

# Fouling Behavior of Camel and Cow Milks Under Different Heat Treatments

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**Abstract** Using a developed laboratory-scale device, different heat treatment conditions were applied on camel and cow milks. After each fouling experiment, photos of stainless steel plates were taken and dry deposit weights were determined. The thermal behavior of camel and cow proteins was studied by electrophoresis (sodium dodecyl sulphate polyacrylamide gel electrophoresis, SDS-PAGE), differential scanning calorimetry (DSC), and free thiol group concentration evolution. The obtained results have shown that heating both camel and cow milks at 70 °C for 2 h generates deposit formation. The fouling rate was more important when heating camel milk than after heating cow milk for all heat conditions except at 90 °C for 2 h. Electrophoresis patterns indicated that after heating camel milk at 90 °C,  $\alpha$ -lactalbumin ( $\alpha$ -la), camel serum albumin (CSA), and  $\kappa$ -casein bands decreased. Bovine serum albumin (BSA) disappeared from the electrophoresis patterns after heating cow milk at 70 °C while  $\beta$ -lactoglobulin ( $\beta$ -lg) and  $\alpha$ -la bands disappeared only at 90 °C. DSC thermograms of camel milk showed that the denaturation temperature of camel proteins is 77.8 °C, lower than that of cow proteins which is 81.7 °C. The results of free thiol group evolution versus temperature and heating time showed that camel protein denaturation starts between 70

and 80 °C. However, for cow milk, the whole denaturation phenomenon happens after heat treatment at 70 °C for 30 min.

**Keywords** Heat treatment · Camel proteins · Fouling · Electrophoresis (SDS-PAGE) · Differential scanning calorimetry (DSC) · Cow proteins

## Introduction

In the dairy industry, every product is heated at least once; thus, heat treatment is by far the main unit operation. Thermal treatments are carried out to ensure food microbiological quality and confer various functional properties to milk products, like thermal stability, gelation, viscosity, foaming, and emulsifying ability (Schmitt et al. 2009; Donato et al. 2009). The thermal processing in heat exchangers induces the fouling of hot surfaces due to the denaturation of whey proteins. Fouling refers to the accumulation of undesirable deposits on hot surfaces of heat exchangers. During the thermal processing of milk, fouling by proteins and minerals is considered to be one major problem for dairy industrials. Extensive research have been dedicated to reduce milk fouling deposition on hot processing surfaces by modifying thermal parameters, adding inhibiting chemicals fouling and modifying the design of the processing equipment. Reducing fouling is related to the control and understanding of  $\beta$ -lactoglobulin ( $\beta$ -lg) denaturation reaction, since, accounting for half of the whey proteins in cow milk (Ayadi et al. 2004, 2007; Schmitt et al. 2007), it is predominant in the fouling phenomenon of milk derivatives. Under heat treatment,  $\beta$ -lg loses its tertiary structure and becomes reactive by exposing a free thiol. On the other hand,  $\beta$ -lg thiol activation causes fouling reactions on the hot surfaces of heat exchangers (Labouré et al. 2004).

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Camel milk represents one of the basic ingredients of human nutrition in many parts of the world, especially in the arid and semi-arid zones. This milk contains all the essential nutrients found in bovine milk (El-Agamy et al. 1998). Camel milk is used as a treatment for a series of diseases. It is also reported to have many potential therapeutic properties (El-Agamy et al. 2009). Camel milk might also be considered as a promising new protein source for children allergic to cow milk protein, and camel milk infant formulae can be taken into account (El-Agamy et al. 2009; Shabo et al. 2005). Kappeler (1998) reported that camel milk is free of  $\beta$ -lg, which is considered one of the major antigens of cow milk proteins responsible for the incidence of hypersensitivity reactions (allergy) in infants (Lara-Villoslada et al. 2005; El-Agamy 2007).

Currently, camel milk is consumed mainly fresh. Therefore, in order to extend its shelf life for long periods, different heat treatments such as pasteurization and sterilization may be applied to camel milk. Heat processing as a means of preserving milk has not been applied to camel milk yet, and up to now, only few studies have investigated the effect of heat treatment on camel whey proteins (Farah 1986; El-Agamy 2000). Knowing that camel milk does not contain  $\beta$ -lg (Farah 1986; Hinz et al. 2012), the understanding of an eventual fouling phenomenon during camel milk heating is of great scientific and technological importance. The detection of potential fouling phenomena and understanding its mechanism during camel milk heating are of major importance for its commercialization. The aim of this study is to quantify fouling phenomena, during different heat treatments of camel and cow milks. It also aims to explain fouling mechanisms and milk protein biochemical modifications by electrophoresis patterns, calorimetric analysis, and free thiol contents determination.

