequences of camel and bovine α − lactalbumin show that the percentage sequence identity and similarity are 69 % and 83 % respectively, due to 39 different residues between both proteins. Ala − C has a considerably more hydrophobic core than the bovine protein at positions 25–35 [8,9]. Both proteins have the same number of cysteine at the same positions and the same number of disulphide bonds (Cys^{6}/Cys^{120}, Cys^{28}/Cys^{111}, Cys^{61}/Cys^{77}, and Cys^{73}/Cys^{91}). Previous studies revealed that Ala − C consists of 123 amino acid residues forming a compact globular structure stabilised by four disulphide bounds and that Ala − C exhibits a high affinity to metal ions, calcium in particular. Ala − C is known also to be richer in essential amino acids and is more digestible than the equivalent bovine protein [10]. However, in contrast to bovine α − lactalbumin (Ala − B), heating Ala − C results in very little protein polymerization/aggregation due to the lack of a free thiol groups [9]. Structurally, Ala − B is an amphiphilic protein which elects it to be a good emulsifying agent to produce protein-stabilised emulsions [11]. When added to oil − water interface, it forms a cohesive interfacial protein layer around the oil droplets, providing steric and electrostatic repulsive or attractive forces between emulsion droplets. The relative stability of emulsions against coalescence is governed by the balance between these forces. Major advances have been made to better understand the adsorption process at the oil interface, the structure and the composition of the adsorbed layer, and the influence of the physical and chemical environment on the emulsifying properties of cow's milk proteins [12,13].

In this respect, understanding the emulsifying properties of camel's milk α − lactalbumin protein under different treatments is of great industrial and scientific interest in order to promote novel and interesting substitutes to existing emulsifiers. The main objective of this work is, therefore, to study the behaviour of camel milk α − lactalbumin protein (Ala − C) at the oil − water interface in a wide range of pH and protein content, and to compare it to bovine α − lactalbumin protein in a low-fat emulsion model.

2. Materials and methods

2.1. Materials

Camel milk used in this study was purchased from a local farm in the region of Tozeur in south Tunisia. Just after milking, 0.02 % of sodium azide (NaN_3) was added to stop bacterial proliferation; then, milk was stored at 4 °C.

Bovine α − lactalbumin (Ala − B) was purchased from Sigma–Aldrich (USP Reference Standard). Protein content was indicated to be 89.88 % (w/w) and this protein was used without further purification. The technical sheet of Ala − B supplied by Sigma Aldrich does not mention any calcium content (apo form).

Local produced rapeseed oil for alimentary use was purchased from a local supplier and used without further purification. Water was produced using a Millipore Milli − Q™ water purification system (Millipore Corp., Milford, MA, USA). All other chemicals used in this study are of reagent grade and purchased from Sigma–Aldrich (USA).

2.2. Camel α-lactalbumin purification

Once the camel milk was skimmed by centrifugation 3800 *g*, 20 min, 7 °C, milk casein was removed by acidic aggregation at pH 4.2 using hydrochloric acid HCl, 1 M, followed by centrifugation at 5000 *g* for 15 min [11]. Supernatant purification was then carried out using a 50 kDa ultrafiltration membrane (VivaFlow 200, Sigma–Aldrich, USA). A pH adjusted water at 6.7 was used to refilter the retentate of the purification. The washing process was carried out six times. Between each cycle, the initial flow of ultrafiltration membrane was restored using a concentrated NaOH solution (10 M) and rinsed with Milli − Q™ water. Once the permeate was collected, it was concentrated using a 5 kDa ultrafiltration membrane (VivaFlow 200, Sigma–Aldrich, USA), and then dialysed against Milli − Q™ water at 4 °C and steady stirring. Water was replaced every 12 h for 4 days [10]. The protein content was 76 % ± 2 %, measured using a total nitrogen analyser (TNM − 1, Shimadzu Corp., Japan) and a nitrogen conversion factor of 6.38. High protein purity was achieved, as the recovery yield of Ala − C was estimated to reach 10.9 % ± 0.2 % of the total α − lactalbumin of camel milk, which represents 0.79 % ± 0.01 % of the total camel milk proteins. A protein profile (sodium dodecyl sulphate polyacrylamide gel (12 % SDS − PAGE)) was achieved at each step of the purification process to test the Ala − C purity [6]. The purified proteins were freeze-dried and stored at 20 °C for further usage. This purification method (after acidification) provides proteins in their apo form (calcium free structure).

2.3. Sample and emulsion preparation

The α − lactalbumin stock solutions (0.4 % w/w, corrected for protein content) were prepared by dispersing Ala − C and Ala − B lyophilized powders in Milli − Q™ water. pH was adjusted to 3.0, 6.0 and 9.0 using either 0.5 M HCl or 0.5 M NaOH, followed by mechanical stirring (550 rpm) at room temperature (23–25 °C) for 90 min. Protein concentration was adjusted to 0.1 %, 0.2 % and 0.4 % (w/w) by dissolving stock solutions with Milli − Q™ water.

Emulsions were prepared by mixing 5 *g* of α − lactalbumin stock solution with 20 % w/w of rapeseed oil within a 50 mL plastic centrifuge tube, followed by homogenisation at 21,500 rpm for 3 min using an Ultraturrax T25 homogenizer (Ika-Werke GmbH, Germany) equipped with a SN25 − 10 G ST tool.

2.4. Emulsion properties

Emulsion stability and activity indexes (ESI and EAI, respectively) were measured according to the modified method of Pearce and Kinsella [14]. Immediately after homogenisation, aliquots of 50 µL were transferred into 10 mL of a pH-adjusted buffer solution containing 0.1 % (w/w) sodium dodecyl sulphate (SDS), and vortexed for 10 s. Optical density was read at 500 nm using an ultraviolet–visible spectrophotometer (Biomate 2S, ThermoFisher Sci., USA) and plastic cuvettes (1 cm path length). After 10 min, a second aliquot of the same emulsion was taken, following the previous procedure. EAI and ESI were then calculated using Eqs. (1) and (2), respectively:

$$\text{EAI}\ (\text{m}^2.\ \text{g}^{-1}) = \frac{2 \times 2.203 \times N \times A_0}{10^5 \times C \times \varphi} \quad (1)$$

$$\text{ESI}\ (\text{min}) = \frac{A_0}{\Delta A}.t \quad (2)$$

where A_0 is the absorbance of the diluted emulsion immediately after homogenisation, N the dilution factor (250), C the weight of protein per volume (g/ml), φ the oil volume fraction in the emulsion (20 %), ΔA the difference of the absorbance between time 0 and time 10 min (A_0 - A_{10}), and t the time interval (10 min).

