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A review of camel β-casein: From purification processes, to bioactivity and techno-functionality

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ABSTRACT

In recent years, interest in pure casein fractions has grown, especially the β-casein due to its physicochemical, biological and techno-functional properties. Camel milk represents a source of β-casein with higher contents than those of cow's milk which makes its isolation more feasible and economical. The present review deals with the current technologies for the purification and isolation of β-casein from camel milk on a laboratory scale. Furthermore, antioxidant, antimicrobial, chaperone, foaming and emulsifying properties of camel β-casein are presented. The examination of the biological activities and technological functionalities of the camel β-casein highlights its potential as a valuable ingredient in the food industry due to its nutritional value which is of significant scientific and industrial interest.

1. Introduction

Caseins are the most abundant protein fraction in milk, representing approximately 80% of its total protein content (Jensen et al., 2012). Specifically, caseins are mainly present in milk gathered as large colloidal aggregates resulting from their supramolecular assembling. These aggregates are known as casein micelles with a mean size of 120–150 nm for micelles in cow's, whereas the average diameter depends from micelles composition and milk origin (Dalgleish, 2011). Caseins performs an important function in mammary secretory cells: its biological function is to carry high amounts of insoluble salt calcium phosphate in the aqueous environment of milk leading to provide nutritional support to infants (Perinelli et al., 2019).

Compositionally, caseins consist of four sub-fractions, α_{S1}-, α_{S2}-, β-, and κ-caseins with molar ratio in cow's milk approximately being 4:1:4:1 (De Kruif & Holt, 2003). These individual caseins are a family of phosphoproteins with molecular weights that range between 19 and 25 kDa and with an average isoelectric point between 4.1 and 5.3 (Liang & Luo, 2020).

The β-casein is a single polypeptide chain with 209 amino acid residues, with opened structure and a molecular mass of 24 kDa representing ~33%–45% of the casein in cow's milk with a concentration that ranges between 9.0 and 11.0 g/L (Farrell Jr et al., 2004; Wal, 1998). It's the second most abundant form of casein in cow's milk.

In particular, great attention has been paid by the scientific community towards the β-casein, due to its high concentration and its particular structural characteristics. Indeed, β-casein, like other casein proteins, are considered intrinsically unstructured proteins due to the lack of tertiary structure with a strong amphiphilic character (Tompa, 2002). It combines with calcium phosphate to create complex aggregates in the form of casein micelles in milk. The β-casein is distinguished by its high content of phosphorylated serine and Pro residues, making it be the most hydrophobic with a high ability of chelating calcium ions (Markoska et al., 2021; Sun et al., 2024).

The β-casein is known by its high genetic variability. Thirteen different genetic variants have been identified up to now including A1, A2, A3, B, C, D, E, F, G, H1, H2, I and J. Among all these genetic variants, A1 and A2 are β-casein the most common variants (Li et al., 2022). Overall, milk that contains only A2 β-casein is called "A2 milk", while milk with only A1 β-casein is called "A1 milk". A2 β-casein is the natural prototype of modern dairy cows, whereas, A1 genetic variation is dominant at present due to natural selection mutation (Osman et al., 2021).

The β-casein fraction of human milk is much higher than that in bovine milk as it represents more than 85% of total caseins and hence, this protein is of interest especially in terms of enrichment for infant

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formulas (Hambraeus & Lönnerdal, 2003). The main difference between human and bovine β-casein is the degree of phosphorylation. Indeed, the bovine β-casein usually occurs in fully phosphorylated forms, while its human counterpart is found in multiphosphorylated forms with a number of phosphate groups that ranges between 0 and 5 groups per molecule. The phosphorylation state of the β-casein in infant formula has the key role in its gastrointestinal digestibility. Overall, dephosphorylation process of bovine β-casein has been suggested to make it closer to its human counterpart (Broyard & Gaucheron, 2015; Ettelaie et al., 2014). The β-casein is considered as an important protein for infant nutrition depending on their phosphorylated degree. Hence, considerable attention has been given by the scientific and industrial communities towards the production of this protein which is regarded as a promising food ingredient due to its abundance and its particular physicochemical structure compared to the other caseins (κ-, α_{S1}- and α_{S2}- caseins).

Studies have found that bovine β-casein is rich in essential amino acids, making it one of the most important sources of amino acids in infant foods (Priyadarshini et al., 2018). The β-casein and its hydrolysis products were reported to be closely associated with human health including immune system diseases, neurological diseases, digestive system diseases and blood system diseases (Chen et al., 2024). For instance, the hydrolyzed β-casein by enzymes from some sources releases bioactive peptides that possess unique physiological functions. Indeed, they showed significant effects on regulating sleep, scavenging free radicals to achieve antioxidant properties, regulating blood pressure of organism angiotensin-converting enzyme (ACE), and regulating immune function. In addition, some common diseases, such as cardiovascular diseases as well as diabetes prevention were also reported to be related to β-casein (Daniloski et al., 2021; Zhou et al., 2021). The digestive peptides produced by bovine β-casein after gastrointestinal digestion can also stimulate phagocytosis by macrophages, enhance anti-infective effects, they can also modulate the composition and diversity of intestinal microbiota and inhibit the colonization of pathogenic bacteria (Guantario et al., 2020; Liu et al., 2022). In addition, β-casein hydrolyzed peptides can promote the expression of intestinal immune barrier mucins in infants and children leading to the reduction of the incidence of intestinal inflammation (Plaisancié et al., 2015).

Furthermore, one important factor when introducing a new ingredient in a product is its availability, its easy separation and its cost of production (Lajnaf al., 2022a). Hence, the identification and isolation of new food ingredients sources is a significant challenge to scientists and food industrials.

Camel milk can be suggested as a new source of the β-casein due the presence of this protein in high content when compared to cow milk (reaching an amount of 65% of total caseins in camel milk), and the low content of the remaining caseins especially κ-casein in camel milk, which makes its isolation easier and more feasible (Kappeler et al., 2003). Furthermore, camel caseins were reported to be less phosphorylated which leads to greater digestibility which could be of great interest for infant's formulas (Kappeler et al., 1998).

This scientific review seeks to provide an overview of the literature relating to camel β-casein proteins. Indeed, it builds on prior reviews of β-casein in camel milk (Almi-Sebbane et al., 2018; Barzegar et al., 2008; Ellouze et al., 2021; Esmaili et al., 2011; Lajnaf et al., 2021) and aims to provide both the scientific and industrial communities with a more comprehensive understanding of camel β-casein, the current technologies for the fractionation and isolation of this protein, its biological activities and technological functionalities.

