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Effect of pH and heat treatment on structure, surface characteristics and emulsifying properties of purified camel β–casein

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Abstract

Oil-in-water emulsions (20%/80%, w/w) were stabilised by two types of β−caseins (1 g/L, w/w) extracted by rennet coagulation from camel and cow's milk, respectively. Both extracts were treated under different ranges of pH (3.0, 6.0 and 9.0) and temperature (25, 65 and 95°C for 15 min) before emulsification. The emulsifying properties of the proteins were studied by surface and interfacial measurements. Results show that the emulsifying activity (EAI) of camel β−casein is higher than the bovine protein. Yet, both proteins exhibited heat stability and no significant effect of temperature was reported. Conversely, a significant effect of pH on camel β−casein was recorded: at pH 6.0, the lowest values of EAI were measured and explained by the formation of micellar protein structure. Under such conditions, camel β−casein is therefore a novel emulsifying protein with high potential to stabilise oil-in-water interfaces which provides numerous applications for the food chemistry field.

Key words

Camel milk, β−casein, emulsion, surface hydrophobicity, interfacial tension, granulometry, dairy protein.

1. Introduction

Camel milk plays an important role in human nutrition; its production has evolved on a broad commercial scale in modern camel farms (FAOSTAT, 2018; Hailu et al., 2016). It consumption is increasingly common in many countries in Asia, Africa and Europe for its nutritious and medicinal properties due to the presence of essential nutrients (protein, fat, lactose, minerals), bioactive molecules, such as lactoferrin and lysozyme (Al Haj & Al Kanhal, 2010; Elagamy, 2000; Figliola et al. 2021; Hailu et al., 2016), and especially for the absence of β−lactoglobulin (Maqsood et al., 2019; Swelum et al., 2021).

Cow's milk proteins, in particular caseins are good emulsifiers that have been widely studied (Dickinson, 2001; Huck-Iriart et al., 2016; Jahaniaval et al., 2000; Liang et al., 2016; McSweeney et al., 2004) and often used as emulsifiers in many food, pharmaceutical and cosmetic applications due to their amphiphilic character and their ability to change their molecular conformation according to pH and temperature operating conditions. The most abundant, β−casein, is found in molecular or aggregated form in solution, based on physiochemical parameters. Its content reaches 36% in cow's milk casein and up to 65% in

camel milk casein. Thus, our interest is particularly oriented to explore the potential emulsifying properties of camel β−casein protein.

Many studies have characterised the molecular structure of this protein in comparison with the bovine one and showed that the molecular weight of camel β−casein (24.65 kDa) is higher compared to that of bovine β−casein (23.58 kDa). The numbers of amino acid residues in camel and bovine β−caseins are 217 and 209, respectively, as reported by Barzegar et al. (2008) and Mohamed et al. (2021). Sequence alignment of bovine and camel β−caseins shows that sequence similarity and identity between these two caseins are 84.5% and 67.2%, respectively (Barzegar et al., 2008). The β−casein has a flexible linear disordered secondary structure and no intramolecular crosslinks. It has also a hydrophilic region at the N-terminal and a hydrophobic region of zero net charge at the C-terminal of the molecule (Li et al., 2016; McCarthy et al., 2014). The amphiphilic molecular nature of these proteins provides very interesting emulsifying properties, as mentioned by several researches (Dalgleish, 2006; Li et al., 2016; Yahimi Yazdi et al., 2014). However, the techno-functional properties of camel milk proteins are poorly understood and hardly studied as in the case of the camel β−casein. In a recent work, Lajnaf et al. (2020) investigated various features of camel β−casein, such as their antioxidant, antimicrobial, emulsifying and physico-chemical properties. Their experimental results displayed an increase in emulsion activity and stability with pH from 5.0 to 9.0 and improved properties in comparison to bovine β−casein.

In the light of these homologies and differences, the aim of this paper is to focus on the emulsifying properties of camel β−casein in a larger range of pH (3.0–9.0) and as a function of heat treatment (up to 95°C), then to compare these properties to those of the bovine β−casein at the oil−water interface, and finally to correlate emulsifying properties to physicochemical properties and molecular structure in order to promote new applications of camel β−casein to the food chemistry field.

