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A laboratory investigation of cow and camel whey proteins deposition 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 wheys. After each deposition experiment, photos of stainless steel plates were taken and dry deposit weights were determined. Proteins denaturation was studied by electrophoresis (SDS-PAGE) and differential scanning calorimetry (DSC). The obtained results have shown that heating both cow and camel wheys at 60 °C does not generate deposit. Furthermore, the heat treatment at above 70 °C was found to cause a severe fouling of stainless steel plate. The electrophoresis patterns indicated that heating at 90 °C caused camel serum albumin's (CSA) band disappearance for both rennet and acid wheys. However, α-lactalbumin's (α-la) concentration decreased versus temperature and heating time. DSC thermograms showed that denaturation temperatures were 73.8 °C for camel rennet whey, 60.5 °C for camel acid whey, 70.5 °C for cow rennet whey and 63.9 °C for cow acid whey. Taken into the count the absence of β-lg in camel milk and based on the obtained results several hypotheses were advanced to explain camel milk fouling during heat treatment.

1. Introduction

Cow milk is very important in the human diet thanks to its balanced basic nutrient composition (proteins, carbohydrates and fats) and high vitamins and mineral contents, including calcium (Haug et al., 2007). In order to ensure a better microbiological quality of milk and increase the duration of its storage for human consumption, dairy industrials have usually applied specific heat treatments, such as pasteurization and sterilization. However, these treatments have a direct influence on the nutritional, biological and functional properties of milk proteins (Changani et al., 1997) and heat exchangers performances (Bansal and Chen, 2006).

Camel milk is one of the main food resources for arid populations. It contains all nutrient components (protein, fat, lactose, minerals) and has a highly biological value due to the higher contents of antimicrobial factors such as lysozyme, lactoferrin and immunoglobulin (El-Agamy et al., 1998). Most of camel milk is consumed in the fresh state. Despite the low production's percentage of camel milk in the world compared to cow milk, the former's preservation has a real importance to avoid its microbial degradation owing to its nutritional specificities. The preservation of camel milk can be achieved using heat treatments such as pasteurization and sterilization processes. However, there are few studies pertaining to the camel milk behavior during heat treatment operations.

Otherwise, the effects of heating bovine milk were intensively studied with special interest in milk microbiological, biochemical and nutritional qualities (Changani et al., 1997), process performances and heat exchanger fouling (Lalande

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et al., 1984; Ayadi et al., 2008). Thus, during the heat treatment of bovine milk, various technological problems, caused by fouling mechanism such as the formation of deposits on heat exchangers surface could happen, inducing the formation of undesirable compounds. Therefore, the reduction of this phenomenon has always been a scientific and technological challenge. Previous studies showed the existence of a great correlation between whey protein denaturation and fouling mechanism (Lametti et al., 1997). One of the proteins responsible for this phenomenon of bovine milk is β-lactoglobulin (β-lg) (Lalande et al., 1984). The unfolded β-lg, an intermediate of the denaturation reaction, is able to aggregate with other proteins or is adsorbed at the deposit layer (Lalande et al., 1984; de Jong, 1997). However, previous studies showed that camel milk is devoid of β-lg (Farah, 1986). To the best of our knowledge, only few studies about the effect of heat treatments on camel milk composition are available in the literature (Farah, 1986; El-Agamy, 2000) and heat exchangers performances. Therefore, the understanding of an eventual fouling phenomenon during camel milk heating is of a great scientific and technological importance. Actually, the detection of potential fouling phenomena and understanding its mechanism during camel milk heating is of a major importance for its commercialization.

The objective of this paper is, therefore, to highlight the existence of a potential deposit formation during the heat treatments of camel rennet and acid wheys, in comparison with cow wheys. The description of this phenomenon was followed by the determination of the whey proteins denaturation degrees in comparison with whey proteins from cow samples.

2. Materials and methods

2.1. 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, a pH (Metrohm pH meter) determination was realized. Then, both milks were skimmed by centrifugation at 3000 *g* during 20 min at 4 °C (Gyrozen 1580MGR, Multi-purpose Centrifuge, Daejeon, Korea). Rennet-wheys were obtained after rennet coagulation of fresh milks at 36 °C in the presence of 1.4 mL L^{-1} of microbial rennet (*M. miehei*, strength = 1:10,000, Laboratories Arrazi, Parachimic, Sfax, Tunisia). Acid-wheys were obtained after the acidification (HCl, 6 M) of fresh milks until pH = 4.6 and 4.3 for cow and camel milks, respectively; the wheys were recovered by centrifugation at 3000 *g* for 20 min at 4 °C (Gyrozen 1580MGR, Multi-purpose Centrifuge, Daejeon, Korea).

2.2. Heat treatment and deposition experiments

An experimental apparatus, at laboratory scale, was conceived to conduct deposition experiments during the heat treatment of wheys from camel and cow milks (200 mL) (Fig. 1). Deposition experiments were realized in inox container (total volume = 500 mL) containing rectangular dismantled plates (types 316 L; 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 3 times. Heat treatment consisted in heating over a hot plate without agitation. After each experiment, the photos of

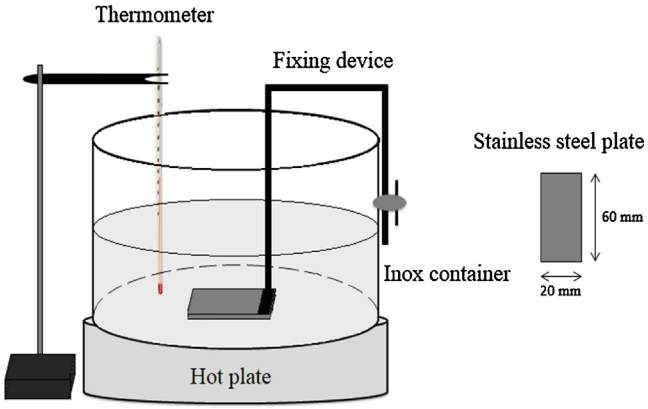

Fig. 1 – Schematic representation of the experimental apparatus to follow deposit generation during heat treatment of camel and cow wheys.

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 deposition are expressed in g of dry matter/cm^2.

2.3. Free thiols content

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 before use. For assay, 1 mL quartz curvets were filled with 840 μL of ultrapure H_2O, 50 μL of the DTNB sodium acetate solution, 100 μL of Tris buffer (1 M, pH = 8.0), and 10 μL whey samples heated at different heat treatments. The curvets were incubated at 37 °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 M^{-1} cm^{-1}), resulting in a concentration of thiols in solution.

2.4. SDS-PAGE electrophoresis

Electrophoresis experiments (sodium dodecyl sulphate polyacrylamide 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 until the change of the 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 protein fractions were estimated by comparing their electrophoretic mobilities with those of marker proteins having molecular weights known.

2.5. Thermal properties: differential scanning calorimetry (DSC) experiments

To improve the signal quality of the DSC analyses, fresh bovine and camel whey samples were previously concentrated using vivaspin 20 tubes (10,000 Da Molecular Weight Cut Off, sactoriusstedim biotech, GmbH, Germany) in a centrifuge unit at 1800 *g* at 20 °C for 2 and 4 h for rennet and acid wheys, respectively. By taking into account the reduction of volume of the retentate, this protocol allowed the concentration of proteins