## Materials and Methods

### Milk Samples

Fresh raw camel milk was obtained from an experimental station located in the south of Tunisia (Douz region in Kébili governorate). Fresh cow milk was purchased from a local breeding located in Sfax region (Sfax governorate). Once arrived to the laboratory at 4 °C, pH (Metrohm pH meter) was determined. Then, both milks were skimmed by centrifugation at 3000g during 20 min at 4 °C (Gyrozen 1580MGR, Multi-purpose Centrifuge, Daejeon, South Korea).

### Heat Treatment and Deposition Experiments

A laboratory-scale device was constructed to conduct fouling experiments during the heat treatment of camel and cow milks (200 mL). Fouling experiments were realized in inox container (total volume=500 mL) containing rectangular dismantled

plates (types 316L; 20 mm×60 mm), placed at the bottom of the recipient, under different heat conditions (durations, 60 and 120 min; temperatures, 60, 70, 80, and 90 °C), and reproduced at least three times. Heat treatment consisted in heating over a hot plate without agitation. After each experiment, the photos of fouled plates were systematically taken, using a digital camera (Samsung EC-ES80, 12 MP, 5× Optical Zoom, USA) performing with no magnification, and dry stainless steel plates were weighed (drying condition, 105 °C/8 h). The results describing fouling are expressed in grams of dry matter per square centimeter.

### SDS-PAGE Electrophoresis

Electrophoresis experiments (sodium dodecyl sulphate polyacrylamide gel electrophoresis, SDS-PAGE) were carried out using a Bio-Rad apparatus (Mini-Protean Tetra Cell) of gels in vertical blocks. The concentration of acrylamide gel was 15 %. Electrophoresis was run at 120 mA when marker color (bromophenol blue) was at 0.5 cm from the anode end of the block (approximately 3 h). SDS-PAGE experiments were realized according to the procedure of Laemmli (1970). The molecular weights of the different proteins were estimated by comparing their electrophoretic mobilities with those of marker proteins having known molecular weights.

### DSC Experiments

To improve the signal quality of the differential scanning calorimetry (DSC) analyses, cow and camel milks were previously concentrated by an ultrafiltration pilot (Pall centramate equipped with a membrane Pall Omega 10-kDa suspended screen OS010C11) at 1.5 bar and 30 °C. Bovine and camel retentates were concentrated about three times, collected, and stored at 4 °C before DSC experiments.

The thermal behaviors of concentrated camel and cow milks were monitored by DSC using a TA Q-1000 calorimeter (TA Instruments, New Castle, DE). Calibration was made with an indium standard (melting point=156.66 °C,  $\Delta H$  melting=28.41 J/g). About 90 mg of the samples was accurately weighed in a hermetic stainless steel pan of 100  $\mu$ L. A pan with 90 mg of distilled water was used as a reference, and both pans were hermetically sealed. The samples were tempered at 20 °C for 5 min and heated from 20 to 160 °C at 5 °C/min. Measurements were performed five times with independent samples. The baselines have not been adjusted.

### Free Thiol Contents

To quantify free thiols, 158 mg (2 mM) of 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) was added to 820 mg of a 50-mM sodium acetate solution, dissolved in 200 mL ultrapure water, and refrigerated until use. For the assay, 1-mL quartz cuvettes

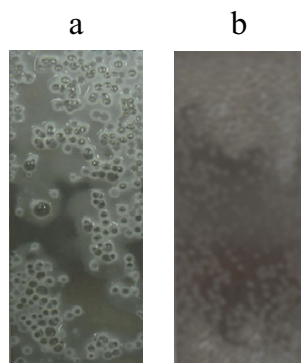
were filled with 840  $\mu\text{L}$  of ultrapure  $\text{H}_2\text{O}$ , 50  $\mu\text{L}$  of the DTNB sodium acetate solution, 100  $\mu\text{L}$  of Tris buffer (1 M,  $\text{pH}=8.0$ ), and 10  $\mu\text{L}$  of camel or cow milks differently heated. The curvetts were incubated at 37  $^\circ\text{C}$  for 5 min and the optical density was measured at 412 nm (Ellmann 1959). The absorbance values for each sample were divided by the molar extinction coefficient of the DTNB mixed disulfide complex ( $13,600 \text{ M}^{-1} \text{ cm}^{-1}$ ) resulting in a concentration of thiols in the solution. The measurements were carried out five times to determine reproducibility.

