

Deposit Generation During Camel and Cow Milk Heating: Microstructure and Chemical Composition

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Abstract The aim of this study is to identify the chemical composition and the microstructure of the deposits obtained after heating camel and cow milks at 80 °C for 60 min using a laboratory-scale device. Like cow milk, camel milk was affected by heat treatment with the reduction of the non-casein nitrogen content reflecting the denaturation of camel whey proteins. The composition of the deposits generated during heating camel and cow milks at 80 °C for 60 min revealed that while camel deposit contained 57 % w/w protein, cow deposit showed a higher protein content of 69 % w/w. The mineral content was 35 % w/w for camel deposit which was higher than that of cow sample, which was 28 % w/w. SEM of both deposits showed a familiar structure of a protein deposit with large clumps composed of smaller aggregates. Camel deposit showed an amorphous structure due to its deficiency in β -lactoglobulin.

Keywords Camel milk · Heat treatment · Denaturation · Whey proteins · Minerals · Deposits

Introduction

Cow milk and some of its derivatives are usually pasteurized before their commercialization (Nelson 2012). Pasteurization is a thermal process whose objective is to lower the concentration of microorganisms in the milk to render it safe to drink (Bansal and Chen 2006). Pasteurization induces the deposit formation on hot surfaces due to the denaturation of whey proteins and mineral deposition. These phenomena are considered to be major problems for dairy industrials. Extensive researches have been dedicated to reduce milk deposit formation on hot processing surfaces by modifying thermal parameters and adding inhibiting chemical deposition.

Early research investigations have focused mainly on fouling phenomenon by dairy products (Burton 1968; Visser and Jeurmink 1997). Burton (1968) reported that milk fouling might be classified in two categories known as type “A” and type “B”. Concerning type A (protein) deposit, it takes place at the temperatures range of 75–110 °C. The deposits are white, soft, and voluminous made up of protein (50–60 %), minerals (30–35 %), and fat (4–8 %). As for type B (mineral) deposit, it occurs at temperatures above 110 °C. These deposits are yellow, hard, and granular containing minerals, mainly calcium phosphate (70–80 %), proteins (15–20 %), and fat (4–8 %). Gotham (1990) stated that at pasteurization temperature, most of the proteins are denatured and the unfolded β -lactoglobulin (β -lg) is the major actor for the deposit formation (de Jong 1997).

Camel milk is one of the basic components of human diet in different parts of the world, mainly in the arid and semi-arid zones. It contains also all the essential nutrients found in bovine milk (protein, fat, minerals, lactose) (El-Agamy et al. 1998). One main difference between both bovine and camel milks is that the latter does not contain β -lg (Felfoul et al. 2015). It is also admitted that casein micelles of camel milk are poor in κ -casein as a consequence of low heat stability of

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Table 1 Detailed composition of casein and whey proteins in camel and cow milks (Kappeler 1998)

		Camel milk	Cow milk
		Relative amount in total casein (%)	
Caseins	α_{S1} -CN	22	38
	α_{S2} -CN	9.5	10
	β -CN	65	39
	κ -CN	3.5	13
		Concentration in milk (mg/L)	
Whey proteins	α -la	>5000	600–1700
	PGRP	370	–
	Lactophorin	954	300
	Lactoferrin	220	140
	β -lg	–	<4000