2. Camel milk caseins and β-casein

2.1. Camel milk caseins composition and structure

Camel milk has become more popular in many countries in the world including Asia Africa and Europe, due to its exceptional nutritional and medicinal properties such as anti-diabetic, hypo-cholesterolemic, hypo-allergenic and anti-cancer properties (Al haj & Al Kanhal, 2010; Hailu et al., 2016; Izadi et al., 2019). Indeed, several researchers have reported the medicinal properties of camel milk through both of *in vitro* and *in vivo* assays. For instance, *in vivo* tests of Korish and Arafah (2013) showed that camel milk possess significant hypo-cholesterolemic and hypoglycemic effects due to its unique composition that is rich in minerals; vitamins, insulin and insulin-like protein. On the other hand, camel α-lactalbumin, when bound to oleic acid, exerted potent anti-cancer activity against four cancer cell lines, particularly breast cancer cells, using *in vitro* assays by inducing selective apoptosis and causing cell cycle arrest (Uversky et al., 2017). Furthermore, camel milk proteins including caseins and whey proteins are reported to be promising alternatives to cow's milk-based hypoallergenic infant formulas due to their low cross-reactivity, as revealed by *in vitro* and *in vivo* tests of Maryniak et al. (2018).

According to the latest statistics of Food and Agriculture Organization of the United Nations FAO-STAT, camel milk production (both species) in the world is reported to be about 4.12 million tons per year representing 0.44% of the total milk production, while cow's milk represents 81% with 753.32 million tons (FAOSTAT, 2022). Nations in African continent are currently expected to be the biggest producer of camel milk worldwide (70% of total camel milk) followed by Asian countries (30% of total camel milk production) (FAOSTAT, 2022).

Literature data have shown that the physicochemical and biochemical composition of camel milk is much closer to that in human milk than that of bovine milk (Al haj & Al Kanhal, 2010). Various compositional differences between both of bovine and camel milk were investigated, such as a higher contents of minerals such as iron, sodium, potassium and magnesium as well as vitamins including A, B-2, C and E. Furthermore, lower amounts of total solids lactose, proteins and fat in camel milk were found compared with cow milk (Al haj & Al Kanhal, 2010; Konuspayeva et al., 2009).

As for milks of other mammalian species, camel milk proteins can be classified according to their solubility into two main components: caseins and whey proteins. Casein fraction is the major protein in camel milk representing 61.8% - 88.5 % of the total proteins (Ereifej et al., 2011). This proportion is similar to that of other mammalian species that belong to Camelidae family. For instance, caseins in llama milk (*Lama glama*) represent approximately 74.5% of total proteins in milk and appear to be similar to that in cow milk with different proportions of protein subclasses (Fernandez & Oliver, 1988; Saadaoui et al., 2014). Meanwhile, Alpaca milk (*Vicugna pacos*) showed lower content of caseins to sheep and camelid milk such as llama ranging between 73% and 72% of the proteins in this milk (Martini et al., 2015). On the other hand, caseins were the most abundant protein Chinese Bactrian Camel milk (*Camelus bactrianus*), ranging from 65% to 80% of total proteins of this milk depending on the lactation period (Zhang, Fu, et al., 2005).

The size distribution shows that camel micelles are greater in diameter than those in cow's milk as the majority of camel micelles range between 200 and 500 nm. These particular characteristics lead to the formation of a less firm coagulum which is obtained from camel milk by during cheese processing (Farah & Ruegg, 1989). Camel casein fraction contains the four known caseins with different proportions and different physicochemical characteristics when compared to bovine caseins. Camel β-casein is the main protein with the highest proportion of 65% followed by the α_{S1}-casein representing 22% of total casein fraction. Meanwhile, the β-casein and the α_{S1}-casein accounted in average for 39% and 38% in bovine casein, respectively. The κ-casein represents 3.5 % of the total camel casein compared with 13 % in cow's milk caseins (Kappeler et al., 2003). On the other hand, Mohamed et al. (2020) confirmed through capillary electrophoreses results that the β-casein is the major casein in camel milk with and average percentage of 67% of total camel caseins, followed by α_{S1}-casein (25.6%), α_{S2}-casein (4.2%) and κ-casein (3.2%). In the same way, Reversed-Phase High--Performance Liquid Chromatography (RP-HPLC) analysis showed that

camel milk caseinates contained the same caseins as bovine caseinates including κ-, α- and β-casein. The β-casein accounted in average 53.4% of total caseins in camel milk and 44.5% in bovine milk. On the other hand, camel caseinates comprises in average 45.5% of α_{S1}- and α_{S2}-casein, while bovine caseinates contain 47.1% of these caseins. The κ-casein content was also significantly lower in camel milk when compared to bovine milk with percentages of 1.1% and 8.4% of total camel and bovine caseinates, respectively (Lajnaf et al., 2022b). These percentages are in close agreement with those of Ryskaliyeva et al. (2018) who noted that the average value of β-casein in camel milk is 53.2% of total caseins in camel milk, while the α_{S1}-, α_{S2}- and κ-casein accounted for 37.9%, 5.8% and 3.6%, respectively. The protein proportions of llama milk have been investigated only to a very limited extent, and conflicting information about the identity and concentration of the constituent proteins exists. Previous studies noted that α-casein and β-casein accounted for 38.5% and 35.4%, respectively of total casein in llama milk (Fernandez & Oliver, 1988). These values are lower than those reported for casein in camel milk. Furthermore, It has to be noted that γ-casein was also identified in llama milk after the use of β-mercaptoethanol in caseins identification experiments (Rosenberg, 2006). On the other hand, the whole casein fraction in Chinese Bactrian camel milk contained about 38% of α_{S1}-casein, 21% of α_{S2}-casein and 41% of β-casein. Among the β-casein fraction, 12% were constituted by κ-casein which was reported to be co-eluted simultaneously with β-casein (Ochirkhuyag et al., 1997). Meanwhile, Zhang, Yao, et al. (2005) found that no protein bands homologues to bovine κ-casein could be detected in the electrophoresis pattern of milk from the Alxa and Gobi Red Bactrian camel milk.

2.2. Molecular and structural characteristics of camel β-casein

The β-casein is the main protein in camel milk with a mean concentration of 15 g/L according to Mohamed et al. (2020) and 15.6 g/L as reported by Kappeler et al. (1998) representing more than the half of total camel caseins according to previous studies. Meanwhile, Omar et al. (2016) found lower values of the β-casein concentration in camel milk, around 12.78 ± 0.92 g/L using capillary electrophoreses method.