## Results and Discussion

### Fouling Evolution of Camel and Cow Milks

Figure 1 shows the photos of plates after the heat treatments at 70  $^\circ\text{C}/2 \text{ h}$  of camel and cow milks. For both milks, the obtained deposit corresponds to that of type « A » described by Burton (1968), i.e., it is voluminous, spongy, and of a whitish color.

Figure 2 describes the dry deposit mass evolution on stainless steel plate surfaces after fouling experiments. Figure 2b shows a classical deposit generation evolution obtained during heat treatment of cow milk, a first period with low deposit quantities followed by an exponential evolution. In fact, during the first period of the run, the fouling process started and the deposit layer thickness was insufficient to be detected by dry deposit weight measurements. After this step, the deposit buildup started and continued to increase with temperature and time. On the other hand, Fig. 2a shows a different shape close to a linear evolution during camel milk heating. The deposit composition and fouling mechanism of cow milk under pasteurized temperature was extensively studied (Lalande et al. 1984; Bansal and Chen 2006; Petit et al. 2013; Bouvier et al. 2014). These fouling phenomena result from the thermal denaturation of whey proteins, mainly  $\beta$ -lg, induced by calcium ions occurring in the bulk fluid during heating (Robbins et al. 1999). The initial adsorbed layers were considered to be prone to sequential protein and calcium phosphate binding.



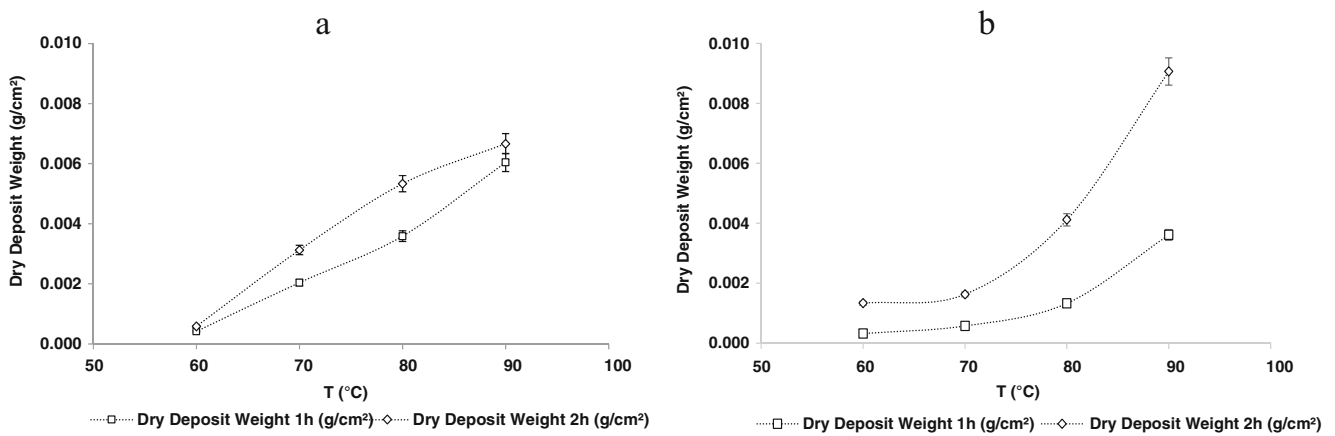
**Fig. 1** Photos of protein deposition during camel (a) and cow (b) milk heating at 70  $^\circ\text{C}$  for 2 h

Moreover, during milk heating, the part of the soluble calcium becomes insoluble owing to the inverse solubility product of calcium phosphate salts with temperature and precipitates as a calcium phosphate salt. Either this precipitate may be formed in solution or it may associate with the already present casein micellar calcium phosphate or with the  $\beta$ -lg aggregated in the serum phase or with both. With time, the precipitate would ultimately form a deposit on the stainless steel wall of the processing equipment. Otherwise, by the interaction with the casein micelles in milk, whey protein fouling is limited to a great extent. Indeed,  $\beta$ -lg is deposited onto these micelles through a linkage with  $\kappa$ -casein present on the micellar surface and may still play a role in fouling as a bridging agent between the casein micelles and the heating surface (Visser and Jeurmink 1997). Based on all these previous studies, the fouling phenomenon is proven to be generated mainly by  $\beta$ -lg denaturation reaction after heating cow milk. For camel milk, early investigations reported the absence of this protein ( $\beta$ -lg) (Farah 1986; Hinz et al. 2012). However, the results presented in Figs. 1 and 2 show the existence of a deposit formation during camel milk heating. The understanding of the deposit mechanism during camel milk heating is of great importance for its preservation by thermal operations such as pasteurization and sterilization, and therefore its commercialization at a wide scale.

