International Dairy Journal 139 (2023) 105563

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Review

Technological properties and biological activities of camel α-lactalbumin – A review

Roua Lajnaf [a, b, *], Hamadi Attia [a], Mohamed Ali Ayadi [c]

[a] Alimentary Analysis Unit, National Engineering School of Sfax, BPW 3038, Sfax, Tunisia
[b] Montpellier University, UMR IATE, Place E. Bataillon, 34095 Montpellier Cedex 5, France
[c] Department of Food Technology, University of Liege –Gembloux Agro-Bio Tech, Passage des Déportés, 2, Gembloux, B-5030, Belgium

ARTICLE INFO

Article history:
Received 2 August 2022
Received in revised form
26 November 2022
Accepted 29 November 2022
Available online 10 December 2022

ABSTRACT

α-Lactalbumin (α-La) enrichment of formulae has received much attention as it is considered as one of the most nutritious food ingredient due to its high content of essential amino acids. Camel milk is deficient in β-lactoglobulin (β-Lg), which is the main whey protein in bovine milk, and therefore represents a source of α-La at a higher concentration than that of bovine milk; this makes the isolation of α-La from camel milk more feasible and economical. Furthermore, the content of essential amino acids in camel α-La is significantly higher than that of the bovine counterpart. This review presents the differences between camel and bovine α-La in a comparative study including extraction processes and antioxidant, antimicrobial, foaming and emulsifying properties. The examination of the biological activities and technological functionalities of camel α-La show it to be a scientifically interesting and industrially relevant ingredient for the food industry due to its nutritional value.

Contents

* Corresponding author.
E-mail addresses: roua.lajnef@enis.tn, roua_lajnaf@yahoo.fr (R. Lajnaf).

https://doi.org/10.1016/j.idairyj.2022.105563
0958-6946/

1. Introduction

α-Lactalbumin (α-La) is one of the major whey proteins; it a highly abundant protein present in the milk of all mammalian species (Barbana & Pérez, 2011; Redington, Breydo, Almehsar, Redwan, & Uversky, 2016). The protein belongs to the superfamily of lysozyme that also includes calcium-binding lysozymes and lysozymes. It is an important protein belonging to the soluble fraction of milk as it contributes significantly to its biological, nutritional and physical characteristics. The biosynthesis of α-La takes place in mammary glands during lactogenesis as it is involved in the last stage of the synthesis of lactose by the association and the modification of the substrate specificity of galactosyltransferase, which could greatly elevate the affinity of the resulting complex for glucose (Barbana & Pérez, 2011; Grobler, Wang, Pike, & Brew, 1994; Redington et al., 2016).

α-La is the major protein in human whey with a concentration of about 2.45 g L^{-1}; whereas it is ranked second in bovine whey after β-lactoglobulin (β-Lg) with a concentration of 0.5 g L^{-1}. Hence, it represents approximately 3.4% of the total milk protein and 20% of the whey proteins (Brew, 2013; Salami et al., 2009; Swaisgood, 1995). It is a globular, calcium metalloprotein of 123 amino-acid residues (Fig. 1) containing one calcium (Ca^{2+}) atom per mole of protein molecule. Calcium is a divalent cation that plays an important role in stabilising the spatial structure of the α-La. The binding of this ion is carried out through acid functions of the Asp residues that are located in positions 82, 87 and 88 of the amino-acid residues in the primary structure of α-La. There is also a second binding site occupied by zinc, with an affinity 105 times lower than that of calcium ion (Permyakov & Berliner, 2000). Thus, α-La exists in both holo- and apo-forms that include calcium-loaded and calcium-depleted forms, respectively (Atri et al., 2010; Lajnaf, Gharsallah, Jridi, Attia, & Ayadi, 2020; Lam & Nickerson, 2015; Svensson, Håkansson, Mossberg, Linse, & Svanborg, 2000).

The α-La consists of two domains: a large α-helical domain and a small β-sheet domain connected by a calcium binding loop as shown by X-ray crystallography (Acharya, Stuart, Walker, Lewis, & Phillips, 1989; Lajnaf, Picart-Palmade, Attia, Marchesseau, & Ayadi, 2017; Permyakov & Berliner, 2000). First, the α-helical domain is composed of two short 3_{10}-helices and three α-helices, meanwhile, the small β-sheet domain is composed of a small three-stranded antiparallel β-pleated sheet, series of loops as well as a short 3_{10}-helix (Redington et al., 2016). The α-helical domain represents a basic lobe with a calculated isoelectric point (pI) of 9.6, whereas the β-sheet domain is an acidic lobe containing the Ca^{2+} binding loop with a pI of 3.7 (McGuffey, Epting, Kelly, & Foegeding, 2005). Both of α-helical and β-sheet domains in the molecular structure of the α-La are separated by a deep cleft (Redington et al., 2016).

The protein structure of the α-La is stabilised by four disulphide bonds with no free thiol groups, unlike the molecular structure of the β-Lg that is characterised by the presence of two disulphide bonds and one free thiol group (Chatterton, Smithers, Roupas, & Brodkorb, 2006; Lajnaf et al., 2020; McGuffey, Otter, van Zanten, & Allen Foegeding, 2007). The positions of the disulphide bonds in the molecular structure of the α-La as well as the calcium binding site are strongly conserved across mammalian species. However, the overall α-La primary sequences are only about 16% conserved amongst different species (Redington et al., 2016). The buried disulfide bonds of the α-La were located in the cysteine residues at the positions Cys_6/Cys_{120}, Cys_{28}/Cys_{111}, Cys_{61}/Cys_{77}, and Cys_{73}/Cys_{91}. The Cys_6/Cys_{120} is reported to be the super-reactive disulphide bond to dithiothreitol at 25 °C and to heating process, because of many reasons such as the its surface exposure, the geometric strain imposed by the native fold and the positive charge distribution in this region (Kuwajima, Ikeguchi, Sugawara, Hiraoka, & Sugai, 1990; McGuffey et al., 2005).

At physiological conditions, the α-La is reported to be in its folded state, whereas it is easily converted into the molten globule state upon loss of calcium, either at low pH, or at high temperatures of the heat treatment, or upon the reduction of disulphide bonds. The molten globule state of the α-La is considered as one of the best-characterised folding intermediates of globular proteins due to the increase the accessibility of the hydrophobic regions of α-La compared with the folded state (Kuwajima et al., 1990; Rösner & Redfield, 2009).

The temperature of the denaturation of the α-La is relatively low (~64 °C), but this process is of 80−90% reversible once the protein is heated at 77 °C. On the other hand, when the α-La is heated at 95 °C for 14 min, the rate of return to the native form decreases by 40% (Chaplin & Lyster, 1986; McGuffey et al., 2005). The dimeric and tetrameric forms of α-La appear after heat treatment (McGuffey et al., 2005). Indeed, at a temperature above 90 °C, α-La undergoes an irreversible denaturation by the cleavage of the intramolecular disulphide bridges inducing the creation of dimeric and tetrameric forms and hence, large protein aggregates are created at 95 °C (Fig. 2). The irreversibility of the denaturation of the α-La was attributed to the breakdown of disulphide bonds during heating at high temperatures leading to free thiol groups. Afterwards, free −SH groups catalyse the intermolecular thiol/disulphide interchange reactions and the formation of soluble oligomers (Chaplin & Lyster, 1986).

Nutritionally, the α-La possesses a high concentration of the essential amino-acids such as Lys, Trp and Cys when compared with caseins and other whey proteins such as β-Lg (Heine, Klein, & Reeds, 1991). Furthermore, it has an important role in immunity as it acts as a powerful antioxidant component in the system of the neonate (Heine et al., 1991; Lien, 2003). Hence, α-La is considered as

```
LALBA_CAMDR     1 KQFTKCKLSDELKDMNGHGGITLAEWICIIFHMSGYDTETVVSNNGNREYGLFQINNKIW
Q28049_BOVIN    1 EQLTKCEVFRELKDLKGYGGVSLPEWVCTAFHTSGYDTQAIVQNNDSTEYGLFQINNKIW
                    * ***    ****  * **  * ** *  ** *****   * **   ************

LALBA_CAMDR    61 CRDNENLQSRNICDISCDKFLDDDLTDDKMCAKKILDKEGIDYWLAHKPLCSEKLEQWQC
Q28049_BOVIN   61 CKDDQNPHSSNICNISCDKFLDDDLTDDIMCVKKILDKVGINYWLAHKALCSEKLDQWLC
                  * *  *  * *** ************** ** ****** ** ****** ****** ** *

LALBA_CAMDR   121 EKW
Q28049_BOVIN  121 EKL
                  **
```

Fig. 1. Sequence alignment of α-lactalbumins from camel milk (LALBA_CAMDR) and bovine milk (Q28049_BOVIN); an asterisk (*) indicates identical residues. Protein sequence alignment is performed by Web Expasy (http://web.expasy.org/sim). Protein sequences are provided by EBI Search (http://www.ebi.ac.uk).

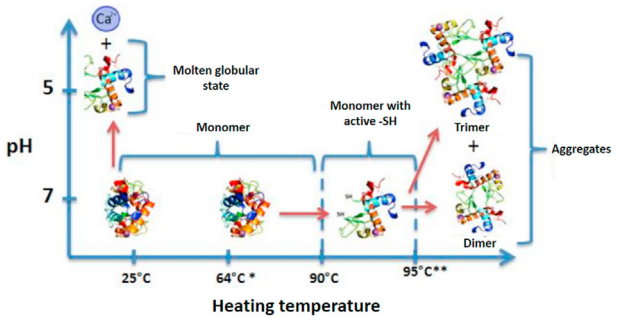

Fig. 2. Influence of extrinsic factors (pH and temperature) on the structure of α-lactalbumin (Chaplin & Lyster, 1986; Matsumura et al., 1994; McGuffey et al., 2005): *, denaturation temperature; **, aggregation temperature.

an important protein for infant nutrition since it is the primary protein fraction in human milk. Therefore, great attention has been paid by the scientific and industrial communities towards the production of α-La as it is considered to be a promising food ingredient due to its significant levels of essential amino-acids and its reversibility and stability upon heating denaturation (Salami et al., 2009).