Camel β-casein (Swiss-Prot accession number Q9YVD0) consists of 217 amino acid residues forming an intrinsically unstructured protein with some structural differences as compared with the bovine one. The number of amino acid residues for camel β-casein is higher than that of bovine β-casein (209 amino acid residues) as well as caprine and ovine β-caseins (207 amino acid residues). Meanwhile, it's lower when compared to that of equine β-casein (226 amino acid residues) (Li et al., 2022; Martin et al., 2011, pp. 821–842; Miclo et al., 2007). The primary sequence of camel β-casein was determined by Kappeler et al. (1998) (Fig. 1) and the gene of this protein as well as its promoter region were sequenced and characterized by Pauciullo et al. (2014). Camel β-casein is characterized by a higher molecular mass and pI value (24.9 kDa; pI 4.66) compared with those of bovine β-casein (23.58 kDa; pI 4.49) (El-Agamy, 2009; Kappeler et al., 1998). Molecular masses of proteins in camel milk were measured by Kappeler et al. (1998) using matrix-assisted Laser Desorption Ionization–mass Spectrometry (MALDI) including phosphorylation. Observed molecular masses of camel β-casein was comparable in that of another camelid specie: *Llama glama* which is determined to be 24.97 kDa, suggesting a high level of sequence homology between both β-caseins (Saadaoui et al., 2014). However, molecular mass of camel β-casein was different when compared to that of β-casein in Chinese Bactrian milk (*Camelus bactrianus*) which ranges between 26 and 31 kDa (Zhang, Fu, et al., 2005). The alignment of the β-casein sequences of bovine and camel milk showed an identity and similarity levels of 67.2% and 84.5%, respectively, between these two sequences, which are reported to be the highest identity and similarity levels among the other camel caseins (Fig. 2) (Barzegar et al., 2008; Lajnaf et al., 2022). Structural characteristics of camel β-casein were characterized using various methods including Fourier-Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR) and fluorescence spectroscopy analysis (Barzegar et al., 2008; Ellouze et al., 2021; Esmaili et al., 2011; Lajnaf et al., 2020, 2022). An analysis of the FTIR spectra of camel and bovine β-casein revealed that their secondary structures are quite similar due the high similarity and identity levels of their sequences. However, the FTIR spectra comparison indicated a difference regarding the peak positions of amide I of camel and bovine β-caseins and hence, a significant difference of the β-sheet structure of these homologous proteins (Cao et al., 2019; Lajnaf et al., 2020). The Proton NMR spectra of camel β-casein showed that methyl signals ranging between 0.7 and 0.9 ppm of the spectra are more pronounced when compared to those in the spectra of bovine β-casein due to the highest contents of Ile residues in random coil regions of the camel β-casein suggesting a higher hydrophobicity when compared to its bovine counterpart (Lajnaf et al., 2020; Salmen et al., 2012). Fluorescence spectroscopy analysis were used by many authors in order to understand the difference between camel and bovine β-casein in term of surface characteristics especially the exposure of hydrophobic amino acids such as Trp, Tyr and Phe. Fluorescence emission spectra of camel β-casein at excitation wavelength of 275 nm as

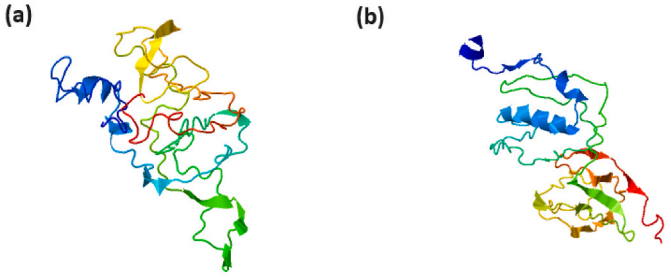

Fig. 2. The 3D structures of the β-caseins in cow's (a) and camel milk (b) as modeled using TASSER server (Yang et al., 2015) (https://zhanggroup.org/I-TASSER/).

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CASB_CAMDR      1 REKEEFKTAGEALESISSSEESITHINKQKIEKFKIEEQQQTEDEQQDKIYTFPQPQSLV
CASB_BOVIN      1 RELEELNVPGEIVESLSSSEESITRINK-KIEKFQSEEQQQTEDELQDKIHPFAQTQSLV
                  ** **     **   ** ******** *** *****  ********* ****  * * ****

CASB_CAMDR     61 YSHTEPIPYPILPQNFLPPLQ-PAVMVPFLQPKVMDVPKTKETIIPKRKEMPLLQSPVVP
CASB_BOVIN     60 YPFPGPIPNS-LPQNIPPLTQTPVVVPPFLQPEVMGVSKVKEAMAPKHKEMPFPKYPVEP
                  *    ***   ****  *  * * *  ***** ** * * **   ** ****    ** *

CASB_CAMDR    120 FTESQSLTLTDLENLHLPLPLLQSLMYQIPQPVPQTPMIPPQSLLSLSQFKVLPVPQQMV
CASB_BOVIN    119 FTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPTVMFPPQSVLSLSQSKVLPVPQKAV
                  *********** *********** * *  ** * * * **** ***** *******  *

CASB_CAMDR    180 PYPQRAMPVQAVLPFQEPVPDPVRGLHPV
CASB_BOVIN    179 PYPQRDMPIQAFLLYQEPVLGPVRGPFPI
                  ***** ** ** *  ****  ****  *
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Fig. 1. Sequence alignment of β-caseins from cow's milk (CASB_BOVIN) and camel milk (CASB_CAMDR) as provided by Ebi serach (http://www.ebi.ac.uk) and performed by Web Expasy (http://web.expasy.org/sim). *: identical residues.

a function of emission wavelength between 285 and 450 nm was so different when compared to that of its bovine counterpart. Indeed, the deficiency in Trp and the higher Tyr contents in camel β-casein when compared to its bovine counterpart led to different emission maximum wavelengths (Lajnaf et al., 2022). Camel β-casein contains 5 Tyr and 10 Phe residues, that are mainly located in the hydrophobic part of its primary structure, which is totally devoid of Trp residue. Meanwhile, bovine the primary structure of β-casein which contains one Trp, 4 Tyr and 8 Phe residues (Esmaili et al., 2011). Barzegar et al. (2008) revealed that camel β-casein the intensity emission spectrum decreased with temperature after heating at 45 °C, 50 °C and 60 °C during 60 min at pH 7.5. On the contrary, Esmaili et al. (2011) reported that thermal treatments at 30 °C and 37 °C increases intensity emission spectrum of the pure camel β-casein due to the exposed hydrophobic patches upon heating.

Fluorescence intensities of pure camel β-casein was also studied as a function of pH variations and thermal treatments. reported to be higher at pH 3 when compared with those at pH 6 and 9 regardless of heating temperature. Thus, this work confirmed that camel β-casein showed a flexible protein conformation with a higher resistance to pH variations but a greater sensitivity to thermal treatments when compared to its bovine counterpart (Ellouze et al., 2021). Indeed, camel β-casein was more protective against a change in pH due to its hidden hydrophobic residues, whereas this protein exposed higher surface hydrophobic residues after intensive heat treatment 95 °C and especially in acidic conditions (Barzegar et al., 2008; Ellouze et al., 2021).