2.5. Emulsion ζ–potential

ζ–potential was measured using a Zetasizer Nano ZS (Malvern Pananalytical, UK). The emulsion samples were diluted at a ratio of 1:100 (v/v). Samples were equilibrated for 120 s before collecting data. The sampling time was fixed at 400 µs. Data was accumulated from 10 sequential readings at 25 °C, and the mathematical model of Smoluchowski [15] was selected to convert the electrophoretic mobility measurements into ζ–potential values using Eq. (3):

$$\zeta = k\left(\pi\eta/\varepsilon\right)U \quad (3)$$

where ζ is the measured ζ–potential (mV), η the viscosity of the

emulsion (Pa.s), ε the dielectric constant (V/m), U the electrophoretic mobility (m^2/Vs), and k a conversion constant.

2.6. Droplet size and microscopic observation

The droplet size distribution was determined using a laser scattering technique (Mastersizer 3000E, Malvern Pananalytical, UK). Just after homogenisation, 1 ml aliquot of each emulsion was gently blended to an equal volume of pH-adjusted buffer containing 1 % sodium dodecyl sulphate SDS to avoid multi-scattering effect and prevent emulsion flocculation. The droplet size distribution of each emulsion was measured at steady agitation 1500 rpm. The Sauter diameter, d_{32}, was used to describe the mean diameter of droplets; this is defined by Eq. (4):

$$d_{32} = \sum n_i d_i^3 \Big/ \sum n_i d_i^2 \quad (4)$$

where n_i is the number of particles of diameter d_i.

Microscopic observations were carried out using an Axiovert 25 inverted microscope (Carl Zeiss GmbH, Germany) equipped with a monochrome Pulnix camera (JAI, Japan, 640 × 480 pixels). Emulsion aliquots of 20 µL were placed onto a microscope slide and carefully covered by a cover slip, avoiding any bubble formation. Micrographs were recorded at ×100 magnification.

2.7. Surface properties

Surface hydrophobicity of Ala − C and Ala − B solutions at different pH and protein concentration was measured according to the modified method of Alizadeh-Pasdar & Li-Chan [16], which uses an 8 − anilino − 1 − naphthalenesulfonate (ANS) probe to interact with hydrophobic moieties on the protein surface to give a fluorescent signal. Each protein solution was diluted 2.5 times of the initial protein concentrations. 20 µL of ANS (8 mM) solution dissolved in a phosphate buffer (50 mM, pH 7.0) was added to 4 mL of each protein solution. The solution was excited at 390 nm, and the emission spectrum was measured from 400 to 600 nm using a spectrofluorimeter Flx (SAFAS, Monaco). The emission and excitation slits were set to 5 nm, and the measurements were performed at 25 °C. The maximum area of the fluorescence spectrum was corrected with the area of the buffer.

The interfacial tension for each protein solution was measured using a K12 tensiometer (Krüss GmbH, Germany) equipped with a platinum Wilhelmy's plate to achieve complete wetting (contact angle θ is 0, i.e. $\cos(\theta) = 1$). Within glass sample cup (40 mm diameter), 5 mL of protein solution were added, followed by the immersion of Wilhelmy's plate; then, an upper layer of colza oil (10 mL) was poured over it. The measurement time was fixed to 2000s. The interfacial tension was obtained by correlating the force F (mN) applied on the immersed plate to the wetted length of the plate L (mm) between the plate and the liquid as expressed by Eq. (5):

$$\sigma\left(\frac{\text{mN}}{\text{m}}\right) = \frac{F}{L}\cos(\Theta) = \frac{F}{L} \quad (5)$$

The resulted data was displayed by the change in tension from the pure fluid value vs. log time (Eq. (6)), which allows an easy comparison of systems of different σ_0 and the visualization of the diffusion rate of proteins at the oil − water interface [17].

$$\Pi(t) = \sigma_0 - \sigma_t \quad (6)$$

In this equation, Π is the surface pressure, σ_t the measured interfacial tension at time t, and σ_0 the interfacial tension of pure fluids.

2.8. Rheological behaviour

Rheological measurements were conducted at 25.0 °C using an AR − G2 rheometer (TA Instruments, USA) equipped with a 40 mm standard steal parallel plate. 2-ml aliquots of freshly prepared emulsion were used per measurement. An up and down strain sweep test was carried out between 0.1 s^{-1} to 1000 s^{-1}. For all measurements a gap distance was fixed at 1000 µm.

2.9. Statistics

All experiments were performed in triplicate and reported as the mean ± standard deviation. A two-way analysis of variance (ANOVA) was applied to test for significance of the main effects, i.e. pH (3.0, 6.0, 9.0) and protein concentration (0.1, 0.2, 0.4 %, w/w), along with their associated interactions, on the physicochemical and emulsifying properties of Ala − C and Ala − B proteins. A quadratic model with a second-order interaction term was assumed to correlate the physicochemical and emulsifying properties of proteins to the main factors, as expressed in Eq. (7):

$$Y_i = a_0 + a_1.C + a_2.pH + a_3.C^2 + a_4.pH^2 + a_5.pH.C \quad (7)$$

where Y_i is the tested response, a_i are the observed effects, and pH and C are the main factors. The significance of the effects is expressed in terms of p-value (p). Statistical analyses were carried out using IBM SPSS Statistics (Ver. 20, IBM, USA).

3. Results and discussion

3.1. Surface properties of bovine and camel α-lactalbumin

3.1.1. Surface hydrophobicity

Fig. 1 shows the evolution of surface hydrophobicity of Ala − C and Ala − B as a function of pH (3.0, 6.0, 9.0) and protein concentration (0.1 %, 0.2 % and 0.4 % w/w). Globally, Ala-C treated proteins present lower surface hydrophobicity values than Ala-B. For both protein types, surface hydrophobicity increases when protein concentration gets higher. However, the effect of protein concentration is more significant for Ala-C than Ala − B proteins. The effect of pH is the same for both

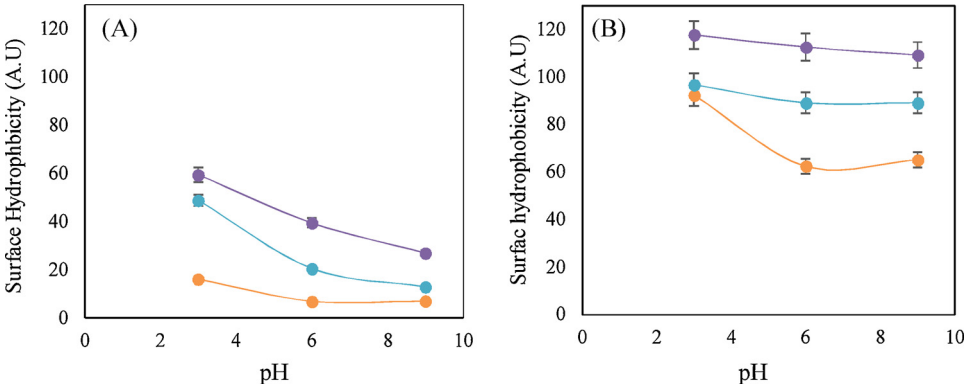

Fig. 1. Surface hydrophobicity (U.A) by ANS fluorescence probe (8 mM) of Ala − C (A) and Ala − B (B) proteins in terms of pH (3.0, 6.0, 9.0) and protein concentration (●) 0.1 %, (●) 0.2 %, (●) 0.4 % (w/w). Data represent the mean ± standard deviation (n = 3).

