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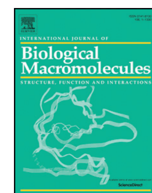
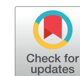


Antioxidant and antibacterial activities, interfacial and emulsifying properties of the apo and holo forms of purified camel and bovine α-lactalbumin



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ABSTRACT

The antioxidant and antibacterial activities of camel and bovine α-lactalbumin (α-La) in both calcium-loaded (holo) and calcium-depleted (apo) forms were investigated and compared. Antioxidant assay showed that camel and bovine α-La exhibited significant Ferric-reducing antioxidant power (FRAP), ferrous iron-chelating activity (FCA) and antiradical activities especially in their apo form. Camel apo α-La also exhibited attractive antibacterial activities against Gram-negative bacteria (*Pseudomonas aeruginosa*) and against fungal pathogens species (*Penicillium bilaiae*, *Aspergillus tamari* and *Aspergillus sclerotiorum*). Likewise, emulsifying properties (emulsification ability (EAI) and stability (ESI) indexes) and the surface characteristics (surface hydrophobicity, ζ-potential and interfacial tension) of the α-La were assessed. Maximum EAI were found at pH 7.0, with higher EAI values for the camel apo α-La (EAI ~19.5 m^2/g). This behavior was explained by its relative high surface hydrophobicity and its greater efficiency to reduce the surface tension at the oil-water interface. Furthermore, emulsions were found to be more stable at pH 7.0 compared to pH 5.0 (ESI ~50%) due to the higher electrostatic repulsive forces between oil droplets at pH 7.0 in consistence with the ζ-potential results. This study concluded that the camel apo α-La has antibacterial, antioxidant, and emulsifying properties in agricultural and food industries.

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1. Introduction

Camel milk is an important product consumed in both raw and fermented forms and provides all the essential nutrients for populations in hot regions of Middle East and Africa [1]. This milk is known to have better functional value when compared to bovine milk due to its therapeutic properties including anti-diabetic hypo-allergic and anticancer properties [2].

Camel milk differs from bovine milk in its physico-chemical composition and the structure of its proteins leading to different biological and techno-functional properties [3,4]. As for other mammalian milks, camel milk proteins can be classified into two major fractions: caseins and whey proteins representing respectively 75.4% and 25.5% (w/w) of the total camel proteins [5].

In bovine whey, the main proteins are the β-lactoglobulin (β-Lg), α-lactalbumin (α-La), serum albumin and the immunoglobulins, representing 55%, 24%, 15% and 5% (w/w), respectively [6]. Compared with bovine whey, camel whey is claimed to be devoid of β-Lg which has been demonstrated to be one of the most dominant cow milk allergen limiting cow's milk consumption and the preparation of infant formulae [7].

Thus, the α-La is the main protein fraction of camel whey, accounts for ~18.8% (w/w) of the total camel milk proteins with a concentration of 2.2 g/L [8–11]. The α-La is a globular small, acidic and hydrophilic calcium metalloprotein present in the soluble fraction of milk of all mammalian species [12]. The main biological function of this protein is to participate in lactose synthase (LS) complex [13]. As the bovine α-La, camel α-La is composed of 123 amino-acid residues, in which 39 residues are different when compared to its bovine counterpart. Consequently, the similarity and identity between these proteins according to the sequence alignment data are 82.9% and 69.1%, respectively [11,14,15].

Furthermore, camel α-La has a molecular weight (MW) of ~14.43 whereas its bovine counterpart has a MW of 14.18 kDa. Its isoelectric

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point (pI) is between 4.1 and 4.8, and therefore in milk (pH ~ 6.5) it is negatively charged [14].

Both α-La have no free thiol groups and 4 buried disulfide bonds with cysteine residues at the same positions (Cys6/Cys120, Cys28/Cys111, Cys61/Cys77, and Cys73/Cys91). Whereas, the conformation of camel α-La shows a higher sensitivity and to the calcium loss and a greater thermal stability when compared to its bovine equivalent [15].

In addition to structural differences between both proteins, camel α-La showed a greater level of digestibility with both enzymes chymotrypsin and trypsin as well as a highest antioxidant activity when compared to the α-La of cow milk [16]. The foaming properties of camel α-La were studied by Lajnaf et al. [17]. No significant difference of the foaming and interfacial properties (at the air-water interface) was found between camel and bovine α-La at neutral pH. On the other hand, these properties were found to increase considerably at acid pH (pH 4.3) where the α-La assumes the molten globular state, thus enhancing its foaming properties [11]. However, only few works exist on the emulsifying and interfacial properties of camel α-La and information on their interfacial behavior depending on pH value will be interesting to determine [18,19].

Most importantly, the paper is dedicated to investigate the differences between camel and bovine α-La in calcium-depleted (apo) and calcium-saturated (holo) states in comparative antioxidant, antimicrobial and emulsifying studies. Antioxidant and antimicrobial activities of the α-La were examined by various *in vitro* assays. Thus, the examination of the biological activities and emulsifying properties of the camel α-La revealed an interesting ingredient for food industry due to its nutritional value, which is of a great scientific and industrial relevance.

2. Material and methods

2.1. Materials

Camel milk samples (*Camelus dromedarius*) used in this work were purchased from a modern camel farm in the south of Tunisia. Fresh cow milk was purchased from a local breeding which is located in the region of Sfax (Tunisia).

Once purchased, both samples were immediately cooled to 4 °C and transported to the laboratory within 24 h. pH values were directly measured (744-pH meter, Metrohm, Herisau, Switzerland) after collecting milk and before storing. The fat was removed by centrifugation (Thermo Scientific Heraeus Megafuge Centrifuge, Germany) at 3000g for 20 min at 4 °C [20] and the skimmed milk was stored at −18 °C before experiments.

2.2. Purification of camel α-La

After defatting milk, the casein fraction of camel milk was separated from the soluble fraction by rennet addition (1.4 mL/L of milk) at 37 °C during 1–2 h [21,22].

The coagulum was centrifuged at 5000*g* for 15 min at 20 °C (Thermo Scientific Heraeus Megafuge Centrifuge, Germany), the curd containing the camel caseins was discarded and the supernatant representing the remaining liquid whey, known as the sweet whey, was kept for the α-La purification. Afterwards, the camel α-La was isolated by ultrafiltration (UF) membrane technology using a tangential filtration mode (Millipore UF systems). A Labscale™ TFF System (USA) with MW cut-off regenerated cellulose membranes of 30 kDa (Millipore Coroperation, Billierca, MA) was applied to separate the α-La from the other camel whey proteins [11,16].

Bovine α-La was isolated from skimmed bovine milk using the method described by previous studies [23,24]. Once isolated, both camel and bovine α-La samples were lyophilized (Bioblock Scientific Christ ALPHA 1–2) for further analysis.

The purity of the isolated α-La was checked by RP-HPLC (Reverse Phase High-Performance Liquid Chromatography) (Agilent 1260 Infinity quaternary LC, Germany) [4,25] using a separation column C18 (Zorbax Eclipse Plus C18, 250 mm length x 4.6 mm, particle size 5 μm, Packing Lot: B14292).

