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## Chapter

# Chemistry of Camel Milk Proteins in Food Processing

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## Abstract

Camel milk and its extracted protein fractions were found to provide various potential techno-functional properties which can be used in the food industry. This chapter summarizes existing knowledge on camel milk protein's chemistry to explain the different reactions and their control for the major processes utilized by the modern milk processing industry. The composition and chemical properties of camel milk proteins including caseins and whey proteins are investigated. The effect of processing upon denaturation, aggregation, and destabilization of milk proteins is updated. Technological consequences of thermal processing as well as techno-functional properties of camel milk proteins are also described in different techno-functional properties including foaming, emulsifying, and gelling properties. This chapter aims to improve camel milk production and consumption worldwide not only in the arid countries and the hot regions.

**Keywords:** camel milk, food industry, caseins whey proteins, food processing, thermal processing

## 1. Introduction

According to recent statistics by the statistics of Food and Agriculture Organization [1], the total population of camels in the world is estimated to be about 38.6 million, with Chad having the largest herd worldwide (8.8 million) followed by Somalia (7.3 million), Sudan (4.9 million), and Kenya (4.7 million) [1]. Camels live mainly in the vast pastoral areas in Asia and Africa, they are divided into two different species belonging to the genus *Camelus*. Dromedary camels (*Camelus dromedaries*) with one-humped and Bactrian camel (*Camelus bactrianus*) with two-humped [2]. Overall, dromedary camels mainly live in desert arid areas including the Middle East, North and East Africa, South West Asia and Australia while Bactrian camels prefer living in cooler areas such as East to Northern China, West Asia, and Southern Russia (Mongolia and Kazakhstan) [2]. Camels are usually considered to be a good source of milk and meat, meanwhile they are used for other purposes such as sports racing and transportation [3].

Camel milk plays a key role in human nutrition, especially in hot regions and arid countries. Indeed, this milk contains all the essential nutrients already found in bovine milk [4, 5]. According to the latest FAO statistics, camel milk production (both species) in the world is reported to be about 3.11 million tons per year representing

0.34% of the total milk production of the world, whereas the cow milk production represents 81.2% of total milk production (746 million tons per year) [6].

In Tunisia, total camel milk production is estimated to be around 1099.64 tons per year, representing only 0.1% of total milk production in Tunisia [1, 7]. Camel milk is popular in Tunisia and consumed as fresh milk as a treatment for a series of diseases such as cancer diseases. The produced camel milk in Tunisia is also dedicated to scientific research in various laboratories and research centers. Indeed, recently, camel milk was also reported to be an efficient treatment for other diseases, such as dropsy, tuberculosis, jaundice, hepatitis, asthma, and leishmaniasis [8]. 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 [9–14].

Unfortunately, camel milk has not been given as much attention in research compared with cow milk because of its relatively limited production and consumption despite its health benefits and therapeutic properties. Most of the research conducted on camels in the past was mainly focused on their physicochemical features. However, recent studies have mainly concentrated on the compositional, characteristics and technological properties of camel milk and its derived proteins. This review covers the recent works on camel milk properties with an emphasis on camel milk proteins. The aim of this chapter is to review the currently available information on Dromedary camel milk properties, composition as well as camel milk proteins: extraction processes, biochemical, and techno-functional properties.

## **2. Protein composition of camel milk**

Overall, milk proteins represent a significant nutritional intake (source of essential amino acids). These proteins represent also a source of important techno-functional properties for the conservation and processing of milk into dairy products for human consumption [15].

The total protein content in camel milk ranges from 21.5 to 49 g/L with an average of 31 g/L of milk [16]. This variation in the composition of camel milk proteins depends not only on the race of the producing female but also on seasonal conditions [17]. For instance, protein contents in camel milk, which was collected from the same breed, were found to vary significantly depending on seasons ranging between 24.8 g/L of proteins in summer to 29 g/L in winter [18].

As with other milk of different mammalian species, dairy proteins are commonly classified according to their solubility in two fractions: caseins (insoluble in acidic medium) and whey proteins (called soluble proteins) (**Table 1**). Indeed, the caseins precipitate at their isoelectric pH which is 4.6 and 4.3 for bovine and camel milk, respectively, while whey proteins remain soluble in these pH values [19–22].

### **2.1 Caseins**

Camel caseins are phosphoproteins that represent the most abundant protein fraction of milk. They occupy 61.8–88.5% of all camel proteins with an average of 75.4% (w/w) against an average content of 80% (w/w) for cow's milk [23].

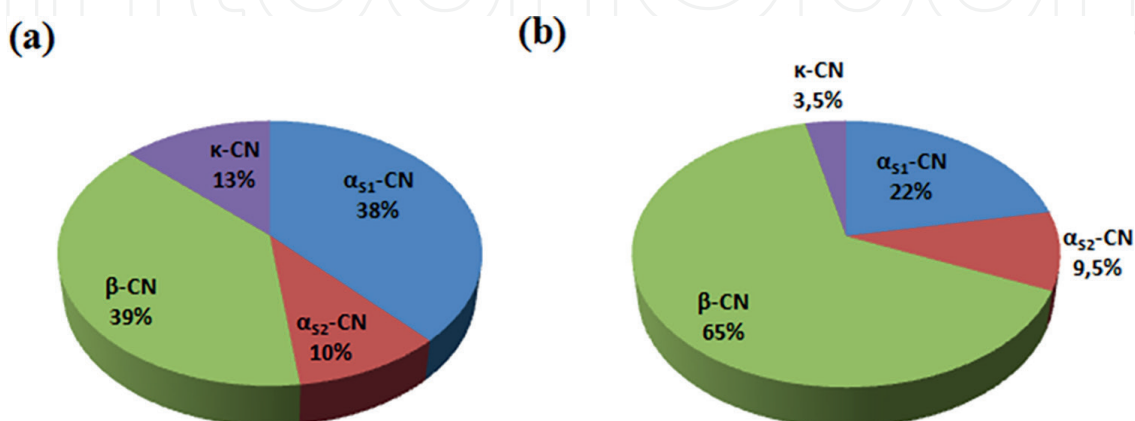
Compositionally, caseins in bovine milk are composed of four caseins including  $\alpha_{S1}$ -,  $\alpha_{S2}$ -,  $\beta$ -, and  $\kappa$ -caseins with a molar ratio of approximately 4:1:4:1 in bovine milk [22].