Table 1
Statistical results from a 2-way ANOVA describing the physicochemical and emulsifying properties of Ala − C and Ala − B proteins as a function of pH and protein concentration.

Factors		*C*	*pH*	C^2	pH^2	***CXpH***
EAI	Ala-C	***	***	**	***	*
	Ala-B	***	*	***	*	NS
ESI	Ala-C	***	NS	***	NS	NS
	Ala-B	*	**	NS	**	***
Viscosity	Ala-C	***	***	***	***	*
	Ala-B	***	**	***	***	***
Surface hydrophobicity	Ala-C	***	**	*	*	*
	Ala-B	*	**	NS	*	*
d_{32}	Ala-C	NS	***	***	***	***
	Ala-B	***	***	***	***	NS

Note that NS means 'not significant', referring to $p > 0.05$, (*) means $0.01 < p \leq 0.05$, (**) $0.001 < p \leq 0.01$, (***) $p \leq 0.001$.

proteins: surface hydrophobicity is significantly higher at pH 3.0 as described by ANOVA in Table 1. However, an increase in surface hydrophobicity always emerges as a function of protein concentration, even though this increase is sometimes small. Moreover, interaction ($pH \times C$) is also significant for both proteins ($p < 0.05$). Despite a similarity rate of 82.9 % of the linear structure between the camel and bovine α − lactalbumin [18] and higher hydrophobic residues on its secondary structure [9], the conformational reorganisation in aqueous solutions of native Ala − C displays far lower surface hydrophobicity than Ala − B (e.g., 7 A.U. vs. 65 A.U. respectively, at pH 6.0); this can be attributed to an enhanced molecular flexibility of camel proteins. As for the pH effect, this evolution is explained by the exposure of hydrophobic moieties at acidic pH, which highlights a partial denaturation enhanced by high protein concentration. This leads to conclude that the camel α − lactalbumin, which has a flexible molecular structure, is affected by the changes of pH and its interaction with protein concentration can undergo a conformational reorganisation to possibly better align at the oil − water interface when emulsified, which confirms our current results in response to pH and heat treatment [19].

3.1.2. Interfacial properties

Interfacial tension between rapeseed oil and treated protein solutions was measured for the lowest protein concentration (0.1 % w/w). At high protein content (0.2 % and 0.4 %, w/w), no diffusion regime was observed. The higher the concentration, the lower the interfacial tension (σ), and the higher the surface pressure (Π). So, the oil − water interface was immediately saturated by the high protein content at the surface, considering the static measurement method of our tensiometer. For that, no data was presented for 0.2 % and 0.4 % of protein content.

The surface pressure (Π) for Ala − C and Ala − B proteins (0.1 % w/w) at different pH (3.0, 6.0 and 9.0) is presented in Fig. 2. For most measurements, Ala − C proteins at the oil − water interface induce steeper and greater increase in surface pressure than Ala − B proteins (3.2 $mN.m^{-1}$ vs. 2.4 $mN.m^{-1}$, respectively). For Ala − C proteins, surface pressure increases faster at pH 6.0 and 9.0 than at pH 3.0, i.e. a greater Π(t) slope is observed. Conversely, for Ala − B proteins, surface pressure is the smallest at pH 6.0. The interfacial tension reflects, in most cases, the amphiphilic state of emulsifiers at the oil − water interface; however, this is not necessarily correlated to the degree of emulsifying activity of the proteins. In fact, the former depends entirely on the droplet formation and the protein structure at the oil − water interface after homogenisation, whereas interfacial tension describes equilibrium conditions.

By observing the results, three regimes of protein adsorption to the interface are distinguished (Fig. 2): (*i*) an induction period in which Π(t) is zero or remains close to zero; (*ii*) a second regime in which a sharp increase in surface pressure is observed; and (*iii*) a region where Π(t) increases slightly (pH 3.0) or even decreases (Ala − B at pH 9.0).

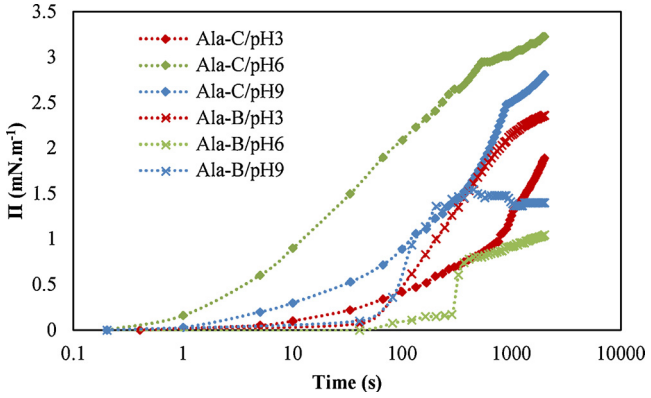

Fig. 2. Surface pressure ($mN.m^{-1}$) vs. log time (s) converted from interfacial tension between rapeseed oil and Ala − C and Ala − B proteins (0.1 % w/w) at different pH (3.0, 6.0, 9.0) using platinum Wilhelmy's plate method.

The decline in surface pressure slope signs the saturation of the first protein layer and the stabilisation of the interface [17,19,20]. For Ala − B proteins, the highest Π value is reported at pH 3.0. The induction time is the shortest at this pH, and the third regime is not achieved within 2,000 s. This result is correlated to the high hydrophobicity of Ala − B proteins previously observed (Fig. 1A). Conversely, for Ala − C proteins which exhibit a far lower surface hydrophobicity, the fastest increase at short time is reported when pH is close to the pI (5.1–5.2), i.e. proteins seem to adsorb more rapidly, whereas the fastest increase in the third region is observed at pH 3.0. So, at 0.1 % protein concentration, the Ala − B proteins make longer time to align at the oil-water interface; this may be attributed to the reduced ability of adsorbed proteins to change their conformation and unfold in response to hydrophilic/hydrophobic side chains, and undergo enhanced interactions with the oil phase.