Overall, 500 μL of the isolated protein were added to 3.7 mL of the solvents A and B in 70:30 ratio (v/v) as follows: solvent A (acetonitrile, water, and trifluoroacetic acid in a ratio 100:900:1, (v/v/v)) and solvent B (acetonitrile, water, and trifluoroacetic acid in a ratio 900:100 (v/v/v)). 20 μL of the filtered mixture (through 0.45 μm nylon filter) were then injected into the column. Once the sample was injected, a gradient was immediately generated by increasing linearly the proportion of solvent B as function of time from 20% to 46% in 40 min for the elution at a flow rate of 1.0 mL/min and at a column temperature of 25 °C. Protein signal was recorded by UV detection at 220 nm wavelength.

Purchased standard (bovine α-La from Sigma Aldrich) was dissolved in deionized water (from Milli-Q system, Millipore, USA) and then diluted in solvent A and solvent B mixture (70:30) as samples separately.

The purity of the isolated α-La was assessed by calculating the area of their peaks in each chromatogram and expressed as percentage of total protein.

2.3. Preparation of apo and holo forms of the α-La

Lyophilized α-La samples were dissolved in deionized water (Milli-Q system, Millipore, USA) at a protein level of 1 g/L. Apo and holo α-La forms were generated by dissolving the native purified protein (1 g/L for protein content) in 20 mM-Tris-HCl buffer at pH 7, containing 3.5 mM-EDTA (Sodium ethylene diaminetetraacetate) and 2 mM-$CaCl_2$, respectively [15,26]. The EDTA-treated α-La samples were then kept under agitation during 60 min at room temperature (21–23 °C) to make sure all bound calcium ions were removed.

The pH of the α-La solutions was adjusted from the initial, to 5.0 or 7.0, using either 1 M HCl or 1 M NaOH [27].

2.4. Antioxidant activities

2.4.1. DPPH assay

The antiradical activity of the purified α-La protein in its holo and apo forms was carried out by the DPPH (1,1-diphenyl-2-picrylhydrazyl) method as described by Bersuder et al. [28]. A volume of 500 μL of each α-La sample at a concentration of 1 g/L was added to 375 μL of ethanol (99%) and 125 μL of DPPH solution 0.02% in ethanol (w/v) as free radical source.

The mixture was shaken under dark during 60 min and the absorbency was then measured at 517 nm using a UV mini 1240, UV/VIS spectrophotometer (SHIMDZU, Kyoto, Japan). Ethanol and BHA (butylated hydroxyanisole) were used as blank and positive control, respectively. DPPH-radical-scavenging-activity was calculated using the equation Eq. (1) and expressed as percentages:

$$DPPH-radical-scavenging-activity=((C+B-S)/C)\times 100 \qquad (1)$$

where C (control) is the absorbance of 500 μL of water, 125 μL of DPPH-ethanol solution and 375 μL of ethanol at $\lambda = 517$ nm; S (sample) is the absorbance of 500 μL α-La sample added to 375 μL of ethanol and 125 μL of DPPH-ethanol solution at $\lambda = 517$ nm, and B (blank) was the absorbance of 500 μL sample extract and 500 μL ethanol at 517 nm.

2.4.2. Ferric-reducing antioxidant power (FRAP)

The FRAP (ferric-reducing antioxidant power) method was assessed according to the procedure described by [29,30]. Briefly, 900 μL of FRAP reagent (containing 300 mM acetate buffer, pH 3.6, 20 mM $FeCl_3$ solution and 10 mM TPTZ (2,4,6-Tripyridyl-s-Triazine) in 40 mM HCl and in a ratio 10:1:1) were mixed with 100 μL of the α-La sample. The mixture was incubated at 37 °C during 20 min. The absorbance was read at $\lambda = 593$ nm using a UV mini 1240, UV/VIS spectrophotometer (SHIMDZU, Kyoto, Japan).

The FRAP was calculated and expressed as micromoles of Trolox equivalent (TE) per g of protein sample (μM-TE/g) using a standard curve of Trolox (0–50 μM).

2.4.3. Ferrous iron-chelating activity (FCA)

The chelating activities of the α-La sample towards Fe^{2+} were measured by the methods of Boyer and McCleary [31] modified by Al-Shamsi et al. [32]. Briefly, 4.7 mL of each protein sample (1 g/L) were added with 0.1 mL of 2 mM $FeCl_2$ and 0.2 mL of 5 mM ferrozine (3-(2-pyridyl)-5,6-bis(4-phenyl-sulphonic-acid)-1,2,4-triazine). The mixture was then incubated during 20 min at room temperature and the optical density was measured against a blank (without protein) at λ = 562 nm.

The ferrous chelating activity (FCA) exhibited by the α-La sample was calculated using the following equation Eq. (2):

$$FCA\ (\%) = \left[1-\left(A_{sample}/A_{blank}\right)\right] \times 100 \tag{2}$$

where A_{sample} and A_{blank} are the absorbance of the α-La sample and control (without protein) reaction respectively at λ = 562 nm.

2.5. Detection of antimicrobial activities

2.5.1. Antibacterial activity

Two Gram-positive (*Enterococcus faecalis* ATCC 25912 and *Staphylococcus aureus* ATCC 25923) and two Gram-negative (*Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922) bacteria were selected for the bacteria test in this work. LB (Luria Bertani) agar plates were inoculated with 100 μL of an overnight culture containing approximately 10^7 CFU (Colony Forming Units)/mL of the respective indicator strain.

100 μL of the culture suspension containing approximately 10^7 CFU/mL were spread over the LB agar plates. The α-La solutions (1 mg/mL) were delivered into wells (5 mm diameter) which are cut into the agar plates [33]. Diffusion was carried out at 4 °C for 2 h. Afterwards, the plates were incubated during 24 h at 37 °C and the inhibition zones were examined. The antibacterial activity of the α-La was evaluated by measuring the diameter (in millimeters) of the inhibition zone around the wells.

2.5.2. Antifungal activity

Antifungal activity of the α-La was evaluated against 4 fungal pathogens species including *Aspergillus sclerotiorum* (MG204869.1), *Aspergillus protuberus* (MH137674.1), *Aspergillus tamarii* (KY828882.1) and *Penicillium bilaiae* (MF681615.1). A volume of 100 μL of each fungal suspension (containing approximately 10^6 conidia per mL) was spread on the surface of the Petri dishes. Wells (5 mm diameter) were punched and filled with 100 μL of the α-La solutions (1 mg/mL). Plates were then incubated at 25 °C during 5 days. The antifungal activity was evaluated by measuring the diameter of inhibition zones around the wells.

2.6. Emulsifying properties

A volume of 20 mL (85%, v/v) of the different α-La samples (1 g/L, pH 5 or 7) was homogenized with 3.5 mL (15%, v/v) of corn oil for 30 s at room temperature using the Ultra-Turrax T25 high-speed mixer (IKA Labortechnik, Germany) performed at 21500 rpm [34]. Afterwards, an aliquot of each freshly created emulsion (100 μL) was pipetted and dispersed into 900 μL of 0.1% SDS (sodium dodecyl-sulfate) solution (w/v). The SDS-emulsion mixture was vortexed during 10 s and the absorbance was measured at λ = 500 nm using a UV mini-1240 PC spectrophotometer.

Emulsions were kept undisturbed and then 100 mL aliquots were taken after 10 min and dispersed into 900 μL of 0.1% (w/v) SDS solution. The absorbance was also measured at λ = 500 nm as described above.