	Cow's milk		Camel's milk	
	Concentration (g/L)	MW	Concentration (g/L)	MW
Fat globules	40	0.1–15 $\mu\text{m}$ (average = 3.78 $\mu\text{m}$ )	12–64 (average = 35)	2.99 $\mu\text{m}$
Caseins micelles	26	100–140 nm (average = 120 nm)	16.3–27.6	260–300 nm (average = 280 nm)
Whey proteins	7	3–6 nm	6.3–8	n.d
$\alpha$ -La	1.2	14 kDa	>5	14.430 kDa
$\beta$ -Lg	3.2	18 kDa	—	—
SA	0.4	66 kDa	3.4	66 kDa
Ig	0.8	150–900 kDa	0.718	80 kDa
Lf	0.1	86 kDa	0.229	75 kDa
Lactose	46	0.35 kDa	24–58 (average = 44)	0.35 kDa
Minerals	7	—	6–9 (average = 7.9)	—
Vitamins	3.2	—	3.7	—

Abbreviations:  $\alpha$ -La:  $\alpha$ -lactalbumin;  $\beta$ -Lg:  $\beta$ -lactoglobulin; SA: serum albumin; Ig: immunoglobulins; Lf: lactoferrin [15].

**Table 1.**  
 Composition of camel milk in comparison with cow's milk.

On the other hand, camel caseins consist of the known four sub-fractions including  $\alpha_{S1}$ -,  $\alpha_{S2}$ -,  $\beta$ -, and  $\kappa$ -caseins with proportions approximately being 22, 9.5, 65, and 3.5%, respectively in bovine milk (**Figure 1**) [25]. Recently, Lajnaf et al. [26] found that camel sodium caseinates contain four caseins at different percentages 1.1, 45.5, and 53.4% for  $\kappa$ -,  $\alpha$ -, and  $\beta$ -caseins, respectively. The caseins of camel milk are homologous to bovine caseins with identity levels that range between 44.6% ( $\alpha_{S1}$ -casein) and 67.2% ( $\beta$ -casein) [27]. The  $\alpha$ - and  $\beta$ -caseins are known as calcium-sensitive caseins or “sensitive calcium caseins” due to their precipitation at a calcium concentration estimated at 30 mM, while  $\kappa$ -casein remains in solution under these conditions.



**Figure 1.**  
 Proportions of the different caseins  $\alpha_{S1}$ -,  $\alpha_{S2}$ -,  $\beta$  and  $\kappa$  of the total caseins of cow's milk (a) and camel's milk (b) [24]. Abbreviation: CN: casein.

Camel milk is distinguished by the low contents of  $\kappa$ -casein as reported by various authors. In the same way, Lajnaf et al. [28] found that no peaks were detected for the  $\kappa$ -casein due to its low concentration which probably makes it obscured by other caseins.

Overall, the comparison of camel and bovine properties revealed that camel milk caseins are less phosphorylated than their bovine counterparts and less negatively charged at neutral pH when compared to bovine caprine caseins [29, 30].

### 2.1.1 $\alpha$ -Casein

The  $\alpha$ -casein, which includes both  $\alpha_{S1}$ - and  $\alpha_{S2}$ -caseins, is the most abundant protein in cow's milk and its concentration in milk is estimated at  $12.8 \pm 2.3$  g/L. However, the concentration of this protein is lower in camel milk (7.6 g/L) [25, 31, 32].

The  $\alpha_{S1}$ -casein, whose concentration is round of 9.5 and 5.3 g/L in bovine and camel milk, respectively, representing 38 and 22% of total bovine and camel caseins, respectively. Bovine  $\alpha_{S1}$ -casein contains 199 amino acid residues with a molecular weight (MW) estimated at 22.9 kDa, while camel  $\alpha_{S1}$ -casein is slightly bigger with 215 amino acids and a MW of 25.8 kDa. The isoelectric point (pI) is estimated to be around 4.26 and 4.40 for the bovine and camel  $\alpha_{S1}$ -caseins, respectively [25, 33]. The differences between camel and bovine  $\alpha_{S1}$ -caseins result in identity and similarity indexes which are around 44.6 and 59.7%, respectively [27]. Bovine  $\alpha_{S1}$ -casein is characterized by the absence of cysteine residues. However, it contains 8 serine residues in phosphorylated form. Due to the presence of a large number of proline residues (9.2 and 8.5% proline respectively for camel and bovine  $\alpha_{S1}$ -caseins, respectively) [33].

The content of  $\alpha_{S2}$ -casein in camel milk is similar to that of cow's milk. It represents 10 and 9.5% of the caseins of bovine and camel caseins, respectively [34]. Recently, this content has been reported as 0.3–3.9 g/L as reported by Mohamed et al. [35]. The primary structure of  $\alpha_{S2}$ -bovine casein has 207 amino acid residues with an MW of 24.4 kDa, while camel  $\alpha_{S2}$ -casein has a lower MW of 22 kDa as it contains 178 residues of amino acids. The pI is estimated respectively at 4.78 and 4.58 for both bovine and camel  $\alpha_{S2}$ -caseins [33].