At long time, it must be pointed out that surface pressure is never stabilised even after 2000 s, except maybe for Ala − C at pH 9.0. Concerning the effect of pH, a comparison between equilibrium values cannot be achieved, and the comparison between values at 2,000 s cannot be used to deduce which pH provides the highest equilibrium surface pressure. This is particularly true for Ala − C because when surface pressure increases as a function of pH, the slope of Πt decreases and vice versa at 2000 s.

In addition, it must be pointed out that surface pressure data in Fig. 2 and surface hydrophobicity in Fig. 1 do not correlate. This result is not surprising, as surface hydrophobicity is related to the protein structure in water, whereas adsorbed proteins are known to unfold and undergo conformational rearrangements at an oil-water or air-water interface.

Finally, it emerges, despite their similar structures, that Ala − B proteins are probably less flexible than Ala − C proteins, so that they adsorb less rapidly at an oil − water interface, especially when pH is close to the pI.

3.2. Emulsifying properties

3.2.1. Emulsifying activity and stability indices

Table 2 presents the emulsifying activity and stability indices (EAI and ESI respectively) of Ala − C and Ala − B stabilised emulsions as a function of pH and protein concentration.

For EAI, a similar evolution between camel and bovine proteins is observed in terms of protein content. Close maximum values of EAI, between 240–260 $m^2.g^{-1}$, are therefore measured. EAI decreases as protein concentration increases from 0.1 % to 0.4 % (w/w). Differences emerge after pH changes from 3.0–6.0 and 9.0 between Ala − C and Ala − B stabilised emulsions. For the emulsions stabilised by bovine proteins (columns B in Table 2), emulsifying activity exhibits very close

Table 2
Emulsifying activity index (EAI) and stability index (ESI) for Ala − C (A) and Ala − B (B) stabilised emulsions as a function of pH (3.0, 6.0 and 9.0), and protein concentration (0.1, 0.2 and 0.4 % w/w). The letters "a", "b" and "c" represent homogenous subsets of different classes.

EAI ($m^2.g^{-1}$)						
C/pH	3.0		6.0		9.0	
	A	B	A	B	A	B
0.1 %	256.1^{c}	243.4^{cb}	86^{ca}	177.3^{ca}	163.3^{cb}	221.4^{cb}
0.2 %	96.4^{bc}	86.4^{b}	20.5^{ba}	92^{ba}	101.5^{b}	111^{b}
0.4 %	49.4^{ac}	50.3^{ab}	10.9^{a}	49.7^{a}	56.4^{ab}	47.4^{ab}
ESI (min)						
C/pH	3.0		6.0		9.0	
	A	B	A	B	A	B
0.1 %	19.4^{ba}	29.7^{ab}	29.4^{ba}	19.8^{a}	26.5^{ba}	26.3^{a}
0.2 %	18^{a}	53.2^{b}	14.3^{a}	19.8^{ba}	16.8^{a}	22.6^{ba}
0.4 %	24.9^{ba}	40.6^{ab}	18.6^{ba}	23.8^{a}	19.8^{ba}	20.6^{a}

trends for the different pH values. A low effect of pH for Ala − B is assessed by ANOVA (Table 1), whereas the effect of protein concentration is highly significant ($p < 0.001$). As already stated, the EAI of Ala − B stabilised emulsions is higher at the protein concentration of 0.1 % (w/w): similar trends had already been observed for cow's milk proteins [21,22]. Actually, EAI depends on the native state of the proteins and on the treatments that have been applied. Another explanation of the effect of protein concentration has been mentioned by Guo and Mu [23]: at low concentrations, protein adsorption at the oil − water interface is diffusion-controlled, while at high concentration, the activation-energy barrier prevents protein migration in a diffusion-dependent manner.

For emulsions stabilised by Ala-C (columns A in Table 2), EAI at pH 6.0 is the lowest, regardless protein concentration. The effects of pH, as well as of protein concentration are significant for camel proteins (Table 1). For higher protein concentrations, 0.2 % and 0.4 % (w/w) respectively, EAI measurements are rather close at both pH 3.0 and 9.0. At 0.1 % protein concentration, EAI is higher for pH 3.0 (256 $m^2.g^{-1}$). This result is in line with the higher surface hydrophobicity of Ala-C observed at acidic pH, even though it contradicts previous results of section 3.1.2 on surface pressure. It seems that the adsorption of Ala − C proteins at the oil–water interface at pH 6.0 and at the static conditions of surface pressure measurements, favoured the reduction of electrostatic repulsion close to their pI (5.1–5.2) which make them unable to form stable interface layers. Thus, the Ala − C proteins may adsorb readily, but keep their globular conformation and cannot prevent droplet coalescence in the high-shear conditions of emulsion preparation.

The emulsifying stability index (ESI), presented in Table 2, is an immediate measurement of the processed emulsions, which indicates their relative stability at short time (after 10 min). Thus, it reflects immediate instability, such as coalescence or/and creaming of oil droplets. Regarding emulsifying stability (ESI), Table 2 shows that ESI values of Ala − B stabilised emulsions are higher at pH 3.0, but closer at pH 6.0 and 9.0 to Ala − C stabilised emulsions. The stability of protein-stabilised emulsions by Ala − B has been abundantly discussed in the literature [11,20,24,25] to be associated with electrostatic repulsion forces between droplets, steric stabilisation by protein tails or loops at the oil droplets surface, and/or high viscosity of the continuous phase. Generally, in these emulsion systems, the creaming stability increases with increasing protein concentration up to a certain concentration, and then remains almost constant [26]. However, for Ala − B stabilised emulsions, emulsion formation is mainly governed by hydrophobic interactions, with a rather stable protein structure which depends on protein concentration, so that stability could proceed through steric interaction at all pH and enhanced electrostatic repulsion far from the pI. Conversely, the stability of Ala − C stabilised emulsions, exhibiting high EAI, is mainly attributed to the associated electrostatic forces at