Emulsifying activity index (EAI, m^2/g) and emulsion stability index (ESI, %) of α-La solutions were determined using Eqs. (3) and (4) suggested by Pearce and Kinsella [35]:

$$EAI\ (m^2/g) = [2 \times 2.303 \times A_{500} \times dilution]/\left[C \times (1-\Phi) \times 10^4\right] \tag{3}$$

$$ESI\ (\%) = [A_{10}/A_0] \times 100 \tag{4}$$

where, A_{500} represents the absorbance of the diluted emulsion at 500 nm, C is the α-La concentration (g/mL), Φ is the oil fraction volume (Φ = 0.15), 100 is the dilution, A_0 and A_{10} represent respectively the absorbance at 500 nm at time zero and after 10 min.

2.7. Interfacial properties

The interfacial tension for camel and bovine α-La solutions was measured using a TSD (Tensiometry System Digital) 971 (Gibertini Elettronica, Italia) equipped with the "Du Noüy methodology" as described in previous works [27,36]. All α-La protein samples were measured at a concentration of 1 g/L.

First, 20 mL of the protein solution were added within a 40 mm diameter glass sample beaker. Then, Du Nüoy ring (20 mm diameter) was immersed followed by the addition of upper corn oil layer (20 ± 0.5 mL). The ring was pulled upwards to stretch the created oil-water interface to calculate the interfacial tension value (mN/m) by determining the maximum force (F_{max}) using the following Eq. (5)

$$\gamma = F_{max}/4\pi R\beta \tag{5}$$

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

2.8. Determination of the hydrophobicity

The surface hydrophobicity of the isolated α-La was measured using the method described by Al-Shamsi et al. [32] and Lajnaf et al. [34]. Briefly, 1 mL of the α-La sample (at a concentration of 1 mg/mL) and 200 μL of 1 mg/mL bromophenol-blue (BPB) were added and kept under agitation during 10 min at room temperature, followed by centrifugation at 2000 *g* for 15 min at 25 °C. The absorbance of supernatants (diluted 1:10 in distilled water) was read at λ = 595 nm using an UVmini-1240 spectrophotometer. A control was prepared using Tris-HCl buffer (20 mM, pH 8.0) instead of protein sample.

The surface hydrophobicity was determined from bound-BPB amounts by Eq. (6):

$$\text{Bound}-\text{BPB}\ (\mu g) = [(\text{Absorbance of control}-\text{Absorbance of sample})/\text{Absorbance of control}] \times 200\ \mu g \tag{6}$$

2.9. Electrical charge (ζ-potential measurements)

The ζ-potential of the camel and bovine α-La solutions was determined at 25 ± 1 °C using a Zetasizer Nano-ZS90 (Malvern Instruments, Westborough, MA). All α-La samples were measured at a concentration of 1 mg/mL.

The ζ-potential (ζ, mV) was evaluated from the electrophoretic mobility (U_E) using Henry's equation (Eq. (7)):

$$U_E = 2\varepsilon\zeta f(k\alpha)/3\eta \tag{7}$$

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

2.10. Optical microscopy of the emulsion

One milliliter of the created emulsion was taken and diluted with 9 mL of 0.1% SDS (w/v). Emulsions were vortexed during 10 s in a Falcon tube [9]. Afterwards, 100 μL were pipetted from the diluted emulsion and placed between lame and lamella to be an optical microscope (Nikon Eclipse E400, Kanagawa, Japan) with a 40× objective magnification and connected to digital camera.

2.11. Statistics

The significance of the main effects of type (apo and holo) and pH (5.0, 7.0) on camel and bovine α-La emulsifying properties (EAI and ESI indices), surface characteristics (ζ-potential, surface hydropohobicity and interfacial tension measurements), antioxidant and antimicrobial activities was tested by three-way analysis of variance (ANOVA). Statistical analyses were performed with IBM-SPSS software (Version 19). All experiments were carried out at least in triplicate and results were reported as the mean ± one standard deviation.

3. Results and discussion

3.1. Purification of camel and bovine α-La

The HPLC chromatograms of solutions containing the isolated α-La from camel and bovine milk are shown in Fig. 1a and b respectively.

The camel α-La percentage increased from ~18.8% in camel milk to ~91.2% after protein isolation (Fig. 1a). On the other hand, the purity of bovine α-La percentage rose from ~4.7% before purification (in cow milk) to ~89.3% after purification (Fig. 1b).

These percentages of the protein purity are consistent with the work of Muller et al. [23], Lajnaf et al. [11] and Salami et al. [16]. For the camel α-La chromatogram (Fig. 1a), a protein fraction protein (F) with a retention time (RT) 18.77 min was still present after the protein purification at a percentage of 8.8%. This peak could correspond to casein traces or the Peptidoglycan Recognition Protein (PGRP) which is a specific whey protein of camel milk [11,37]. As also seen in Fig. 1b, two protein fractions with RT of 26.08 min and 30.23 min still remained after bovine α-La purification. These protein fractions were suggested to be identified as caseinomacropeptide (RT 26.08 min) and the β-Lg (RT 30.23 min) representing ~5.8% and ~4.9% respectively of the total protein isolate.

3.2. Antioxidant properties

3.2.1. DPPH free radical-scavenging activity

The DPPH radical scavenging activity is most frequently utilized assay to measure the overall antioxidant activity of food ingredients and processed food products. The DPPH radical scavenging activity of the isolated camel and bovine α-La are shown in Table 1.

The DPPH rates (in %) found for camel α-La were 25.16 ± 2.84% and 37.33 ± 4.03% for the holo state and apo state, respectively, while for bovine α-La, values of 15.08 ± 1.50% and 28.16 ± 2.82% were obtained for the holo and apo states, respectively. Thus, Camel α-La exhibited significantly greater antioxidant activity when compared to bovine α-La in both holo and apo states ($p < 0.05$).

Furthermore, significant improvement in DPPH radical scavenging activity was observed in apo form as compared to the holo form in both camel and bovine α-La, with the highest activity in apo camel α-La ($p < 0.05$). These findings are consistent with those of Salami et al. [16] who found that camel α-La carried higher antioxidant activity rather than its bovine counterpart in both native and molten globular states. Overall, proteins have antioxidant activity through amino acid residues including cysteine, tryptophan and methionine. These residues are involved in free radical scavenging in proteins as they possess the highest antioxidant activity compared to the other amino acids [38]. Furthermore, not only the amino acid composition of proteins is important in scavenging the free radicals but also their positioning and their accessibility [39].

Sequence comparison of both α-La shows higher amount of these amino acids of camel α-La in agreement with [14,16]. Thus, the highest DPPH activity of camel α-La in both apo and holo states can be explained the higher content of the solvent-exposed amino acids available for scavenging of free radicals and the different conformational features between both α-La especially after calcium removal.

3.2.2. Ferric-reducing antioxidant power (FRAP)

Another method for measuring the antioxidant potential of the purified camel and bovine α-La is the ferric-reducing antioxidant power: FRAP assay. Ferric-reducing power of a protein is an estimation of its ability to reduce Fe^{3+} to Fe^{2+} by electron-donation from electron-rich amino acid side chains. This measurement is determined using a redox-linked colorimetric reaction. Samples with higher FRAP values are more able to donate electrons or hydrogen and to interrupt free radical chain reactions [40].