It is well known that the  $\alpha_{S2}$ -casein is the most hydrophilic of the other caseins. It has 11 residues of phosphorylated serines and is characterized by the presence of two cysteine residues (residues 36 and 40) forming intramolecular disulfide bridges. This casein is found in partly milk in dimeric form, the two polypeptide chains of which are connected by two disulfide bridges [25]. Its secondary structure contains 32%  $\alpha$ -helix and 30%  $\beta$ -sheets leading to a more organized and structured conformation when compared to those of  $\alpha_{S1}$ -casein. Similarly to other milk proteins, the differences between camel and bovine  $\alpha_{S2}$ -caseins result in identity and similarity indexes which are around 58.3 and 69.2%, respectively [27].

### 2.1.2 $\beta$ -Casein

The  $\beta$ -casein is the main protein in camel milk with a concentration that ranges between 12.8 and 15 g/L representing 65% of the total caseins of camel milk according to Kappeler et al. [34]. However, recent works noted a minimization of its proportion to 53.4% [26], 44.8%, and even 30% according to Felfoul et al. [36] and Ereifej et al. [23], respectively.

The  $\beta$ -caseins of cow's and camel's milk showed differences in their structures and physicochemical characteristics. In fact, bovine  $\beta$ -casein is composed of 209 amino



acid residues with an MW of 23.5 kDa and a pI of 4.49, while camel  $\beta$ -casein is slightly bigger than its bovine counterpart as it contains 217 amino acids leading to an MW of 24.9 kDa and a pI of 4.66. The rates of similarity (84.5%) and identity (67.2%) of the  $\beta$ -caseins are higher than those found for other caseins as reported by Lajnaf et al. [27] and Barzegar et al. [37].

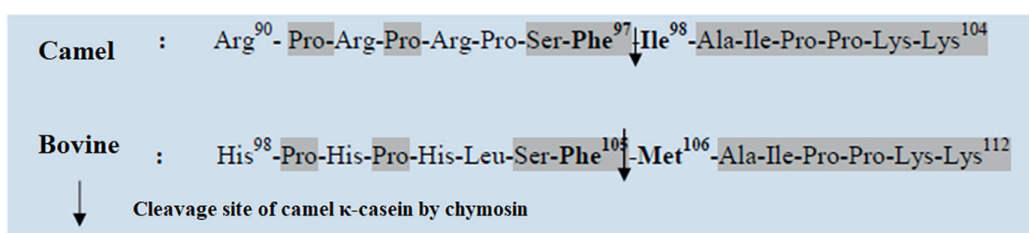
The  $\beta$ -casein, the most hydrophobic of all the caseins, is characterized by a very high amphipolar character. Indeed, it has a C-terminal part (residues 136–209) which is very rich in hydrophobic amino acids, while its N-terminal part is hydrophilic and contains phosphorylated residues (residues 1–40) providing additional negative charges to the molecule. This protein is also characterized by the absence of disulfide bridges, which gives it significant resistance to heat treatment.  $\beta$ -Casein is classified as an intrinsically unstructured protein thanks to the large number of proline residues (16.7%) preventing the formation of secondary structures. Due to its particular structure (unordered, high hydrophobicity, relatively low molecular mass and absence of disulfide bridges), this casein is often at the origin of the properties sought in “stabilizing” protein food ingredients used in the dairy industry [38].

### 2.1.3 $\kappa$ -Casein

The  $\kappa$ -casein is the key milk protein that is involved in the rennet coagulation process of milk. Among caseins, the concentration of  $\kappa$ -casein ( $4.4 \pm 0.3$  g/L) was found the lowest representing 13% of bovine caseins. However, it is found in camel milk at a content four times lower than that of cow’s milk varying from 0.1 to 2.4 g/L representing 3.5% of caseins or even 1.1% [26, 29, 35]. Bovine  $\kappa$ -casein has 169 amino acid residues with an MW of 18.9 kDa and a pI of 3.97, while camel  $\kappa$ -casein is composed of 162 amino acids with a molecular mass of 18.2 kDa and a pI of 4.11 [33]. These differences between both camel and bovine  $\kappa$ -caseins result in similarity and identity of 58.4 and 66.3%, respectively.

Similarly to  $\beta$ -casein,  $\kappa$ -casein has a particular amphipolar structure with a C-terminal part that contains highly hydrophilic residues and a hydrophobic N-terminal part. It is also characterized by a low calcium binding capacity due to the presence of a single phosphorylation site at position 149.

It is well known that the partial hydrolysis of bovine  $\kappa$ -casein by chymosin takes place at the peptide bond 105(Phe)-106(Met) leading to the release of a very hydrophilic peptide: the caseinomacropeptide (64 amino acids—molecular mass 6.7 kDa) and the formation of paracasein  $\kappa$ , which is very hydrophobic and insoluble. The cleavage site of camel  $\kappa$ -casein by chymosin is located at position 97(Phe)-98(Ile) (Figure 2) and leads to the release of a macropeptide with a molecular mass of 6, 77 kDa which is comparable to bovine macropeptide [29].



**Figure 2.**  
Cleavage sites of camel and bovine  $\kappa$ -caseins by chymosin [15].

## 2.2 Whey proteins

Whey proteins represent the second protein milk fraction representing 20–25% (w/w) of total milk proteins depending on the milk origin [39]. Camel whey proteins accounted on average for 24.51% of the total protein ranging between 11.49 and 38.82% of total milk proteins [23]. Overall, extracted camel whey after acid precipitation of caseins at pH 4.3 has a white color compared to the yellowish color of bovine whey. This is due to the low content of riboflavine in camel whey [2].

