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Effect of the demineralization process on the physicochemical and biochemical properties of camel and bovine cheese-wheys

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Abstract

Cheese-whey is a valuable byproduct of the dairy industry, rich in various nutritional components such as minerals, lactose, and proteins. Whey proteins, often used in concentrate form, are widely applied in the food industry due to their diverse chemical, physical, and techno-functional properties. This study aimed to investigate the physicochemical composition and biochemical characteristics of camel and bovine whey after partial demineralization at a laboratory scale. Camel whey exhibited lower pH values compared to bovine whey, while showing comparable levels of total solids, ash, and lactose, but significantly higher protein content. Analysis of both types of whey, before and after dialysis filtration, demonstrated partial demineralization, a significant reduction in lactose content, and a decrease in β-lactoglobulin levels in bovine whey. These findings suggest that demineralized camel and bovine whey hold significant potential for applications in the agricultural and food industries.

Introduction

Whey is the main by-product of casein or cheese production; it is of great importance in the dairy industry, representing a global production of ~ 200 million tons. Approximately half of the worldwide whey production is treated and transformed into a variety of food and feed products. 50% of this amount is used directly in its liquid form, while 30%is used as powdered cheese-whey, 15% as lactose and the rest as whey-protein concentrates. Whey contains more than half of the total solids initially present in the whole milk, including whey proteins (representing 20% of the total protein in whole milk), most of the lactose, water-soluble vitamins and minerals. Therefore, whey can be considered a valuable by-product with various applications in the food and pharmaceutical industries (Barba, 2021). The demand is increasing for whey proteins fabrication due to the high functional and nutritional values with application in food ingredients industry. Indeed, whey proteins have become the most employed proteins in food formulations due to their excellent functional characteristics including emulsification (Nishanthi *et al.*, 2017).

The demineralization process of whey involves the removal of excess minerals, such as calcium, magnesium, and phosphate, from whey and ultrafiltration permeates. This process is necessary to reduce the mineral content, which can limit the commercial use of whey by-products in various applications (Hoppe and Higgins, 1992). By lowering the mineral load from an initial concentration of 7.3 g/L, demineralization helps expand the range of possible uses for whey and permeates, facilitates further processing, and can also help eliminate undesirable components (Marx *et al.*, 2019). Hence, the demineralization process of whey results in a demineralized whey fraction, which is currently used in food production for infants and children, as well as for special purposes including confectionery, bakery products, meat products and pharmaceutical products (Khramtsov *et al.*, 2017).

Camel milk has recently become more popular in many countries in Asia, Europe and Africa due to its claimed nutritional value and therapeutic properties including anti-cancer, anti-diabetic and hypo-allergic properties (Lajnaf *et al.*, 2024). Compared to bovine whey, the soluble fraction of camel milk is devoid of β-lactoglobulin (β-Lg) which has been considered as one of the most dominant bovine milk allergens in whey, representing more than the half of whey proteins and limiting the use of this milk for the preparation of infant formulae (Ereifej *et al.*, 2011). Thus, α-lactalbumin (α-La) is the major protein of camel whey, representing 52.68% and 20% of camel and bovine whey proteins, respectively with a concentration of 2.2 g/L in camel milk (Ereifej *et al.*, 2011; Omar *et al.*, 2016). Furthermore, camel whey proteins are considered as a rich source of lactoferrin, immunoglobulins, lysozyme, peptidoglycan recognition protein (PGRP), serum albumin acidic and basic sub-units (Hailu *et al.*, 2016).