### Electrophoresis Patterns and DSC of Camel and Cow Milks

The gel electrophoretic patterns of raw and heated camel and cow milks for different heat treatment conditions ( $T, t$ ) are shown in Fig. 3. It is observed that the major protein bands present in cow milk correspond to bovine serum albumin (BSA),  $\alpha_{s1,2}$ - and  $\beta$ -caseins,  $\beta$ -lg, and  $\alpha$ -lactalbumin ( $\alpha$ -la). The most abundant whey protein in cow milk is  $\beta$ -lg, which is not detected in camel milk. This result is in agreement with previously reported findings (Farah 1986; Ereifej et al. 2011). For raw camel milk, the major bands correspond to camel serum albumin (CSA),  $\alpha_{s1,2}$ -,  $\beta$ -, and  $\kappa$ -caseins, peptidoglycan recognition protein (PGRP), and  $\alpha$ -la.

Visually, the bands of camel proteins decreased with temperature and heating time. Pasteurization temperature (70  $^\circ\text{C}$ ) caused no visible changes in camel protein gel patterns (Fig. 3a). Increasing temperature to 80  $^\circ\text{C}$ , CSA band becomes less intense than at 70  $^\circ\text{C}$  and starts to disappear after heating camel milk for 60 min at 80  $^\circ\text{C}$ . A similar observation was noticed with casein bands. Figure 3 shows that the effect of the temperature increases on camel milk was mild on  $\beta$ - and  $\alpha_{s1,2}$ -caseins and it was drastic on  $\kappa$ -casein. Camel  $\alpha$ -la's band remained constant after heating camel milk at 70 and 80  $^\circ\text{C}$ . However, at 90  $^\circ\text{C}$ ,  $\alpha$ -la and CSA bands as well as  $\kappa$ -casein decreased. When cow milk was heated at 70  $^\circ\text{C}$ , BSA disappeared from the electrophoresis pattern.  $\beta$ -lg and  $\alpha$ -la bands remained constant at 80  $^\circ\text{C}$ , but disappeared after the