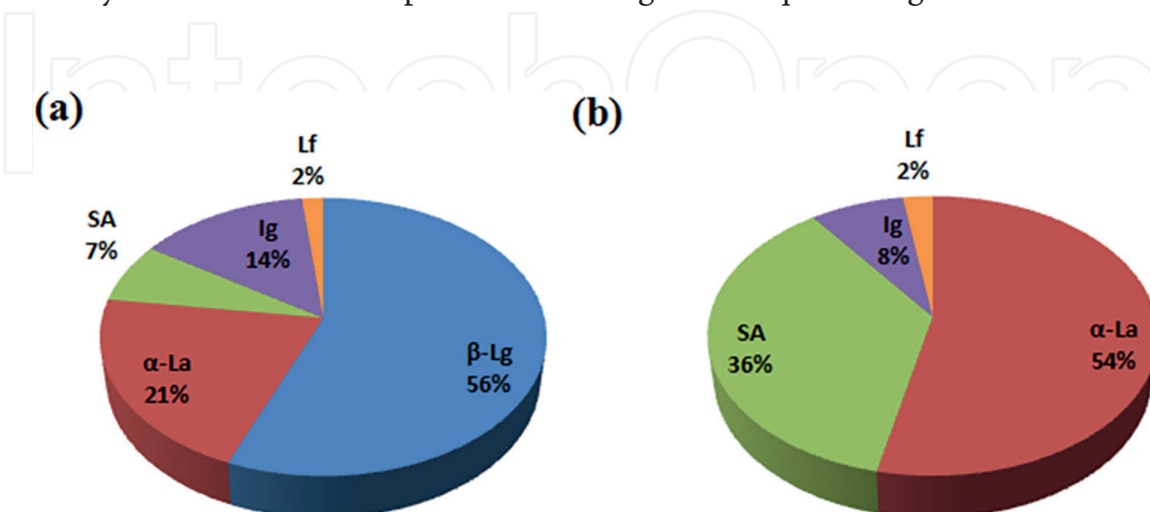
Generally, the protein composition of whey varies according to the mammalian specie. For instance, the soluble fraction of cow's milk, the protein composition is thoroughly studied:  $\beta$ -lactoglobulin is the main protein (~55%), followed by  $\alpha$ -lactalbumin (~25%), the albumin serum (SA) (15%), and finally the immunoglobulins (5%) (**Table 1**). Camel whey is distinguished by the total absence of  $\beta$ -lactoglobulin similar to human milk [28, 33, 34, 40]. Thus,  $\alpha$ -lactalbumin is the major protein of camel whey 50–54% of all of the globular proteins in this milk, this protein is followed by camel serum albumin (CSA) (36%), lactoferrine (2%), and immunoglobulins (8%) (**Figure 3**) [33].

Several works have shown that camel whey contained other specific protein components such as the PGRP (Peptidoglycan Recognition Protein), lactophorine, Wap (Whey Acidic Protein), and CWBP (Camel Whey Basic Protein) [29, 33, 41].

### 2.2.1 $\alpha$ -Lactalbumin

The  $\alpha$ -lactalbumin ( $\alpha$ -La) is the major protein in camel whey as the  $\beta$ -lactoglobulin which is the major protein in bovine whey is totally absent [27, 28, 36, 40, 43]. The concentration of this protein in camel milk is significantly higher than that of cow's milk (1.08 g/L) [32] as it ranges between 2.1 g/L according to Omar et al. [32] and 5 g/L according to El-Agamy [33].

The primary sequence of camel  $\alpha$ -La was determined by Beg et al. [42]. As its bovine counterpart, camel  $\alpha$ -La is composed of 123 amino acids, in which 39 residues are different when compared to bovine  $\alpha$ -La. Consequently, the similarity and identity levels between these proteins according to the sequence alignment data are



**Figure 3.** Proportions of the different whey proteins of cow's milk (a) and camel's milk (b) according to El-Agamy [33]. Abbreviations:  $\beta$ -Lg:  $\beta$ -lactoglobulin,  $\alpha$ -La:  $\alpha$ -lactalbumin, SA: serum albumin, Ig: immunoglobulins, Lf: lactoferrin.

82.9 and 69.1%, respectively according to Salami et al. [24]. MW and pI of camel  $\alpha$ -La (MW = 14.43 kDa and pI = 4.87) are slightly higher than those of bovine  $\alpha$ -La [43, 44].

Similarly to its bovine counterpart, camel  $\alpha$ -La has a high affinity for the  $\text{Ca}^{2+}$  ion with a higher exposure of hydrophobic groups upon calcium depletion than the bovine  $\alpha$ -La [45, 46]. In terms of nutritional properties, several studies have shown that camel  $\alpha$ -La is characterized by a higher digestibility than that of bovine milk, as well as greater antioxidant activity with respect to Ferric-reducing antioxidant power, iron chelating, and antiradical activities especially in their apo forms [44]. This protein presented in its apo form great antibacterial and antifungal properties toward various pathogenic species [43, 44].

### 2.2.2 Camel serum albumin

Serum albumin (SA) protein is a whey protein characterized by its relatively high MW. Indeed, bovine serum albumin (BSA) consists of 583 amino acids with an MW of 66.4 kDa, its primary sequence was determined Hirayama et al. [47]. It has 17 intramolecular disulfide bridges and a free thiol group. On the other hand, camel serum albumin (CSA) was identified by SDS-PAGE as a similar protein to its bovine counterpart with the same MW (66 kDa) [15, 29].