Table 1. Chemical composition of camel and bovine whey as affected by dialysis filtration

Phyisicochemical composition	Bovine whey	Filtrated bovine whey	Camel whey	Filtrated camel whey
pH value	6.35 ± 0.04[a]	6.47 ± 0.06[b]	6.10 ± 0.01[c]	6.40 ± 0.01[a]
Acidity (°D)	15.50 ± 0.70[a]	3.50 ± 0.70[b]	20 ± 0.20[c]	5.5 ± 0.71[d]
Conductivity (mS/cm)	26.4 ± 0.01[a]	2.5 ± 0.04[b]	34.35 ± 0.21[c]	4.18 ± 0.01[d]
Total solids (%)	5.96 ± 0.02[a]	2.68 ± 0.10[b]	5.72 ± 0.35[a]	1.10 ± 0.13[c]
Ash (%)	0.83 ± 0.1[a]	0.62 ± 0.1[b]	0.87 ± 0.05[a]	0.39 ± 0.11[c]
Proteins (g/L)	2.47 ± 0.06[a]	1.42 ± 0.08[b]	4.79 ± 0.11[c]	3.53 ± 0.21[d]
Lactose (g/L)	45.79 ± 0.37[a]	2.87 ± 0.01[b]	45.08 ± 1.46[a]	3.80 ± 0.07[c]

[a-d]samples represented with different letters are significantly different from each other ($p < 0.05$). error bars show the standard deviations of mean values of physicochemical characteristics (ph value, acidity, conductivity, total solids, ash, proteins and lactose).

The separation and fractionation of cheese-whey proteins have facilitated the production of high-quality whey protein supplements, which are now a key output of the dairy industry (Huma *et al.*, 2015) . However, achieving efficient and cost-effective whey protein separation remains a significant challenge. It is crucial to ensure that the proteins retain their native structure and biological activities during separation process. This research aims to investigate the impact of partial demineralization of camel and bovine cheese-wheys using dialysis membrane on their physicochemical and biochemical characteristics.

Materials and methods

Milk samples

Fresh raw camel milk samples were collected from 20 healthy Dromedary camel females (*Camelus dromedarius*) ranging between 2 and 12 months into lactation from the south of Tunisia (region of Gabes), while fresh bovine milk was obtained from a local breed in the region of Sfax (Tunisia).

Bovine and camel whey preparation

Bovine and camel cheese-wheys were extracted from skimmed milk after enzymatic coagulation at 37 °C for 1–2 h in the presence of microbial rennet enzyme with amounts of 0.35 and 1.4 mL enzyme/L bovine and camel milk, respectively, followed by centrifugation at 3000 × g for 20 min at 20 °C using Thermo Scientific Heraeus Megafuge Centrifuge, Germany (Lajnaf *et al.*, 2020).

Filtration of whey

Bovine and camel wheys were filtered by dialysis against deionised water (Milli-Q system, Millipore, USA) at (sample/water, 1:100) for 24 h at 4 °C using dialysis tubing cellulose membrane (12 kDa MWCO, Sigma–Aldrich) and with continuous stirring (Virgen-Ortíz *et al.*, 2012). The distilled water was replaced after the first hour.

Physicochemical analysis

Physico-chemical composition of camel and bovine whey before and after filtration was systematically determined according to the AOAC Official Method (AOAC, 1984). Specific conductivity of whey was determined using the method of conductivity measurements of the EXPERT-002 conductometer (Khramtsov *et al.*, 2017).

The demineralization rate (DR), based on conductivity analysis was then calculated by considering the specific conductivity detected in whey samples at the beginning (C_i in mS/cm) and the end (C_f in mS/cm) of the demineralization process according to Equation (1) (Beaulieu *et al.*, 2020):

$$DR = \left(1 - \frac{Cf}{Ci}\right) \times 100 \quad (1)$$

The mineral reduction rate based on the conductivity measurement was then calculated with the same equation as for the DR (Equation (1)), considering the ash content (%) at the beginning and the end (C_i and C_f, respectively) of the demineralization process (Beaulieu *et al.*, 2020).

All analytical determinations of chemical analysis were performed in triplicate (n = 3). Data were expressed as mean ± standard deviation.