3. Isolation of camel β-casein

Camel β-casein is not yet purchased commercially as purified protein despite its abundant content in camel milk. Therefore, purification processes of this protein were previously investigated and reported on laboratory scale operating under various selected process parameters.

Camel milk is distinguished by its lower content in κ-casein and the different molecular structure of this protein when compared to its bovine counterpart leading to different rennet coagulation. Indeed, camel κ-casein was found to have a different hydrolysis site by chymosin. Indeed, chymosin hydrolyzes camel κ-casein at the Phe97-Ile98, while the hydrolysis site on bovine κ-casein is Phe105-Met106 (Fig. 3) (Kappeler et al., 1998). Coagulation of camel milk through the action of rennet requires a higher quantity. Hence, camel rennet caseins were obtained after rennet coagulation of fresh milks at 37 °C in the presence of rennet content which is four times higher than that used to coagulate cow's milk (0.35 and 1.4 mL of rennet per liter of bovine and camel milks, respectively) (Felfoul et al., 2015; Lajnaf et al., 2019). Camel caseins can also be separated in camel milk through acid precipitation. However, camel caseins precipitate from camel skim milk upon acidification to 4.3 at 20 °C (Felfoul et al., 2015; Lajnaf et al., 2019; Wangoh et al., 1998).

Numerous production technologies were previously reported and described to obtain pure β-casein fractions from camel caseins. The choice of the suitable isolation method to use is mainly determined by the required purity levels, the potential use of the protein and the production scale (Table 1). Overall, chromatographic methods are efficient in obtaining camel β-casein samples with a high purification level but with low yield. Furthermore, chromatographic methods have the disadvantage of limited possibility for scaling-up. Therefore, the production of large quantities of β-casein is needed to be used as ingredient in food industry. The use of other purification methods such as selective solubilization is recently becoming more common. Indeed, this process presents many advantages such as mild operating conditions, efficiency in separation and easy scaling up (Huppertz et al., 2006). Isolation processes of camel β-casein are summarized in Table 1, which gives the β-casein purity, yield and process parameters as presented by various authors.

3.1. Selective solubilization

The β-casein is commonly purified through some methods that use the cold solubilization of this protein, starting with rennet casein as raw material (Huppertz et al., 2006; Le Magnen & Maugas, 1995; Ward & Bastian, 1996). Indeed, the β-casein is the most hydrophobic among the other caseins (α- and κ-casein). This protein tends to dissociate from casein micelles when hydrophobic bonds are weaker even at its pI (Huppertz et al., 2006). Selective solubilization of β-casein is carried out by weakening of hydrophobic bonds at low temperature values (Law, 1996). Therefore, cooling milk leads to dissociate some caseins especially the β-casein which can be separated simply from β-casein-depleted phase by physical process such as centrifugation and filtration. Cold solubilization was used by Huppertz et al. (2006) for the separation of the β-casein fraction from cow's milk at laboratory scale. Lajnaf et al. (2021) modified the process of Huppertz et al. (2006) in order to purify β-casein from camel milk in a comparative study with cow's milk. In this work, skim camel milk was used as raw material (Table 1). First, it was renneted and held for 1–2 h at 37 °C. The coagulum was separated by centrifugation (5000g at 20 °C for 20 min). Whey was removed and the curd obtained was washed and kept with water at 80 °C during 5 min to enable the action of the added rennet, followed by centrifugation (5000g at 20 °C for 15 min). Cold water (5 °C) was finally added to the heated curd and held for up to 24 h to solubilize camel β-casein. The obtained supernatant of the centrifugation after cold solubilization (5000 g for 15 min at 5 °C) represented the purified camel β-casein with a high purity level (81.5%) compared with the purity of bovine β-casein (72.4%) obtained by the same authors (Lajnaf et al., 2021). Similar results were obtained by Ellouze et al. (2021) who used the same modified and achieved a comparable camel β-casein purity of 95% with a recovery of 73% and 51% for camel and bovine β-caseins, respectively.

3.2. Chromatographic techniques

In recent decade, industrial demand for purified individual caseins, especially the β-casein, has increased. Therefore, several purification methods, either at small or large scale, have been developed for the isolation of this protein.

For camel β-casein, chromatographic methods which are used for both of isolation and analytical purposes were used to separate it from

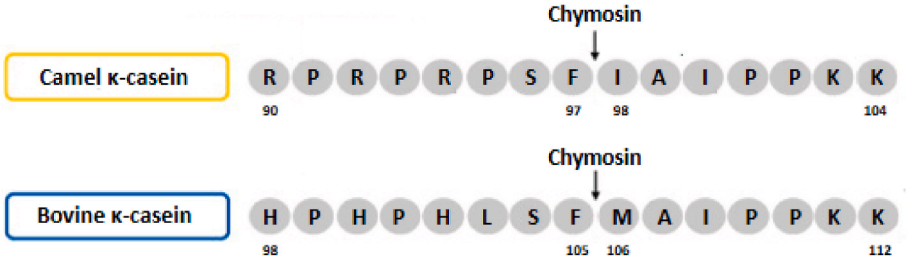

Fig. 3. Chymosin hydrolysis sites in bovine κ-casein and camel κ-casein for the rennet coagulation as first step of the purification of both bovine and camel β-caseins.

Table 1
Purification processes of β-casein from camel milk.

Casein raw material	β-casein separation		β-casein purity/ yield	Reference
	Separation principle	Process parameter		
Renneted skim camel milk: caseins curd obtained after centrifugation at 5000*g* at 20 °C for 15 min	Cold solubilization	Incubation of caseins curd in cold water at 5 °C during 24 h Centrifugation at 5 °C (5000 g for 15 min at 5 °C)	Purity: 81.5%	Lajnaf et al. (2016, 2020) and Lajnaf et al. (2021)
Defatted camel milk with a total removal of whey after rennet coagulation	Cold solubilization	Incubation of heated caseins curd (at 80 °C during 5 min) in cold water at 5 °C during 24 h Centrifugation at 5 °C (5000 g for 15 min at 5 °C)	Purity: 95% Yield: 75%	Ellouze et al. (2021)
Skimed camel milk after removing whey at pH 4.6	Ion-exchange chromatography	DEAE-cellulose column equilibrated with 20 mM phosphate buffer pH 6·6, containing 4m urea, 35 mM EDTA, 10 mM β-mercaptoethanol and 1 mM phenyl- methanesulfonyl fluoride	Purity: 95%–97%	Barzegar et al. (2008), Esmaili et al. (2011) and Salami et al. (2011)
Skimed camel milk after casein precipitation at pH 4.3 using HCl (1M)	FPLC	FPLC separation with a Q-Sepharose HiPrep column which is equilibrated with 5 column volumes of 0.02 M imidazole buffer (pH 7) containing 4 M urea	Yield of 43% of the total β-casein in camel milk	Almi-Sebbane et al. (2018)