BSA and CSA were reported to have similar concentrations  $\sim 0.4$  g/L with different proportions among whey proteins (1.5 and 7% of total bovine and camel whey proteins fractions, respectively). However, the contents of CSA are higher in camel colostrum with concentrations greater than 3.4 g/L [48].

### 2.2.3 Minor camel whey proteins

Lactoferrin is a glycoprotein that belongs to the transferrin family. It contains two binding sites for iron cations and more preferentially the ferric ion ( $\text{Fe}^{3+}$ ). This ability to scavenge iron persists even at low pH values in the stomach and intestines, to deplete free iron which could slow down bacterial growth in the intestines [29]. The concentration of lactoferrin in milk varies according to the producing animal species and according to the stage of lactation. Camel milk is very rich in lactoferrin compared to the milk of other mammalian species. This richness is a form of adaptation to difficult living conditions for young camels to make them more resistant to infections [49].

Camel lactoferrin is composed of 689 amino acids with an MW of 75.3 kDa. The primary sequence of camel lactoferrin has a similarity level of 91.6% with its bovine and human counterparts and 91.3% with porcine lactoferrin. It is a basic protein with a pI of around 8.14 (compared to a value of 8.18 for bovine lactoferrin) [33].

PGRP or "Peptidoglycan Recognition Protein" is part of a family of proteins described recently. It is known for its action on gram-positive bacteria as well as other microorganisms such as nematodes. This inactivation of pathogens is carried out by the binding of this protein to the peptidoglycan of the bacterial membrane, hence its name "Peptidoglycan recognition protein" or PGRP [50]. PGRP is a protein that is not detected in cow's milk. It was isolated from camel milk by Kappeller et al. [50]. It is a protein which is characterized by its low molecular mass (19.11 kDa) containing 172 amino acids. The PGRP of camel milk is a basic protein, it is very rich in Arg residues whereas it is poor in Lys. It is found in camel milk at a concentration of 1.74 g/L [32]. The pI of camel PGRP is 8.73 which is higher than that of human PGRP (pI = 7.94). The similarity level between PGRP in camel milk and human milk is around 91.2% [33]. The PGRP content



increases in camel milk in case of infection of the mammary glands. Also, the high level of PGRP in camel milk at the start of lactation contributes to the protection of the mammary gland as well as the transmission of immunity to the newborn [50].

Camel Whey Basic Protein or CWBP (Camel Whey Basic Protein) is also a protein specific to camel milk. It was identified from camel whey by SDS-PAGE electrophoresis [51] and by ion exchange chromatography [48]. This protein, of relatively low MW (20 kDa), has a unique structure and has no analogy with other dairy proteins. It has been demonstrated in the whey of camelids of the dromedary and bacterial species.

WAP or Whey Acidic Protein is a soluble protein found in the milk of certain mammalian species including rabbits, pigs, rodents, camelids and humans. WAP is a whey protein found at a concentration of 0.157 g/L in camel milk. It contains 117 amino acids with an MW of 12.56 kDa. WAP consists of two domains with four disulfide bridges with a pI of 4.5 [33]. Thus, camel milk contains the highest rate of natural bioactive components, which explains its long shelf life compared to cow's milk [33].

### **3. Effect of processing on chemistry of camel milk proteins**

Thermal treatments are important food processes including in most dairy industries to obtain bacteriologically safe final products and to extend their shelf life. However, a number of structural modifications have been reported and noted in the milk protein components depending on temperature time, and rate of heating. For instance, Singh [52] reported that a range of large heterogeneous protein aggregates of milk proteins occurred in heat-treated milk. Indeed, the association of heat-induced milk proteins which are occurring under different heating conditions has been extensively studied by various authors [53].

Overall, both caseins and whey proteins in heat-treated milk are engaged in protein denaturation. Furthermore, the formation of intermolecular disulfide bridges is mostly responsible for heat-induced protein association in milk. Thermal protein denaturation has been acknowledged as the first step of the reactions leading to the aggregation of the disulfide-linked milk proteins. The resulted thiol groups of cysteine residues which are appearing in unfolded proteins, can initiate thiol-disulfide exchange reactions within hydrophobically-linked protein aggregates. On the other hand, self-aggregation of heat-denatured  $\beta$ -lactoglobulin in cow's milk, and heat-induced association of various whey proteins and their aggregates with caseins have been investigated and explained according to this mechanism [54].

#### **3.1 Effect of processing on caseins**

Similarly to cow's milk, camel milk proteins are significantly affected by thermal treatment processing. However, only few studies about the effect of heat treatments on camel milk proteins including caseins and whey proteins are available in the literature [55].

First, Felfoul et al. [36] found using LC-MS and SDS PAGE electrophoreses techniques that after heating camel milk at 80°C for 60 min, various significant modifications in protein composition were observed.

Indeed, these authors noted that fresh camel milk contains  $\alpha$ -La, PGRP, CSA, and caseins proteins as major proteins. In the same way as bovine milk, the thermal treatment of camel milk at 80°C for 60 min caused various significant modifications in proteins including whey proteins and caseins. However, camel  $\alpha_{S2}$ -,  $\beta$ -, and  $\gamma$ -caseins

concentrations have not been significantly modified by heat treatment similarly to bovine caseins. Other study revealed that the effect of the heating temperature increases on camel milk was mild on  $\beta$ -casein and both  $\alpha$ S1- and  $\alpha$ S2-caseins, whereas it was drastic on  $\kappa$ -casein. Indeed, electrophoretic bands of whey proteins including CSA and  $\alpha$ -La as well as  $\kappa$ -casein decreased at 90°C [56].