In addition, one important factor when introducing a new ingredient in a product is its availability and its cost of production, and the identification of new food ingredients sources will be a new challenge in the food industry. Thus, when compared with cow milk, camel milk can be considered as a source of the α-La due the presence of this protein in high content and the absence of the β-Lg in camel milk, which makes its isolation more feasible and economical (Salami et al., 2009).

2. Camel milk and α-lactalbumin overview

Camel milk has a very important role in human nutrition, especially in the arid countries and hot regions of the world. Indeed, this milk contains all the essential nutrients already found in bovine milk including proteins, fats, minerals and vitamins (El-Agamy, Abou-Shloue, & Abdel-Kader, 1998; Karue, 1998). According to FAO statistics (FAOSTAT, 2020), the overall camel milk production in the world is reported to be about 3.15 million tons per year representing 0.4% of the total milk production of the world. Meanwhile, cow milk production represents around 81% of total milk production with a quantity of 718 million tons per year (FAOSTAT, 2020).

Camel milk was reported to be an efficient treatment for other diseases including such as dropsy, tuberculosis, jaundice, hepatitis, asthma and leishmaniosis. Camel milk has also other potential therapeutic properties, such as anti-carcinogenic, anti-diabetic, and anti-hypertensive, and has been recommended to be consumed by children who are allergic to bovine milk (El-Agamy, Nawar, Shamsia, Awad, & Haenlein, 2009; El-Fakharany et al., 2017; Uversky, El-Fakharany, Abu-Serie, Almehdar, & Redwan, 2017). Camel milk has been found to be much closer in its physico-chemical and biochemical composition to human milk than cow milk, hence, various compositional differences between camel and bovine milk were revealed, such as a higher concentrations of vitamins A, B-2, C and E, as well as minerals including sodium, potassium, magnesium, iron, copper and zinc. Meanwhile, lower concentrations of lactose, proteins, cholesterol and fat in camel milk were found compared with cow milk (Al Haj & Al Kanhal, 2010; Redington et al., 2016; Sakandar et al., 2018).

Camel milk is endowed with a unique composition of protein components compared with milk from other mammalian species (Hailu et al., 2016). First, the percentage of caseins among total proteins was similar for bovine and camel milk (80% and 75.49% for bovine and camel milk, respectively) (Ereifej, Alu'datt, Alkhalidy, Alli, & Rababah, 2011; Walstra, Wouters, & Geurts, 2005). The casein fraction is characterised by the presence of the four known milk caseins α_{S1}-, α_{S2}-, β- and κ-caseins; however, the relative proportions in camel milk were 26:4:67:3 (w/w) for of α_{S1}-, α_{S2}-, β- and κ-caseins, respectively, very different from those in bovine milk at 38:12:36:10 (w/w), respectively (Huppertz, Fox, & Kelly, 2018; Mohamed, Johansson, Lundh, Nagy, & Kamal-Eldin, 2020). Therefore, these differences in casein composition between both of camel and bovine milk are considered to be responsible for its technological properties, including the weak gelation properties when used to make dairy products such as yoghurt and cheese (Mohamed, Ayyash, & Kamal-Eldin, 2022).

The main whey proteins in cow milk are β-Lg, bovine serum albumin (BSA) and α-La, whereas, camel milk whey proteins include α-La, camel serum albumin (CSA) and other fractions not reported in bovine milk such as peptidoglycan recognition protein (PGRP), lactoferrine and camel whey basic protein (CWBP) (Lajnaf, Picart-Palmade, Attia, Marchesseau, & Ayadi, 2022; Merin et al., 2001; Mohamed et al., 2022; Momen, Salami, Alavi, Emam-Djomeh, & Moosavi-Movahedi, 2019). A major difference is that camel milk whey lacks β-Lg, which constitutes about 55% of bovine milk whey proteins (Swaisgood, 1995).

Therefore, camel α-La is the major whey protein of camel milk because of the absence of β-Lg that is the major whey proteins in the milk of almost all mammalian species including cow, sheep, goat, donkey and mare (Hazebrouck, 2016). Camel α-La accounts for 19.7% (w/w) of total camel milk proteins and 84% (w/w) of total camel whey proteins (Lajnaf et al., 2022) with an average concentration varying between 2.1 g L^{-1} (Omar, Harbourne, & Oruna-Concha, 2016) and 3.5 g L^{-1} (Kappeler, Farah, & Puhan, 2003) or even 5 g L^{-1} (El-Agamy, 2009), which is significantly higher than that in cows' milk (1–1.5 g L^{-1}; Hazebrouck, 2016). Camel α-La presents differences in its structural characteristics compared with its bovine counterpart leading to diverse therapeutic properties, biological activities and techno-functional properties (Fig. 3).

Thus, the current paper briefly overviews the purification processes of camel α-La, followed by a comprehensive review on the most important biological activities and techno-functional including foaming and emulsifying properties of this protein. The examination of the technological and biological properties of the camel α-La reveals an interesting ingredient for food industry suggesting this protein as a possible alternative protein in

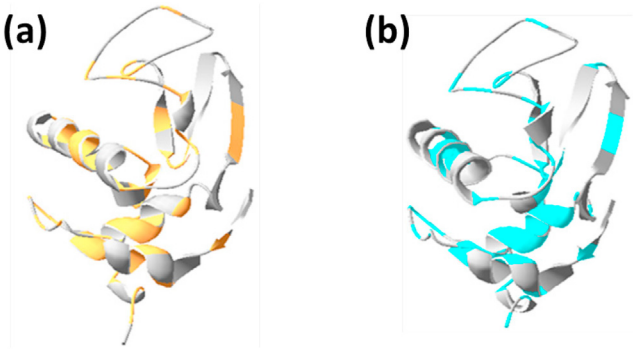

Fig. 3. The secondary structure of α-lactalbumin from (a) bovine milk and (b) camel milk. The hydrophobic parts were stained orange for bovine α-lactalbumin and blue for camel α-lactalbumin. The three-dimensional structure was assessed with "swiss pdb viewer software" (http://swissmodel.expasy.org).

manufacture of infant formulas due to its nutritional value, which is of a great scientific and industrial relevance.

3. Purification processes

Overall, the primary source of the α-La is whey, regardless of milk origin. Indeed, whey is a by-product of cheese making where other components can be found including proteins, lactose, minerals vitamins, and even traces of milk fat. Unlike bovine α-La, camel α-La is not yet sold commercially as purified protein despite its abundant content in camel milk. Therefore, purification processes of this protein were previously investigated on laboratory scale operating under selected process parameters.

There are numerous production technologies that were previously described to obtain pure α-La fractions from whey. The choice of the suitable technology to use is determined mainly by the quantity of α-La present in whey before purification, the required purity levels and the production scale. Bovine α-La was previously isolated from milk using various techniques including chromatography/gel filtration, precipitation/aggregation technologies, membrane separation and enzymatic hydrolysis (Kamau, Cheison, Chen, Liu, & Lu, 2010). Therefore, the extraction of α-La from bovine whey with high purity and extraction yields is not a simple task whether laboratory scale or pilot scale because of the presence of the β-Lg in bovine at high concentrations in bovine whey (the amount of β-Lg in milk is approximately 2.5 times higher than that of α-La in bovine whey). Furthermore, bovine whey proteins β-Lg and α-La present similar physico-chemical characteristics including molecular mass, pI and heat denaturation, which induce difficulties in their separation (Geng, Tolkach, Otte, & Ipsen, 2015).

The purification process of the camel α-La is easier leading to obtain the better resolution of the separation when compared with the isolation processes of bovine α-La. There are numerous production technologies that were previously described to obtain pure α-La fractions from camel whey, where the choice of the suitable technology to use is determined mainly by the quantity of α-La present in whey before purification, the required purity levels and the production scale. Chromatographic methods and membrane technologies are the main purification techniques that were previously used to purify camel α-La from milk leading to different efficiency levels, whereas precipitation/aggregation technologies as well as enzymatic hydrolysis were not yet used in the literature. Chromatographic methods are overall efficient in obtaining α-La samples with a high purification level and a low yield. Thus, there is a need to produce large quantities of α-La to be used in food industry, which inevitably requires further attention. On the other hand, the utilisation of membrane technologies for separating, purifying and concentrating proteins from aqueous streams is a topic of growing interest. Indeed, the advantages of membrane processing include a low energy requirement, mild operating conditions, separation efficiency and easy scaling up (Alsobh, Zin, Vatai, & Bánvölgyi, 2022).

3.1. Chromatographic techniques

Overall, chromatographic techniques have been used for partitioning protein mixtures according to their affinity towards either mobile or stationary phases. However, this technique was usually combined with other separation principles including charge, hydrophobic interactions, metal chelating, acidity, basicity and adsorption affinities. Bovine α-La was previously isolated from other milk proteins using a gel filtration column. For instance, it was separated from β-Lg using Sephadex G-50 gel filtration as they co-eluted in anion-exchange chromatography. Thus, this method was reproducible and showed high purity and well-preserved antigenicity of bovine α-La (Neyestani, Djalali, & Pezeshki, 2003).