Reversed-phase high-performance liquid chromatography (RP-HPLC) analysis

RP-HPLC (Agilent 1260 Infinity quaternary LC, Germany) was used to separate and identify the main proteins from camel and bovine whey- and milk-derived proteins before and after filtration using the method of Lajnaf *et al.* (2022). A C18 column (Zorbax Eclipse Plus C18, 250 mm length × 4.6 mm, particle size 5 µm, Packing Lot #: B14292) was used for whey protein separation. The RP-HPLC analysis was performed using a Shimadzu SPD6A-UV detector measuring the optical density. Quantitative estimation of the main camel and bovine whey proteins was performed by calculating the peak area of each protein.

Results and discussion

Chemical composition of camel and bovine wheys

The physicochemical composition of camel and bovine whey was determined in this study, as shown in Table 1. Camel whey has lower pH values and higher acidity compared to bovine whey. The reason behind difference in pH and acidity of the whey is the difference between camel and cow milk. Indeed, previous researches noted that pH of camel milk is similar to that of sheep milk, but significantly lower than bovine milk (Sawaya *et al.*, 1984). Furthermore, pH values and acidity of wheys are mainly attributed to other number of factors including the lactation stage, colostrum, diseases, etc. (Huma *et al.*, 2015). However, pH values significantly increased while acidity decreased after membrane dialysis regardless of the whey origin, which is explained by the dilution with

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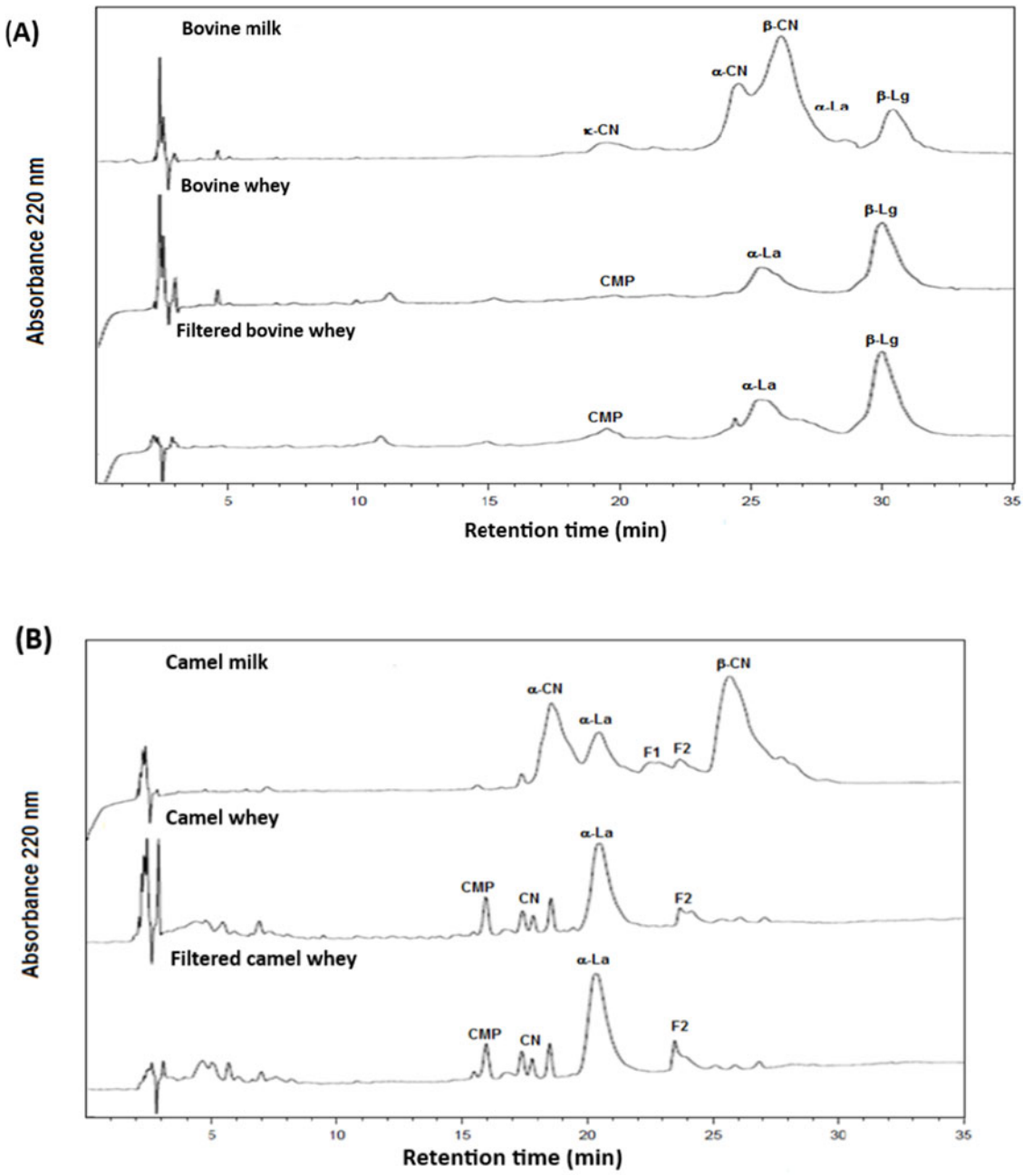