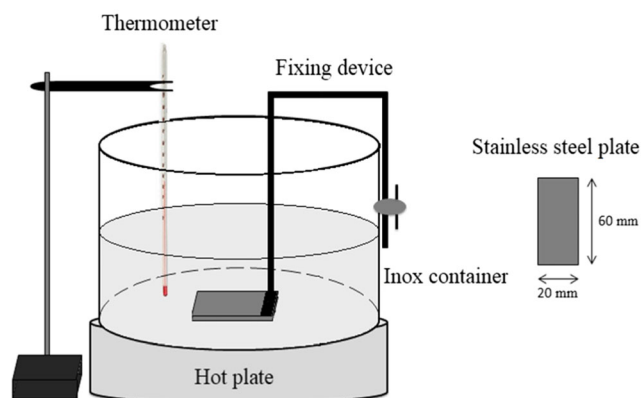
PGRP peptidoglycan recognition protein

camel milk especially at high temperatures. The detailed composition of casein and whey protein fractions in camel and cow milks is shown in Table 1 with reference to Kappeler (1998). Most of camel milk is freshly consumed, and its preservation can be achieved using heat treatments such as pasteurization. However, there are few studies reporting the camel milk behavior during heat treatment. Only few publications related some changes in the biochemistry of camel milk. The denaturation of whey proteins, mainly camel serum albumin (CSA) and α -lactalbumin (α -la), was also described (Levieux et al. 2006). Recently, Felfoul et al. (2015) have investigated the fouling behavior of camel and cow milks under different heat treatments and have demonstrated the existence of a deposit formation after heating camel milk at 80 °C for 60 min. This study aims at the identification of the chemical composition and the microstructure of the obtained deposits from camel milk. A comparison with cow milk was performed in parallel.

Materials and Methods

Camel and Cow Milks

Fresh raw camel and cow milks were obtained from farms located in the south of Tunisia (Douz region in Kébili governorate, Tunisia) and in Brittany (EARL-Richard farm, Saint Rémy du Plain, France), respectively. Once arrived at the laboratory at 4 °C, a pH (744-pH meter, Metrohm SA CH-9101, Herisau, Switzerland) determination was realized. Then, both milks were skimmed by centrifugation at 3000g during 20 min at 4 °C (Gyrozen 1580MGR, Multi-purpose Centrifuge, Daejeon, Korea).

**Fig. 1** Schematic representation of the experimental apparatus to follow deposit generation during heat treatment of camel and cow milks

Deposit Generation Experiments

A laboratory-scale device was constructed to conduct deposition experiments during the heat treatment of camel and cow milks (200 mL) (Fig. 1). Deposition experiments were conducted in a stainless steel container (total volume = 500 mL) containing rectangular plates (types 316 L; 20 × 60 mm), placed at the bottom of the container, at 80 °C for 60 min. Both temperatures of the plates and of the milks were held constant at 80 °C for 60 min. Before each of the deposition experiment, the stainless steel plates were dried at a temperature of 103 ± 0.2 °C for 15 min to be used later once cooled at room temperature. The experiments were reproduced at least three times. Heat treatment consisted of heating over a hot plate without agitation. After each experiment, the plates were dismantled and a sample of wetted deposit (drained in order to remove residual milk traces) was scraped off in order to be immediately analyzed for chemical composition. Then, the plates were cleaned with tap water and rinsed with distilled water for further uses. After each heating experiment, heat-treated camel and cow milks were immediately cooled at 4 °C for physicochemical analyses.

Physicochemical Analyses of Fresh and Heat-Treated Milks and Their Corresponding Deposits

The pH values of the fresh and heat-treated milks were determined using pH meter (Hanna Instruments, Portugal) connected to an electrode 406 M 6 (Mettler Toledo, France). The milk fat was determined according to International Dairy Federation (IDF) (152 A 1997). The dry matter of milks and deposits were determined by drying 5 g of milk sample and 1 g of deposits at 103 °C for 7 h in a capsule containing sand according to IDF (21 B 1987). The ash content of milk and deposits were determined according to NF V04-208 (1989). The total nitrogen content

(TN) of milk and deposits, non-casein nitrogen (NCN), and non-protein nitrogen (NPN) fractions were prepared according to IDF (20 B 1993). For the NCN determination, milks were acidified to pH 4.3 (camel milk) or 4.6 (cow milk) with a mixture of 10 % (v/v) acetic acid and 1 M acetate buffer. For the NPN determination, about 40 mL of 15 % (v/v) trichloroacetic acid was added to 10 mL of milk. The NCN and NPN samples were filtered through Whatman papers (Whatman Int. Ltd., Maidstone, UK) N°42 and 40, respectively. In fresh camel and cow milks, the NCN content corresponds to whey proteins, proteose peptone, while NPN corresponds to creatin, urea, and free amino acids. TN, NCN, and NPN contents were determined by Kjeldhal method (IDF 20 B 1993). The whey protein nitrogen (WPN) and the denatured WPN (%) were determined using the following calculations (Hattem et al. 2011):

$$\text{Whey Protein Nitrogen (WPN)} = \text{NCN} - \text{NPN}$$

$$\text{Denatured WPN}(\%) = \frac{\text{WPN}_{\text{raw}} - \text{WPN}_{\text{heated}}}{\text{WPN}_{\text{raw}}} * 100$$

Cation concentrations (calcium, magnesium, sodium, and potassium) were determined by atomic absorption spectrometer (Varian 220FS SpectrAA, Les Ulis, France). Phosphate concentration was determined by ion chromatography (Gaucheron et al. 1996). Mineral contents in the deposits were analyzed from the ashes obtained after 5 h at 550 °C (AFNOR 1989 NF V04 208). Lactose content in the deposits was determined according to Dubois et al. (1956).