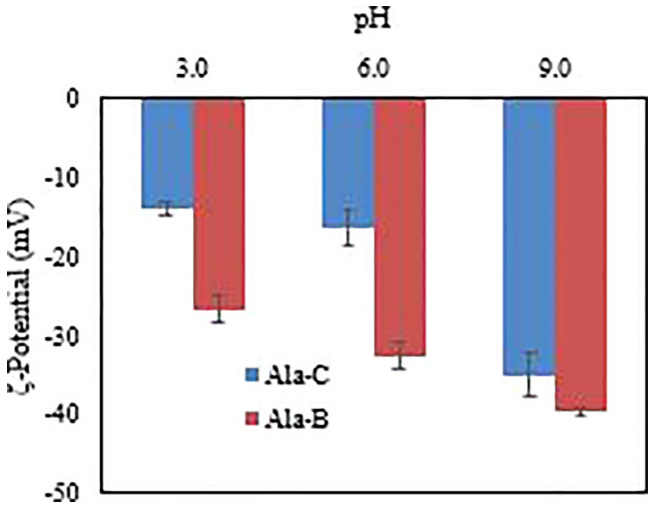

Fig. 3. ζ − Potential (mV) of Ala − C and Ala − B stabilised emulsions (diluted to 1:100) at different pH (3.0, 6.0 and 9.0). Data represent the mean ± standard deviation (n = 3).

pH 3.0 and 9.0, whereas ESI at pH 3.0 is biased by the lower EAI value in this condition. The consequence is that the effect of pH is insignificant in the ANOVA table (Table 1).

As a conclusion, Ala − C and Ala − B proteins present dissimilar behaviours in the emulsion formation process and in the mechanism of emulsion stability, but in both cases, increasing protein concentration does not significantly improves EAI and ESI.

3.2.2. *Emulsion ζ − potential*

The stability of Ala − C and Ala − B stabilised emulsions was further assessed through ζ − potential measurements presented in Fig. 3. In this figure, the ζ − potential of Ala − B stabilised emulsions departs more from 0 than Ala − C stabilised emulsions, as it remains between − 26.7 mV and − 39.5 mV and between − 14 mV and − 35 mV, respectively. Bhattacharjee [27] reported that highly stable emulsion could be observed when ζ − potential reaches at least ± 30 mV. In our work, electrostatic repulsions are involved in emulsion stability, especially at pH 9.0 and 6.0 where ζ − potential is more negative. Low absolute charge at pH 3.0 for both emulsions (− 14.0 mV and − 26.7 mV, respectively) is consequent to positive charged proteins at the oil droplets surface, but proteins are not involved alone in the stability, which explains why the sign of ζ − potential does not change at the pI of proteins. Another explanation for the negative ζ − potential of α − lactalbumin stabilised emulsions at pH < pI was reported by Wiacek and Chibowski [28], suggesting that for this specific protein (α − lactalbumin in comparison with β − casein and serum albumin of cow's milk), the pI was drastically shifted from 5.1–2.2 due to protein conformation change after adsorption at the oil interface. In this work, pH was maintained above pH 3.0; thus, the actual pI of this protein after conformation changes to obtain positive ζ − potential values was definitely not reached.

For Ala − B stabilised emulsions, ESI was higher at pH 3.0 (Section 3.2.1), whereas ζ − potential was closer to 0 in Fig. 3; this highlights that emulsion stability is therefore mainly assessed by other mechanisms, such as steric hindrance or/and surface hydrophobicity. Similarly, the correlation between ζ − potential and ESI appears to be weak for Ala − C. But oil droplets are, however, electrostatically charged, which stabilise them by repulsive forces at longer time.

3.3. *Droplet size analysis*

Fig. 4 shows the volume-based size distribution as well as microscopic observations for Ala − C and Ala-B stabilised emulsions at