2. Materials and methods

2.1 Materials

Camel (*Camelus dromedaries*) milk and cow (*Bos taurus*) milk used in this study were purchased from a local farm in the region of Touser in south Tunisia and in the region of Clermont-Ferrand in the centre of France, respectively. Just after milking, 0.02% of sodium azide (NaN_3) was added to stop bacterial proliferation; then, milk was stored at 4°C. The

β–casein proteins of camel and cow's milk were obtained according to the method described below (section 2.2).

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 Methods

2.2.1 β–Casein extraction

The extraction of β–casein from camel and cow's milk was carried out following the modified method of (Huppertz et al., 2006). Briefly, caseins of both types of milk were precipitated by rennet coagulation (active chymosin ≥ 520 mg/l, C.P.F., France) at 35°C for 90 and 60 min for camel and cow's milk, respectively. A first centrifugation at 5,000 *g* for 15 min followed by inactivation of the enzyme at hot water (80°C for 5 min) were carried out. The resulting casein curd was then washed and suspended in cold water at an equivalent volume to the discarded whey. The curd-cold water mixture thus obtained was kept at 5°C for 24 hours at steady stirring, then centrifuged at 5,000 *g* for 15 min at 5°C. The β–casein recovered after filtration of the supernatant was freeze-dried and stored for further use.

2.2.2 Chemical characterization of the β–casein extracts

The protein content was evaluated using a total nitrogen analyzer (TNM-1, Shimadzu Corp., Japan). This allows the specific detection of nitrogen by chemiluminescence according to the EN 12260 standard. The protein solutions were previously diluted to a total nitrogen content lower than 100 ppm (optimal detection range). The protein content is given by Eq. (1):

$$C\ (\text{mg. L}^{-1}) = TN \times f \times d \qquad (1)$$

where C is the protein mass concentration (mg/L), TN is the measured total nitrogen content (ppm), f is the conversion factor equal to 6.38 and d is the dilution factor.

Lactose content was measured using the modified Dubois method. Briefly, 500 µL of previously diluted sample was added to an equal volume of 5% (w/v) phenol and 2.5 mL of pure sulfuric acid. The mixture was incubated for 10 min without stirring, then a second incubation was performed for 30 min at 35°C after rigorous stirring. The optical density was

measured at a wavelength of 438 nm. The lactose content was determined after projection on a 0.1g/L glucose standard prepared under the same conditions.

Protein profile was performed according to the modified method of Laemmli (1970). 20 µL of each fraction was mixed with an equal volume of buffer solution (10% (w/w) SDS, 0.5 M Tris-HCl (pH 6.8), 2% (w/w) glycerol, 0.5 M β-mercaptoethanol, and 0.1% (w/w) bromophenol blue). The mixture was then heated at 95°C for 5 min, and then 20 µl of sample was loaded into the concentration gel wells. Electrophoretic migration was performed with a Bio-Rad apparatus (Mini-Protean Tetra Cell, BioRad Laboratories, USA). The migration gel was composed of 4% acrylamide concentration gel and 15% separation gel (Ereifej et al., 2011), was then subjected to 120 V electric current for 2 hours. The gels were stained under agitation for 20 minutes with a solution containing 0.1% (w/w) Brilliant Blue R-250 Coomassie Blue in a 10:40:50 solution of acetic acid, ethanol, and water (v:v:v) and then decolorized for 4 hours in a solution containing 14:10:76 ratio of acetic acid, ethanol, and distilled water (v:v:v). The molecular weights of the different proteins were obtained using protein markers (Promega Corporation, USA) with molecular weights ranging from 10 to 200 kDa, prepared under the same conditions as the samples. Quantification was performed using an appropriate densitometric software, provided by GelQuant.NET (biochemlabsolutions.com).

2.2.3 Sample and emulsion preparation

The β–casein stock solutions (0.1% w/w, corrected for protein content) were prepared by dispersing lyophilized powders in Milli–Q™ water by mechanical stirring (550 rpm) at cold temperature (5-7°C) for 90 min. pH was adjusted to 3.0, 6.0 and 9.0 using either 0.5 M HCl or 0.5 M NaOH. Heat treatment was followed at 65 and 95°C for 15 min; then, treatment was stopped by ice bath to room temperature (23-25°C). Other samples of β–casein solutions were kept at room temperature without heat treatment.

Emulsions were prepared by mixing 5 g of β–casein solutions with 20% w/w of rapeseed oil within 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 SN25-10G ST tool.