On the other hand, Lajnaf et al. [26] investigated the effect of different heating temperatures on extracted camel sodium caseinates at neutral pH. RP-HPLC results of these authors showed that both bovine and camel caseins peaks including  $\kappa$ -casein,  $\alpha$ -casein and  $\beta$ -casein remained almost intact upon heating at 70 and 80°C for 30 min. However, higher temperatures (90 and 100°C) significantly affected camel casein peaks especially  $\alpha$ -casein and  $\beta$ -casein, which decreased significantly at these temperatures. Furthermore, the degradation of caseins is synchronized by the appearance of new protein fractions after heating at 90°C for 30 min. In the same way, new peptides were generated upon heating from the parent caseins. Thus, the heat treatment of camel caseinates solutions results in the degradation polymerization of proteins as well as the liberation of several peptides due to protein degradation [26].

### **3.2 Effect of processing on whey proteins**

The effect of processing on camel whey proteins especially thermal processing as well as the acidification process is being studied by many researchers in recent scientific works who are interested in the valorization of camel milk and its consumption as a new alternative of bovine whey especially due to the total absence of the  $\beta$ -lactoglobulin in camel milk.

First, the work of Felfoul et al. [21] was considered as the first study leading to understanding the chemistry of camel whey proteins upon heating and at different pH levels as they studied the effect of different heating temperatures on sweet and acid camel whey. These authors noted that protein denaturation started after heating whey for 30 min for all temperatures. The whole phenomenon happened during 30 min of heating. The obtained results by these authors have shown that heating both bovine and camel whey at 60°C does not generate any denaturation phenomena as it is already observed by Laleye et al. [40]. The electrophoresis patterns showed also that heating camel whey at 90°C during 30 min CSA band disappearance for both rennet and acid wheys. On the other hand,  $\alpha$ -La concentration decreased as a function of heating temperature.

As previously reported, the major camel whey proteins are  $\alpha$ -La, CSA, and PGRP [36, 41, 48, 50, 57]. These proteins were significantly affected by heat treatment at 80°C for 60 min as revealed by Felfoul et al. [36]. Indeed, the corresponding peak of CSA decreased significantly after heating at this temperature while camel  $\alpha$ -La and PGRP have completely disappeared from the HPLC-UV chromatograms. Indeed, these authors found that the concentration of CSA in fresh camel milk was decreased by 42%, while PGRP concentration decreased by 68%, whereas, there was 100% of  $\alpha$ -La disappeared from camel milk. Thus, the most heat-sensitive whey protein in camel milk obviously corresponds to camel  $\alpha$ -La followed by PGRP and CSA [21, 36]. In the same way, Lajnaf et al. [28] found that the chromatographic peak of the  $\alpha$ -La began to decline after the heat treatment at 70°C for 30 min, it decreased significantly when the heating temperature raises from 80 to 100°C for 30 min. Thus, the reduction of the chromatogram peaks is the consequence of the protein denaturation and aggregation upon heating [28]. However, for bovine milk, the peaks of the  $\alpha$ -La and the  $\beta$ -Lg started immediately diminished after the heat treatment at 80°C for 30 min.

The peak of  $\beta$ -Lg totally disappeared after heating at 90 and 100°C for 30 min, unlike the  $\beta$ -Lg dimer peak that increased due to the creation of heat-induced disulfide-bonded dimers as intermediates in the whey proteins aggregation [28].

On the other hand, differential scanning calorimetry (DSC) thermograms of Felfoul et al. [21] showed that denaturation temperatures of camel  $\alpha$ -La were 73.8°C in camel rennet whey and 60.5°C for camel acid whey. Atri et al. [45] noted that denaturation temperatures of purified camel  $\alpha$ -La are 71.7 and 39.6°C in its holo (calcium loaded) and apo (calcium depleted) forms. Indeed, the absence of  $\beta$ -lactoglobulin in camel milk whose denaturation temperatures are 79.6 and 83.4°C in sweet and acid bovine wheys, respectively resulted from different denaturation and aggregation phenomena during heat treatment [21].

Other scientific works have shown that the combination of heating treatment and acidification of camel wheys induced an immediate disappearance of the  $\alpha$ -La and the appearance of several intermediate protein species including dimers, trimers of  $\alpha$ -La. These protein species were formed during heating and before aggregation [20]. These authors have found that acid wheys carried higher denaturation levels compared to sweet wheys regardless of heating temperature value. These findings confirmed that acid whey is characterized by a higher thermal sensitivity than the sweet one with the higher thermal sensitivity of camel whey proteins compared to bovine whey proteins especially at neutral conditions [20]. In the same way, Laleye et al. [40] noted that camel milk whey proteins are slightly more susceptible to heat denaturation than bovine whey proteins regardless of pH level. This behavior can be explained by the particular structure of camel  $\alpha$ -La, especially in acidic conditions. Lajnaf et al. [41] reported that the open structure of the camel  $\alpha$ -La molecule and the reduced electrostatic repulsion of this protein near its pI are all factors that could promote the creation of large aggregates. In the same way, Lajnaf et al. [57] observed that the purified camel  $\alpha$ -La isolated from camel milk was more flexible in acidic conditions, regardless of heating temperature, due to the reduced negative charge of this protein and its molten globular state at low pH values.

Recently, Lajnaf et al. [43] reported that there are various structural differences between the camel and bovine  $\alpha$ -La as a function of different denaturing conditions in food processing including pH, heating temperature, and guanidine hydrochloride mediated. Camel  $\alpha$ -La showed higher stability toward thermal treatments and pH-mediated denaturation. However, it was less stable toward guanidine-mediated denaturation with a fast aggregation and a more disordered structure when compared to its bovine counterpart [43, 58].