other caseins including α_{S1}-, α_{S2}- and κ-caseins. First, Ion-exchange chromatography was used as a purification technique by Barzegar et al. (2008), Esmaili et al. (2011) and Salami et al. (2011) for the separation of the β-casein fractions at laboratory scale (Table 1). Camel casein fraction (obtained after an acid precipitation at pH 4.6) was washed, lyophilized and incubated at 20 °C. Afterwards, camel caseins were dissolved in sodium acetate buffer and mixed with containing 10 mM of β-mercaptoethanol and 4M urea the obtained mixture was applied to the equilibrated on anion-exchange column DEAE-cellulose for isolation purpose which is pre-equilibrated with 20 mM imidazole buffer (Barzegar et al., 2008). Camel β-casein fractions were then detected on Sodium Sodecyl Sulfate (SDS) Polyacrylamide Gel Electrophoresis (PAGE) by Coomassie blue staining and their purity was judged to be greater than 97% as revealed by SDS PAGE electrophoreses as an analytical technique used in this research work (Barzegar et al., 2008; Esmaili et al., 2011). The β-casein was also purified from camel using cation-exchanger by the Fast Protein Liquid Chromatography (FPLC) as an isolation technique in order to investigate the antibacterial activity of its peptic hydrolysate (Almi-Sebbane et al., 2018). The recovery of β-casein obtained using this method was about 43% of the total β-casein of the used camel skim milk, (purity not specified). First, camel caseins were obtained after acid precipitation at pH 4.3, they were then lyophilized, mixed with buffer containing urea and β-mercaptoethanol and subjected to the used isolation technique: FPLC separation with a Q-Sepharose column equilibrated with imidazole buffer (pH 7) containing 4 M urea (Almi-Sebbane et al., 2018). Afterwards, the identity and the purity of the eluted casein fractions were established by comparing both of SDS and urea PAGE electrophoregrams of each FPLC fraction which was used as analytical technique (Almi-Sebbane et al., 2018).

It can be concluded that these methods were reported to be reproducible leading to obtain the camel β-casein with well-preserved structural characteristics and biological properties. However, the main drawbacks of these method are that they are not easily scaled-up to produce large quantities and the obtained fractions are not suitable for human consumption, since these methods involve applications of high concentrations of both of urea and β-mercaptoethanol.

Finally, there is a need to produce large quantities of camel β-casein as it is the main protein in camel milk using purification methods which can result suitable protein for human consumption and that are applicable for production on an industrial scale.

4. Functionality of camel β-casein

4.1. Antioxidant properties

Antioxidants from plants are widely accepted as natural antioxidants in human health. Meanwhile, animal-derived proteins from milk, meat and eggs have been described and reported as a source of antioxidants. In particular, products derived from milk proteins, such as caseinates and whey protein isolate have been extensively tested for their antioxidant potential *in vitro* (Cervato Benvenuto Cestaro, Giovanna, 1999; Corrochano et al., 2019). Overall, it has been previously reported that caseins of camel milk showed significant antioxidant properties which were evaluated by many tests such as ferrous reducing powers (FRAP), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2-2-Azinobis-3ethylbenthiazoline-6-sulphonic acid (ABTS) radical scavenging activity tests (Al-Shamsi et al., 2018; Kumar et al., 2016). Antioxidant properties of pure camel β-casein were previously investigated as a function of protein concentration and in comparison with its bovine counterpart (Esmaili et al., 2011; Lajnaf et al., 2021; Salami et al., 2011). ABTS radical scavenging activity of purified camel β-casein at a concentration of 20 mM and at pH 7.4 was explored by Esmaili et al. (2011). These authors reported that this protein showed significant ABTS radical scavenging activities of camel milk were greatly enhanced after interactions with curcumin. Furthermore, Salami et al. (2011) reported that chymotrypsin -generated β-casein protein hydrolysates were the most effective in scavenging the ABTS radicals when compared with Pepsin and Trypsin due to the primary structure of this protein which plays an important role in its antioxidant activities.

On the other hand, antioxidant properties of camel β-casein were investigated in comparison with its bovine counterpart à different protein concentrations (0.1, 1 and 5 g/L) (Lajnaf et al., 2021). For instance, camel β-casein exhibited greater DPPH radical scavenging activities and metal-chelating activity especially at high protein concentration (5 g/L in this study) due to its to its highest hydrophobicity index among all milk proteins and to its primary sequence of this protein which plays the key role in antioxidant activities. Indeed, camel β-casein has greater antioxidant amino acids content as Met, Tyr, Leu, Ile and Pro when compared to bovine β-casein (Lajnaf et al., 2021). Therefore, camel milk has higher antioxidant activity due to its high concentration of β-casein as the highest contents of antioxidant amino acid residues in this protein as compared with bovine β-casein.

Similarly to camel milk proteins, goat milk proteins also have significant antioxidant properties (ALKaisy et al., 2023). Indeed, Ahmed et al. (2015) confirmed that goat's milk caseins exhibited potent scavenging activities against both of superoxide and DPPH radicals after enzymatic hydrolysis using pepsin. This study is the first to demonstrate the production of antioxidant peptides from caprine caseins using pepsin hydrolysis, which offers a viable alternative to waste utilization in the cheese industry.

4.2. Antibacterial and antifungal properties

The antimicrobial peptides and peptides which are derived from milk present the advantage of being obtained from harmless and inexpensive

source. Therefore, there is a growing interest in their utilization in food industry as food grade biopreservatives or/and as health-promoting food supplements (Almi-Sebbane et al., 2018).

To date, camel caseins are able to generate peptides with various biological activities, such as antioxidant properties, especially after enzymatic hydrolysis. However, only a few studies investigated the antibacterial and anti-fungal activities of camel caseins as well as their hydrolysates (Almi-Sebbane et al., 2018; Jrad et al., 2015; Kumar et al., 2016; Lajnaf et al., 2021). First, Jrad et al. (2015) found that native caseins 20–40 g/L detained significant antibacterial activities towards Gram-positive and Gram-negative bacteria as *Escherichia coli* and *Listeria innocua*. However, similarly to pure bovine β-casein, camel protein was reported to have no antibacterial effect against.

Gram-positive and Gram-negative bacteria such as *Escherichia coli*, *Staphylococcus aureus, Enterococcus faecalis* and *Pseudomonas aeruginosa* even at a protein concentration of 5 g/L (Lajnaf et al., 2021). Hence, the antimicrobial activities of camel caseins as reported by Jrad et al. (2015), can not only be attributed to other camel caseins including α_{S1}-, α_{S2}- and κ-casein, but also to higher protein concentration. On the other hand, bioactive peptides obtained from β-casein after enzymatic hydrolysis using pepsin have been reported to exert considerable antibacterial activities against *Escherichia coli, Staphylococcus aureus* and *Listeria innocua* after ultrafiltration through membranes with a 1 kDa cut-off. Mass spectrometry analysis of the β-casein hydrolysates revealed that there are two putative antibacterial fragments with around 50% homology with casecidins 15 and 17 from its bovine counterpart (Almi-Sebbane et al., 2018).