For physicochemical analyses, deposit generation experiments were performed at least three times. The humid deposits, obtained after heating camel and cow milks at 80 °C for 60 min, were collected and the mixture was homogenized manually using a spatula. A sample of the same mixture was then taken for each physicochemical analysis. The sampling was reproduced three times for each physicochemical analysis performed.

Scanning Electron Microscopy

For this analysis, the deposits obtained after camel and cow milk heating at 80 °C for 60 min were not scraped off to avoid their destructions. The fouled plates were used directly after cutting a portion of the plates. The portions of the fouled plates were prepared using the technique described by Vétier et al. (1988).

Then, the prepared portions were fixed in glutaraldehyde 2.5 % (v/v) buffer cacodylate Na 0.1 M pH 7.3–7.4 for 1 h 30 min at room temperature and washed four times with buffer cacodylate Na 0.1 M pH 7.3–7.4 for 15 min, dehydrated using acetone gradient (60, 70, 80, 90, 95, and 100 %, v/v) for 15 min for each bath, then dried using the CO₂ critical point Leica EM CPD300 (Leica Microsystems, GmbH Hernalser Hauptstrasse 219 A-1170, Vienna, Austria) and metalized using Leica EM, ACE200.

The samples were observed and analyzed by scanning electron microscope JSM-7100F to Field Emission (JEOL Ltd, Tokyo Japan). The SEM experiment was repeated two times. The most significant photographs were presented.

Table 2 Physicochemical characteristics of camel and cow milks before and after heat treatment (60 min at 80 °C)

	Camel milk		Cow milk	
	Fresh	Heated	Fresh	Heated
pH	6.51 ± 0.00 ^a	6.40 ± 0.00 ^a	6.70 ± 0.00 ^b	6.76 ± 0.05 ^b
Dry matter (%)	9.13 ± 0.01 ^a	9.16 ± 0.11 ^b	9.46 ± 0.00 ^c	9.73 ± 0.21 ^d
Protein (%)	3.39 ± 0.01 ^a	3.69 ± 0.02 ^b	3.73 ± 0.00 ^c	3.60 ± 0.00 ^d
NCN (g/L)	4.02 ± 0.01 ^d	1.89 ± 0.01 ^b	3.38 ± 0.01 ^c	1.29 ± 0.01 ^a
NPN (g/L)	0.42 ± 0.01 ^c	0.47 ± 0.01 ^d	0.35 ± 0.01 ^a	0.37 ± 0.01 ^b
WPN (g/L)	3.6	1.42	3.03	0.92
Denatured WPN (%)	60.56		69.64	
Lactose (%)	4.20 ± 0.33 ^a	4.41 ± 0.77 ^b	4.81 ± 0.84 ^c	5.11 ± 0.54 ^d
Ashes (%)	0.95 ± 0.00 ^c	1.00 ± 0.00 ^d	0.81 ± 0.01 ^a	0.85 ± 0.00 ^b
Calcium (g/L)	0.96 ± 0.04 ^c	0.88 ± 0.00 ^c	1.17 ± 0.06 ^b	1.09 ± 0.00 ^a
Magnesium (g/L)	0.083 ± 0.00 ^c	0.085 ± 0.00 ^c	0.12 ± 0.01 ^a	0.12 ± 0.00 ^b
Sodium (g/L)	0.78 ± 0.00 ^a	0.85 ± 0.01 ^b	0.45 ± 0.01 ^c	0.51 ± 0.00 ^d
Potassium (g/L)	2.20 ± 0.05 ^a	2.26 ± 0.01 ^b	1.67 ± 0.03 ^c	1.74 ± 0.01 ^d

Averages ± standard deviation (SD) of three replicates. Values within the same row with different superscript letters differed significantly by Duncan's multiple-range test ($p < 0.05$)

Results and Discussion

Physicochemical Characterization of Fresh and Heat-Treated Milks

Table 2 presents the physicochemical characteristics and the distribution of N-fractions of both fresh and heat-treated camel and cow milks. The values are in accordance with data reported previously in the literature (Attia et al. 2000; Al-Haj and Al-Kanhal 2010).