For camel α-La, chromatographic methods were used to separate it from other camel milk proteins including CSA, lactoferrin, PGRP and casein traces. First, camel α-La was isolated through cation-exchanger by fast protein liquid chromatography to investigate the radical-scavenging properties of camel proteins towards a stable radical cation (El-Hatmi, Jrad, Khorchani, Dari, & Girardet, 2014). Indeed, cation-exchange chromatography enabled the production of the α-La enriched fraction from camel whey proteins that was not retained on the column.

A reverse-phase high-performance liquid chromatography system was also used to fractionate the α-La from camel milk using a C18 column (Waters Associates). After defatting milk (3000 × *g*, 30 min) and caseins removal (high-speed centrifugation 14,000 × *g*, 40 min), the obtained whey was applied to a Sephadex G-100 column (2.3 × 90 cm) equilibrated with the dialysis buffer whose absorbance was monitored at 254 nm (Redington et al., 2016). In the same way, camel α-La using chromatographic technique through Sephadex-G50 gel filtration column. The camel casein-free preparation (after an acid precipitation at pH 4.6) was then treated with ammonium sulphate (45%), and incubated at 4 °C for 24 h. After centrifugation and dialysis, the obtained mixture was applied to the DEAE−cellulose column equilibrated with 50 mM phosphate buffer, pH 7.6 and the obtained the proteins fractions were applied into Sephadex-G50 gel filtration column to separate α-La from CSA (Uversky et al. (2017). These methods were reported to be reproducible leading to obtain a pure fraction of proteins with a high purity and well-preserved biological properties and structural characteristics of camel α-La (Redington et al., 2016; Uversky et al., 2017). Camel α-La was also purified from camel whey using gel permeation chromatography through a Sephadex G100 column that was equilibrated in 0.02 M Tris−HCl buffer pH 8.4 (Levieux, Levieux, El-Hatmi, & Rigaudière, 2006). This chromatographic method led to a pure fraction of camel α-La with a purity of 95%; the method was combined with the ion exchange chromatography on Q-Sepharose Fast Flow to remove minor contaminants and to increase resolution of the obtained protein.

Finally, chromatographic methods are efficient for the purification of camel α-La achieving a high purity (>95%). However, these methods are reported to be usually expensive and hence they were restricted to laboratory scale for the production of pure proteins isolates. Furthermore, the overall yield and the efficiency of the method depend greatly on the number of steps in the purification protocol. Therefore, there is a need to produce large quantities of camel α-La as it is the main whey protein in camel milk using suitable methods which are applicable for production on an industrial scale.

3.2. Membrane separation

Membrane separation techniques are attractive for milk proteins isolation due to their relatively easy scale-up and low processing costs when compared with chromatographic techniques previously described which make it a commercially suitable extraction method especially when it is compared with chromatographic techniques. However, the primary step in purification which consists of whey/casein proteins fractionation and separation makes the difference between the previously reported methods.

The purification of bovine α-La using membrane has been the subject of numerous scientific works. Nevertheless, the efficient membrane separation should give low content of contaminants in the α-La solution (such as β-Lg, bovine serum albumin and immunoglobulins) and an enhanced ratio of α-La/β-Lg (Kamau et al.,

2010). For instance, ultrafiltration (UF) membranes have been used for the purification and concentration of α-La from whey protein concentrate with both an enhanced purity and a satisfactory yield of 90% recovery in the permeate (Muller, Daufin, & Chaufer, 1999).

Some studies already showed that camel α-La could be successfully isolated by means of UF at on a small scale (Atri et al., 2010; Ellouze et al., 2020; Lajnaf et al., 2017; Salami et al., 2009), in which whey solutions prepared from camel skim milk either by acid precipitation, rennet coagulation or ammonium sulphate fractionation. First, camel α-La can be purified simply through a two-step purification procedure using both of ammonium sulphate fractionation (26.4%) to precipitate caseins and UF membrane of 30 kDa molecular mass cut-off to obtain camel α-La. The obtained permeate of the UF membrane represented the purified camel α-La with a high purity level (>98%) (Atri et al., 2010; Salami et al., 2009).

However, the use of ammonium sulphate fractionation in the method of Salami et al. (2009) limits its commercial suitability as it is unsuitable for large scale preparation (Amersham Biosciences, 1999).

Thus, have also purified camel α-La can be purified from camel milk by the combination of UF (using a 30 kDa or 50 kDa molecular mass cut-off) with either acid precipitation of camel caseins at pH 4.2 (Ellouze et al., 2020) or rennet coagulation by the addition of 1.4 mL of microbial rennet per litre of milk followed by an incubation at 37 °C (Lajnaf et al., 2017; Table 2). The quantity of the added rennet is four times higher than that added to cows' milk due to the lower content of κ-casein in camel milk compared with bovine milk (Ramet, 2001). The obtained permeate can be concentrated using another UF process with a 5 kDa UF membrane (Ellouze et al., 2020).

This method is efficient as it would allow the valorisation of the camel whey by-product of camel cheese industry as an interesting ingredient in food industry. The UF technique was a valuable tool to obtain camel α-La with a relative high purity level (>90%) although electrophoresis of the purified fraction of camel α-La showed some contamination (possibly by PGRP). However, the main drawbacks of membrane separation for purification of camel α-La are the decline in proteins transmission during UF processes as a consequence of fouling, concentration polarisation, protein adsorption, and various protein−protein interactions as well as the rejection rate. The high rejection rate as a consequence of membrane fouling which can be considered as a limiting factor for this purification process. Membrane fouling could be reduced through the combination of UF with other processes including back-pulsing process to clean membrane.

4. Characteristics of camel α-lactalbumin

4.1. Molecular structure of α-lactalbumin

Camel α-La (Swiss-Prot accession number P00710) consists of 123 amino acid residues forming a compact globular structure that is stabilised by four disulphide bonds as α-La structures of other mammalian species (Si Ahmed Zennia et al., 2015).

The primary sequence of camel α-La was determined by Beg, von Bahr-Lindström, Zaidi, and Jörnvall (1985) (Fig. 1). Camel α-La is characterised by a higher molecular mass and pI value (14.43 kDa; pI 4.87) compared with those of bovine α-La (14.18 kDa; pI 4.65) (Table 1; El-Agamy, 2009). The alignment of the α-La sequences of cow and camel milk showed an identity and similarity levels of 69.1% and 82.9%, respectively, between these two sequences, with 39 different residues (Fig. 1; Table 1) (Atri et al., 2010). The identity level between camel and bovine α-La was the highest among all camel milk proteins such as β-, α_{S1}-, α_{S2}- and κ-caseins as reported by Lajnaf et al. (2022). Camel α-La was modelled with the automated protein structure homology-modeling server http://swissmodel.expasy.org/ using the template of the PDB code 1hml (human α-La) (Si Ahmed Zennia et al., 2015).

4.2. Physical and chemical properties of α-lactalbumin

Structural properties of purified camel α-La were characterised using various methods including circular dichroism and fluorescence spectroscopy analysis (Atri et al., 2010; Lajnaf et al., 2022; Redington et al., 2016). The secondary structure of camel and bovine α-la were reported to be very similar in their apo and holo states in agreement with Fig. 3 (Atri et al., 2010). Holo α-La forms were more structured than their apo forms regardless of the milk origin of the protein, suggesting that the removal of calcium from camel α-La causes pronounced structural changes in tertiary and secondary structures similarly to bovine α-La. On the other hand, camel α-La has a high affinity for the Ca^{2+} ions with a higher exposure of hydrophobic groups upon calcium depletion compared with bovine α-La (Atri et al., 2010; Si Ahmed Zennia et al., 2015). Indeed, calcium removal from camel α-La induced a reduction in α-helix content and an elevation in the β-structures. Therefore, the creation of non-native β-sheets in denaturing conditions as well as the exposure of hydrophobic amino-acids upon calcium depletion gives to the α-La the ability to penetrate biological membranes leading to a significant cytotoxic activity (Atri et al., 2010, 2011).

Fluorescence spectroscopy analysis revealed that camel α-La has a different molecular structure with different fluorescence emission spectra and a lower fluorescence intensity when compared with those of its bovine counterpart. A greater surface hydrophobicity of camel α-La was also observed when compared with that of bovine α-La due to the higher hydrophobicity of N-terminal part of the α-helical domain of its molecular structure (Atri et al., 2010). On the other hand, primary sequence comparison of bovine and camel α-La shows greater hydrophobicity of the amino-acid sequence 25−35 in the hydrophobic core. The primary structure of camel α-La has an extra Trp residue characterised by a higher exposure upon calcium depletion leading to a large difference between the fluorescence emissions spectra of camel and bovine α-La in their apo and holo states. However, Trp residues in bovine α-La are more solvent accessible than those of camel protein, which could explain the different fluorescence spectra (Redington et al., 2016).

The variation in the fluorescence intensity of camel and bovine α-La as a function of pH and heating temperature showed that camel α-La is greatly affected by pH variation and heating temperatures. Fluorescence intensity of pure camel α-La was reported to be higher at pH 3 compared with that at pH 6 and 9, while it increased at high heating temperatures (90 °C) regardless of pH value due to the higher exposure of hydrophobic moieties of the proteins molecules at high heat treatment, which highlights the partial denaturation enhanced in acidic conditions (Ellouze et al., 2020; Ellouze, Vial, Attia, & Ayadi, 2019).

Thermal denaturation of bovine and camel α-La were previously investigated to determine the behaviour of this protein towards heating process for the food preservation. Melting temperature of bovine and camel α-La are reported to be around 54 °C and 61 °C, for bovine and camel proteins, respectively (Breydo, Almehdar, El-Fakharany, Redwan, & Uversky, 2018). In the same way, camel α-La showed a higher thermostability in the presence or in the absence of bound ion calcium compared with its bovine counterpart. However, thermal denaturation of bovine α-La in the presence of calcium is clearly more cooperative than that of camel α-La (Redington et al., 2016).