In addition to its antimicrobial activity, camel β-casein is also characterized by its important antifungal activities against two against two fungal species of *Aspergillus* (*Aspergillus tamarii* and *Aspergillus sclerotiorum*) at a protein concentration of 5 g/L. Thus, camel β-casein showed a great ability to enable the synthesis of the fungal cell wall and to disturb fungal membrane structure resulting the cell lysis of fungal species. On the other hand, the same work evidenced that bovine β-casein didn't show any antifungal activities under the same experimental conditions (Lajnaf et al., 2021). These findings showed that pure mammalian milk proteins exhibited significant antimicrobial properties in their native state without enzymatic hydrolysis. For instance, the α_{S2}-casein from goat milk at a protein concentration of 5 g/L showed important antimicrobial effects against various Gram-positive and Gram-negative bacteria, including *Listeria monocytogenes*, *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhi*, and *Shigella flexneri*. Thus, caprine α_{S2}-casein was considered as an important antimicrobial agent (Triprisila et al., 2016).

4.3. Chaperone activities

In most general sense, chaperones from protein's family are molecules that assist the folding of partner molecules through a combination of mechanisms including the unfolding of the misfolded substrate and the prevention of its aggregation especially in denaturation conditions. These properties are overall achieved due to their binding to the exposed hydrophobic parts of the proteins in their denatured/unfolded states (Guagliardi et al., 1995; Todd et al., 1996). Several reports have been published about disordered proteins that displayed significant chaperone activities such as caseins (α- and β-caseins) which are considered as potent inhibitors of fibril formation by κ-casein (Thorn et al., 2005). The β-casein is classified in the category of intrinsically unstructured proteins and considered as a mobile disordered molecule leading to important chaperone activities (Cases et al., 2005; Zhang, Fu, et al., 2005).

Chaperone activities of camel β-casein were investigated in comparison with bovine β-casein using alcohol dehydrogenase as a substrate (Barzegar et al., 2008). This study confirmed that camel β-casein presented significant chaperone activities, whereas these activities were 4-fold lower than that of bovine β-casein due to the different molecular structure of both homologous proteins. Indeed, intrinsic fluorescence studies confirmed that camel β-casein is dotted with lower hydrophobic surfaces at all studied temperatures (45 °C, 50 °C and 60 °C) when compared to its bovine counterpart leading to weaker interactions of camel β-casein with the aggregation-prone pre denatured molecular species of the substrate ADH, which resulted in lower chaperone activity when compared to bovine protein.

4.4. Polyphenol encapsulation

Previous studies have evidenced that caseins are excellent carriers for bioactive components with hydrophobic nature such as curcumin. Indeed, curcumin, which is considered as the most researched bioactive compound, was previously encapsulated in bovine caseins nanoparticles using the spray dry technique leading to an improvement of its solubility and biological activities (Nair & Gopi, 2020).

Esmaili et al. (2011) confirmed that camel β-casein can also be used for curcumin encapsulation similarly to its bovine counterpart. These authors reported that camel β-casein interacts with curcumin through hydrophobic interactions leading to increase its solubility 2500 fold. Furthermore, the presence of camel β-casein enhanced the cytotoxicity of curcumin to human leukemia cell line K-562 as well as its antioxidant activities (Esmaili et al., 2011).

4.5. Digestibility and allergenicity

Caseins are known to be sensitive to enzymatic hydrolysis as they are more rapidly hydrolyzed than globular whey proteins due to their greater flexibility and open structures (Dupont & Tomé, 2020). Since digestibility and bioactivity of proteins are very important when they are being used as ingredient in food products, enzymatic digestibility of camel caseins was studied in comparison with bovine caseins (Al-Shamsi et al., 2018; Salami et al., 2008, 2011). First, Salami et al. (2008) reported that both camel and bovine caseins share the same digestibility level toward chymotrypsin which was higher than trypsin due to the greater number of potential hydrolytic sites in the primary structures of casein targeted by chymotrypsin regardless of milk origin. These authors noted that camel β-casein showed a higher resistance to tryptic-than to chymotryptic-enzymatic digestion and an overall lower digestibility toward the studied enzymes when compared to α_{S1}-casein in camel milk. Indeed, hydrolysis of camel α_{S1}-casein by chymotrypsin was complete in 5 min whereas minor portion of camel β-casein remained intact even after 15 min of hydrolysis (Salami et al., 2008). On the other hand, Al-Shamsi et al. (2018) noted noticeable digestion of all caseins after hydrolysis using bromelain, alcalase and papain, especially using alcalase and papain. Quantitative analysis of different camel milk caseins revealed that β-casein and κ-casein were significantly degraded in all camel milk hydrolysates after 4 and 6 h, while small amounts of α-caseins were still found after 6 h of hydrolysis. Degradation percentage β-casein ranged between 95.6% and 100% confirming that this casein is highly digestible by proteases compared to the other proteins in camel milk (Al-Shamsi et al., 2018).

Cow's milk allergy (CMA) is one of the most common food allergies especially in children representing 10%–40% of the total food allergies and 15% of total anaphylactic reactions. Caseins are considered as one of the major allergens in cow's milk which are called Bos d8 (Bos d9) consisting of α_{S1}-casein (Bos d9), α_{S2}-casein (Bos d10), β-casein (Bos d11) and κ-casein (Bos d12) with an allergenic activity of 57 % of total allergic patients (Peñas et al., 2006). Among the known four caseins in cow's milk, the β-casein is considered as highly allergenic as 92% of sera from patients allergic to whole caseins present IgE directed against this protein (Bernard et al., 1998). Therefore, β-casein epitopes might be responsible for clinical symptoms in allergic reactions to bovine caseins. Previous studies have evaluated the clinical use as milk substitution of milk from different mammalian species including sheep milk, goat milk, mare milk, donkey milk and camel milk. Mares and donkeys produce a

similar type of milk, with no significant differences in the proteins, lactose, lipids and minerals contents. Furthermore, the composition of equine's milks is more similar to that of human milk due to the low levels of proteins, caseins (40%–45% of total proteins), the high levels of lactose (Verduci et al., 2019). Therefore, many clinical studies indicated the high tolerability of donkey's and mare's milk by infants who are suffering from CMA and who were intolerant to hypoallergenic formulas including extensively hydrolyzed milk formulas, soy protein-based infant formula and even amino acid infant formulas (Souroullas et al., 2018). For instance, the clinical trials of Carroccio et al. (2000) who studied a group of 21 children with CMA who underwent daily treatment with a donkey's milk based diet (210–250 mL/kg/day) revealed that 86% these patients showed a clinical tolerance and negative Radio-Allergo-Sorbent Test (RAST) results. Furthermore, 52% of patients who tolerated donkey's milk became cow's milk-tolerant during this study leading to conclude that donkey's milk can be used for the treatment of CMA in children (Carroccio et al., 2000). In the same way, Curadi and Giampietro (2001) proved the palatability and adequacy of mare's milk to children with CMA through a simple trial administered mare's milk to a group of 25 children with CMA. Indeed, twenty-four children with CMA (96%) liked and tolerated this milk and only one patient presented positive skin prick test results. These findings suggested that equine's milk (mare's and donkey's milk) can be regarded as a suitable alternative for children with IgE-mediated CMA (Lajnaf et al., 2023; Souroullas et al., 2018).