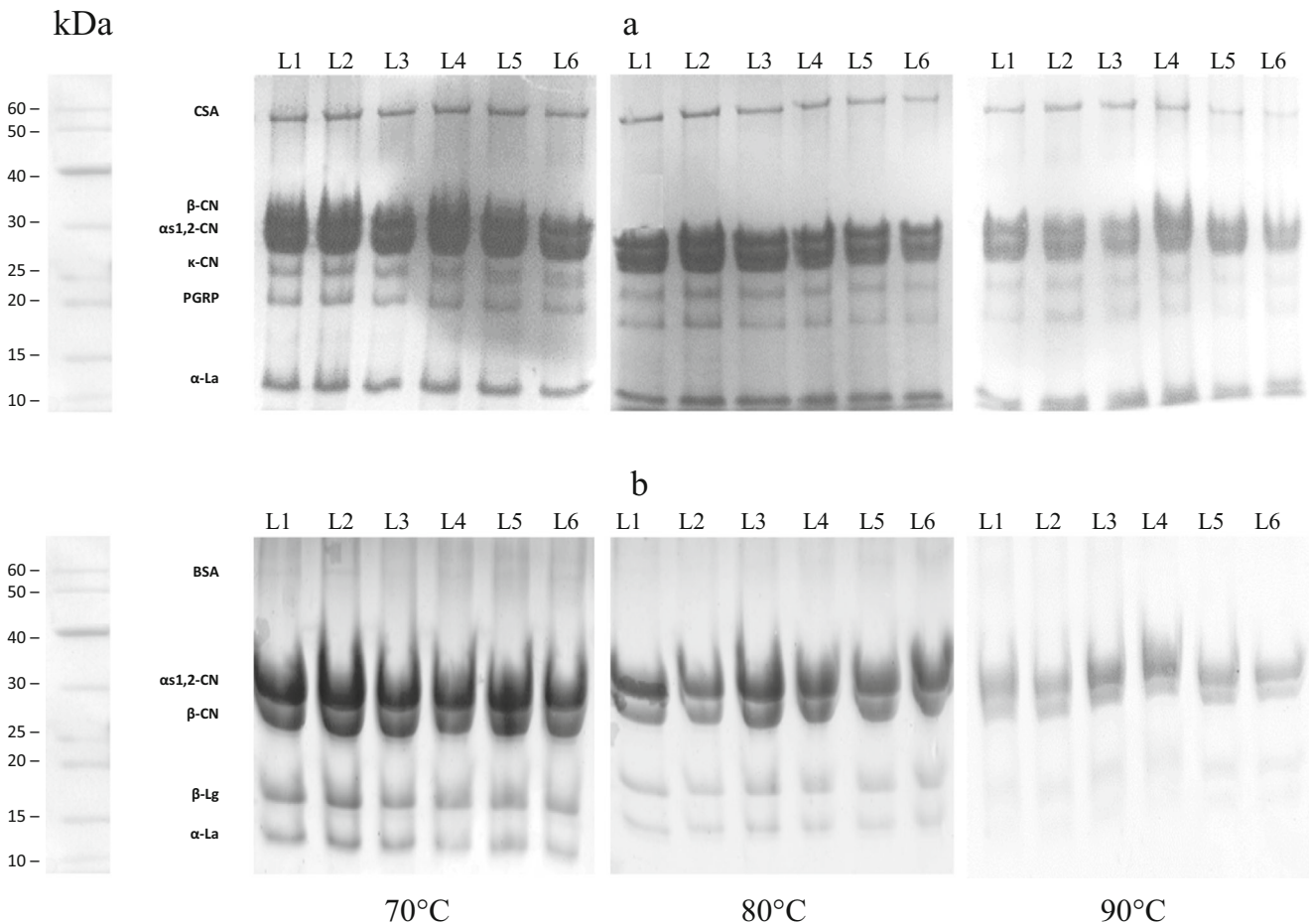


**Fig. 2** Evolution of dry deposit weight versus temperature during 1 and 2 h of **a** camel milk and **b** cow milk

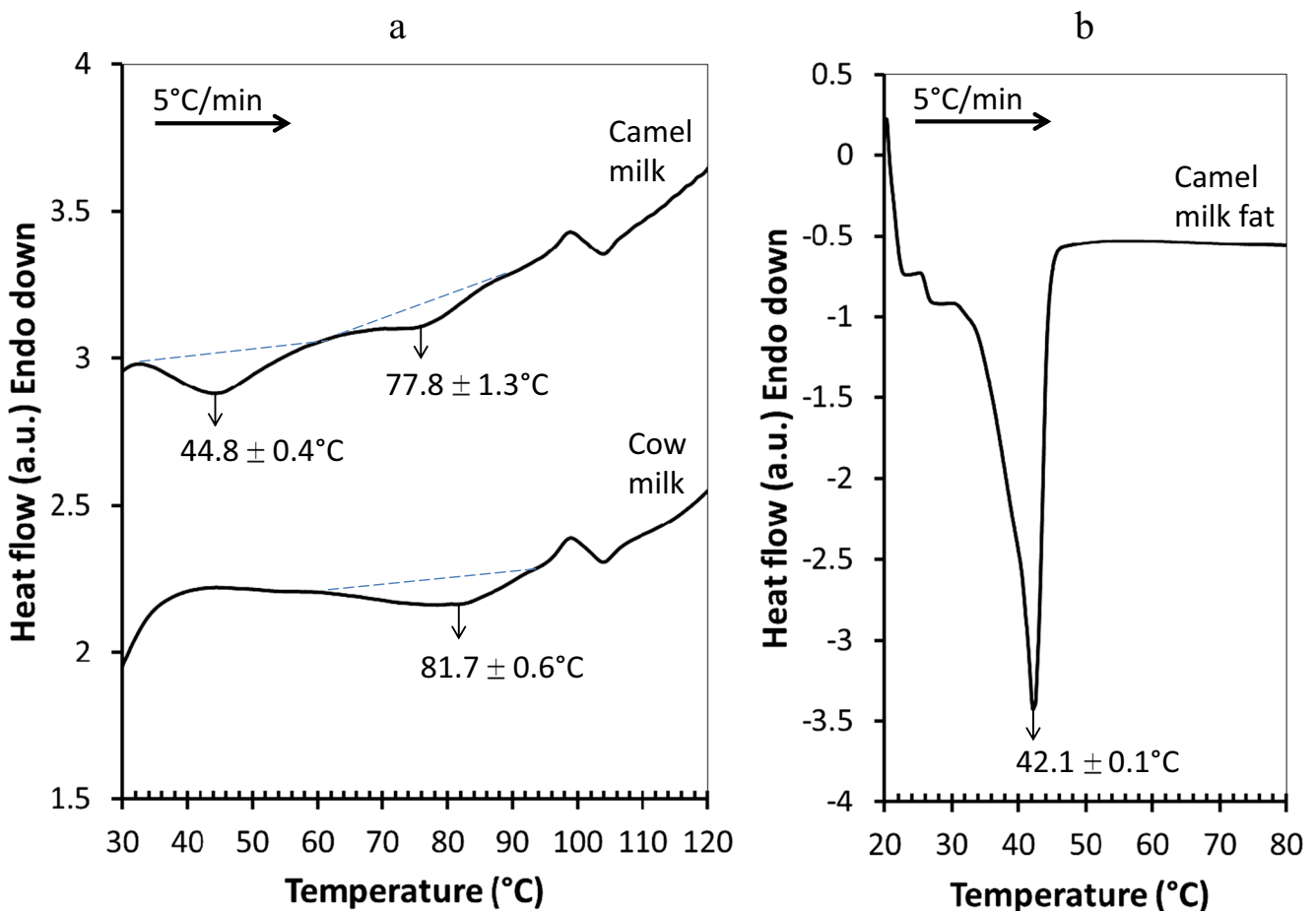
heat treatment of 90 °C (Fig. 3). Based on these findings, it was confirmed that  $\beta$ -lg is the major actor responsible of fouling generation during cow milk heating. For camel milk, the CSA remained the most affected protein by heat treatment followed by  $\alpha$ -la. Both proteins can therefore be responsible