Figure 1. RP-HPLC chromatograms recorded at 220 nm for bovine and camel milk and whey protein fractions (chromatograms A and B, respectively). Abbreviations are: β-CN, β-casein; α-CN, α-casein; CN, caseins; α-la, α-lactalbumin; β-lg, β-lactoglobulin; F, protein fraction; CMP, caseinomacropeptide.

deionized water upon filtration. Total solids content of both wheys didn't vary much from each other (~5.9%), in agreement with the results of the study by Zouari *et al.* (2020), carried out with acid and sweet wheys from camel and cow's milk. Similarly, both camel and bovine whey exhibited the same ash contents (~0.82%) in accordance with the values of Wangoh *et al.* (1998). However, these values were significantly reduced after dialysis filtration, confirming the demineralization of whey after dialysis filtration with mineral reduction rate of 25.3% and 55.2% for bovine and camel whey, respectively (Table 1). In the case of proteins, a significant difference was observed, with higher protein concentrations in camel whey than in bovine whey. The protein concentrations in sweet bovine and camel whey samples were 2.47 ± 0.06 and 4.79 ± 0.11 g/L of whey, respectively. These values are lower than those reported by (Hailu *et al.*, 2016) and Zouari *et al.* (2020). No significant differences in the lactose concentration were observed between bovine and camel wheys (~ 45 g/L), in accordance with the finding of Bouhaddaoui *et al.* (2019), who reported that fat contents ranged from 45 to 56 g/L in camel whey with an average of 49.8 g/L and 42 g/L for camel and bovine milk, respectively. However, dialysis filtration of whey reduced effectively lactose contents in both wheys with the reduction rate of 93.7% and 91.6% for bovine and camel wheys, respectively. Therefore, dialysis filtration proved to be a valuable tool for the reduction of lactose content in whey regardless of the milk origin. Finally, Table 1 also shows the dependence of the specific conductivity of cheese-whey

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on the origin of milk and the degree of its demineralization. Indeed, the highest value obtained for the camel whey with specific conductivity was 34.35 ± 0.21 mS/cm, followed by the bovine whey with 26.4 ± 0.01 mS/cm and the lowest value for the cheese-whey with conductivity of 2.5 ± 0.04 mS/cm and 4.18 ± 0.01 mS/cm for bovine and camel wheys, respectively. In the whey compartment, the decrease in conductivity was higher for bovine whey than camel whey, which corresponded to DR levels of 87.8% versus 84.2% for bovine and camel wheys, respectively. These findings are in agreement with those of Khramtsov *et al.* (2017) who reported that the demineralization causes significant decrease to the specific conductivity of whey. These authors noted that the process of demineralization of whey was completed when the specific conductivity value reached approximately 1–1.5 mS/cm. Meanwhile, the demineralization process of acid wheys in the study of Beaulieu *et al.* (2020) resulted in DR values that ranged between 20.32% and 77.23% depending on membrane configuration, experimental conditions and raw materials.