The structural differences between the camel and bovine α-La as a function of various denaturing conditions including pH, heating temperature and guanidine hydrochloride mediated unfolding

Table 1
Physico-chemical characteristics of camel and bovine α-lactalbumins.[a]

Species	α-Lactalbumin property					
	Mass (kDa)	pI	Amino acids	Concentration (mg L^{-1})	Similarity	Identity
Cow	14,186	4.65	123	500−1700	82.9%	69.1%
Camel	14,430	4.87	123	2100−5000		

[a] Abbreviation: pI, isoelectric point. From El-Agamy (2009) and Lajnaf et al. (2022).

were also investigated (Redington et al., 2016). Bovine and camel α-La are differently affected by pH, temperature, guanidine hydrochloride in the presence or lack of calcium. Camel α-La showed a higher stability towards heating and pH-mediated denaturation, whereas it was less stable towards guanidine-mediated denaturation with a fast aggregation and a more disordered structure compared with bovine α-La as confirmed by an in silico study (Redington et al., 2016).

5. Biological properties of camel α-lactalbumin

5.1. Antioxidant activities

Overall, it has been reported that camel milk proteins detained significant antioxidant activity which were evaluated by reducing power and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity tests, iron ferrous chelating activities and ferrous reducing powers (Al-Shamsi, Mudgil, Hassan, & Maqsood, 2018; Jrad et al., 2015; Kumar, Chatli, Singh, Mehta, & Kumar, 2016; Salami et al., 2010).

Antioxidant properties of camel α-La were investigated by various authors as a function of the conformational states of the protein and in comparison with bovine α-La. Camel α-La exhibited greater 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), DPPH radical scavenging activities, metal-chelating activity and ferric-reducing antioxidant power (FRAP) when compared with its bovine counterpart due to its higher content of antioxidant amino-acid residues that are involved in free radical scavenging; proteins present antioxidant activity through antioxidant amino-acid residues including Trp, Met and Cys (Lien, 2003; Salami et al., 2009). Furthermore, camel α-La showed greater amounts of solvent-exposed antioxidant amino-acids that are available for scavenging of free radicals.

Antioxidant properties of camel α-La were enhanced after the calcium removal either with ethylene glycol tetra acetic acid (EGTA) or with EDTA treatments (Lajnaf et al., 2020; Salami et al., 2009). This behaviour was attributed not only to the solvent-exposed amino-acids that are available for antioxidant activities including hydrophobic and aromatic amino-acids, but also the different conformational features between camel α-La and bovine α-La.

On the other hand, antioxidant properties of camel α-La were investigated in comparison with other camel whey proteins. For instance, camel α-La showed higher scavenging activities towards ABTS radicals when compared with other camel proteins fractions such as lactoferrine. Indeed, the differences between ABTS radical scavenging activity between camel α-La and lactoferrine are attributed to the different amino acid residues composition and the presence of an additional Trp residue in the primary sequence of camel α-La, whereas lactoferrine is characterised by the presence of basic amino acid residues including Lys and Arg that are reported to not be efficient free-radical scavengers (El-Hatmi et al., 2014; Salami et al., 2009). Therefore, camel whey proteins were considered are a source of hydrolysates with significantly higher free radical-scavenging properties when compared with bovine whey protein hydrolysates. This behaviour was attributed to the high concentration of camel α-La in camel whey as well as the highest contents of antioxidant amino acid residues in this protein when compared with its bovine counterpart (Salami et al., 2010).

5.2. Antimicrobial activities

In previous studies it has been indicated that camel milk has an antimicrobial effect against both of Gram-positive and Gram-negative bacteria, including *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*, *Listeria innocua*, and *Salmonella typhimurium* (Al Haj & Al Kanhal, 2010). This inhibitory activity was attributed to the presence of antimicrobial substances in camel milk such as caseins, β-casein, α-La, lactoferrin, immunoglobulins and lactoperoxidase (Jrad et al., 2015; Lajnaf et al., 2020). Enzymatic treatment usually improves the antimicrobial activities of bovine and camel whey proteins towards pathogenic bacteria such as *E. coli*. Camel whey proteins showed higher antibacterial activities when compared with bovine proteins due to the difference in the protein composition of whey samples and the dominance of α-La in camel whey (Salami et al., 2010).

Similarly to other pure bovine milk proteins such as β-Lg, pure bovine α-La was reported to have no antibacterial effect against Gram-positive and Gram-negative bacteria including *E. coli*, *S. aureus*, *Lactobacillus casei* and *Lactobacillus acidophilus* (Sedaghati, Ezzatpanah, Mashhadi Akbar Boojar, & Tajabadi Ebrahimi, 2014). In the same, native α-La from human milk had no bactericidal effect against *Streptococcus pneumoniae*. However, this protein could be converted to the active bactericidal form after the removal of calcium ion by EDTA treatment becoming active against *S. pneumoniae* (Håkansson Mossberg, Linse & Svanborg, 2000). Antimicrobial activities of purified camel α-La were recently investigated as a function of the conformational states (apo and holo states) and compared with those of bovine α-La. Camel Holo α-La had no bactericidal activity against Gram-positive or Gram-negative bacteria including *E. coli*, *S. aureus*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*, and no antifungal activity against *Aspergillus sclerotiorum*, *Aspergillus tamarii*, *Penicillium bilaiae* and *Aspergillus protuberus*. However, camel α-La exhibited significant antibacterial and antifungal properties after calcium depletion at a protein concentration of 1 g L^{-1}, unlike its bovine counterpart. Camel apo α-La exhibited moderate antibacterial activities in vitro against *P. aeruginosa* and strong antifungal activities against two species of *Aspergillus* (*A. tamarii* and *A. sclerotiorum*) (Lajnaf et al., 2020). Therefore, although monomeric camel α-La is inactive with no bactericidal properties, it could be converted to the active form under different denaturing conditions such as calcium removal leading to various antibacterial and antifungal properties. Considering the antimicrobial activity result, camel α-La could be used as a natural additive to prevent foods from microbial growth during processing and preservation especially dairy products.

5.3. Digestibility and allergenicity

Digestibility is an important biological property of food proteins. Overall, structural changes in proteins have a great influence on

their digestibility; it may have both desirable and undesirable effects on the digestibility of the ingested proteins and hence, their nutritional quality and availability of essential amino-acids (Mohammadian & Madadlou, 2018).

Since digestibility and bioactivity of food proteins are very important when they are being used as a food ingredient, enzymatic digestibility of the purified camel α-La was studied in comparison with bovine α-La. The differences in the hydrolysis of camel and bovine α-La in their native and molten globular state using individual digestive enzymes including pepsin chymotrypsin and trypsin evidenced that camel α-La has higher digestibility with both enzymes trypsin and chymotrypsin leads when compared with its bovine counterpart. Camel and bovine α-La possess the same number of cleavage sites for chymotrypsin, then the target residues might be available in greater density on the surface of the camel α-La molecule. However, both α-La exhibited similar sensitivity to pepsin enzyme (Salami et al., 2009).

Food allergy is a major health problem affecting approximately 5–8% of pediatric population at the age below 3 years and 1–2% of the adult population (Bu, Luo, Chen, Liu, & Zhu, 2013). Epidemiological studies noted that animal food allergens, especially cow milk proteins allergy, was the most prevalent food allergy for young children, meanwhile, plant-based food allergens are more prevalent in adults (Just, Beaudouin, Deschildre, & Renaudin, 2017). Cows, milk protein allergy (CMPA) represents 10–40% of the total food allergies and 15% of anaphylactic reactions. Some of milk proteins are potentially allergenic and called “Bos d” followed by a number according to the protein type (Apweiler et al., 2004). α-La is one of the most common allergens in cow milk, which is called Bos d4 with an allergenic activity of 12% of total allergic patients (D'Auria et al., 2018). Several studies have evaluated the clinical use of milk from different mammalian species including goat milk, sheep milk, donkey milk, mare milk and camel milk. Especially to children suffering from cows' milk allergy, camel milk represents a promising cow milk alternative due to its deficiency in β-Lg, which is one of the main food allergens in cow milk. Moreover, camel and cows' wheys showed a low cross-reactivity through an animal (Brown Norway rats) due to the highest contents of α-La in camel whey compared with its bovine counterpart, indicating a low protein similarity. Thus, camel α-La could be a promising alternative to cow milk based hypoallergenic infant formulas (Maryniak, Hansen, Ballegaard, Sancho, & Bøgh, 2018).

Camel α-La is suggested to have interesting hypoallergenic properties for children with cows' milk protein allergy; however, comprehensive human studies (in vivo or in vitro studies) on the antigenic characteristics of the pure camel α-La are missing. Thus, there is a clear need to increase our general understanding of the suitability of camel milk proteins as hypoallergenic cow's milk-based infant formulas.

5.4. Cytotoxic effect on cancer cells

In addition to its antioxidant and antimicrobial activities, recent studies have shown that α-La is even able to bind fatty acids including oleic acid and linoleic acid (Atri et al., 2011). Overall, binding of oleic acid to α-La leads to the conversion of this protein into the biologically active form named “human α-La made lethal to tumour cells” (HAMLET). This complex is characterised by its ability to induce death of undifferentiated cells and tumour cells, while it spares healthy and mature cells (Svensson et al., 2000). Several in vitro and in vivo studies showed that the HAMLET complex is distinguished by a highly selective apoptotic activity against tumour cells. On the other hand, similar anticancer activities were reported for the oleic acid-α-La complexes made with α-La isolated from milk of other mammalian species, including cow, goat and camel milk giving raise to BAMLET, GAMLET and CAMLET for bovine, goat and camel α-La made lethal to tumour cells, respectively (Breydo et al., 2018; Uversky et al., 2017).