for deposit generation after heating camel milk, alone or by interaction with other proteins and minerals.

Figure 4a depicts the DSC curves recorded during the heating of concentrated camel and cow milks. In both cases, the recorded thermal phenomena were endothermic and



**Fig. 3** SDS-PAGE electrophoresis patterns of camel and cow milk proteins treated at 70, 80, and 90 °C for different heating durations. *L1*, unheated milk; *L2*, heated milk (0 min); *L3*, milk heated (30 min); *L4*, milk heated (60 min); *L5*, milk heated (90 min); *L6*, milk heated (120 min)



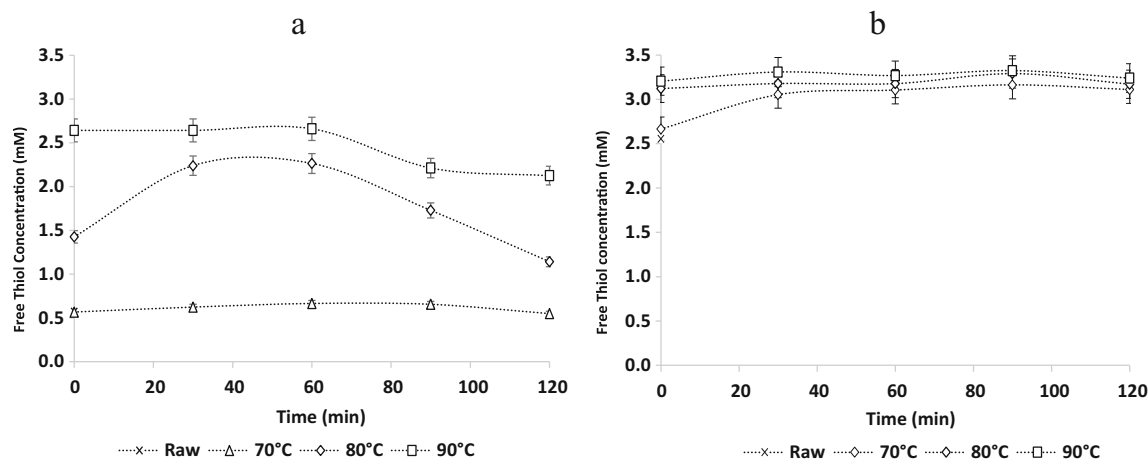
**Fig. 4** Differential scanning calorimetry curves recorded during heating at 5 °C/min of (a) concentrated camel and cow milk, and (b) camel milk fat

attributed to the unfolding of globular proteins during heat treatment, which requires a heat absorption to break the intramolecular bonds (non-covalent, disulfide...). Hence, the endotherms recorded during milk heating were interpreted as the denaturation of the proteins found in milks.