After heat treatment, significant changes were observed between camel and cow milks mainly for the NCN and the NPN contents. The amounts of NPN in raw camel and cow milks were 0.42 and 0.35 g/L, respectively. The NPN content was higher for fresh camel milk than for fresh cow milk (Table 2). This was due to the fact that camel milk is known to contain enzymes and free amino acids in higher concentrations than cow milk (Taha and Kielwein 1989; Kappeler 1998; El-Agamy et al. 2009). After heat treatment, these contents increased significantly and were equal to 0.47 and 0.37 g/L. With regard to Hassan et al. (2009), they noted no significant difference in NPN contents between raw and heat-treated camel milks at 85 °C for 5 min ($p < 0.05$). As for Hattem et al. (2011), they showed that the NPN content of raw camel milk is significantly higher than that of heat-treated camel milk ($p < 0.05$) for different heat conditions used (63 °C/30 min; 80 °C/30 min; 90 °C/30 min; 72 °C/15 s). The significant increase in the NPN contents was due to the forced thermal treatment used in this study (80 °C/60 min) as well as the presence of heat-induced protein degradation products in the heat-treated milks.

The amounts of NCN in raw camel and cow milks were 4.02 and 3.38 g/L, respectively (Table 2). These values

showed a pronounced difference between camel and cow milks. After heating both milks, the NCN values decreased significantly to reach 1.89 and 1.29 g/L for camel and cow milks, respectively (Table 2). This agrees with the results found by Hassan et al. (2009) and Hattem et al. (2011). The significant decrease in the NCN contents was due to heat-induced denaturation of whey proteins as well as the formation of new interactions between themselves and with caseins. These results were in accordance with those described by Levieux et al. (2006). It is noteworthy that the denaturation reaction of β -lg in cow milk is one reason for the significant decrease in the NCN content. This result may be explained by the higher concentration of free thiol groups (-SH) after heating cow milk than when heating camel milk (Felfoul et al. 2015).

Table 2 shows that thermal denaturation was higher for bovine whey proteins than for camel whey proteins (60 and 69 % for camel and cow whey proteins, respectively). The results found in this study were in agreement with those reported by Farah (1986) and Wernery et al. (2003).

Chemical Composition of the Deposits

Bansal and Chen (2006) claimed that good understanding of all constituents of the milk product before and after thermal treatment will help with the characterization of the deposit itself, as well as the process of deposition as a whole. The chemical composition of the deposits obtained after the heat treatment of camel and cow milks revealed significant differences (Table 3). In fact, dry matter content is significantly higher for camel deposit (37 %) than for that of cow (35 %). However, Table 2 shows that cow milk has higher dry matter content than that of camel. This result suggests that camel milk components (mainly proteins and minerals) have higher

Table 3 Chemical composition of deposits obtained after heat treatment of camel and cow milks (60 min at 80 °C)

	Camel samples		Cow samples	
	Raw milk	Deposit	Raw milk	Deposit
Dry matter (%)	9.13 ± 0.01 ^A	37.24 ± 0.69 ^b	9.46 ± 0.00 ^B	35.90 ± 0.86 ^a
Protein (%/DM)	3.39 ± 0.01 ^A	57.43 ± 1.56 ^a	3.73 ± 0.00 ^B	68.96 ± 3.13 ^b
Lactose (%/DM)	4.20 ± 0.33 ^A	1.71 ± 0.01 ^b	4.81 ± 0.84 ^B	1.31 ± 0.01 ^a
Ashes (%/DM)	0.95 ± 0.00 ^B	34.89 ± 0.03 ^b	0.81 ± 0.01 ^A	27.67 ± 0.67 ^a
Protein/mineral	3.57	1.65	4.60	2.49
Calcium (%/DM)	0.96 ± 0.04 ^A	0.27 ± 0.07 ^a	1.17 ± 0.06 ^B	0.25 ± 0.10 ^a
Magnesium (%/DM)	0.083 ± 0.00 ^A	0.034 ± 0.01 ^a	0.12 ± 0.01 ^B	0.052 ± 0.01 ^b
Sodium (%/DM)	0.78 ± 0.00 ^B	0.29 ± 0.12 ^b	0.45 ± 0.01 ^A	0.19 ± 0.12 ^a
Potassium (%/DM)	2.20 ± 0.05 ^B	0.57 ± 0.07 ^b	1.67 ± 0.03 ^A	0.41 ± 0.25 ^a
Phosphorus (%/DM)	–	1.64 ± 0.34 ^a	–	1.61 ± 0.12 ^a