2.2.4 Emulsion properties

Emulsion stability and activity indexes (ESI and EAI, respectively) were measured according to the method used in our previous work (Ellouze et al., 2020). EAI and ESI were then calculated using Eqs. (2) and (3), respectively

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

$$\text{ESI}\ (\text{min}) = (\text{A}_0/\Delta\text{A}) \times \text{t} \quad (3)$$

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 10 min (A_0-A_{10}), and t the time interval (10 min).

2.2.5 Emulsion ζ–potential

The 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 and the sampling time was fixed at 400 µs. Data was accumulated from 10 sequential readings at 25°C, and the mathematical model of Smoluchowski was selected (Sze et al., 2003) to convert the electrophoretic mobility measurements into ζ–potential values

2.2.6 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 (1,500 rpm). The Sauter diameter, d_{32}, was used to describe the mean diameter of droplets; this is defined as:

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

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.

2.2.7 Surface properties

Surface hydrophobicity of β–casein proteins treated at different pH and temperature was measured according to the modified method of Alizadeh-Pasdar & Li-Chan (Alizadeh-Pasdar & Li-Chan, 2000), which uses an 8–anilino–1–naphthalenesulfonate (ANS) probe to interact with hydrophobic moieties on protein surface to give a fluorescent signal. Each protein solution was diluted to a final protein concentration of 0.08%. 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(θ)=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 rapeseed oil (10 mL) was poured over it. The measurement time was fixed to 2000 s. 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):

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

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 (Beverung et al., 1999; Wu et al., 1999).

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

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.

2.2.8 Rheological behaviour

Rheological measurements were conducted at 25°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. A flow test was carried out between 0.1 s^{-1} and 1,000 s^{-1} of shear rate. For all measurements, a gap distance was fixed at 1000 µm.

2.2.9 *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 temperature treatment (25°C, 65°C, 95°C), along with their associated interactions, on the physicochemical and emulsifying properties of β–CNC and β–CNB 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):

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

where Y_i is the tested response, a_i are the observed effects, and pH and T 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).

The significance of the effects is expressed in terms of the p-value (p) and is represented in supplementary materials.

3 Results and discussion

3.1 Chemical characterization of the β–casein extracts

Protein extracts of β–CN from cow's milk and camel milk (β–CNB and β–CNC, respectively) obtained according to the described protocol of (Huppertz et al., 2006) were characterized and results are presented. The chemical composition (Table 1) and the protein profile (Fig. 1) of the two obtained extracts (C1 and C4) as well as milk caseins before extraction (C2 and C3) show that the protein contents of the two β–CN extracts are 73% and 51% for camel and cow's milk, respectively. The mineral and the lactose contents (Table 1) are higher for β–CNB than for β–CNC. The extraction yield of β–CN is therefore higher for camel milk considering its high β–CN content compared to cow's milk.

The protein profile (Fig. 1) displays a large band of β–CN protein in both extracts (molar mass of 23~25 kDa). Besides, a slight band appears at the range of 18~19 kDa and is identified to the κ–CN (columns C1 and C4). Table 2 summarizes the different proportions of the extracted proteins (β–CN and κ–CN) which are in order of 87% and 12% for bovine extract (C1) and of 93% and 6% for cameline extract (C4), respectively. Similar results were obtained by Huppertz et al. (2006). The extraction of the bovine β–CN with the described method is, therefore, accompanied by a small percentage of κ–CN. However, at low concentrations of proteins (as in the case of this study: 0.1%), the presence of κ–CN is thus considered negligible.

3.2 Emulsifying properties of the β–casein

The emulsifying activity and stability indexes (EAI and ESI) of the prepared oil-in-water emulsions stabilized by the β–CN extracts of cow's and camel milk at the concentration of 1 g/L are represented in terms of pH and temperature in Fig. 2.

The EAI (Fig. 2A) of β–CN depends on pH and treatment temperature as well as on the milk type. According to ANOVA, **only the effect of pH is statistically significant on camel β–CN ($p \leq 0.01$). Therefore, there is no significant effect of temperature on both types of β–CN where the EAI is almost constant vs. temperature**. However, EAI is higher for the β–CNC than the β–CNB whatever the temperature (maximum values of 122 vs. 98.5 $m^2.g^{-1}$, respectively) at pH 3 and pH 9, whereas the opposite behaviour usually emerges at pH 6 which is close to the isoelectric point of the proteins.