The DSC curves of camel milk sample (Fig. 4a) reveals a two-step endotherm, one at lower temperature (44.8 °C) and another at higher temperature (77.8 °C). The endotherm occurring at lower temperature is due to the melting of residual fat in camel milk (Fig. 4b), which is in accordance with those obtained by Karray et al. (2004). Besides, this peak represents the main difference observed between cow and camel milks. Indeed, this peak does not exist in cow DSC curve, which may be attributed to the difference in the nature of fat composition between cow and camel milks. Camel milk is very difficult to cream due not only to the small size of fat globules but also to the presence of agglutinins (Farah and Rüegg 1991). The second endotherm occurring at 77.8 °C is due to denaturation of many proteins mainly camel whey proteins since casein molecules are not strongly modified at this temperature level of heat. Since camel milk is deficient in  $\beta$ -lg, the presence of one single transition at 77.8 °C suggests the overlapping of thermal denaturation peaks of  $\alpha$ -la and CSA (Fig. 4a). Similarly,

cow milk sample is also investigated using DSC (Fig. 4a), whose curve shows one single endothermic peak. The thermogram gives denaturation temperature at 81.7 °C. This endotherm probably corresponds to the denaturation of a proteins mixture. No thermal transition of individual cow proteins was observed as in the case of camel milk. Atri et al. (2010) have demonstrated that in both holo and apo states, the stability of camel  $\alpha$ -la is greater than that of bovine  $\alpha$ -la. On the other hand, El-Agamy (2000) has reported that camel proteins are relatively heat stable and this stability is due to the absence of  $\beta$ -lg. As for Levieux et al. (2006), they have confirmed that purified camel immunoglobulins, CSA, and  $\alpha$ -la are denatured at 67.2, 73, and 77.5 °C, respectively. With respect to Paulsson et al. (1985), they have reported the stability of purified bovine  $\alpha$ -la,  $\beta$ -lg, and BSA at pH 6.6 and observed only one peak at temperature lower than 80 °C. Laleye et al. (2008) have indicated that the denaturation temperatures are 109 and 106 °C for camel and bovine wheys, respectively.

The comparison between the DSC curves of camel and cow milks shows that camel proteins are more heat sensitive than cow proteins. The denaturation temperature of camel proteins is 77.8 °C, lower than that of cow proteins which is 81.7 °C (Fig. 4a). This challenges about the protein



**Fig. 5** Evolution of free thiol concentration of camel (a) and cow (b) milks at different heat treatment conditions (time and temperature)

denaturation development in the deposits formed just after heating camel and cow milks at different heat treatment conditions. Regarding the dry deposit mass evolution findings (Figs. 1 and 2), we have shown that when heating camel and cow milks at 60 °C, no deposit was formed on the stainless steel plates; at 70 °C, the deposit begins to appear, and the plates were completely fouled after heating camel and cow milks at 80 °C. It was also demonstrated in Fig. 2 that the deposition rate was higher when heating camel milk than after heating cow milk. These results are further confirmed by the DSC findings since the denaturation temperatures of camel and cow proteins reached their maximum at 77.8 and 81.7 °C, respectively (Fig. 4).

### Free Thiol Contents

The evolution of free thiol concentrations after heating camel and cow milks under different thermal conditions is shown in Fig. 5. When milk is subjected to heat treatment, protein denaturation happens and significant changes occur in the -SH and -SS- groups of the proteins (Hoffmann and Van Mill 1997). It is observed that free thiol group contents changed differently for camel and cow milks with heat treatment intensity (Fig. 5).

The comparison between both camel and cow milks reveals that camel milk presents significantly lower free thiol concentrations compared to cow milk for all heat conditions. Initially, the number of cysteine and methionine residues (2.56 mM) on the protein chains of cow milk is higher compared to that of camel milk (0.57 mM). In fact, camel  $\alpha$ -la (8 cysteine and 3 methionine residues) has no free thiol group but four SS groups, CSA (14 cysteine and 3 methionine residues) has seven SS groups, and camel  $\alpha$ <sub>s2</sub>-casein (2 cysteine residues) has one SS group. On the other hand,  $\beta$ -lg (4 methionine and 5 cysteine residues) has 1 free thiol group and 2 SS groups, bovine  $\alpha$ -la (8 cysteine and 1 methionine residues) has 4 SS groups, and BSA (5 methionine and 34 cysteine

residues) has 17 SS groups. A lower free sulfhydryl group SH content reflects the formation of intermolecular disulphide bonds or other oxidation products (Donovan and Mulvihill 1987). Moreover, heating time has a significant effect on the exposure of thiol groups (Saffon et al. 2013).