As shown in Table 1, FRAP values of the α-La samples were significantly improved after calcium removal. Thus, the calcium-depleted apo-form of α-La samples showed a significantly higher FRAP value when compared with their holo counterparts regardless of the α-La origin ($p < 0.05$). FRAP value of camel apo α-La (11.74 ± 0.32 μM-TE/g of proteins) was significantly higher rather than bovine apo α-La (10.41 ± 0.23 μM-TE/g of proteins), but no significant differences were observed between camel and bovine holo α-La (~7.5 μM-TE/g of proteins).

The FRAP value of purchased bovine α-La in previous studies was found to be similar to that of purified bovine α-La in our study. It achieved a value of 8.19 ± 1.19 μM-TE/g of proteins [41].

Furthermore, after the removal of calcium bound ion, the α-La has been found to change in conformation to a more open structure [27]. Possibly, this structure could enhance reducing activity towards ferric

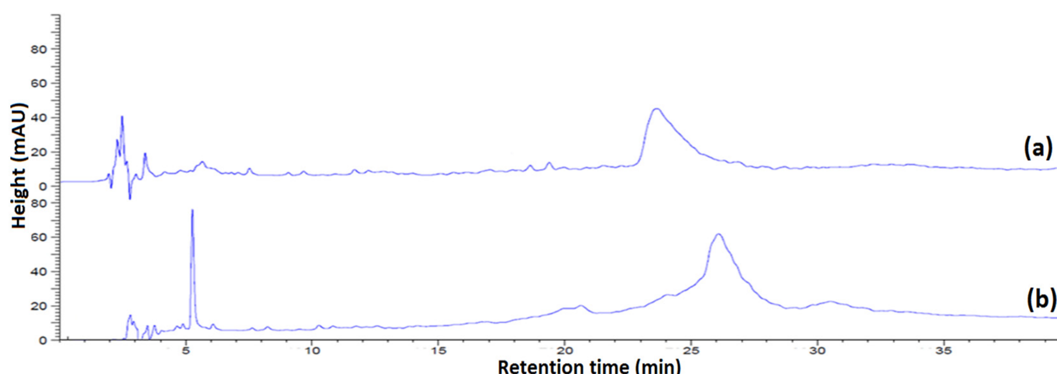

Fig. 1. HPLC chromatograms recorded at 220 nm for purified camel and bovine α-La (chromatograms a and b, respectively).

Table 1
Antioxidant properties: DPPH (DPPH-radical-scavenging-activity), The FRAP (ferric-reducing antioxidant power) and FCA (ferrous chelating activity) of camel and bovine apo and holo α-La.

Protein	α-La form	FRAP (μM-TE/g of protein)	DPPH (%)	FCA (%)
Bovine α-La	Holo α-La	7.31 ± 0.21^a	15.08 ± 1.50^a	13.58 ± 7.11^a
	Apo α-La	10.41 ± 0.23^b	28.16 ± 2.82^b	68.40 ± 5.56^b
Camel α-La	Holo α-La	7.24 ± 0.50^a	25.16 ± 2.84^b	15.21 ± 3.12^a
	Apo α-La	11.74 ± 0.32^c	37.33 ± 4.03^c	81.27 ± 4.55^c

a-c Different letters in the same column indicate significant differences ($p < 0.05$) between protein fractions.

ions, and suppress their pro-oxidant effect. The open structure of both α-La have then higher FRAP values, due to their exposed electron-rich side chains.

3.2.3. Ferrous iron-chelating activity (FCA)

Ferrous iron-chelating activity (FCA) is among the most important antioxidant properties. This activity involves studying and determining the ability of the tested compound to chelate the metal ions. Among metal ions, ferrous ions (Fe^{2+}) are associated with cells oxidative damage through the generation of hydroxyl radical which contributes to oxidative stress and results in lipid peroxidation [42]. As depicted in Table 1, no significant difference was observed between Fe^{2+} chelating capacity values of holo camel and bovine α-La (FCA ~14%). Whereas, when calcium was depleted, FCA values of both α-La significantly increased with higher values for the camel α-La (FCA ~81.27%) ($p < 0.05$). The increase in FCA values of α-La, after calcium removal, could be due to increased exposure of certain aromatic and hydrophobic amino acid residues, which actively participated in metal chelation [32]. Higher metal-chelating activity of proteins is attributed to the better accessibility of the antioxidant amino acid residues to the metal ions [43].

α-La has two chelating sites for minerals: the first one binds Ca^{2+} and the second is specific for Zn^{2+}, but it is able to bind Co^{2+} and Cu^{2+} [44]. Baumy and Brule [45] noted that the α-La has a high affinity to several bivalent cations such as Ca, Mn, Zn, Cu, Mg and Fe. Indeed, the binding ability of α-La is 6.0 of Fe^{2+} ions per protein molecule at pH 6.6.

3.3. Antimicrobial activities

Antimicrobial activity results of camel and bovine α-La are presented in Table 2. The bovine α-La (holo and apo forms) and camel holo α-La had no bactericidal activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa* and no antifungal activity against *Penicillium bilaiae*, *Aspergillus tamari*, *Aspergillus sclerotiorum* and *Aspergillus protuberus*.

The same trends were reported for bovine α-La by Håkansson et al. [33] and Sedaghati et al. [46] who found that native α-La exhibited no antimicrobial activity on Gram-positive and Gram-negative bacteria.

On the other hand, the analyzed apo camel α-La, exerted significant antibacterial activities towards *Pseudomonas aeruginosa* with inhibition zones of 15 ± 0.5 mm. Furthermore, apo camel α-La exhibited antifungal action on *Penicillium bilaiae*, *Aspergillus tamari* and *Aspergillus sclerotiorum* with inhibition zones of 9 ± 1.2, 16 ± 0.8 and 15 ± 0.5 mm, respectively.

In the same way, Svensson et al. [47] reported that the monomeric α-La is inactive, whereas, it could be converted to the active form under different conditions as calcium removal. Håkansson et al. [33] confirmed that human α-La must be in its apo state in order to convert to the active form. They found that EDTA-treated apo α-La was active against *Streptococcus pneumoniae* at a concentration of 0.5 mg/mL. However, apo camel α-La had non-inhibitory effect on *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Aspergillus protuberus*.

In addition to its antimicrobial activities, recent studies have shown that camel α-La is even able to bind oleic acid leading to the creation of a complex (named CAMLET: camel α-La made lethal to tumor cells). This complex exerted a potent anticancer activity against four cancer cell lines particularly breast cancer cells through the induction of selective apoptosis and causing arrest of the cell-cycle [48]. On the other hand, both camel and bovine α-La in their complexes with oleic acid are characterized by reduced tertiary structure and high levels of native secondary structure [49]. However, camel α-La was shown to be more disordered and possessed stronger aggregation propensities when compared to bovine α-La. The structural differences between the camel and bovine α-La were preserved and in some cases increased in their oleic acid complexes, which increased in its lethality against cancer cells [49,50].

3.4. Emulsifying properties

The EAI measures how well the tested proteins can coat the surface of an oil droplet within a dilute emulsion, while the ESI gives an estimate of the emulsion's relative stability after a pre-determined time [35].

EAI and ESI values of the purified α-La as a function of the milk origin (bovine and camel milk), pH value (5.0 and 7.0) and conformational states (apo and holo states) at a protein concentration of 1 g/L are shown in Fig. 2. The electronic microscopy images of the created emulsions are shown in Fig. 3. Thus, findings indicated that α-La proteins at pH 7.0, coated the oil-droplets better than those at pH 5.0 with higher EAI values of apo camel α-La proteins especially camel one (EAI ~19.5 and 17.2 m^2/g for apo camel and bovine α-La, respectively at pH 7.0) (Fig. 2a). Fig. 3 showed also that the oil-droplet diameter visually decreased with the increase of pH level during emulsion preparation. On the other hand, in acidic conditions, better emulsification activity values were obtained with the bovine α-La when compared to its camel counterpart in both apo and holo states.