## **4. Effect of processing on camel milk protein functionality**

### **4.1 Foaming properties**

The foaming and stabilizing properties of camel milk as well as its protein fractions were investigated by different authors [28, 41, 59–61]. First, Lajnaf et al. [26, 28] studied the effect of different heating temperatures ranging between 70 and 100°C on skimmed camel milk as well as extracted sodium caseinates. These authors noted that for the camel milk and sodium caseinates, heating improved significantly the foamability in comparison with that of bovine milk and bovine caseinates, with better foaming capacity achieved after a heat treatment at 90 and 100°C due to the presence of higher amounts of  $\beta$ -casein in camel milk. Indeed, this

protein is well known as a mobile disordered protein due to its particular flexible structure [62]. However, lower foam stability of camel milk and camel caseinates foams is observed due to the different protein composition of both milk proteins especially the absence of  $\beta$ -Lg and the lower amounts of  $\kappa$ -casein [26, 28]. On the other hand, the stability of foam formed from skimmed camel and bovine milk increased significantly with increasing preheating temperatures up to 90°C, above which lower foam stability is observed [28]. While for camel sodium caseinates, foam stability increased as a function of heating temperature even at 100°C [26]. The increase in foaming properties of camel milk is attributed to an increase in the hydrophobic interactions due to an exposure of hydrophobic groups, which are already buried inside the globular structure of whey proteins [28, 63]. Furthermore, this behavior can be explained by the increase in the adsorption velocity and the diffusion of milk proteins upon heating at the air-water interface as confirmed by Dickinson [64]. In the same way, heat treatment significantly ameliorated the foaming properties of camel and bovine sodium caseinates especially at high-temperature values (90 and 100°C for 30 min).

Parallely, the heating process affects the physicochemical properties of caseins including the increase of surface hydrophobicity due to the greater exposure of buried hydrophobic groups and the increase of the ability to reduce the interfacial tension at the air-water interface. The heating process also decreases the electronegative charge of proteins leading to a greater flexibility and hence, higher foaming properties of heated milk proteins [26].

The foaming properties of camel whey proteins are significantly affected by different stabilizing food processing, especially thermal processing and acidification. This behavior is mainly explained by scientists as camel milk is totally deficient in  $\beta$ -Lg because it is well-known that this protein plays a key role in the process of protein aggregation in bovine whey solution [60].

Camel whey foaming properties are reported to depend on both pH value and thermal treatments. Camel whey solutions showed the best foamability closed off the isoelectric point of camel  $\alpha$ -La (around pH 4.3), regardless of heating temperature. Thermal treatments at 70°C significantly improved the foaming properties of both bovine and camel acid wheys. However, the stability of foam greatly increased upon heating only for the acid camel whey [41]. Acid camel whey is distinguished by its exceptional ability to create foams with the greatest foam stability if compared to other whey, with an increase of these properties after a heat treatment. Hence, the lack of  $\beta$ -Lg in camel whey leads to exceptional foaming properties of this whey, especially with the combination of preheating and preacidification before the creation of the foams [41]. In the same way, the foamability of camel  $\alpha$ -La in solution was maximal in acid conditions, near its effective pI. Indeed, at this acid pH, the protonation of the negative groups decreased the electrostatic repulsions of the  $\alpha$ -La and induced a partial denaturation with the release of its chelated calcium. The obtained molten globular state enhanced the foaming properties of this protein. Heating processes improved the stability of the foam which is created by camel  $\alpha$ -La due to the presence of aggregated proteins at the air-water interface. Aggregates are reported to contribute to improving foam stability whereas, they slowed the adsorption of proteins and the creation of foam [43, 57]. In addition to the heating process, the effect of the spray drying process on the techno-functional of camel milk proteins was investigated by Zouari et al. [65] noted the low denaturation extent of camel and bovine milk proteins powders participated in the enhancement of their foaming capacity and stability [65].



## **4.2 Emulsifying properties**

Emulsification is a common food process in the food industry, it is encountered with mayonnaise sauces, cream, soups, butter, and margarine [66]. Overall, oil-in-water emulsions are produced by the homogenization process of oil and aqueous phases in the presence of emulsifiers which are adsorbed onto the surfaces of oil-droplets leading to the reduction of the interfacial tension and emulsion creation. In the food industry, the most common emulsifiers used are milk proteins including caseins such as  $\beta$ -casein which is the most surface-active dairy protein, and whey proteins including  $\beta$ -Lg and  $\alpha$ -La [62, 66]. The effect of food processing on the ability of camel milk proteins to create and stabilize emulsions was studied by different authors [20, 60, 67]. First, Lajnaf et al. [20] reported that camel whey emulsifying properties depended on both pH level and the degree of denaturation of these proteins after a heat treatment. Higher emulsifying activity stability was obtained for sweet whey especially the sweet camel whey due to the presence of electrostatic repulsive forces between proteins. However, acidification reduces these repulsive forces leading to the reduction of emulsifying properties of milk proteins.

Laleye et al. [40] reported the lower emulsifying properties of pre-acidified camel whey when compared to bovine whey due to the pronounced aggregation of camel whey protein molecules. Indeed, the aggregation behavior of camel whey proteins at lower pH values is associated to the high content of the  $\alpha$ -La [40]. Furthermore, thermal treatments of camel whey proteins at 70 and 90°C improved the emulsifying properties of these proteins, especially in acidic conditions due to the denaturation and aggregation of proteins. Indeed, the size of whey proteins' aggregates is higher in acidic conditions than in neutral pH due to the minimized electrostatic repulsion between neighboring proteins molecules leading them to interact and aggregate. These aggregates are characterized by a greater ability to stabilize foam and emulsion compared to native proteins [20, 68].