The CAMLET complex exerted a significant anticancer activity against four cancer cell lines especially breast cancer cells by the induction of selective apoptosis leading to stop the cell-cycle (Uversky et al., 2017). Camel α-La was reported to present a more disordered structure when compared with bovine α-La, which increased its ability to complex with oleic acid leading a higher lethality against cancer cells (Breydo et al., 2018). The intrinsic disorder propensities of α-La from milk of various mammalian species showed a compact ordered structure containing functionally important short regions of intrinsic disorder. Indeed, intrinsically disordered food proteins are characterised by an important multifunctionality and hence, a multitude of physiological functions including anticancer properties (Permyakov et al., 2016; Uversky et al., 2016). Thus, the higher intrinsic disorder of camel α-La when compared with its bovine counterpart contributes to its functionality and its anticancer properties as it does to the majority of other proteins.

Structural properties of α-La-oleic acid and α-La-linoleic acid complexes were studied and compared. Camel α-La was partially unfolded when it was complexed with linoleic acid, whereas, oleic acid did not significantly change the structure of protein. Although the structures of α-La-oleic acid complex and α-La-linoleic acid complex at 60 °C were different, these two complexes showed similar cytotoxic effects to human prostate cancer cells. However, the complex of α-La-oleic acid at 60 °C lost its specific biological activity toward tumor cells once it is renatured after denaturation (Atri et al., 2011).

6. Techno-functional properties of camel α-lactalbumin

6.1. Foaming and interfacial properties at the air/water interface

Milk foams are defined as multiphasic colloidal systems in which the created air bubbles are dispersed and stabilised by the surface-active protein components of milk (Dickinson, 2003; Lajnaf et al., 2022). Milk foams are important in various food products including ice creams, chocolate mousses and whipped creams. Therefore, the research of new foam-forming and stabilising agents continues to develop promising food ingredients characterised of both of health benefits and techno-functional properties (Murray, 2020).

Camel α-La has a great potential as a natural foam stabiliser in food industry due to its particular structure as presented by various authors (Ho, Zou, & Bansal, 2021; Lajnaf et al., 2017, 2022; Laleye, Jobe, & Wasesa, 2008; Maqsood et al., 2019).

First, camel α-La was reported to be the predominant protein at the air–water interface upon camel whey foaming regardless of pH level. Indeed, the lack of β-Lg in camel milk leads to the dominance of camel α-La at the air–water interface of camel. Thus, camel α-La plays a key role in competitive adsorption of camel milk proteins, meanwhile, the other proteins (CSA, camel proteins fractions F and lactoferrin) have been suggested to have a minor role in the creation and stabilisation of foams made from camel milk proteins (Laleye et al., 2008). In the same way, the absence of β-Lg in camel whey allows camel α-La to adsorb more easily and rapidly at the air–water interface leading to lower film rigidity compared with that made by bovine whey. Furthermore, the evolution of the surface tension curves of rennet camel whey and the pure camel α-La were very similar. Therefore, the foaming and interfacial properties at the air–water interface of the soluble fraction of camel milk are maintained by camel α-La due to its higher content as it represents approximately 84% of the total camel whey proteins. However,

camel α-La showed the lowest foaming and interfacial properties when compared with bovine β-Lg and β-casein, despite its predominance at the air−water interface, because of its considerable amounts of α-helix, β-sheet, and the intra-molecular disulphide bridges which lead to a more structured protein compared with flexible caseins. Furthermore, hydrophobic residues of α-La proteins are buried in the interior of the protein molecule. Hence, more energy is needed to unfold the native structure of the α-La and longer time is taken to fully adsorb at an air−water interface when creating foams when compared with other milk proteins such as caseins (Lajnaf et al., 2022; Laleye et al., 2008).

Foaming properties of camel milk proteins are reported to be influenced by several factors, including protein isolation process, heating temperature, pH level, protein concentration and finally the method of foaming. Camel and bovine proteins including α-La showed similar surface-active properties at the air−water leading to similar foaming capacity regardless of pH values (from pH 2 to pH 10). These findings suggested that the structural differences between camel and bovine α-La did not affect their foaming properties (Maqsood et al., 2019).

On the other hand, pH (4.3 and 6.5) and heating temperature (20 °C, 70 °C and 90 °C for 30 min) were reported to affect considerably foaming and interfacial properties of camel α-La at the air−water interface. Foamability and interfacial properties of camel α-La solution were maximal in acid conditions at room temperature at a protein concentration of 1.7 g L^{-1} (Table 2). In acidic conditions, the protonation of the negative groups decreased the electrostatic repulsions of the α-La and induced a partially denaturation with the release of its chelated calcium leading to the molten globular state. This molecular state is obtained at pH values less than 5 and characterised by a higher tensioactivity leading to greater foaming properties of the protein when compared with the native state at neutral pH (Dickinson & Matsumura, 1994; Lajnaf et al., 2017; Matsumura, Mitsui, Dickinson, & Mori, 1994). Increased temperature treatment (i.e., either 70 °C or 90 °C for 30 min) significantly improved the foaming properties of camel α-La solution and its ability to unfold at the air−water interface. At neutral pH, heat treatment was found to improve foamability, and affected the physico-chemical properties of camel α-La by increasing its thiol group contents and decreasing its electronegative charge. However, foamability of the purified camel α-La in acidic conditions decreased after the heating process because of the proteins denaturation and aggregation at this pH level. Despite the reduction of acidified α-La upon heating, foams were more stable after a thermal treatment at pH 4.3 than at 6.5, due to higher levels of protein aggregation at low pH (Fig. 4, Table 3). Thus, aggregates contributed to improve the stability of foams but reduced the adsorption of proteins and the creation of foam.

These findings will be certainly beneficial to dairy processors, as they are helpful for evaluating the potential for commercial use of camel milk foam in various dairy products including cappuccino-style beverages and ice cream. Thus, pilot-scale results of foaming properties are still needed to confirm the strong potential of camel α-La as foaming agent for potential applications in industrial foam production.

6.2. Emulsifying and interfacial properties at the oil/water interface

Emulsification is a common operation in food, cosmetic and pharmaceutic industries. It is encountered with various food products mayonnaise, soups, cream, sauces, salad dressings, margarine and butter. Overall, emulsions are created by the homogenisation of two immiscible liquids which are mechanically agitated whereby one liquid of them becomes dispersed as droplets

Table 2
Purification processes of camel α-lactalbumin (α-La) from milk.

Whey raw material	α-La separation			Reference
	Separation principle	Process parameter	Purity/yield	
Defatted camel milk with a removed part of caseins after high-speed centrifugation (14,000 × *g* for 40 min)	Chromatography and gel filtration	Sephadex G-100 column equilibrated with the dialysis buffer and reverse-phase high-performance liquid chromatography on a C18 column	–	Redington et al. (2016)
Defatted camel milk with a total removal of caseins after an acid precipitation at pH 4.2	Chromatography and gel filtration	Cation-exchanger by fast protein liquid chromatography. Detection of proteins at 280 nm.	–	El-Hatmi et al. (2014)
Skimmed camel milk after removing caseins at pH 4.6	Chromatography and gel filtration	DEAE−cellulose column equilibrated with 50 mM phosphate buffer and Sephadex-G50 gel filtration column	–	Uversky et al. (2017)
Camel whey obtained after acid precipitation	Chromatography and gel filtration	Gel permeation chromatography on a Sephadex G100 column equilibrated in 0.02 M Tris−HCl buffer pH 8.4	>95%	Levieux et al. (2006)
Skimmed camel milk after casein precipitation using ammonium sulphate (26.4%)	Membrane separation	UF using UF membrane of 30 kDa cut-off	>98%	Salami et al. (2009)
Skimmed camel milk after casein precipitation using ammonium sulphate (26.4%)	Membrane separation	UF using membrane of 30 kDa cut-off. UF of obtained permeate on 10 kDa UF membrane	>97%	Atri et al. (2010)
Skimmed camel milk with a removed casein by acidic aggregation at pH 4.2	Membrane separation	UF using a 50 kDa UF membrane. Permeate concentrated using a 5 kDa UF membrane	Yield of 10.9% of the total α-La of camel milk	Ellouze et al. (2020)
Sweet whey obtained after rennet coagulation for 2 h at 37 °C by rennet addition (1.4 mL L^{-1} camel milk)	Membrane separation	UF using a UF membrane of 30 kDa cut-off (Amicon bioseparations model)	Protein purity of 90% and extraction yield of 47.3%	Lajnaf et al. (2017)
Sweet whey obtained after coagulation for 1−2 h at 37 °C by rennet addition (1.4 mL L^{-1} camel milk)	Membrane separation	UF using regenerated cellulose membranes, cut-off 30 kDa, tangential filtration mode	Protein purity of 91.2%	Lajnaf et al. (2020)

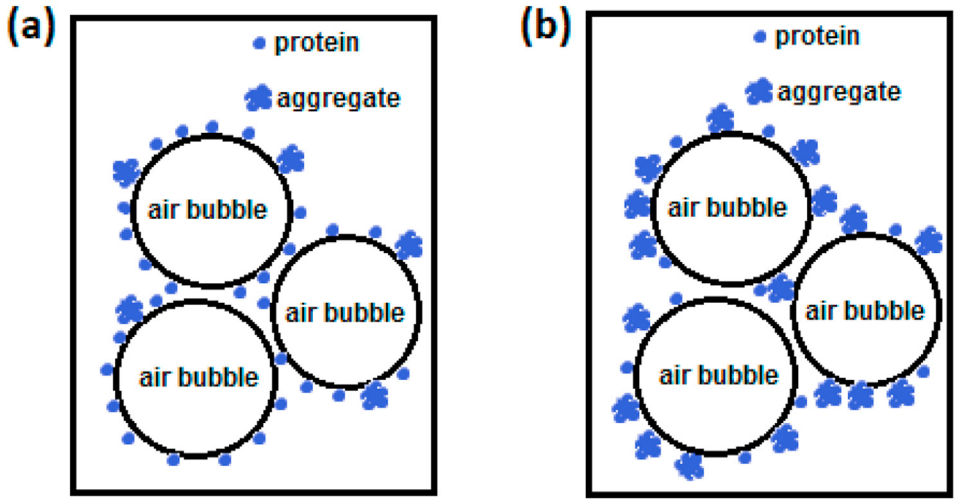

Fig. 4. Schematic presentation of camel α-lactalbumin adsorption after thermal treatment of proteins (a) at near-neutral pH (pH 6.5) and (b) under acidic conditions (pH 4.3) (Lajnaf et al., 2017).

within the other. During homogenisation, emulsifiers are adsorbed onto the surfaces of freshly formed droplets leading to the reduction of the interfacial tension and hence, to the droplet disruption.