Statistical analyses showed that camel α-La experienced a much larger magnitude difference in EAI values ($p < 0.05$). For instance, camel α-La increased from 6 m^2/g at pH 5.0 in both holo and apo states to 15.8 and 19.5 m^2/g at pH 7.0 (difference of 9.8 and 13.5 m^2/g for camel holo and apo α-La, respectively), whereas bovine holo α-La increased from 11.2 m^2/g at pH 5.0 to 15.8 m^2/g at pH 7.0 (difference of

Table 2
Antimicrobial activities (zone of inhibition in mm) of camel apo and holo α-La.

Protein	α-La form	Antibacterial activities	Antifungal activities		
Microbial species	–	*Pseudomonas aeruginosa*	*Penicillium bilaiae*	*Aspergillus tamarii*	*Aspergillus sclerotiorum*
Camel α-La	Holo α-La	–	–	–	–
	Apo α-La	15 ± 0.5 mm	9 ± 1.2 mm	16 ± 0.8 mm	15 ± 0.5 mm

Bovine α-La exhibited no antimicrobial activity.

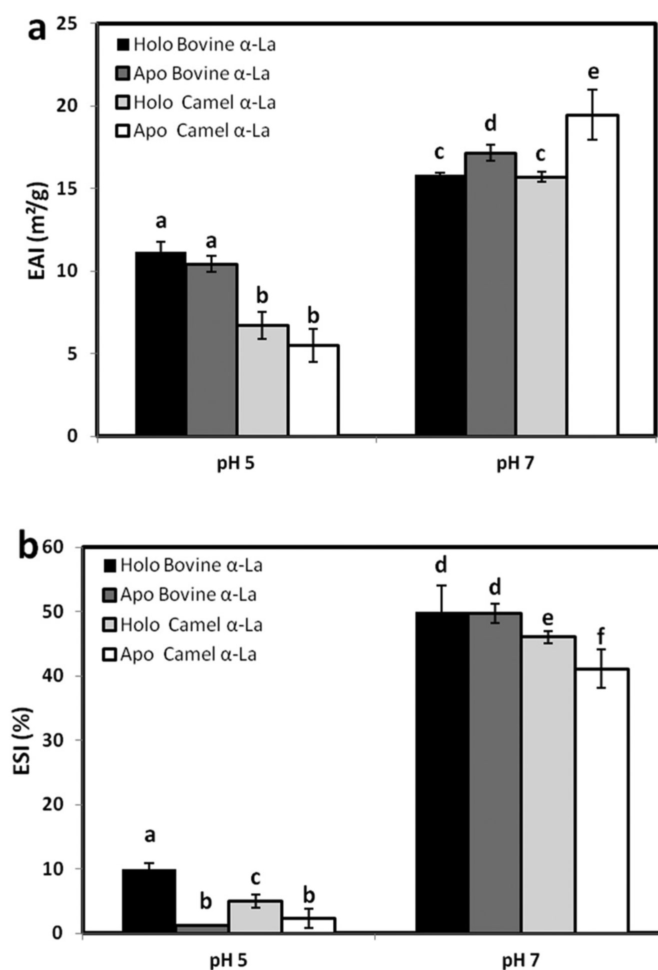

Fig. 2. Emulsifying Activity Index (EAI) (a) and Emulsion Stability Index (ESI) (b) of camel and bovine apo and holo α-La, at a protein concentration of 1 g/L and as function of pH value (5.0 and 7.0). [a–e]Samples represented with different letters are significantly different from each other ($p < 0.05$). Error bars show the standard deviations of mean values of EAI and ESI.

4.6 m^2/g), and apo bovine α-La increased from 10.4 m^2/g at pH 5.0 to 17.2 m^2/g at pH 7.0 (difference of 6.8 m^2/g).

These results are in agreement with those of Lam and Nickerson [27] carried out with bovine α-La. These authors have found that the EAI values of holo and apo bovine α-La were higher at pH 7.0 than pH 5.0 due to electrostatic-repulsive charges which may help the α-La obtain a more open structure when compared to acidic conditions. Indeed, electrostatic repulsive forces lead proteins to spread out across an oil-droplet surface which enhances its coverage.

Furthermore, electrostatic repulsion keeps the α-La protein from aggregation resulting in a better adsorption to the oil-droplet surface [27,36]. Besides, previous studies reported that apo bovine α-La is suggested to be more open than the holo α-La due to the absence of the bound calcium ions possibly allowing the protein to be more flexible and to reorient with greater ease at the oil-water interface [27].

A similar behavior was observed by Ellouze et al. [18] for camel α-La suggesting that the effect of pH level is significant for camel α-La. These authors found that EAI values of camel α-La were lower at pH 6.0 than pH 9.0 due to the proximity of the pI, where protein molecules were found to carry the lowest negative charge. Thus, the reduction of the electrostatic repulsion forces close to the pI makes the α-La unable to form stable interface layers at the oil-water interface [18].

ESI values of both camel and bovine α-La were significantly higher at pH 7.0 than those at pH 5.0 regardless of the protein form and origin and in agreement with previous findings for bovine α-La [27]. Bovine α-La gave the highest stability of emulsions at pH 7.0 reaching 50% for both holo and apo states, followed by camel holo α-La (ESI ~46%) and then camel apo α-La (ESI ~41%) (Fig. 2b). On the other hand, at pH 5.0, ESI values of holo bovine α-La were significantly higher than those of holo camel α-La (ESI ~10% and 5% for holo bovine and camel α-La, respectively). Furthermore, emulsions made with apo camel and bovine α-La at pH 5.0 were found to be unstable (ESI < 2%).

Similarly, Lam and Nickerson [27] reported a similar increase of bovine α-La ESI values at neutral pH values. Indeed, the higher net charge of the α-La at pH 7.0 would increase the electrostatic-repulsive forces between protein films surrounding oil-droplets leading to the creation of more stable emulsion, than at pH 5.0 where films would have reduced electronegative charges since the α-La is near its pI value. Indeed, Electrostatic repulsion preserves droplets from flocculating which enhances the emulsion stability of α-La. Contrarily, at pH values close to the pI of the protein, the magnitude of the electrical charge on the oil-droplets surface would be decreased. Hence, the negative charge of whey protein is insufficient to generate electrostatic-repulsive forces between the created oil-droplets of the emulsion leading to droplets aggregation forming large flocculates [51].

For the camel whey proteins, Lajnaf et al. [34] reported that ESI values of sweet whey were significantly higher than those of acid whey regardless of heating temperature value. On the other hand, bovine α-La was found to have higher emulsion stability when compared to camel α-La reaching an emulsion stability value of 53 min. This stability could proceed through steric interaction in all pH levels and enhancing the electrostatic repulsion far from the pI [18,19].