Up to now, camel milk is considered as a new protein source for nutrition for children with CMA due to its deficiency in β-lactoglobulin and its low contents in α_{S1}-casein (El-Agamy et al., 2009). Moreover, camel and cow's milk proteins showed a low sequence identity levels when compared to other mammalian milk proteins (sheep, goat, mare and donkey milk proteins) ranging between 40.5% (a_{S1}-casein, Bos d9) and 53.5% (κ-casein, Bos d12), except for β-casein (Bos d11) which is characterized by the highest identity level of 63.8% among other caseins (Lajnaf et al., 2023). Thus, the sequence alignment between camel and bovine β-caseins reveals the presence of 4 domains of 8 continuous identical amino acids which could induce a significant risk of cross-reaction (Fig. 1) (Hazebrouck, 2016). Immunoblotting assays of bovine and camel milk developed with IgE from sensitized brown rats were performed by Maryniak et al. (2018) in order to investigate the specificity of the responses towards milk proteins of both mammalian species. These authors found that, among all caseins, antibodies reacted most pronounced with the camel milk β-casein fraction.

Therefore, camel β-casein can cross-react with bovine anti-β-casein antibodies, whereas, comprehensive human studies (*in vitro* and *in vivo* studies) on the antigenic properties of the isolated camel β-casein are still missing. Thus, there is a clear need to increase our general understanding of the suitability of camel β-casein as an ingredient in hypoallergenic formulas for patients with CMA.

4.6. Emulsifying properties

Emulsions are mixture of two immiscible liquids which are mechanically agitated leading dispersion of droplets within the other (Lam & Nickerson, 2015). Emulsions are encountered in many food products in food industry including soups, mayonnaise, margarine, sauces, cream, salad dressings and butter (Guzey & McClements, 2006). Milk proteins are well known by their excellent emulsion-creating and stabilizing properties. Indeed, during emulsion formation, milk protein molecules are rapidly adsorbed at the oil-water surfaces of the newly created oil droplets to form a protein film. Hence, conformational changes of protein structure were likely to occur once they are adsorbed, and the resulting steric stabilizing layer protects fine droplets against coalescence (Cases et al., 2005).

Emulsifying properties of camel β-casein varied greatly depending on pH level and heating temperature. First, emulsifying properties of camel and bovine β-caseins were investigated in a comparative study as a function of pH level to understand the effect of acidification on the competitive adoption of camel milk proteins at the oil-water interface. Lower values of emulsion activity and stability indexes (EAI and ESI, respectively) of camel and bovine β-caseins in acidic conditions (pH 5) were reported after an increase in the oil droplet size compared to those at pH 7 and 9. This is believed to be due to the pronounced precipitation of casein protein molecules at acid pH value. Furthermore, emulsions which are stabilized by proteins were more stable at neutral pH values due to the higher electrostatic repulsive forces between the created oil droplets under these conditions (Dickinson & Matsumura, 1994). On the other hand, camel β-caseins molecules coated the oil-droplets better than its bovine counterpart with higher EAI values, meanwhile, bovine β-casein showed a higher ability to stabilize emulsions especially at neutral pH values. Indeed, camel β-casein carried the highest surface hydrophobicity and greatest ability to reduce the interfacial tension at the oil-water interface (Lajnaf et al., 2021).

On the other hand, previous studies noted that acidification of caseins dominates the interfacial behavior over preheating at the oil-water interface. Thus, the combination of acidification and heating of caseins before creation of the emulsion gives results similar to acidification (Mellema & Isenbart, 2004). In the same way, Ellouze et al. (2021) compared the emulsifying properties of camel and bovine β-caseins fractions obtained by cold solubilization of renneted skimmed milk. These authors found that camel β-casein is characterized by higher emulsifying activities (EAI index) that those of its bovine counterpart especially at pH 9 regardless of heating temperature. However, camel protein showed higher sensitivity to pH variations than bovine with lower values of EAI at pH 6. Furthermore, heat treatment at 65 °C and 95 °C for 15 min was found to enhance the ability of camel β-casein to stabilize emulsions especially at pH 6 and to reduce these properties in its bovine counterpart. This behavior was explained by the conformational changes in the β-casein molecules either by intermolecular associations and aggregations, or by thermal distortions that reduces their ability to stabilize oil-in-water interfaces (Ellouze et al., 2021).

Finally, these researches confirmed the strong potential of camel β-casein as emulsifier agent for potential applications in industrial emulsion-based food production regardless of heating temperature pH and value.

4.7. Foaming properties

Foams are defined as colloidal systems in which air bubbles are dispersed in an aqueous continuous phase (Damodaran, 1997). Foam production is a common operation in food processing as it is encountered with many products such as ice cream, mousses, toppings, meringue, marshmallows and aerated chocolate (Borcherding et al., 2008). Milk proteins are widely used for creation and stabilization of foamed food products. Therefore, foaming properties of solutions containing different milk proteins has been investigated under various physicochemical conditions including pH, thermal treatment and ionic strength (Borcherding et al., 2008; Marinova et al., 2009; Zhang et al., 2004).