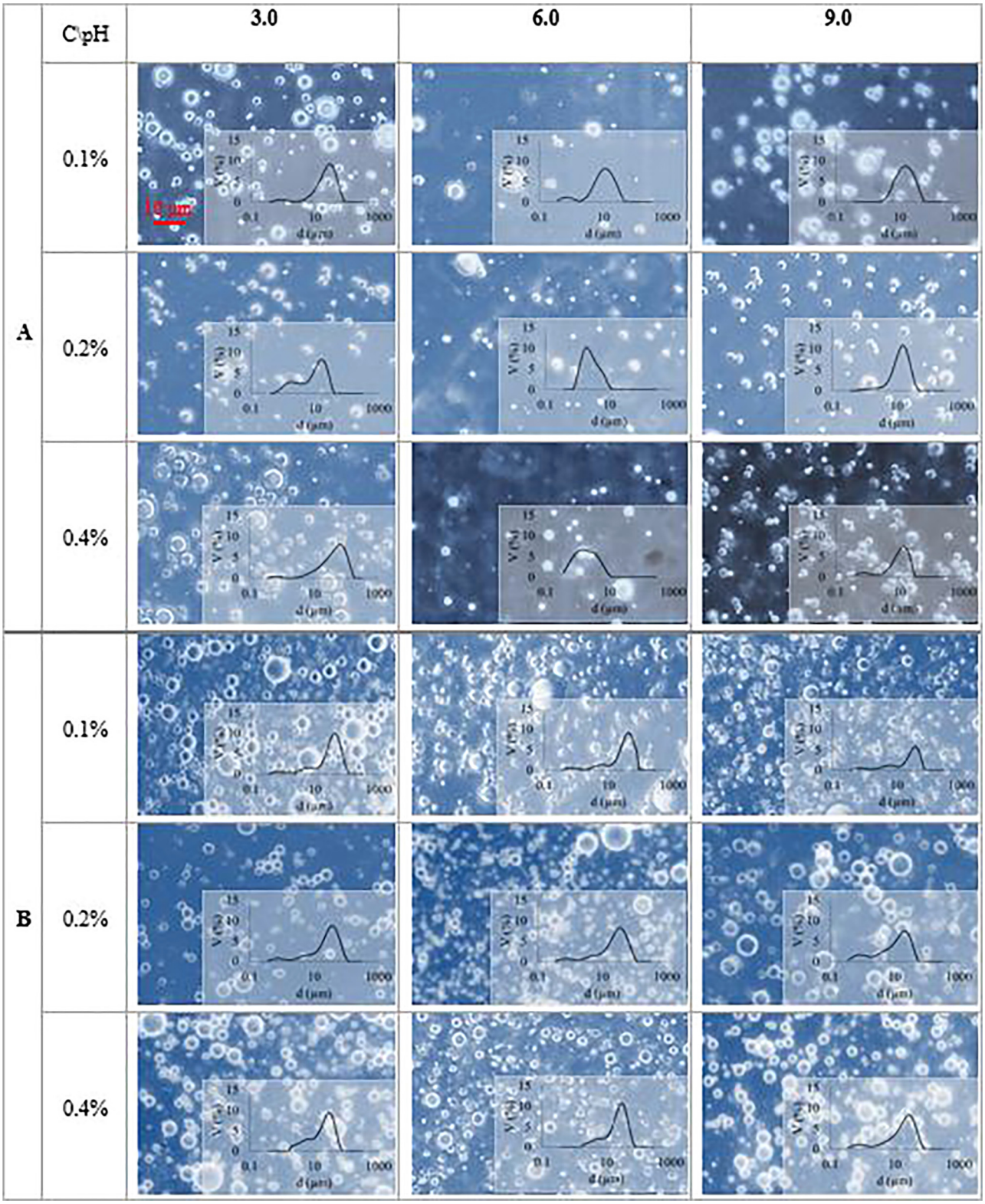

Fig. 4. Micrographs and volume-size distribution (%) of oil droplet diameters (μm) stabilised by Ala – C (A) and Ala – B (B) proteins at different pH (3.0, 6.0, 9.0) and protein concentration (0.1, 0.2, 0.4 % w/w).

different pH and protein concentration. The evolution of droplets size diameter (d_{32}) of the processed emulsions is also summarized in Fig. 5.

Overall, the order of magnitude of d_{32} is similar for both types of emulsions, varying between 2 μm and 13 μm. Mainly, bimodal distributions are attributed to Ala – B emulsions (Fig. 4B), while unimodal ones occur only for Ala – C emulsions (Fig. 4A). For nearly all the protein concentrations, the average diameter of oil droplets stabilised by Ala – B is maximized at pH 6.0; on the contrary, it is usually minimized at pH 6.0 with Ala – C proteins (Fig. 5). This result is supported by microscopic observations (Fig. 4A): these highlight higher droplet density number for Ala – C emulsions at both pH 3.0 and 9.0 with similar diameters, while a very low droplet number density is observed at

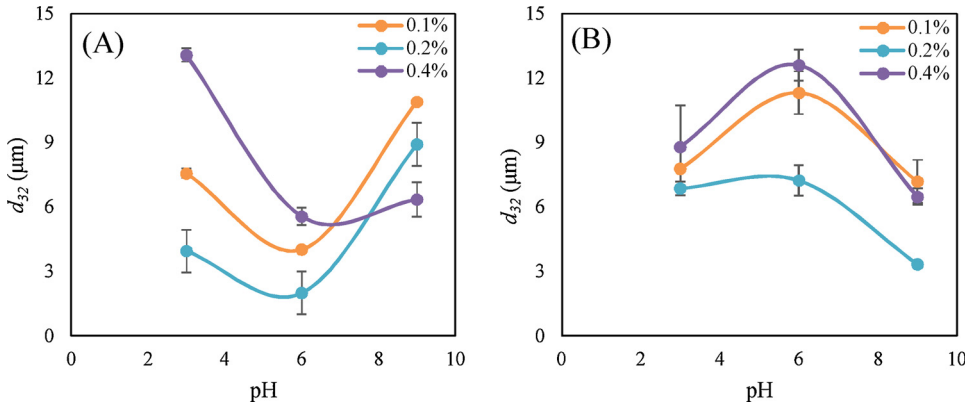

Fig. 5. Sauter diameter d_{32} (µm) for Ala − C (A) and Ala − B (B) stabilised emulsions as a function of pH (3.0, 6.0, 9.0) and protein concentration (0.1, 0.2, 0.4 % w/w). Data represent the mean ± standard deviation (n = 3).

pH 6.0. It results that only the smallest droplets could be formed and stabilised at a pH close to pI, which can be explained by poor EAI of Ala − C at this pH. The ANOVA model indicates that the effect of pH and the interaction ($pH \times C$) are the most significant for Ala − C stabilised emulsions (Table 1). The comparison of micrographs between Ala − B and Ala − C also highlights a higher droplet number density with Ala − B stabilised emulsions in comparison to Ala − C. It was also reported in the literature that a high concentration of proteins increases the coverage of oil droplet, inhibiting droplet from aggregation [29]; this is the case after increasing the protein concentration from 0.1 % to 0.2 % (w/w), where droplet sizes decrease from 7.6 µm to 3.95 µm, and from 11 µm to 7.2 µm for Ala − C at pH 3.0 and Ala − B at pH 6.0, respectively. Larger droplets appear at 0.1 % (w/w) of protein content due to protein coverage limitation. However, at 0.4 % (w/w) of protein concentration, an unexpected increase in droplets size explained by a protein aggregation and interaction due to interface over-saturation: This can also be observed from micrographs, showing homogeneous dispersion at 0.2 % (w/w) of protein concentration, while larger clusters and aggregates due to flocculation can be seen at 0.4 % (w/w) concentration, as Ala − B emulsions present a lower ζ − potential in section 3.2.

Finally, Ala − C proteins seem able to stabilise smaller droplets and to produce emulsions with higher droplet density number, except close to the pI. Larger droplets are stabilised when protein concentration is increased, which also favours droplet flocculation for both proteins.

3.4. Emulsion rheology

The apparent viscosity (η) was measured vs. shear rate between 0.1 and 1,000 s^{-1}, and the evolution of viscosity of Ala − C and Ala − B stabilised emulsions at fixed shear rate (10 s^{-1}) is presented in terms of pH and protein concentration in Fig. 6.

First, all the studied emulsions exhibit a nearly Newtonian behaviour at high shear rate, and non-Newtonian trends at low shear rate. The emulsion viscosity ranges between 1.2 mPa.s and 40 mPa.s and between 1.4 mPa.s and 71 mPa.s for Ala − B and Ala − C stabilised emulsions, respectively. For Ala − B stabilised emulsions (Fig. 6B), viscosity decreases with increasing pH from 6.72 mPa.s at pH 3.0–1.2 mPa.s at pH 9.0. For Ala − C stabilised emulsions (Fig. 6A), viscosity is higher at pH 3.0 and 9.0 than pH 6.0, regardless of the protein concentration. The effect of pH is more significant on Ala − C than on Ala − B stabilised emulsions for which the role of the $pH \times C$ interaction prevails (Table 1). For Ala − C, the decline in viscosity between pH 3.0 and 6.0 results from friction reduction between oil droplets, as droplet density decreases (Fig. 4) even though d_{32} decreases (Fig. 5). Contrary to Ala − C, the viscosity values of Ala − B stabilised emulsions at pH 3.0 and 6.0 are usually close, followed by a decrease when pH is increased. Regarding the concentration effect, 0.2 % (w/w) of protein content leads to significantly viscous emulsions for both proteins with a strong effect of protein concentration ($p < 0.001$). Thus, the smallest d_{32} values usually correspond to the highest viscosity values at constant pH, except at pH 9.0. This corresponds to an expected behaviour in the non-Newtonian region due to velocity field distortion around each droplet, as explained by Amin et al. [30]: once the droplets are encapsulated, the viscosity remains high due to a rigid and well-structured viscoelastic film around the oil droplets. However, when pH is changed, viscosity is also strongly affected by emulsion stability; for example, Ala − C emulsions at pH 6.0 which exhibit a very low EAI in Table 2 also display the lowest viscosity despite their low d_{32} values. While viscosity increases with protein content when concentrations changes from 0.1 % to 0.2 % (w/w), as expected, it decreases for a further increase to 0.4 % (w/w) protein when d_{32} increases at the same time.