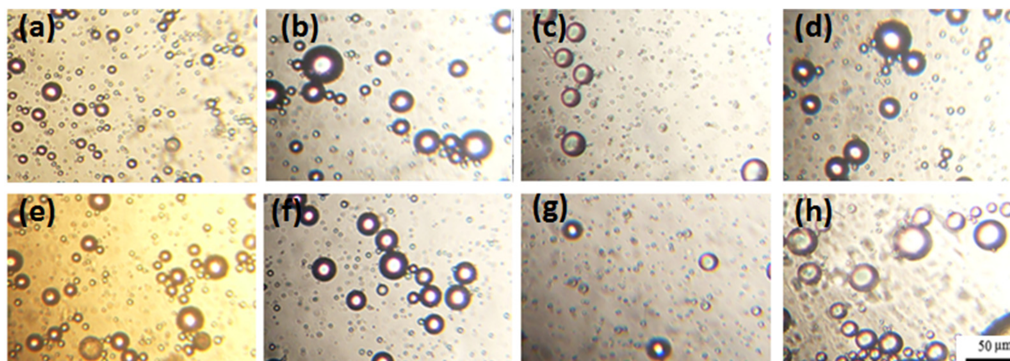

Fig. 3. Microscopy images of oil-in-water emulsions stabilized by bovine α-La (a: holo α-La pH 7.0; b: holo α-La pH 5.0; c: apo α-La pH 7.0; d: apo α-La pH 5.0) and camel α-La (e: holo α-La pH 7.0; f: holo α-La pH 5.0; g: apo α-La pH 7.0; h: apo α-La pH 5.0). The experiments were performed in 20 mM Tris-HCl buffer, pH 7.0, at 25 °C at a protein concentration of 1 g/L. Bars for magnification.

Table 3
Surface properties: ζ-potential measurements (mV), surface hydrophobicity (μg of BPB-bound) and the interfacial tension (mN/m) of camel and bovine apo and holo α-La as function of pH (5.0 and 7.0). The experiments were performed in 20 mM Tris-HCl buffer, pH 7.0, at 25 °C at a protein concentration of 1 g/L.

Protein	α-La form	ζ-Potential (mV)		Surface hydrophobicity (μg of BPB-bound)		Interfacial tension (mN/m)	
pH value	–	pH 5	pH 7	pH 5	pH 7	pH 5	pH 7
Bovine α-La	Holo α-La	-3.5 ± 0.5^{a}	-19.4 ± 0.7^{c}	46.3 ± 3.1^{a}	14.9 ± 2.5^{d}	15.5 ± 0.5^{a}	22.9 ± 0.7^{d}
	Apo α-La	-4.4 ± 1.2^{a}	-19.7 ± 0.7^{c}	54.1 ± 2.1^{b}	33.1 ± 4.1^{e}	13.4 ± 1.2^{b}	21.5 ± 0.6^{e}
Camel α-La	Holo α-La	-2.1 ± 0.4^{b}	-17.9 ± 0.2^{d}	55.8 ± 2.1^{b}	15.1 ± 4.5^{d}	13.1 ± 0.9^{b}	22.1 ± 0.5^{de}
	Apo α-La	-1.9 ± 0.5^{b}	-16.6 ± 0.5^{e}	63.9 ± 3.1^{c}	42.9 ± 1.2^{a}	10.3 ± 0.5^{c}	17.6 ± 0.7^{f}

a-g Different letters in the same measurement indicate significant differences ($p < 0.05$) between protein fractions.

3.5. Surface characteristics

3.5.1. Surface hydrophobicity

The surface hydrophobicity of bovine and camel α-La as a function of pH value (pH 7.0 and 5.0) and the conformational states (apo and holo states) is shown in Table 3. Overall, the surface hydrophobicity of camel α-La was significantly higher than that of its bovine counterpart.

Indeed, at pH 7.0, the BPB-bound amounts were 14.9 ± 2.5 μg/mL and 33.1 ± 4.1 μg/mL for holo and apo bovine α-La, respectively, and 15.1 ± 4.5 μg/mL and 42.9 ± 1.2 μg/mL for holo and apo camel α-La, respectively. At pH 5.0, the isolated α-La proteins carried a higher BPB-bound amounts when compared to those at neutral pH regardless of the milk origin. The maximum hydrophobicity values were achieved with the apo α-La in these conditions where BPB-bound amounts of camel and bovine apo α-La reached 63.9 and 54.1 μg/mL of the protein solution, respectively. In support of these results, Atri et al. [15] reported that camel α-La shows a greater surface hydrophobicity when compared to its bovine counterpart in both apo and holo states at pH 7.5. Indeed, primary structure of camel α-La contains more hydrophobic amino acids such as tryptophan. Furthermore, after calcium removal, the camel α-La showed a greater surface hydrophobicity due to the increase in tryptophan residues exposure to solvent and the greater hydrophobicity of the N-terminal part of the α-helical domain of the protein.

In the same way, Lajnaf et al. [34] have found that surface hydrophobicity was greater for acid whey than sweet whey especially after heating treatment. Indeed, whey proteins have a more open structure with exposed hydrophobic moieties in acidic conditions rather than at neutral pH. Lam and Nickerson [36] confirmed that, whey proteins molecules were highly negatively charged at neutral pH. This may repel BPB from binding to the protein. On the contrary, the reduction of the electrostatic repulsion near whey protein's pI and their open structure could promote protein-BPB interactions.

3.5.2. Determination of ζ-potential

Surface charge values (or ζ-potential) for bovine and camel α-La (holo and apo states) at a protein concentration of 1 g/L in response to pH value (5.0 and 7.0) were measured and given in Table 3. At pH 7.0, the ζ-potential values were ~−19.4 ± 0.7 and ~−19.7 ± 0.7 mV for holo and apo bovine α-La, respectively and ~−17.9 ± 0.2 and ~ −16.06 ± 0.5 mV for holo and apo camel α-La, respectively. At pH 5.0, both camel and bovine α-La carried a low net charge regardless of the conformational state (apo or holo) (~−4 mV and ~−2 mV for bovine and camel α-La, respectively).

The low net surface charge at pH 5.0 is believed to be associated with the proximity to α-La's pI as reported by Lam and Nickerson [27]. Camel α-La carried lower negative charge compared to bovine α-La in agreement with previous studies [34,52]. Hence, camel whey proteins carried lower negative charge than bovine proteins suggesting that this difference can be mainly attributed to the pI of both α-La (4.65 and 4.87 for bovine and camel α-La, respectively).

3.5.3. Interfacial properties

The interfacial tension between corn oil and the α-La solutions (at a concentration of 1 g/L) in response to pH values (5.0 and 7.0) and conformational states (apo and holo states) are shown in Table 3. Overall, both camel and bovine α-La were found to significantly reduce the interfacial tension at oil-water interface from 29.1 mN/m ($p < 0.05$).

At pH 7.0, findings indicate that the apo form of both α-La are more efficient than the holo form in reducing the interfacial properties at the oil-water interface, with lower surface tension values of the camel apo α-La (21.5 and 17.6 mN/m for bovine and camel α-La, respectively). These results are in agreement with the highest EAI values observed with the same solution of camel apo α-La at pH 7.0 (Section 3.4). Dickinson and Matsumura [53] and Ibanoglu and Ibanoglu [54] reported that the treatment with EDTA leads to better tensioactive properties by changing the conformation of α-La due to the increased flexibility and rate of unfolding of the protein.