These results are coherent with those of Lee et al. (2004) and Pérez-Fuentes et al. (2017) which described the adsorption of the β–CNB on hydrophobic surfaces and its dependence on the effect of ionic force, including pH. Indeed, the bovine (β–CN, of amphiphilic nature, changes it conformation with high ionic forces, which alters its adsorption kinetics at the interface.

Regarding ESI (Fig. 2B), the average value of emulsions stability is about 15±2 min, except for two β–CNC stabilized emulsions for which ESI is between 30 and 50 min when treated at pH 6.0-65°C and 95°C, respectively, and except for a third emulsion stabilized by β–CNB (ESI about 31 min when treated at pH 9.0-25°C). However, these trends seem to correlate with low EAI values, i.e., emulsions where the oil phase is poorly dispersed. **The effects of pH as well as the temperature are significant only for the emulsions stabilized by camel β–CN ($p \leq 0.001$). At pH values of 3.0 and 9.0, it is noted that stability decreases with the increasing**

treatment temperature of β–CN. This decrease is more pronounced for bovine than cameline β–CN for which stability is affected immediately after homogenization process. While the emulsifying activity (EAI) of treated proteins (65 and 95°C) is independent of the applied thermal treatment (Fig. 2), the emulsifying stability (ESI) gets lower as the applied temperature is higher. This suggests changes in the β–CN conformation either by intermolecular aggregations and associations, or by thermal distortions that diminish their ability to stabilize oil-in-water interfaces. At pH 6.0, a reverse behaviour is observed: an increase in emulsifying stability (ESI) with the increase of the applied heat treatment. This behaviour is more pronounced for β–CNC (from 14.6 to 49.2 min) than for β–CNB (from 14.5 to 16.3 min). This is due to the low activity previously observed for β–CNB and/or to irreversible molecular interactions (denaturation), allowing emulsion stability at these conditions.

3.3 Interfacial properties of β–caseins

In order to understand the mechanisms involved in the emulsification of camel milk β–CN and the differences of molecular structure compared to bovine β–CN, the analysis of surface hydrophobicity and interfacial tension was carried out.

3.3.1 Surface hydrophobicity

To characterise the surface hydrophobicity of the β–CN from camel and cow's milk, the extrinsic fluorescence of the ANS chromophore fixed on its surface hydrophobic amino acid resides after treatment at different pH and temperature was measured. The results are displayed in Fig. 3. **Thus, the surface hydrophobicity of both proteins is greater at pH 3.0 than at other pH (6.0 and 9.0), regardless of the temperature of heat treatment. Nevertheless, the β–CNB are more hydrophobic under these conditions than β–CNC. At pH 6.0 and 9.0, the surface hydrophobicity of β–CNC and β–CNB is almost equal.** ANOVA shows that the pH effect is more significant for the β–CNC than the β–CNB. The effect of temperature is significant only for β–CNC. **This result proves that both types of β–CN have flexible protein conformation that depends on pH and that the β–CNB is relatively more resistant to heat treatment.**

In the literature, it has been reported that the intrinsic hydrophobicity of the β–CNB is usually lower than the β–CNC (Atamer et al., 2017; Barzegar et al., 2008; Esmaili et al., 2011),

which does, however, not contradict our results. **This should be due to the presence of five residues of Tyrosine and eight residues of Phenylalanine, mainly located in the hydrophobic part. However, the primary structure of β–CNC is devoid of Tryptophan residues, while β–CNB contains a Tryptophan residue, although four Tyrosine residues are missing.** In addition, intrinsic fluorescence due to these residues was shown to be more important at a neutral pH (7) than at lower pH (5). Consequently, the 3D conformation of the two proteins is different**, but the exposure of the hydrophobic parts on their surfaces is also less important for β–CNC. This means that the β–CNC is more protective against a change in pH (hidden hydrophobic residues), but not vs. temperature (Barzegar et al., 2008; Li et al., 2016, 2019) for which this structure is relaxed, exposing higher surface hydrophobicity after intensive heat treatment (95°C) and especially at acid pH (3.0). At pH above the isoelectric point (pH 6.0 and 9.0) where electrostatic charges are negative, the measured surface hydrophobicity is temperature-independent, and this is similar for both types of proteins, which agrees with the data of this work.**

3.3.2 Surface pressure

The study of the interfacial tension of β–CN at the oil-water interface was carried out and the results expressed in term of surface pressure are represented in Fig. 4A and 4B for camel and bovine proteins respectively.