Momen et al. [60] studied the effect of the heating process at 85°C for 15 min in a temperature-controlled water bath on the created emulsions. These authors noted that the emulsions prepared with camel whey proteins did not show any visible aggregation or gelation after heat treatment, whereas emulsions prepared by bovine whey proteins formed a gel-like structure in different protein concentrations. Indeed, the limited heat-induced modification in the conformational structure of camel whey proteins confirmed that these proteins are not very sensitive to heat-induced disulfide bridging and hydrophobic interactions. Thus, this study showed the technological viability of camel whey protein for the fabrication of high-protein emulsion. In the same way, the emulsifying properties of camel  $\alpha$ -La were less sensitive to various thermal treatments at 95°C. This behavior was explained by the higher conformational flexibility of this protein which increased with temperature, contrary to its bovine homologous protein [67, 69]. Indeed, bovine  $\alpha$ -La enhanced emulsion stability as a function of pH and heat treatment, due to hydrophobic interactions and a more rigid molecular structure compared to camel  $\alpha$ -La [69]. Furthermore, a higher surface coverage of the oil droplets was obtained for camel apo  $\alpha$ -La which carried the highest ability to reduce the surface tension values at the oil-water interface when compared to bovine  $\alpha$ -La in its holo and apo states. The stability of the created emulsions seemed greatest at neutral pH due to the presence of the electrostatic repulsive forces between the adsorbed  $\alpha$ -La molecules contrary to these molecules in acidic conditions. These conditions reduced these repulsive forces leading to the decrease of emulsifying properties of camel  $\alpha$ -La [43, 44].



Ellouze et al. [70] reported that camel milk  $\beta$ -casein showed an enhanced ability to form softer emulsions and to stabilize oil droplets in acidic conditions, regardless of heat treatment compared to bovine  $\beta$ -casein. However, the heating process affects the interfacial properties of  $\beta$ -casein. Indeed, the ability of this protein to create emulsions did not show any effect upon heating, whereas, stabilization of emulsified oil droplets with  $\beta$ -casein is higher without the heating process as proteins retain their native structure with no thermal denaturation, which allows intramolecular hydrophobic interactions and hence, the maintenance of a stable protein film around the created oil droplets. On the other hand, surface pressure was higher in acidic conditions for camel  $\beta$ -casein and after a thermal treatment at 95°C. This phenomenon is explained by hydrophobic interactions and a relaxed structure allows proteins to be more cohesive under the applied treatments [70]. In the same way, emulsifying properties of camel  $\beta$ -casein solutions depended on pH level with or without thermal processing. Preacidification affects the physicochemical properties of camel  $\beta$ -casein by increasing the surface hydrophobicity and also decreasing the negative charge and the efficiency to reduce the interfacial tension. Therefore, casein precipitation decreases the emulsifying properties of camel  $\beta$ -casein and its ability to create and stabilize emulsions [70].

### 4.3 Gelling properties

The effect of different food processing on gelling properties of camel milk was described by few scientific works. First, Zouari et al. [71] studied the effect of the acidification process on the gelation of camel milk and found that the gelation behavior of camel milk is mainly controlled by the pI and hydrophobic interactions. These authors found that the intermolecular interactions between different camel milk caseins are higher and stronger when compared with those in bovine milk. The effect of thermal processing on the quality of fermented camel milk products including yogurt and cheese is not completely investigated. The fermentation process of milk into yogurt requires pre-heating in order to denature the whey proteins and form disulfide bridges between these proteins and  $\kappa$ -casein, leading to improved yogurt structure. Manufacturing yogurt from camel milk is difficult and the yogurt curd produced from camel milk is fragile and has a thin consistency because of the presence of bioactive antimicrobial components including PGRP and lactoferrin [2]. Furthermore, the different compositions of camel milk whey proteins, such as its lack of  $\beta$ -lactoglobulin and the predominance of  $\alpha$ -lactalbumin are also the reasons for the fragile structure of camel milk yogurt [72]. Pasteurization process of the camel at temperatures higher than 65°C for 30 min results in the manufacturing of camel cheeses with significantly weaker gels [73, 74]. Furthermore, high-pressure processing of milk at 350 MHz for 5 min produces harder cheese than pasteurization treatment at 65°C for 30 min [72]. Finally, further studies are needed to understand and to explain the effect of various food processing, especially thermal ones on gelling properties of camel milk.

## 5. Conclusion

Camel milk is different in its composition from that of cow's milk including fats, minerals, lactose, and proteins. The main differences in camel milk proteins composition are the total absence of the  $\beta$ -Lg and the low amount of  $\kappa$ -casein, leading to confirm

that  $\beta$ -casein and  $\alpha$ -La are the major proteins in colloidal and soluble fractions of camel milk, respectively. Camel milk proteins show different behavior when compared to bovine proteins. For instance, previous studies noted that heating treatment of milk significantly affects  $\alpha$ -La followed by PGRP and CSA, with a relative thermal sensitivity of whey proteins when compared to caseins. However, thermal treatment of camel caseinates leads to the degradation and denaturation of individual caseins including  $\alpha$ -casein and  $\beta$ -casein which is probably associated with liberation of resulted peptides. Different techno-functional properties of camel milk proteins are significantly affected by food processes including thermal processes and nonthermal such as acidification. For instance, the combination of acidification and thermal treatments improves the foaming properties of whey proteins, while these processes reduced emulsifying properties of camel whey proteins.

Finally, this chapter investigates the interesting techno-functional properties and the chemical of camel milk proteins as a function of different food processes. Hence, this could confirm the strong potential of camel milk for potential applications in the food, pharmaceutical, and cosmetic industries.

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