Finally, it emerges that despite their differences in droplet size and density in Section 3.3, emulsions stabilised by Ala − C present a significantly higher viscosity at pH 3.0 and 9.0 than with Ala − B, which confers a higher long-term stability to emulsions stabilised by Ala − C. But both types of emulsions exhibit very low viscosity values at pH 6.0.

4. Conclusions

The main findings of the comparison between camel and bovine α − lactalbumin emulsifying properties are summarized as follows: (1) The emulsifying activity of Ala − C proteins is more significantly affected by pH change than that of Ala − B proteins; (2) The measurement of surface properties shows that protein hydrophobicity is more involved in controlling emulsification process, as ζ − potential was also more negative using Ala − B; a higher emulsifying stability is therefore granted for Ala − B stabilised emulsions; (3) The concentration effect as well as the pH − concentration interaction significantly influence the emulsifying properties of Ala − C. However, the lowest protein concentration of 0.1 % (w/w) showed always the highest emulsifying activity, whereas medium concentration (0.2 % w/w) afforded the best emulsion properties, i.e. small droplet size and high emulsion viscosity. These facts suggest an unbalance in the two dominant forces (the electrostatic effect and configurational entropy) that drive conformational rearrangement at emulsion interfaces toward hydrophobic repulsion.

Author contribution statement

Maroua ELLOUZE: Investigation, formal statistics, Data curation, Writing- Original draft preparation, **Roua LAJNAF and Ahmed ZOUARI**: Methodology, Software, **Hamadi ATTIA**: Resources, **Mohamed Ali AYADI and Christophe VIAL**: Supervision reviewing and validation.

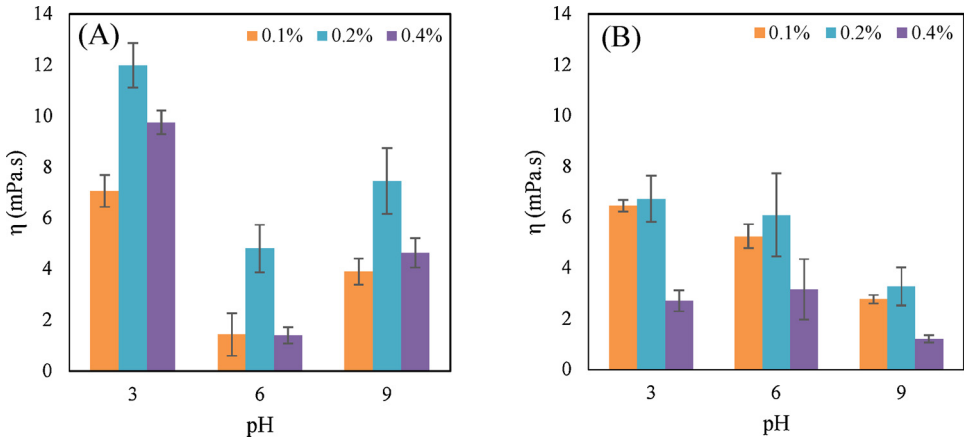

Fig. 6. Apparent viscosity (Pa.s) at a fixed shear rate ($10\,s^{-1}$) of Ala − C (A) and Ala − B (B) stabilised emulsions in terms of pH (3.0, 6.0, 9.0) and protein concentration (0.1, 0.2, 0.4 % w/w) using 40 mm standard steal parallel plate.

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References

[1] H. El-Hatmi, J.M. Girardet, J.L. Gaillard, M.H. Yahyaoui, H. Attia, Characterisation of whey proteins of camel (Camelus dromedarius) milk and colostrum, Small Rumin. Res. 70 (2007) 267–271.

[2] Y.-T. Hu, Y. Ting, J.-Y. Hu, S.-C. Hsieh, Techniques and methods to study functional characteristics of emulsion systems, J. Food Drug Anal. 25 (2017) 16–26, https://doi.org/10.1016/j.jfda.2016.10.021.

[3] R. Lajnaf, L. Picart-Palmade, E. Cases, H. Attia, S. Marchesseau, M.A. Ayadi, The foaming properties of camel and bovine whey: the impact of pH and heat treatment, Food Chem. 240 (2018) 295–303, https://doi.org/10.1016/j.foodchem.2017.07.064.

[4] R. Lajnaf, L. Picart-Palmade, H. Attia, S. Marchesseau, M.A. Ayadi, Foaming and adsorption behavior of bovine and camel proteins mixed layers at the air/water interface, Colloids Surf. B Biointerfaces 151 (2017) 287–294, https://doi.org/10.1016/j.colsurfb.2016.12.010.

[5] S. Momen, M. Salami, F. Alavi, Z. Emam-Djomeh, A.A. Moosavi-Movahedi, The techno-functional properties of camel whey protein compared to bovine whey protein for fabrication a model high protein emulsion, LWT. 101 (2019) 543–550, https://doi.org/10.1016/j.lwt.2018.11.063.

[6] I. Felfoul, C. Lopez, F. Gaucheron, H. Attia, M.A. Ayadi, A laboratory investigation of cow and camel whey proteins deposition under different heat treatments, Food Bioprod. Process. 96 (2015) 256–263, https://doi.org/10.1016/j.fbp.2015.09.002.

[7] A. Conti, J. Godovac Zimmermann, L. Napolitano, J. Liberatori, Identification and characterization of two alpha-lactalbumins from Somali camel milk Camelus dromedaries, Milchwissenschaft 40 (1985) 673–675.

[8] M.S. Atri, A.A. Saboury, R. Yousefi, M. Dalgalarrondo, J.-M. Chobert, T. Haertlé, A.A. Moosavi-Movahedi, Comparative study on heat stability of camel and bovine apo and holo α-lactalbumin, J. Dairy Res. 77 (2010) 43–49, https://doi.org/10.1017/S0022029909990367.