Surface pressure depends on protein's type as well as on pH and treatment temperature. For the β–CNC solutions (Fig. 4A), surface pressure is greater than for the β–CNB (2.45 and 1.87 mN.m, respectively), *i.e.* the protein extract from camel milk reduces the interfacial tension between water and rapeseed oil more than the cow's milk extract. This reduction is greater at pH 3.0-25/95°C and at pH 9.0-95°C for β–CNC; the effects of pH and temperature are statistically significant ($p < 0.001$) for β–CNC, but for β–CNB, the influence of pH and temperature is reduced and more complex.

The results also show that the increase in temperature affects the interfacial properties of β–CN. This effect is more pronounced at 25°C and 65°C for the β–CNB where the reduction in interfacial tension between the two phases (oil/water) is greater than at high temperature (95°C). This agrees with the evolution of emulsifier activity. Although the EAI (Fig. 2) of the β–CN is not influenced by thermal treatment, stabilization of emulsified oil droplets (ESI) with β–CNB is higher at 25°C where proteins retain their native

structure (no thermal denaturation), which allows intramolecular hydrophobic interactions and thus the maintenance of a stable protein film around the oil droplets. For β–CNC, surface pressure is higher at pH 3.0 after high heat treatment (95°C). This result is consistent with the previously measured surface hydrophobicity (Fig. 3). Hydrophobic interactions and relaxed structure allow proteins to be more cohesive under the applied treatments.

3.4 Characterization of the β–caseins stabilized emulsions

Oil-in-water emulsions stabilized by the bovine and cameline β–CN were characterized in terms of surface charge, droplet size distribution, and apparent viscosity.

3.4.1 Surface charge

The ζ–potential (Fig. 5A and B), which reflects the surface charges of the oil droplets emulsified by β–CNC and β–CNB, does not exceed -30 mV for both types of proteins. The electrostatic forces, which maintain the relative stability of the emulsion, as described above, remain weak. The pH effect is significant for both types of β–CN, but not the temperature effect. However, the interaction effect of both factors ($pH \times T$) is statistically significant for the β–CNB emulsions.

For β–CNC stabilised emulsions (Fig. 5A), the ζ–potential is maximum at pH 9.0 for the different temperature. Besides, at pH 6.0 the ζ–potential is lower than at pH 3.0 especially after heat treatment (65°C and 95°C). This result, as well as that on the emulsifying activity index (Fig. 2A), reveals that surface charges are the most involved in the protein adsorption at the oil-water interface. **The electrostatic interactions between the charged motes of the β–CN proteins at their relaxed micellar structure induce high emulsifying activity (EAI). The micellar structure is therefore, formed at higher protein concentration (0.1% w/v) than the critical micellar concentration (CMC 0.05% w/v); then, it is relaxed by the effect of a high pH (9.0).** These findings were also described by Pérez-Fuentes et al., (2017) for the adsorption of bovine β–CN on hydrophobic surfaces.

For the β–CNB stabilized emulsions (Fig. 5B), the ζ–potential decreased with increasing pH from -13 to -27.5 mV for untreated proteins (25°C) but increased for treated proteins from -29 to -18.8 mV. These results are close to those reported by McCarthy et al. (2013) for an emulsion stabilized by β–CNB at 2% (w/w) concentration. These authors showed that the ζ–potential

was estimated to be ~ -30 mV at pH > 6 and ~ -20 mV at pH 5 (McCarthy et al., 2013). Therefore, the impact of temperature causes a reversal evolution of these results. **Thus, heat-treated β–CNB proteins lose their surface charges and adopt a compact structure. Protein interactions and aggregation are mainly the cause of the previously observed decrease in emulsifying stability (ESI).** These trends are primarily due to differences in the structure and amino acid composition of the two studied proteins (Lam & Nickerson, 2013).