RP-HPLC profile

Fig. 1A and B show that RP-HPLC chromatograms of bovine and camel milk protein fractions, respectively. The characterized protein fractions which are derived from bovine and camel wheys are skimmed milk, native whey and filtrated whey. For bovine milk, five major peaks with retention times (RT): 17.8 min, 22.4 min, 23.9 min, 26.1 min and 27.8 min) were detected and identified as κ-casein (∼4.4%), α-casein (∼20.5%), β-casein (∼59%), α-La (∼1.7%) and β-Lg (∼14.4%). Meanwhile, six major protein peaks were identified in camel milk (Fig. 1B). These peaks corresponded to κ-casein (∼1.4%), α-casein (∼27.1%), α-La (∼14.7%), protein fractions (F1 and F2) (∼2.3 and 1.6%) and β-casein (∼52.9%) with RT of 16.3 min, 18.3 min, 20.2 min, 22.2 min, 23.3 min and 25.3 min, respectively. Thus, chromatograms showed that β-casein is the main protein of the colloidal fraction of bovine and camel milk representing more than the half of total bovine and camel proteins in accordance with the results of Kappeler *et al.* (1998). Camel milk also presented ahigher content of α-casein and lower amounts of κ-casein when compared to bovine milk proteins in agreement with the findings of Lajnaf *et al.* (2022) and Omar *et al.* (2016). As expected, no peak corresponding to β-Lg was detected in camel milk and whey in agreement with previous researches (Ereifej *et al.*, 2011). Meanwhile, β-Lg is the main protein of bovine whey as shown in chromatograms (Fig. 1A) followed by α-La representing ∼ 64.9% and ∼ 32.6% of the total whey proteins, respectively. It is also possible to observe a third peak of protein fraction in bovine whey with an RT of 18.3 min, which is identified as caseinomacropeptide (CMP). Camel α-La was found to be the main protein in the camel whey accounting for 62 % of the total camel and whey. Fig. 1B, shows three other main peaks in camel whey with RT of 14.18 and 23.5 min camel whey, suggested to be identified as the CMP (∼8.8%), traces of caseins (∼13.7%), and camel serum albumin-lactoferrin (∼15.5%) in agreement with Ereifej *et al.* (2011)

Chromatograms showed that dialysis filtration didn't affect significantly the composition of bovine and camel whey as they show all peaks previously observed in native whey. However, the proportions of proteins have changed upon filtration. For instance, the β-Lg contents were significantly reduced to 55.8% of total whey proteins, while the percentage of α-La raised to 64.3% in agreement with the findings of Huma *et al.* (2015). This could be of great interest to food industry, as the level of β-Lg, the main allergen in milk has been significantly reduced.

Conclusion

The aim of the present work was to investigate the effect of partial demineralization by dialysis filtration on physicochemical composition and biochemical characteristics of bovine and camel whey in comparative study. The overall results presented previously showed that, camel whey exhibited higher pH values and lower acidity compared to bovine whey. Conversely, camel whey showed similar levels of total solids, ash and lactose compared to bovine whey, with higher protein content than that of bovine whey. Analysis of camel and bovine whey before and after dialysis filtration indicated that the reduction in minerals, lactose, and specific conductivity led to the partial demineralization of whey with DR levels of 92.7% and 84.2% for bovine and camel wheys respectively as well as significant reduction of lactose content (>90%). Therefore, this reduction is of great interest to the dairy industry as it allows the production of delactosed whey specifically for patients suffering from lactose intolerance. RP-HPLC results indicated that proportions of proteins have changed upon filtration including the reduction of β-Lg, the main allergen of bovine milk. Finally, the studies presented in this communication show that gram quantities of demineralized whey from camel and bovine milk can readily be obtained from liter quantities of these milk, but some modifications may be required in scale-up to a process using hundreds or thousands of liters of milk.

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