Milk proteins are one of the most common emulsifiers used in the food industry. The most common emulsifiers used in the food industry are proteins, they are considered as the most surface-active

Table 3
The effect of physico-chemical conditions (heating and pH level) on the techno-functional properties (foaming and emulsifying properties) of camel α-lactalbumin (α-La).

Techno-functional property	Operating conditions	Results	Reference
Foaming properties	Protein concentration of 2 mg mL^{-1} and pH value of 7.	The predominance of α-La protein at the air–water interface upon camel whey foaming regardless at pH 5 and 7. Foaming properties of camel whey are maintained by camel α-La.	Laleye et al. (2008)
	Protein concentration 1.7 g L^{-1}; heating treatment at 20 °C, 70 °C and 90 °C, 30 min, pH 6.5 and 4.3.	Better foam at pH 4.3 relative to 6.5. Higher ability to stabilise foams after heat treatment at 70 °C and 90 °C especially at pH 4.3 due to proteins aggregates	Lajnaf et al. (2017)
	Protein concentration of 1% (w/v), and pH values adjusted to 2, 4, 6, 8 and 10.	Structural differences between camel and bovine α-La did not affect their foaming properties leading to similar behaviour of foaming properties for camel and bovine wheys.	Maqsood et al. (2019)
	Protein concentration 0.5 g L^{-1}, pH 7.	Similar foaming and interfacial behaviour at the air/water interface compared with bovine α-La and similar foaming and interfacial properties at the air/water interface compared with camel whey. Good foaming properties, but poor ability to stabilise foams: foam half-life lower than 30 s.	Lajnaf et al. (2022)
Emulsifying properties	Protein concentration of 2 mg mL^{-1}, pH values of 5 and 7.	Lower emulsifying properties of camel whey proteins in acidic conditions because of the pronounced aggregation of camel α-La which exhibited low solubility at pH 5.	Laleye et al. (2008)
	Protein concentrations from 3 to 8% (w/v). Heat treatment at 85 °C for 15 min in a temperature controlled water bath.	Higher stability of emulsions made by camel whey proteins after heating process compared with bovine proteins especially camel α-La due to its particular structure characterised by presence of four buried disulphide bridges and the absence of free thiol groups.	Momen et al. (2019).
	Protein concentration 1 g L^{-1}; pH 5 and 7.	Better emulsification activity and stability values at pH 7.0 relative to 5.0. Higher ability to coat oil droplets for camel apo α-La compared with camel holo α-La.	Lajnaf et al. (2020)
	Protein concentration 2 g L^{-1}; heating treatment at 25 °C, 65 °C and 95 °C, 15 min, pH 3.0, 6.0 and 9.0.	The enhancement of the emulsifying and interfacial properties of camel α-La at pH 3.0 and 9.0 and after thermal treatments at 65 °C and 95 °C for 15 min due to the greater molecular flexibility of the protein.	Ellouze et al. (2019)
	Different protein concentrations (0.1%, 0.2% and 0.4%, w/w); pH 3.0, 6.0 and 9.0.	The reduction of the emulsifying properties at high protein concentration especially at pH 6.0, despite the highest ability of the camel α-La to reduce the oil–water interface.	Ellouze et al. (2020)

agents in formulated emulsion systems (Lam & Nickerson, 2015a,b).

Emulsifying properties of bovine α-La has been studied in terms of its emulsion activity and stability indexes (EAI and ESI indexes, respectively) as well as the interfacial tension including surface tension and viscoelastic modulus (Hunt & Dalgleish, 1994; Lam & Nickerson, 2015b; Zhai et al., 2012). Overall, α-La has been reported to change its secondary and tertiary structure upon adhering to the oil−water interface to enhance emulsion stability leading to a preferencial adsorption of α-La over the β-Lg especially in acidic conditions (Zhai et al., 2012).

Emulsifying properties of camel α-La varied considerably depending on pH level, heating temperature and the conformation states including calcium-loaded and calcium-depleted states as summarised in Table 2. First, emulsifying properties of camel and bovine wheys in a comparative study were investigated as a function of pH values to understand the effect of acidification on the competitive adoption of camel milk proteins at the oil−water interface. Lower emulsifying properties of camel whey proteins in acidic conditions were reported after an increase in the droplet size and a formation of a more noticeable creamy layer within 1 h for camel milk compared with bovine whey. This is believed to be due to the pronounced aggregation of camel whey protein molecules (Laleye et al., 2008). The low emulsifying stability of the emulsion made by camel whey at pH 5 revealed an aggregation phenomenon of the protein molecules including camel α-La that exhibited low solubility at pH 5. In the same way, bovine and camel α-La molecules coated the oil-droplets better at pH 7 than those at pH 5 with higher EAI values of apo α-La proteins, especially camel that carried the highest ability to reduce the interfacial tension at the oil−water interface (Lajnaf et al., 2020; Laleye et al., 2008). Indeed, emulsions were more stable at neutral pH due to the higher electrostatic repulsive forces between oil droplets at this pH level, especially for bovine α-La that presented higher ESI values when compared with its camel counterpart in both holo and apo states (Dickinson & Matsumura, 1994). Higher emulsifying properties of camel α-La were also found at pH 3 when compared with both of pH levels 6 and 9 with a higher viscosity of the created emulsions. Thus, high proteins concentrations showed low emulsifying activity, especially near its isoelectric point despite the highest ability of the camel α-La to reduce the oil−water interface under these conditions (Ellouze et al., 2020). While acidification destabilised emulsions created by camel α-La, the EDTA treatment led to better tensioactive properties by changing the proteins conformation and the increase of its flexibility and hence, its ability to create oil-in-water emulsions.

Emulsifying properties of camel and bovine whey proteins were also studied as a function of heating temperature. Emulsions prepared with camel whey proteins were more stable against thermal treatment when compared with bovine whey proteins. Camel whey proteins were more stable than bovine whey proteins, especially camel α-La. Indeed, camel α-La gives whey a higher heat stability due to its particular structure characterised by presence of four buried disulphide bridges and the absence of free thiol groups (Momen et al., 2019). On the other hand, it is notable that the combination of heating and acidification of whey proteins before creation of the emulsion gives results similar to acidification. For instance, Heat treatment at 65 °C and 95 °C for 15 min was found to enhance emulsifying properties of camel α-La as well as its ability to reduce the surface tension at the air−water interface due to the greater exposure of its buried hydrophobic groups especially at pH 3. However, the oil-in-water emulsion made by camel α-La was less stable at pH 6 after heating treatment due to the reduced hydrophobic interactions and the minimised electrostatic repulsive forces between the created oil droplets (Ellouze et al., 2019).

Finally, these findings confirmed the strong potential of camel α-La as emulsifier agent for potential applications in industrial emulsion production regardless of pH value and heating temperature. Previous studies have demonstrated the obvious influence of pH and heating temperature on the physicochemical properties and oil-water interfacial properties of camel α-La, but no study has revealed the effect of ionic strength on the competitive adsorption of this protein at the oil−water interface while creating emulsions.

7. Conclusion

In the present paper, we reviewed the techno-functional and biological properties of camel α-La that can be obtained by various purification processes including the UF and Sephadex-G50 gel filtration column. The biological activities showed that camel α-La exhibited higher antioxidant activities than its bovine counterpart with respect to DPPH and ABTS scavenging activity, FRAP and iron chelating activities, especially in its apo forms due to the higher content of anti-oxidant amino acid residues (Trp, Cys and Lys) and the different conformational features between camel and bovine α-La. Furthermore, camel apo α-La showed significant in vitro antibacterial and antifungal activities against bacteria such as *P. aeruginosa* and against three fungal pathogens species including *A. tamarii*, *A. sclerotiorum* and *P. bilaiae*. In addition to its antimicrobial activities, camel α-La was reported to bind oleic acid leading to the creation of the CAMLET complex, which exerted potent anticancer activities against cancer cell lines including breast cancer cells by inducing a selective apoptosis and causing arrest of the cell-cycle.

The techno-functional properties of the purified camel α-La are influenced by several variables including pH value, heating temperature and the conformational state. Camel α-La was found to foam better in acidic conditions relative to neutral pH, unlike the emulsifying properties that decreased in pH 5. This behaviour is attributed to the electrostatic repulsion that preserves droplets from flocculating and enhances the emulsion stability of camel α-La. On the other hand, heat treatments enhanced the ability of camel α-La to stabilise emulsions and foams.