Averages ± standard deviation (SD) of three replicates. Values within the same row with different superscript lowercase letters differed significantly ($p < 0.05$) for deposit samples. Values within the same row with different superscript uppercase letters differed significantly ($p < 0.05$) for milk samples

adherence ability on the plate surface than cow milk components.

Lactose content was also demonstrated to be significantly higher in the case of the deposit generated after heating camel milk (1.7 %) than that for cow milk (1.3 %). To the best of our knowledge, the lactose presence in the deposit obtained after heating milk has not been yet discussed in the literature (Nelson 2012).

Table 3 indicates that the camel deposit contained about 57 % (w/w) protein against 69 % (w/w) for cow deposit. The amount of mineral deposit was much less than that of protein for both deposits; for the camel sample, the amount of mineral deposit was 35 % (w/w) against 28 % (w/w) for cow sample. The ratio of protein/mineral was higher for cow deposit (2.5) than for camel deposit (1.6). This agrees with the results found by Boxler et al. (2013) who studied the composition of cow milk fouling deposits on diamond-like carbon-coated surfaces at pasteurization and UHT temperatures using an electrical heater. Besides, Table 2 indicates that 61 and 70 % of camel and cow whey proteins, respectively, were denatured due to heating. From these results, it was then deduced that camel deposit is mainly generated by both protein denaturation and mineral precipitation.

The mineral composition of the deposits generated after heating camel and cow milks was listed in Table 3. The obtained results indicate the presence of calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), and phosphorus (P). K and Na amounts were higher in camel deposit. Nevertheless, cow deposit contains more Mg than its camel counterpart (Table 3). Moreover, Ca and P are the main minerals deposited during deposition experiment in a massic ratio of 6:1 P:Ca for both deposits. Table 2 shows that the difference of Ca content between raw and heat-treated camel and cow milks was about 0.08 %. On the other hand, Table 3 indicates that Ca contents in the deposits generated after heating camel and cow milks were significantly similar, 0.27 and 0.25 %, respectively. These values were considerably higher than 0.08 %, presented earlier in Table 2. This means that the total amount of Ca deposited on the plate's surface is directly related not only to its precipitation due to heat treatment but also to the casein micelles destabilization after the heat treatment of both milks. Andritsos et al. (2002) studied the calcium phosphate deposit formation on the hot surfaces from simulated cow milk ultrafiltrate. These authors have indicated that calcium phosphate precipitate at pH 6.7 from a temperature of 42–44 °C. With regard to Boxler et al. (2014) who studied the composition of milk fouling deposits under pulsed flow conditions, they have shown that calcium phosphate are present on the solution at the temperature of 62 °C. These findings confirmed the obtained results in this study since we proceed with an intense heat treatment

of 80 °C for 60 min. It is well known that in milk fouling, the constituents of deposition proceed through a complex process in which both whey protein aggregation and calcium phosphate formation in the bulk fluid are to be accounted for (Sadeghinezhad et al. 2013). Robbins et al. (1999) proved that protein aggregation and calcium phosphate thermal instability appeared together at high temperatures which is the case of our study (80 °C).

Photos and Microstructure of the Deposits

Visual Observation

Figure 2 reports the photographs corresponding to the visual observation of the aggregates formed on the plates after heating camel and cow milks. For both samples, the obtained deposits corresponded to that of type « A » described by Burton (1968), i.e., voluminous, spongy with a whitish color. Moreover, the visual comparison of the deposits shows that there was a pronounced difference in structure between both samples. Indeed, camel deposit presented larger craters than that of cow deposit. The deposit obtained during camel milk heating was denser than that of cow milk, and the stainless steel plate was completely covered.