[9] S. Si Ahmed, A. Zennia, F. Mati, Y. Saulnier, G. Verdier, G. Chiappetta, L. Mulliert, J. Miclo, J.-M. Vinh, Girardet, Identification by FT-ICR-MS of Camelus dromedarius α-lactalbumin variants as the result of nonenzymatic deamidation of Asn-16 and Asn-45, Food Chem. 187 (2015) 305–313, https://doi.org/10.1016/j.foodchem.2015.04.036.

[10] M. Salami, R. Yousefi, M.R. Ehsani, S.H. Razavi, J.-M. Chobert, T. Haertlé, A.A. Saboury, M.S. Atri, A. Niasari-Naslaji, F. Ahmad, A.A. Moosavi-Movahedi, Enzymatic digestion and antioxidant activity of the native and molten globule states of camel α-lactalbumin: possible significance for use in infant formula, Int. Dairy J. 19 (2009) 518–523, https://doi.org/10.1016/j.idairyj.2009.02.007.

[11] R. Lajnaf, L. Picart-Palmade, H. Attia, S. Marchesseau, M.A. Ayadi, The effect of pH and heat treatments on the foaming properties of purified α-lactalbumin from camel milk, Colloids Surf. B Biointerfaces (2017), https://doi.org/10.1016/j.colsurfb.2017.05.002.

[12] R.S.H. Lam, M.T. Nickerson, Food proteins: a review on their emulsifying properties using a structure–function approach, Food Chem. 141 (2013) 975–984, https://doi.org/10.1016/j.foodchem.2013.04.038.

[13] E. Dickinson, Milk protein interfacial layers and the relationship to emulsion stability and rheology, Colloids Surf. B Biointerfaces 20 (2001) 197–210, https://doi.org/10.1016/S0927-7765(00)00204-6.

[14] K.N. Pearce, J.E. Kinsella, Emulsifying properties of proteins: evaluation of a turbidimetric technique, J. Agric. Food Chem. 26 (1978) 716–723, https://doi.org/10.1021/jf60217a041.

[15] D.J. McClements, Protein-stabilized emulsions, Curr. Opin. Colloid Interface Sci. 9 (2004) 305–313, https://doi.org/10.1016/j.cocis.2004.09.003.

[16] N. Alizadeh-Pasdar, E.C.Y. Li-Chan, Comparison of protein surface hydrophobicity measured at various pH values using three different fluorescent probes, J. Agric. Food Chem. 48 (2000) 328–334, https://doi.org/10.1021/jf990393p.

[17] C.J. Beverung, C.J. Radke, H.W. Blanch, Protein adsorption at the oil/water interface: characterization of adsorption kinetics by dynamic interfacial tension measurements, Biophys. Chem. 81 (1999) 59–80, https://doi.org/10.1016/S0301-4622(99)00082-4.

[18] M.S. Atri, A.A. Saboury, R. Yousefi, M. Dalgalarrondo, J.-M. Chobert, T. Haertlé, A.A. Moosavi-Movahedi, Comparative study on heat stability of camel and bovine apo and holo α-lactalbumin, J. Dairy Res. 77 (2010) 43–49, https://doi.org/10.1017/S0022029909990367.

[19] M. Ellouze, C. Vial, H. Attia, M.A. Ayadi, Camel α − lactalbumin at the oil − water interface: effect of pH and heat treatment on the structure, surface characteristics and emulsifying properties, LWT. 116 (2019) 108550, , https://doi.org/10.1016/j.lwt.2019.108550.

[20] V. Mitropoulos, A. Mütze, P. Fischer, Mechanical properties of protein adsorption layers at the air/water and oil/water interface: a comparison in light of the thermodynamical stability of proteins, Adv. Colloid Interface Sci. 206 (2014) 195–206, https://doi.org/10.1016/j.cis.2013.11.004.

[21] R.S.H. Lam, M.T. Nickerson, The effect of pH and temperature pre-treatments on the physicochemical and emulsifying properties of whey protein isolate, Lwt - Food Sci. Technol. 60 (2015) 427–434, https://doi.org/10.1016/j.lwt.2014.07.031.

[22] J. Zhai, S.V. Hoffmann, L. Day, T.-H. Lee, M.A. Augustin, M.-I. Aguilar, T.J. Wooster, Conformational changes of α-Lactalbumin adsorbed at oil–water interfaces: interplay between protein structure and emulsion stability, Langmuir. 28 (2012) 2357–2367, https://doi.org/10.1021/la203281c.

[23] Q. Guo, T.H. Mu, Emulsifying properties of sweet potato protein: effect of protein concentration and oil volume fraction, Food Hydrocoll. 25 (2011) 98–106, https://doi.org/10.1016/j.foodhyd.2010.05.011.

[24] R.S.H. Lam, M.T. Nickerson, The effect of pH and temperature pre-treatments on the structure, surface characteristics and emulsifying properties of alpha-lactalbumin, Food Chem. 173 (2015) 163–170, https://doi.org/10.1016/j.foodchem.2014.09.078.

[25] S. Tcholakova, N.D. Denkov, I.B. Ivanov, B. Campbell, Coalescence stability of emulsions containing globular milk proteins, Adv. Colloid Interface Sci. 123 (2006) 259–293, https://doi.org/10.1016/j.cis.2006.05.021.

[26] H. Singh, A. Ye, Interactions and functionality of milk proteins in food emulsions, Milk Proteins, Elsevier, 2014, pp. 359–386, https://doi.org/10.1016/B978-0-12-405171-3.00012-X.

[27] S. Bhattacharjee, DLS and zeta potential – What they are and what they are not? J. Control. Release 235 (2016) 337–351, https://doi.org/10.1016/j.jconrel.2016.06.017.

[28] A.E. Wiącek, E. Chibowski, Zeta potential and droplet size of n-tetradecane/ethanol (protein) emulsions, Colloids Surf. B Biointerfaces 25 (2002) 55–67, https://doi.org/10.1016/S0927-7765(01)00304-6.

[29] C. Chang, F. Niu, Y. Su, Y. Qiu, L. Gu, Y. Yang, Characteristics and emulsifying properties of acid and acid-heat induced egg white protein, Food Hydrocolloids 54 (Part B) (2016) 342–350, https://doi.org/10.1016/j.foodhyd.2015.09.026.

[30] S. Amin, G.V. Barnett, J.A. Pathak, C.J. Roberts, P.S. Sarangapani, Protein aggregation, particle formation, characterization & rheology, Curr. Opin. Colloid Interface Sci. 19 (2014) 438–449, https://doi.org/10.1016/j.cocis.2014.10.002.