There is still little information in the literature regarding the in vivo digestibility and allergenicity of camel α-La. Assessment of in vitro digestibility using both of trypsin and chymotrypsin enzymes showed greater degree of hydrolysis of camel α-La than of the bovine protein regardless of the conformational state of proteins. Reports on the allergenicity of camel milk proteins showed that there was a weak cross-reactivity between whey proteins from camel and bovine milk especially α-La. However, further studies of the pure camel α-La are needed for confirmation of the consumption of this protein as an alternative for children with cows' milk allergy.

Finally, this review highlighted the important biological and techno-functional properties of the purified camel α-La compared with its bovine counterpart which may suggest camel α-La as a possible alternative protein in manufacture of dairy products and infant formulae; whereas, further study on the digestibility and the allergenicity of the protein is required.

Declaration of competing interest

None.

Acknowledgements

This work was supported by the Ministry of Scientific Research and Technology of Tunisia.

References

Acharya, K. R., Stuart, D. I., Walker, N. P. C., Lewis, M., & Phillips, D. C. (1989). Refined structure of baboon α-lactalbumin at 1.7 Å resolution: Comparison with C-type lysozyme. *Journal of Molecular Biology, 208*, 99–127.

Al Haj, O. A., & Al Kanhal, H. A. (2010). Compositional, technological and nutritional aspects of dromedary camel milk. *International Dairy Journal, 20*, 811–821.

Al-Shamsi, K. A., Mudgil, P., Hassan, H. M., & Maqsood, S. (2018). Camel milk protein hydrolysates with improved technofunctional properties and enhanced antioxidant potential in in vitro and in food model systems. *Journal of Dairy Science, 101*, 47–60.

Alsobh, A., Zin, M. M., Vatai, G., & Bánvölgyi, S. (2022). The application of membrane technology in the concentration and purification of plant extracts: A review. *Periodica Polytechnica Chemical Engineering, 66*, 394–408.

Amersham. (1999). *Protein purification handbook*. Uppsala, Sweden: AB Amersham Pharmacia Biotech.

Apweiler, R., Bairoch, A., Wu, C. H., Barker, W. C., Boeckmann, B., Ferro, S., et al. (2004). UniProt: The universal protein knowledgebase. *Nucleic Acids Research, 32*, D115–D119.

Atri, M. S., Saboury, A. A., Moosavi-movahedi, A. A., Goliaei, B., Alijanvand, H. H., Sharifzadeh, A., et al. (2011). Structure and stability analysis of cytotoxic complex of camel α-lactalbumin and unsaturated fatty acids produced at high temperature. *Journal of Biomolecular Structure and Dynamics, 28*, 919–928.

Atri, M. S., Saboury, A. A., Yousefi, R., Chobert, J., Haertle, T., & Moosavi-Movahedi, A. A. (2010). Comparative study on heat stability of camel and bovine apo and holo α-lactalbumin. *Journal of Dairy Research, 77*, 43–49.

Barbana, C., & Pérez, M. D. (2011). Interaction of α-lactalbumin with lipids and possible implications for its emulsifying properties - A review. *International Dairy Journal, 21*, 727–741.

Beg, O. U., von Bahr-Lindström, H., Zaidi, Z. H., & Jörnvall, H. (1985). The primary structure of α-lactalbumin from camel milk. *European Journal of Biochemistry, 147*, 233–239.

Brew, K. (2013). α-Lactalbumin. In P. L. H. McSweeney, & P. F. Fox (Eds.), *Advanced dairy chemistry* (pp. 261–273). San Diego, CA, USA: Springer.

Breydo, L., Almehdar, H. A., El-Fakharany, E. M., Redwan, E. M., & Uversky, V. N. (2018). Not all AMLETs are made equal: Complexes of cow and camel α-lactalbumin with oleic acid show different structure and stability. *Journal of Biomolecular Structure and Dynamics, 36*, 4405–4412.

Bu, G., Luo, Y., Chen, F., Liu, K., & Zhu, T. (2013). Milk processing as a tool to reduce cow's milk allergenicity: A mini-review. *Dairy Science & Technology, 93*, 211–223.

Chaplin, L. C., & Lyster, R. L. J. (1986). Irreversible heat denaturation of bovine α-lactalbumin. *Journal of Dairy Research, 53*, 249–258.

Chatterton, D. E. W., Smithers, G., Roupas, P., & Brodkorb, A. (2006). Bioactivity of β-lactoglobulin and α-lactalbumin—Technological implications for processing. *International Dairy Journal, 16*, 1229–1240.

D'Auria, E., Mameli, C., Piras, C., Cococcioni, L., Urbani, A., Zuccotti, G. V., et al. (2018). Precision medicine in cow's milk allergy: Proteomics perspectives from allergens to patients. *Journal of Proteomics, 188*, 173–180.

Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids, 17*, 25–39.

Dickinson, E., & Matsumura, Y. (1994). Proteins at liquid interfaces: Role of the molten globule state. *Colloids and Surfaces B: Biointerfaces, 3*, 1–17.

El-Agamy, E. I. (2009). Bioactive components in camel milk. *Bioactive Components in Milk and Dairy Products, 107*, 159–192.

El-Agamy, E. I., Abou-Shloue, Z., & Abdel-Kader, Y. I. (1998). Gel electrophoresis of proteins, physicochemical characterization and vitamin C content of milk of different species. *Alexandria Journal of Agricultural Research, 43*, 57–70.

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

El-Fakharany, E. M., El-Baky, N. A., Linjawi, M. H., Aljaddawi, A. A., Saleem, T. H., Nassar, A. Y., et al. (2017). Influence of camel milk on the hepatitis C virus burden of infected patients. *Experimental and Therapeutic Medicine, 13*, 1313–1320.

El-Hatmi, H., Jrad, Z., Khorchani, K., Dari, A., & Girardet, J.-M. (2014). Fast protein liquid chromatography of camel α-lactalbumin fraction with radical scavenging activity. *Emirates Journal of Food and Agriculture, 26*, 309–316.

Ellouze, M., Lajnaf, R., Zouari, A., Attia, H., Ayadi, M. A., & Vial, C. (2020). Camel α–lactalbumin at the oil–water interface: Effect of protein concentration and pH change on surface characteristics and emulsifying properties. *Colloids and Surfaces B: Biointerfaces, 189*. Article 110654.

Ellouze, M., Vial, C., Attia, H., & Ayadi, M. A. (2019). Camel α-lactalbumin at the oil-water interface: Effect of pH and heat treatment on the structure, surface characteristics and emulsifying properties. *LWT, 116*. Article 108550.

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

FAOSTAT. (2020). *Crops and livestock products*. https://www.fao.org/faostat/en/#data/QCL.

Geng, X. L., Tolkach, A., Otte, J., & Ipsen, R. (2015). Pilot-scale purification of α-lactalbumin from enriched whey protein concentrate by anion-exchange chromatography and ultrafiltration. *Dairy Science & Technology, 95*, 353–368.

Grobler, J. A., Wang, M., Pike, A. C., & Brew, K. (1994). Study by mutagenesis of the roles of two aromatic clusters of α-lactalbumin in aspects of its action in the lactose synthase system. *Journal of Biological Chemistry, 269*, 5106–5114.

Hailu, Y., Hansen, E. B., Seifu, E., Eshetu, M., Ipsen, R., & Kappeler, S. (2016). Functional and technological properties of camel milk proteins: A review. *Journal of Dairy Research, 83*, 422–429.

Håkansson, A., Svensson, M., Mossberg, A., Sabharwal, H., Linse, S., Lazou, I., et al. (2000). A folding variant of α-lactalbumin with bactericidal activity against *Streptococcus pneumoniae*. *Molecular Microbiology, 35*, 589–600.

Hazebrouck, S. (2016). Laits de chèvre, d'ânesse et de chamelle: une alternative en cas d'allergie au lait de vache? *Innovations Agronomiques, 52*, 73–84.

Heine, W. E., Klein, P. D., & Reeds, P. J. (1991). The importance of α-lactalbumin in infant nutrition. *Journal of Nutrition, 121*, 277–283.

Ho, T. M., Zou, Z., & Bansal, N. (2021). Camel milk: A review of its nutritional value, heat stability, and potential food products. *Food Research International, 2012*. Article 110870.

Hunt, J. A., & Dalgleish, D. G. (1994). Effect of pH on the stability and surface composition of emulsions made with whey protein isolate. *Journal of Agricultural and Food Chemistry, 42*, 2131–2135.

Huppertz, T., Fox, P. F., & Kelly, A. L. (2018). The caseins: Structure, stability, and functionality. In R. Y. Yada (Ed.), *Proteins in food processing* (pp. 49–92). Kidlington, UK: Woodhead Publishing.

Jrad, Z., El-Hatmi, H., Adt, I., Khorchani, T., Degraeve, P., & Oulahal, N. (2015). Antimicrobial activity of camel milk casein and its hydrolysates. *Acta Alimentaria, 44*, 609–616.

Just, J., Beaudouin, E., Deschildre, A., & Renaudin, J.-M. (2017). *Allergies alimentaires: Nouveaux concepts, affections actuelles, perspectives thérapeutiques*. Amsterdam, the Netherlands: Elsevier Health Sciences.

Kamau, S. M., Cheison, S. C., Chen, W., Liu, X. M., & Lu, R. R. (2010). α-Lactalbumin: Its production technologies and bioactive peptides. *Comprehensive Reviews in Food Science and Food Safety, 9*, 197–212.

Kappeler, S. R., Farah, Z., & Puhan, Z. (2003). 5′-Flanking regions of camel milk genes are highly similar to homologue regions of other species and can be divided into two distinct groups. *Journal of Dairy Science, 86*, 498–508.

Karue, C. N. (1998). The dairy characteristics of the Kenyan camel. *Colloques-CIRAD*, 55–60.