Scanning Electron Microscopy

The morphological analysis obtained by SEM (Fig. 3) of fouled stainless steel plates after heat treatment of camel and



Fig. 2 Photos of the deposits obtained after heat treatment at 80 °C during 60 min of camel (a) and cow (b) milks

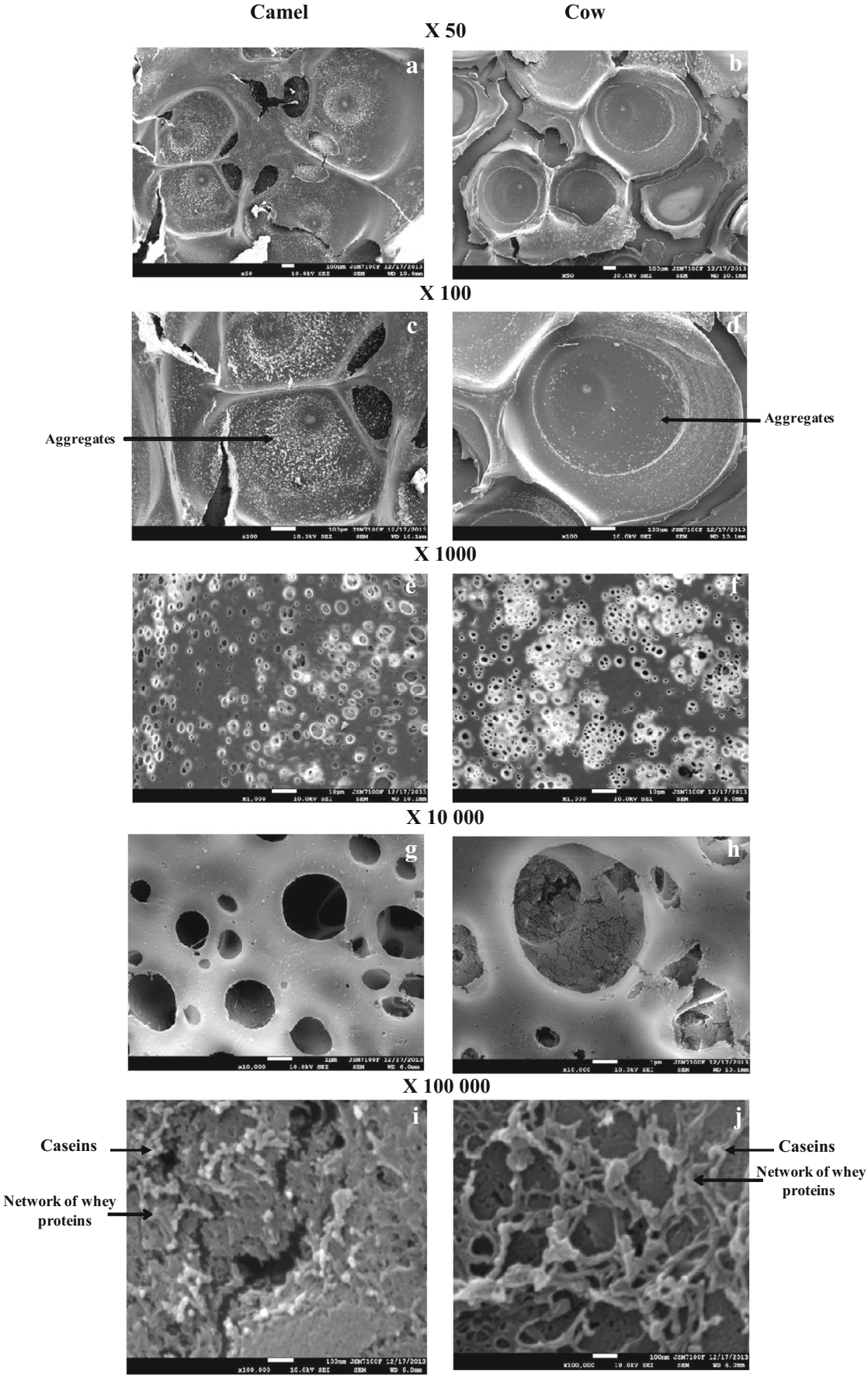


Fig. 3 Scanning electron micrographs of the deposits obtained after heat treatment of 80 °C during 60 min of camel and cow milks. Scale bars are given in each case

cow milks were taken at the same magnifications for both samples ($\times 50$, $\times 100$, $\times 1000$, $\times 10,000$, and $\times 100,000$).