No significant difference was observed between surface tension values of camel and bovine holo α-La (γ ~ 22 mN/m). Lajnaf et al. [17] reported that camel and bovine α-La have the same efficiency to reduce the surface tension at the air-water interface, whereas, the adsorbed layer made with bovine α-La is more rigid when compared to that of camel α-La suggesting that a stronger protein-protein interactions (hydrophobic, hydrogen, and electrostatic interactions) for the bovine protein at the air-water as compared to camel one.

At pH 5.0, the order of effectiveness to reduce the surface tension at the oil-water interface was: camel apo α-La (γ = 10.3 ± 0.5 mN/m) > camel holo α-La (γ = 13.1 ± 0.9 mN/m) = bovine apo α-La (13.4 ± 1.2 mN/m) > bovine holo α-La (15.5 ± 0.5 mN/m). pH level plays a key role in the adsorption of the α-La at the interface. Indeed, at neutral pH the electrostatic repulsion between the α-La molecules leads to greater difficulties in aligning at the oil-water interface to create a viscoelastic film. Whereas, near its pI, the α-La carried less of negative charge allowing for higher interactions and better adsorption to the interface [27,36]. On the other hand, camel whey proteins exhibited the highest effectiveness to reduce the surface tension at the oil-water interface in acidic conditions despite their lower EAI values in these conditions. Hence, the interfacial behavior of camel whey proteins at the oil-water interface is mainly maintained by the camel α-La due to its high hydrophobicity [34]. Ellouze et al. [18] observed that the α-La isolated from camel milk exhibited the fastest increase in the surface pressure at the oil-water interface when pH is close to the pI of the protein, allowing it to adsorb more rapidly.

4. Conclusion

The emulsifying properties, *in vitro* antioxidant and antibacterial activities of purified camel and bovine α-La were compared in this study. The experiments were performed in the presence of saturating concentrations of calcium ($CaCl_2$) as well as in the presence of EDTA, yielding to the holo and apo forms of α-La.

Results showed that both camel and bovine α-La exhibited significant antioxidant activity especially with respect to FRAP, iron chelating and antiradical activities in their apo forms. Furthermore, camel apo α-

La showed moderate antibacterial activities *in vitro* against Gram-bacteria (*Pseudomonas aeruginosa*) and against fungal pathogens species (*Penicillium bilaiae*, *Aspergillus tamari* and *Aspergillus sclerotiorum*).

Camel and bovine α-La emulsifying properties depended on the pH level and the conformational states of the protein (apo or holo states). A Higher surface coverage of the oil droplets (EAI) was obtained for apo α-La especially the camel apo α-La which carried the highest ability to reduce the surface tension values at the oil-water interface. On the other hand, the stability of the created emulsions seemed greatest at neutral pH due to the presence of the electrostatic repulsive forces between α-La molecules as confirmed by the ζ-potential measurements. Finally, these results confirmed the strong potential of camel α-La especially in its apo state for potential applications in food, pharmaceutical and cosmetic industries.

CRediT authorship contribution statement

Roua Lajnaf: Conceptualization, Methodology, Software, Visualization, Investigation, Writing-Original draft preparation, reviewing and editing.

Houda Gharsallah: Conceptualization, Visualization, Investigation, Software.

Mourad Jridi: Conceptualization, Visualization, Investigation.

Hamadi Attia: Supervision.

M.A. Ayadi: Conceptualization, Supervision, Reviewing and Editing.

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References

[1] M. Khalesi, M. Salami, M. Moslehishad, J. Winterburn, A.A. Moosavi-Movahedi, Biomolecular content of camel milk: a traditional superfood towards future healthcare industry, Trends Food Sci. Technol. 62 (2017) 49–58.

[2] E.I. El-Agamy, M. Nawar, S.M. Shamsia, S. Awad, G.F.W. Haenlein, Are camel milk proteins convenient to the nutrition of cow milk allergic children? Small Rumin. Res. 82 (2009) 1–6.

[3] Y. Hailu, E.B. Hansen, E. Seifu, M. Eshetu, R. Ipsen, S. Kappeler, Functional and technological properties of camel milk proteins: a review, J. Dairy Res. 83 (2016) 422–429.

[4] R. Lajnaf, A. Zouari, I. Trigui, H. Attia, M.A. Ayadi, Effect of different heating temperatures on foaming properties of camel milk proteins: a comparison with bovine milk proteins, Int. Dairy J. 104 (2020).

[5] K.I. Ereifej, M.H. Alu'datt, H.A. Alkhalidy, I. Alli, T. Rababah, Comparison and characterisation of fat and protein composition for camel milk from eight Jordanian locations, Food Chem. 127 (2011) 282–289.

[6] Swaisgood H.E., in: O. R. Fennema (Ed.) 3rd ed., Food Chem., Marcel Dekker, New York, NY 1996, pp. 841–878.

[7] Y. Uchida, M. Shimatani, T. Mitsuhashi, M. Koutake, Process for Preparing a Fraction Having a High Content of α-Lactalbumin From Whey and Nutritional Compositions Containing Such Fractions, US Patent, vol. 5, 1996 503–864 (Urbisinov).

[8] U. Merin, S. Bernstein, R. Yagil, C. Van Creveld, P. Lindner, N. Gollop, Short communication, A comparative study of milk serum proteins in camel (*Camelus dromedarius*) and bovine colostrum, Livestock Production Sci 67 (2001) 297–301.

[9] L.C. Laleye, B. Jobe, A.A.H. Wasesa, Comparative study on heat stability and functionality of camel and bovine milk whey proteins, J. Dairy Sci. 91 (2008) 4527–4534.

[10] A. Omar, N. Harbourne, M.J. Oruna-Concha, Quantification of major camel milk proteins by capillary electrophoresis, Int. Dairy J. 58 (2016) 31–35.

[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 Surfaces B Biointerfaces 156 (2017) 55–61.

[12] R.L. Hill, K. Brew, Lactose synthetase, Adv. Enzymol. Relat. Areas Mol. Biol. 43 (1975) 411–490.

[13] V. Calderone, M.G. Giuffrida, D. Viterbo, L. Napolitano, D. Fortunato, A. Conti, K.R. Acharya, Amino acid sequence and crystal structure of buffalo α-lactalbumin, FEBS Lett. 394 (1996) 91–95.

[14] O.U. Beg, H. von Bahr-Lindström, Z.H. Zaidi, H. Jörnvall, The primary structure of alpha-lactalbumin from camel milk, Eur. J. Biochem. 147 (1985) 233–239.

[15] M.S. Atri, A.A. Saboury, R. Yousefi, J. Chobert, T. Haertle, A.A. Moosavi-Movahedi, Comparative study on heat stability of camel and bovine apo and holo α-lactalbumin, J. Dairy Res. 77 (1) (2010) 43.

[16] M. Salami, R. Yousefi, M. Reza, S. Hadi, J. Chobert, A. Akbar, M. Sadat, A. Niasari-naslaji, F. Ahmad, T. Haertle, 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.

[17] 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 Surfaces B Biointerfaces. 151 (2016) 287–294.

[18] M. Ellouze, R. Lajnaf, A. Zouari, H. Attia, M.A. Ayadi, C. Vial, Camel α-lactalbumin at the oil-water interface: effect of protein concentration and pH change on surface characteristics and emulsifying properties, Colloids Surfaces B Biointerfaces 189 (2020).

[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-Food Sci. Technol. 116 (2019) 108550.