3.4.2 Emulsion granulometry and microscopic observations

The emulsion characterisation was also achieved by granulometry measurements of both types of β–CN stabilised emulsions. The size distributions of the oil droplets stabilised by β–CNB and β–CNC are bimodal (Fig. 6A and 6B, respectively). Two major populations of droplets are then present; the first one (small droplets) with maximum size of 2 µm peaks at about 1% of the emulsion volume; the second (large droplets) is centred around 35 µm in size and peaks up to 13% in volume. The size droplet homogeneity on microscopic observations of the β–CNC (Fig. 6A) emulsions is lower than the β–CNB (Fig. 6B) in terms of the different pH and temperature values. It also emerges from the micrographs that emulsions stabilized by β–CNC are finer at pH 3 and pH 9, but not at pH 6, than those stabilised by β–CNB. **These observations reveal important flexibility of the β–CNC vs. the operating conditions by allowing them to adopt different conformations and configurations at the oil-water interface. At acidic pH (3.0), the fraction of large droplets increases slightly with the temperature of heat treatment. Similarly, at pH 9.0 where the droplets size is the smallest for the unheated proteins (25°C), this fraction increases more significantly after heating.** Nevertheless, at pH 6.0, the highest volume of large droplets is observed at 25°C with a peak about 56 µm where the proteins are at their compact micellar shape. **Otherwise, the droplets size decreases after heat treatment due to the dissociation of their micellar structures. For β–CNB stabilised emulsions, droplet size also increases with temperature.**

In parallel, the Sauter diameter (d_{32}) of the β–CNC stabilised emulsions (Fig. 6C) at pH 6.0 display the highest values. While for the β–CNB (Fig. 6D) stabilised emulsions, the highest values are observed at pH 3.0, which confirms microscopic observations. The effect of pH is more significant for the β–CNB than the β–CNC, as well as the effect of the interaction ($pH \times T$) (Table 1). Microscopic observations of both types of emulsions highlight also that at high temperature, oil droplets stabilized by β–CNC (Fig. 6A) are closer in terms of size (low

span), particularly at pH 9.0 where the smallest size is observed after high heat treatment (95°C), which confirms the temperature effect previously discussed.

Finally, β–CNC seem to be more efficient to prepare fine emulsions under acidic and alkaline conditions, whereas β–CNB must be preferred at neutral pH.

3.4.3 Emulsion viscosity

The rheological behaviour of the different prepared emulsions was evaluated, and the viscosity measurements are represented in Fig. 7. Both types of emulsions represent a non-Newtonian flow, as the viscosity is higher at low shear rates and decreases at high shear rates (where it remains constant). For the β–CNC (Fig. 7A), viscosity varies between 5 and 65 mPa.s while those of β–CNB (Fig. 7B) varies between 3 and 330 mPa.s. This latter high viscosity is due, first, to hydrophobic forces of protein's surface at acid pH (Fig. 3). Besides, it was reported (Ellouze et al., 2019; Maldonado-Valderrama et al., 2008; Seta et al., 2014) **that the β–CNB proteins adsorb rapidly to the oil-water interface compared to globular proteins due to its flexible and random molecular conformation.** Moreover, the β–CNB emulsions viscosity is closely related to the oil droplets size. In such ranges, as reported previously (Ellouze et al., 2019, 2020), the larger the droplets are, the higher viscosity is, resulting from cohesion forces between droplets.

Similarly, for β–CNC (Fig. 7A) stabilized emulsions, despite the lower viscosity in comparison with the β–CNB, such a correlation is observed, where at low shear rate, viscosity is relatively higher at pH 6.0 due to the low emulsifying activity earlier discussed (section 3.2), which results in bigger droplets at pH 3.0 due to the high surface hydrophobicity (Fig. 3).

4 Conclusion

The investigation of the emulsifying properties of camel milk β–casein allowed to identify the main factors acting on the formation and the stabilization of oil/water interfaces. The comparison with bovine protein showed important discrepancies explained mainly by differences in molecular composition and structure. The presence of phospho-serine residues in camel β–casein provides the thickness and steric stabilizing properties of the absorbed layer surrounding the oil droplets. This protein shares some main characteristics with bovine β–casein in terms of number of hydrophobic residues, surface hydrophobicity plots and number of serine and threonine. Nevertheless, the resulting emulsifying properties has shown its ability

to adopt a micellar structure in such concentration ranges. The consequences are that camel milk β−caseins present an enhanced ability to form softer emulsions and to stabilize small droplets under acidic conditions, regardless of heat treatment as well as at pH 9 after heat treatment compared to bovine β−casein.

The stabilization mechanisms highlighted in this study will allow a wider exploitation of camel β−casein according to the treatment conditions in various pharmaceutical, food or cosmetic applications.

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