Kumar, D., Chatli, M. K., Singh, R., Mehta, N., & Kumar, P. (2016). Antioxidant and antimicrobial activity of camel milk casein hydrolysates and its fractions. *Small Ruminant Research, 139*, 20–25.

Kuwajima, K., Ikeguchi, M., Sugawara, T., Hiraoka, Y., & Sugai, S. (1990). Kinetics of disulfide bond reduction in α-lactalbumin by dithiothreitol and molecular basis of superreactivity of the Cys6-Cys120 disulfide bond. *Biochemistry, 29*, 8240–8249.

Lajnaf, R., Gharsallah, H., Jridi, M., Attia, H., & Ayadi, M. A. (2020). Antioxidant and antibacterial activities, interfacial and emulsifying properties of the apo and holo forms of purified camel and bovine α-lactalbumin. *International Journal of Biological Macromolecules, 165*, 205–213.

Lajnaf, R., Picart-Palmade, L., Attia, H., Marchesseau, S., & Ayadi, M. A. (2017). The effect of pH and heat treatments on the foaming properties of purified α-lactalbumin from camel milk. *Colloids and Surfaces B: Biointerfaces, 156*, 55–61.

Lajnaf, R., Picart-Palmade, L., Attia, H., Marchesseau, S., & Ayadi, M. A. (2022). Foaming and air-water interfacial properties of camel milk proteins compared to bovine milk proteins. *Food Hydrocolloids, 126*. Article 107470.

Laleye, L. C., Jobe, B., & Wasesa, A. A. H. (2008). Comparative study on heat stability and functionality of camel and bovine milk whey proteins. *Journal of Dairy Science, 91*, 4527–4534.

Lam, R. S. H., & Nickerson, M. T. (2015a). The effect of pH and temperature pretreatments on the physicochemical and emulsifying properties of whey protein isolate. *LWT - Food Science and Technology, 60*, 427–434.

Lam, R. S. H., & Nickerson, M. T. (2015b). The effect of pH and temperature pretreatments on the structure, surface characteristics and emulsifying properties of α-lactalbumin. *Food Chemistry, 173*, 163–170.

Levieux, D., Levieux, A., El-Hatmi, H., & Rigaudière, J.-P. (2006). Immunochemical quantification of heat denaturation of camel (*Camelus dromedarius*) whey proteins. *Journal of Dairy Research, 73*, 1–9.

Lien, E. L. (2003). Infant formulas with increased concentrations of α-lactalbumin. *American Journal of Clinical Nutrition, 77*, 1555S–1558S.

Maqsood, S., Al-Dowaila, A., Mudgil, P., Kamal, H., Jobe, B., & Hassan, H. M. (2019). Comparative characterization of protein and lipid fractions from camel and cow milk, their functionality, antioxidant and antihypertensive properties upon simulated gastro-intestinal digestion. *Food Chemistry, 279*, 328–338.

Maryniak, N. Z., Hansen, E. B., Ballegaard, A. S. R., Sancho, A. I., & Bøgh, K. L. (2018). Comparison of the allergenicity and immunogenicity of camel and cow's milk – A study in brown Norway rats. *Nutrients, 10*. Article 1903.

Matsumura, Y., Mitsui, S., Dickinson, E., & Mori, T. (1994). Competitive adsorption of α-lactalbumin in the molten globule state. *Food Hydrocolloids, 8*, 555–566.

McGuffey, M. K., Epting, K. L., Kelly, R. M., & Foegeding, E. A. (2005). Denaturation and aggregation of three α-lactalbumin preparations at neutral pH. *Journal of Agricultural and Food Chemistry, 53*, 3182–3190.

McGuffey, M. K., Otter, D. E., van Zanten, J. H., & Allen Foegeding, E. (2007). Solubility and aggregation of commercial α-lactalbumin at neutral pH. *International Dairy Journal, 17*, 1168–1178.

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

Mohamed, H., Ayyash, M., & Kamal-Eldin, A. (2022). Effect of heat treatments on camel milk proteins – A review. *International Dairy Journal, 133*. Article 105404.
Mohamed, H., Johansson, M., Lundh, Å., Nagy, P., & Kamal-Eldin, A. (2020). Caseins and α-lactalbumin content of camel milk (*Camelus dromedarius*) determined by capillary electrophoresis. *Journal of Dairy Science, 103*, 11094–11099.
Mohammadian, M., & Madadlou, A. (2018). Technological functionality and biological properties of food protein nanofibrils formed by heating at acidic condition. *Trends in Food Science & Technology, 75*, 115–128.
Momen, S., Salami, M., Alavi, F., Emam-Djomeh, Z., & Moosavi-Movahedi, A. A. (2019). The techno-functional properties of camel whey protein compared to bovine whey protein for fabrication a model high protein emulsion. *LWT, 101*, 543–550.
Muller, A., Daufin, G., & Chaufer, B. (1999). Ultrafiltration modes of operation for the separation of α-lactalbumin from acid casein whey. *Journal of Membrane Science, 153*, 9–21.
Murray, B. S. (2020). Recent developments in food foams. *Current Opinion in Colloid & Interface Science, 50*. Article 101394.
Neyestani, T. R., Djalali, M., & Pezeshki, M. (2003). Isolation of α-lactalbumin, β-lactoglobulin, and bovine serum albumin from cow's milk using gel filtration and anion-exchange chromatography including evaluation of their antigenicity. *Protein Expression and Purification, 29*, 202–208.
Omar, A., Harbourne, N., & Oruna-Concha, M. J. (2016). Quantification of major camel milk proteins by capillary electrophoresis. *International Dairy Journal, 58*, 31–35.
Permyakov, E. A., & Berliner, L. J. (2000). α-Lactalbumin: Structure and function. *FEBS Letters, 473*, 269–274.
Permyakov, E. A., Permyakov, S. E., Breydo, L., Redwan, E. M., Almehdar, H. A., & Uversky, V. N. (2016). Disorder in milk proteins: α-Lactalbumin. Part C. Peculiarities of metal binding. *Current Protein and Peptide Science, 17*, 735–745.
Ramet, J.-P. (2001). *The technology of making cheese from camel milk (*Camelus dromedarius*) (FAO Animal Production and Health Paper 113)*. Rome, Italy: Food & Agriculture Organisation.
Redington, J., Breydo, L., Almehdar, H. A., Redwan, E. M., & Uversky, V. N. (2016). α-Lactalbumin: Of camels and cows. *Protein and Peptide Letters, 23*, 1072–1080.
Rösner, H. I., & Redfield, C. (2009). The human α-lactalbumin molten globule: Comparison of structural preferences at pH 2 and pH 7. *Journal of Molecular Biology, 394*, 351–362.
Sakandar, H. A., Ahmad, S., Perveen, R., Aslam, H. K. W., Shakeel, A., Sadiq, F. A., et al. (2018). Camel milk and its allied health claims: A review. *Progress in Nutrition, 20*, 15–29.
Salami, M., Moosavi-Movahedi, A. A., Ehsani, M. R., Yousefi, R., Haertlé, T., Chobert, J.-M., et al. (2010). Improvement of the antimicrobial and antioxidant activities of camel and bovine whey proteins by limited proteolysis. *Journal of Agricultural and Food Chemistry, 58*, 3297–3302.
Salami, M., Yousefi, R., Ehsani, M. R., Razavi, S. H., Chobert, J.-M., Haertlé, T., et al. (2009). Enzymatic digestion and antioxidant activity of the native and molten globule states of camel α-lactalbumin: Possible significance for use in infant formula. *International Dairy Journal, 19*, 518–523.
Sedaghati, M., Ezzatpanah, H., Mashhadi Akbar Boojar, M., & Tajabadi Ebrahimi, M. (2014). β-Lactoglobulin and α-lactalbumin hydrolysates as sources of antibacterial peptides. *Journal of Agricultural Science and Technology, 16*, 1587–1600.
Si Ahmed Zennia, S., Mati, A., Saulnier, F., Verdier, Y., Chiappetta, G., Mulliert, G., et al. (2015). Identification by FT-ICR-MS of *Camelus dromedarius* α-lactalbumin variants as the result of nonenzymatic deamidation of Asn-16 and Asn-45. *Food Chemistry, 187*, 305–313.
Svensson, M., Håkansson, A., Mossberg, A.-K., Linse, S., & Svanborg, C. (2000). Conversion of α-lactalbumin to a protein inducing apoptosis. *Proceedings of the National Academy of Sciences, 97*, 4221–4226.
Swaisgood, H. E. (1995). Protein and amino acid composition of bovine milk. In R. G. Jensen (Ed.), *Handbook of milk composition* (pp. 464–468). London, UK: Academic Press.
Uversky, V. N., El-Fakharany, E. M., Abu-Serie, M. M., Almehdar, H. A., & Redwan, E. M. (2017). Divergent anticancer activity of free and formulated camel milk α-lactalbumin. *Cancer Investigation, 35*, 610–623.
Uversky, V., Permyakov, S. E., Breydo, L., Redwan, E. M., Almehdar, H. A., & Permyakov, E. A. (2016). Disorder in milk proteins: α-Lactalbumin. Part B. A multifunctional whey protein acting as an oligomeric molten globular "oil container" in the anti-tumorigenic drugs, liprotides. *Current Protein and Peptide Science, 17*, 612–628.
Walstra, P., Wouters, J. T. M., & Geurts, T. J. (2005). *Dairy science and technology*. Boca Raton, FL, USA: CRC Press.
Zhai, J., Hoffmann, S. V., Day, L., Lee, T.-H., Augustin, M. A., Aguilar, M.-I., et al. (2012). Conformational changes of α-lactalbumin adsorbed at oil–water interfaces: Interplay between protein structure and emulsion stability. *Langmuir, 28*, 2357–2367.