[20] I. Felfoul, J. Jardin, F. Gaucheron, H. Attia, M.A. Ayadi, Proteomic profiling of camel and cow milk proteins under heat treatment, Food Chem. 216 (2017) 161–169.

[21] 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.

[22] 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.

[23] A. Muller, B. Chaufer, U. Merin, G. Daufin, Purification of alpha-lactalbumin from a prepurified acid whey: ultrafiltration or precipitation, Lait 83 (2003) 439–451.

[24] A. Muller, B. Chaufer, U. Merin, G. Daufin, Prepurification of alpha-lactalbumin with ultrafiltration ceramic membranes from acid casein whey: study of operating conditions, Lait 83 (2003) 111–129.

[25] Z. Yüksel, Y.K. Erdem, Detection of the milk proteins by RP-HPLC, GIDA-Journal Food. 35 (2010) 5–11.

[26] M. Svensson, A. Håkansson, A.K. Mossberg, S. Linse, C. Svanborg, Conversion of alpha-lactalbumin to a protein inducing apoptosis, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 4221–4226.

[27] 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.

[28] P. Bersuder, M. Hole, G. Smith, Antioxidants from a heated histidine-glucose model system. I: investigation of the antioxidant role of histidine and isolation of antioxidants by high-performance liquid chromatography, J. Am. Oil Chem. Soc. 75 (1998) 181–187.

[29] I.F.F. Benzie, J.J. Strain, [2] Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration, Methods Enzymol, Elsevier 1999, pp. 15–27.

[30] D. Kumar, M.K. Chatli, R. Singh, N. Mehta, P. Kumar, Antioxidant and antimicrobial activity of camel milk casein hydrolysates and its fractions, Small Rumin. Res. 139 (2016) 20–25.

[31] R.F. Boyer, C. J. McCleary, Superoxide ion as a primary reductant in ascorbate-mediated ferretin iron release, Free Radic. Biol. Med. 3 (1987) 389–395.

[32] K.A. Al-Shamsi, P. Mudgil, H.M. Hassan, S. Maqsood, Camel milk protein hydrolysates with improved technofunctional properties and enhanced antioxidant potential in *in vitro* and in food model systems, J. Dairy Sci. (2017) 1–14.

[33] A. Håkansson, M. Svensson, A. Mossberg, H. Sabharwal, S. Linse, I. Lazou, B. LoÈnnerdal, C. Svanborg, A folding variant of α-lactalbumin with bactericidal activity against Streptococcus pneumoniae, Mol. Microbiol. 35 (2000) 589–600.

[34] R. Lajnaf, I. Trigui, O. Samet-Bali, H. Attia, M.A. Ayadi, Comparative study on emulsifying and physico-chemical properties of bovine and camel acid and sweet wheys, J. Food Eng. 109741 (2019).

[35] K.N. Pearce, J.E. Kinsella, Emulsifying properties of proteins: evaluation of a turbidimetric technique, J. Agric. Food Chem. 26 (1978) 716–723.

[36] 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.

[37] S.R. Kappeler, C. Heuberger, Z. Farah, Z. Puhan, Expression of the peptidoglycan recognition protein, PGRP, in the lactating mammary gland, J. Dairy Sci. 87 (2004) 2660–2668.

[38] R.J. Elias, D.J. McClements, E.A. Decker, Antioxidant activity of cysteine, tryptophan, and methionine residues in continuous phase β-lactoglobulin in oil-in-water emulsions, J. Agric. Food Chem. 53 (2005) 10248–10253.

[39] I. Arcan, A. Yemenicioğlu, Antioxidant activity of protein extracts from heat-treated or thermally processed chickpeas and white beans, Food Chem. 103 (2007) 301–312.

[40] A. Alemán, E. Pérez-Santín, S. Bordenave-Juchereau, I. Arnaudin, M.C. Gómez-Guillén, P. Montero, Squid gelatin hydrolysates with antihypertensive, anticancer and antioxidant activity, FRIN 44 (2011) 1044–1051.

[41] A.R. Corrochano, Y. Sariçay, E. Arranz, P.M. Kelly, V. Buckin, L. Giblin, Comparison of antioxidant activities of bovine whey proteins before and after simulated gastrointestinal digestion, J. Dairy Sci. 102 (2019) 54–67.

[42] L. Tang, Y. Zhang, Z. Qian, X. Shen, The mechanism of Fe^{2+}-initiated lipid peroxidation in liposomes: the dual function of ferrous ions, the roles of the pre-existing lipid peroxides and the lipid peroxyl radical, Biochem. J. 352 (2000) 27–36.

[43] M. Salami, A.A. Moosavi-Movahedi, F. Moosavi-Movahedi, M.R. Ehsani, R. Yousefi, M. Farhadi, A. Niasari-Naslaji, A.A. Saboury, J.-M. Chobert, T. Haertlé, Biological activity of camel milk casein following enzymatic digestion, J. Dairy Res. 78 (2011) 471–478.

[44] K. Murakami, L.J. Berliner, A distinct zinc-binding site in the alpha-lactalbumins regulates calcium binding. Is there a physiological role for this control? Biochemistry 22 (1983) 3370–3374.

[45] J.J. Baumy, G. Brule, Binding of bivalent cations to α-lactalbumin and β-lactoglobulin: effect of pH and ionic strength, Lait 68 (1988) 33–48.
[46] M. Sedaghati, H. Ezzatpanah, M. Mashhadi Akbar Boojar, M. Tajabadi Ebrahimi, β-Lactoglobulin and α-lactalbumin hydrolysates as sources of antibacterial peptides, J. Agric. Sci. Technol. 16 (2014) 1587–1600.
[47] M. Svensson, H. Sabharwal, A. Håkansson, A.-K. Mossberg, P. Lipniunas, H. Leffler, C. Svanborg, S. Linse, Molecular characterization of α–lactalbumin folding variants that induce apoptosis in tumor cells, J. Biol. Chem. 274 (1999) 6388–6396.
[48] V.N. Uversky, E.M. El-Fakharany, M.M. Abu-Serie, H.A. Almehdar, E.M. Redwan, Divergent anticancer activity of free and formulated camel milk α-lactalbumin, Cancer Investig. 35 (2017) 610–623.
[49] L. Breydo, H.A. Almehdar, E.M. El-Fakharany, E.M. Redwan, V.N. Uversky, Not all AMLETs are made equal: complexes of cow and camel α-lactalbumin with oleic acid show different structure and stability, J. Biomol. Struct. Dyn. 36 (2018) 4405–4412.
[50] J.M. Redington, L. Breydo, H.A. Almehdar, E.M. Redwan, V.N. Uversky, α-Lactalbumin: of camels and cows, Protein Pept. Lett. 23 (2016) 1072–1080.
[51] D.J. McClements, Food Emulsions: Principles, Practices, and Techniques, CRC, 2004.
[52] S. Momen, M. Salami, Z. Emam-Djomeh, E. Hosseini, N. Sheibani, A.A. Moosavi-Movahedi, Effect of dry heating on physico-chemical, functional properties and digestibility of camel whey protein, Int. Dairy J. 86 (2018) 9–20.
[53] E. Dickinson, Y. Matsumura, Proteins at liquid interfaces: role of the molten globule state, Colloids Surfaces B Biointerfaces 3 (1994) 1–17.
[54] E. Ibanoglu, Ş. Ibanoglu, Foaming behaviour of EDTA-treated α-lactalbumin, Food Chem. 66 (1999) 477–481.