As can be seen in Fig. 3c and d, the presence of protein aggregates with 10- μ m thickness was observed. Camel protein aggregates were much denser than those of cow (Fig. 3e and f). Caseins are present as white spheres in both samples as shown in Fig. 3g and h. The presence of fatty globules is very clear in camel deposit as shown in Fig. 3g. This result indicates that camel fatty globule membrane is resistant to heat treatment (Fig. 3g). The SEM's of the obtained deposits revealed the familiar structure of a protein deposit with large clumps (100-nm thickness) composed of smaller aggregates (Fig. 3i and j). Figure 3i reveals that the deposit generated during heating camel milk shows a more amorphous structure than that of cow milk (Fig. 3j). The whey protein networks were also obvious in both figures and were smaller for camel sample than for that of cow.

The results indicate that the difference in structure could be due to the difference in the composition between both deposits generated during heating camel and cow milks. Table 3 shows that the cow deposit contained more proteins than camel deposit. β -Lg, which is the major whey protein present in cow milk and responsible for cow deposit formation, is absent in camel milk with reference to many researchers (El-Agamy et al. 2009; Felfoul et al. 2015). Hence, the absence of this protein could be the major factor responsible for the amorphous structure of the camel deposit. In fact, the protein network was not well structured as shown in Fig. 3i. On the other hand, mineral content was higher for camel deposit than that of cow. It is reported that with high concentrations of minerals, the deposit layer becomes more compact (de Jong 1997), which could be in relation with our study regarding camel deposit (Fig. 3i). Moreover, the protein content is higher than that of minerals for both deposit samples (Table 3).

In a recent study, we showed that free thiol group content was significantly higher for cow milk than for camel milk in both fresh and heated states (Felfoul et al. 2015). This difference in the degree of covalent bonding, between active sulfhydryl (–SH) groups exposed on the protein surface, which have taken place to form the protein aggregates, could explain the difference in structure between both samples. Thereby, by considering this –SH reactivity, the probability of aggregation in heat-treated camel milk is lower than in heat-treated cow milk. From Fig. 3j, the cow deposit consists mainly of β -lg which has one free –SH group and two S-S bridges. Indeed, the structure is well defined and the presence of calcium phosphate particles is clear in Fig. 3j. The mineral particles are embedded in the proteinaceous matrix. This observation is in agreement with those of Visser and Jeurnink (1997) who

studied the milk fouling in the dairy industry. The deposit formation on the hot surfaces caused by cow milk heating results from the complex interaction between caseins, whey proteins (mainly β -lg), and minerals, especially Ca. Generally, when cow milk is heated, proteins partially unfold and then aggregate to form a three-dimensional network that entraps water through capillary forces (Ayadi et al. 2004). As a result of these reactions between whey proteins and other components, some explanation can be proposed. β -Lg aggregates both to itself and with κ -casein in casein micelles. The amount of β -lg attached to the micelle increases with temperature and Ca ion concentration (Robbins et al. 1999). Otherwise, for camel milk, α -la constitutes the major whey protein. This protein does not contain a free –SH group but four S-S bridges. After the heat treatment of camel milk at 80 °C for 60 min, these S-S bridges would supposedly be broken so that α -la could associate with other proteins. However, the aggregates formed after heating camel milk seemed to be not as structured as that of β -lg in cow milk (Fig. 3i).

Conclusion

This study was carried out to compare the composition and the microstructure of deposits generated during heating camel and cow milks at 80 °C for 60 min. The data have shown significant differences between fresh and heat-treated camel and cow milks mainly in NCN and NPN contents. Significant differences in chemical composition of both camel and cow deposits were also observed. Indeed, the camel deposit contained about 57 % (w/w) protein against 69 % (w/w) for cow deposit. The amount of mineral deposit for camel sample was 35 % (w/w) against 28 % (w/w) for cow deposit. The SEMs of both deposits have indicated that the structures of both deposits consisted of large clumps of 100 nm protein aggregates. Based on this experimental study, the effects of heat treatment, which are well known for cow milk, cannot be directly extrapolated to camel milk due to the qualitative and quantitative differences in terms of the composition of both milks. These results could be a database to the understanding of deposition phenomena during camel milk heating.

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