Figure 5a shows that after heating camel milk at 70 °C for all durations, free thiol groups are not detected. Heated camel milk at 70 °C has a free thiol content equal to that of raw material. This means that camel protein denaturation has not started at 70 °C yet, which confirms the previously obtained DSC results. However, with the increase in temperature to 80 °C, three phases were observed. The concentration of free SH increased dramatically in the first few minutes of heating reflecting sulfhydryl group exposure. A second step with a constant free thiol SH content for camel milk at 80 °C during 30 min was observed. Then, a dramatic decrease was noticed after 60 min of heating. At this level, all sulfhydryl groups were exposed and aggregation phenomenon was noticed. At 90 °C, free -SH concentrations reached a maximum value of 2.66 mM. This result can be explained by the camel protein thermal denaturation, mainly  $\alpha$ -la which undergoes a considerable change in conformation during denaturation (Atri et al. 2010), and  $\kappa$ -casein induced by calcium ions. Figure 5b indicates that protein denaturation started after heating cow milk for 30 min for all temperatures. The whole phenomenon happens during 30 min of heating. An exhaustion of cow proteins was deduced after heating cow milk at different temperatures for 30 min, which may be explained by the thermal denaturation of  $\beta$ -lg. Furthermore, Fig. 5 shows that cow protein denaturation is faster compared to that of camel proteins. This result is confirmed by the DSC results of both milks. Indeed, the onset denaturation temperature of cow proteins is 60.4 °C against 69.7 °C for camel proteins (Fig. 4).

With respect to dry deposit mass evolution results (Fig. 2), we have shown the existence of an initiation phase after the heat treatment of cow milk from 60 to 70 °C. However, this phase does not exist when heating camel milk due to the initial higher

concentrations of free thiol groups for cow milk than for camel milk. The role of the free thiol groups in fouling is apparent not only on  $\beta$ -lg aggregation and subsequent adsorption after heating cow milk but also on the adsorption rate. The latter's  $\beta$ -lg on the surface, which refers to the fouling rate, seems to be proportional to the concentration of activated protein molecules, of which the thiol group plays a key role (Visser and Jeurmink 1997). The results of free thiol group evolution versus temperature and heating time (Fig. 5) show that camel protein denaturation starts between 70 and 80 °C heat treatment which is confirmed by the DSC results (77.8 °C). Otherwise, we have already demonstrated that the deposit amount formation (Fig. 2) was more important when heating camel milk than after heating cow milk for all heat conditions except at 90 °C for 2 h. This result could be contributed to the fact that camel  $\alpha$ -la and CSA might have a more important adherence ability than  $\beta$ -lg in cow milk. Indeed, every denatured whey protein is able to adhere on the hot surfaces during milk heat treatment, alone or by interaction with the casein micelles, mainly  $\kappa$ -casein. Early studies showed that  $\kappa$ -casein and denatured  $\beta$ -lg (two disulfide bridges and one SH free sulfhydryl group) could react with each other via thiol-disulfide exchange (Lowe et al. 2004). The thiol SH groups of both  $\beta$ -lg and BSA lead to the intermolecular disulfide bond formation between the whey proteins (Oldfield et al. 2005).

## Conclusion

The obtained results have shed the light on the deposition phenomenon during the heat treatment of camel and cow milks under different heat conditions. In this study, we confirm that  $\beta$ -lg is the major actor responsible for fouling phenomenon for cow milk, and we demonstrate that  $\alpha$ -la and CSA denaturation reactions are involved in generating deposit formation after heating camel milk. It was also shown that cow protein denaturation is faster compared to that of camel proteins in spite of the heat sensitivity of camel proteins over bovine proteins. With reference to these findings, we suppose that  $\alpha$ -la and CSA have a more important ability to adhere on hot surfaces during camel milk heating than  $\beta$ -lg in cow milk. Based on this experimental study, future research works will be dedicated to study the thermal behavior of isolated individual camel proteins.

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