For camel milk, β-casein was found to play a key role in the great foaming properties that characterized this milk. It plays the main role in stabilizing camel milk foams due to its particular secondary structure (Lajnaf et al., 2020). Therefore, camel β-casein has a great potential as natural stabilizer of foams in food industry as revealed by various authors (Al-Shamsi et al., 2018; Lajnaf et al., 2016, 2022). First, camel β-casein showed similar foamability to that of bovine counterpart with lower foam stability values at protein concentration 0.5 g/L and at pH 7. Camel was reported to be the predominant protein at the air-water interface alone in binary mixture with camel α-lactalbumin as reported by Lajnaf et al. (2016). Indeed, these authors found foamability and foam stability of camel β-casein and α-lactalbumin binary mixtures increased with an increasing content of the β-casein up to a certain degree (25% of α-lactalbumin and 75% of β-casein) and then stayed

constant. Indeed, camel β-casein is considered as the most surface-active protein in camel milk protein due to its particular disordered structure and its high hydrophobicity. Therefore, camel β-casein is the first adsorbed and predominant protein at the air-water interface when compared with other proteins (whey proteins and caseins) (Lajnaf et al., 2016). Interfacial studies of camel milk proteins showed purified camel β-casein showed the highest efficiency in reducing the surface tension at air-water interface when compared to caseins, whey α-lactalbumin and skimmed camel milk. These findings lead to note that the β-casein is the most surface active protein of camel milk and has the main role in the creation and stabilization of camel milk foams at neutral pH. Meanwhile, viscoelastic modulus values of camel milk film proteins while creating foams are dominated by β-casein which maintains the rigidity of the protein film at the air-water interface (Lajnaf et al., 2022). Therefore, the adsorption layers of camel milk proteins are modeled as follows: first, β-casein polypeptide is adsorbed at the air-water interface as inner adjacent layer in a "train" and outer layer extending into the aqueous phase as a "tail" or "loop" following by adsorption of α-lactalbumin monomers leading to an increased the stiffness of the previously created film as the β-lactoglobulin is totally deficient in camel milk (Dickinson et al., 1993; Lajnaf et al., 2022). Foaming properties of camel β-casein are reported to be influenced by several factors including pH level and heating temperature. Camel and bovine β-caseins at a protein concentration of 0.5 g/L showed similar interfacial properties at the air-water resulting similar foaming capacity values regardless of pH values (5 and 7). These findings revealed that foaming properties decreased in acidic conditions because of acid precipitation and the lower surface protein coverage. the interfacial properties of bovine and camel β-casein indicated that when pH decreased, the initial adsorption rate values of β-casein were consequently reduced and the ability of protein to align at the air–water interface was also changed. Findings indicate that varying pH affected the physicochemical properties of the bovine and camel β-casein by decreasing the surface negative charge and intrinsic fluorescence. These results were more pronounced on the bovine β-casein which suggested a higher acid-sensitivity of this protein when compared to its camel counterpart according to our previously unpublished study.

Finally, these findings confirmed the strong potential of camel β-casein as foaming agents for potential applications in food, pharmaceutical and cosmetic industries (Fig. 4). Incorporation tests of camel β-casein in some foamed dairy products are still needed to confirm the strong potential of this protein foaming in industrial foam production.

5. Conclusion

Camel β-casein is a promising food ingredient due to its clean-label status and multifunctional application. The β-casein could be successfully purified from camel milk by means chromatographic techniques and cold solubilization with a purity exceeding 90% on a small scale. Camel β-casein presents an exceptional combination of biological and techno-functional properties especially as a foaming and emulsifier agent. In the long term, camel β-casein could be of great interest, especially for its biological properties including antioxidant and antifungal properties. The application of techno-functional food ingredient as camel β-casein in emulsions and foams has a higher short-term commercial relevance to dairy industries.

However, further study on the allergenicity and the digestibility of this protein is required. Furthermore, pilot-scale results of isolation of camel β-casein are still needed to confirm the efficiency of the used methods to obtain pure fractions of this protein.

CRediT authorship contribution statement

Roua Lajnaf: Writing – review & editing, Writing – original draft,

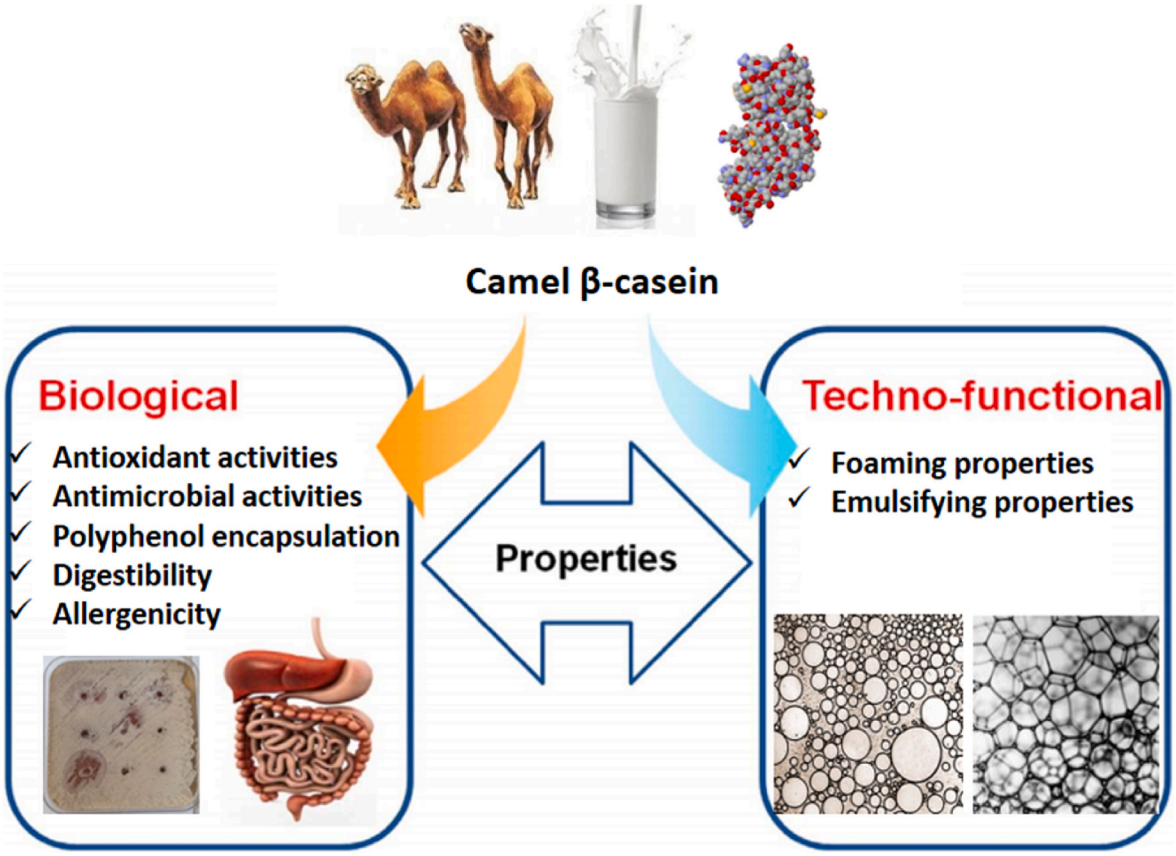

Fig. 4. Most important techno-functional and biological properties of camel β-casein.

Visualization, Software, Methodology, Investigation, Conceptualization. **Hamadi Attia:** Validation, Supervision. **Mohamed Ali Ayadi:** Visualization, Validation, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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