



Physicochemical and microbiological study of fresh cream and fermented butter (Smen) made from camel milk

Saïd Mosbah^{1*}, Ghania Annou², M'hamed Bouricha³, Safia Mekkaoui⁴, Boudjenah-Haroun Saliha¹

¹Laboratory of Research on Phoeniculture, Kasdi Merbah University, Ouargla, 30000, Algeria

²Laboratory for the Protection of Ecosystems in Arid and Semi-Arid Zones, Kasdi Merbah University, Ouargla, 30000, Algeria

³Laboratory of Biogeochemistry of Desert Environments, Kasdi Merbah University, Ouargla, 30000, Algeria

⁴Saharan Bioresources Laboratory: Preservation and Valorization, Université Kasdi Merbah-Ouargla 30.000 Algérie

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Abstract

Smen is the most used local product by Algerian nomadic peoples, which is produced by the spontaneous fermentation of camel milk. The aim of this study was to evaluate the physicochemical and microbiological properties of fresh cream separated from camel milk and the changes that occurred after the fermentation and maturation of Smen. The Smen is produced after the churning of fermented camel milk. Furthermore, the fresh cream is separated directly by centrifugation of raw camel milk. Butter yield and fat recovery efficiency show a decrease after Smen ripening. Physicochemical analyzes of Smen showed a significantly ($p < 0.05$) higher acid value (8.5 mg KOH/g) and ash (1.16 %); lower total solids (60.8 %) and fat (50.5 %) than fresh cream (2.08 mg KOH/g, 0.38 %, 68.2 %, 63.6 % respectively). The milk fermentation and the manufacturing process of Smen interfere with the microbiological properties by increasing lactic bacteria flora and yeast and absence of fecal flora and pathogenic bacteria such as *Staphylococcus aureus* and Sulfite-reducing clostridia. The fermentation process modifies the nutritional and hygienic properties of milk fat, in which Smen became higher health benefits than fresh cream by the growth of lactic bacteria flora and the synthesis of bioactive molecules.

* Corresponding Author: Saïd Mosbah ✉ smmms2010@gmail.com

Introduction

Camel milk is generally consumed in a raw state, but due to the limitation of means of cold storage in many rural areas in African countries, the milk is stored at room temperature, which allows them to ferment quickly by the natural lactic flora (Heita and Cheikhoussef, 2014).

Nomads of Algerian Sahara "Tuaregs", improve the conservation quality of camel milk by transforming it into derived products, such as fermented cheese (Klila) and clarified butter (Smen). Smen is made from fermented butter, which is separated from spontaneously fermented milk by the churning method (Iradukunda *et al.*, 2018). This product can be stored for years depending on metrological conditions in tightly closed earthenware pots. Camel's Smen has been used for many therapeutic purposes, such as gastrointestinal discomfort and skin problems. The therapeutic properties of camel milk and its products were attributed to the fact that camels graze on various plant species rich in bioactive molecules (Seifu, 2007).

During the fermentation process of food, yeast and bacteria convert carbohydrates to many metabolites like organic acid, alcohol and carbon dioxide.

These metabolites enhance a food's flavor profile, nutritional value, as well as serve the preservation purpose. In addition, the bioactive compounds resulting from this process give richness to food products by improving therapeutic and medicinal properties (Ewe and Loo, 2016).

Fermented dairy products, such as yogurt, kefir, butter and Smen, are products providing functional components, such as prebiotic substances synthesized by lactic acid bacteria. These products present an important current food industry trend and increase consumer demand for their health benefits (Bourrie *et al.*, 2016).

To this end, the present study aims to evaluate the physicochemical and microbiological characteristics

of fresh cream separated from camel milk and the changes obtained after the maturation of Smen as a traditional product.

Materials and methods

Fresh cream and smen preparation

Fifteen fresh raw camels' milk samples (*Camelus dromedarius*) were obtained from a local farm in the area of Ouargla (Southeastern Algeria). Samples of milk were collected (500 ml of each one), kept refrigerated and transported to our laboratory within 4 h. Fresh cream and fermented camels' butter (Smen) were manufactured in our laboratory according to the traditional method described by Samet-Bali *et al.*, (2009) and Berhe *et al.*, (2013) with some modifications.

Fresh cream is obtained by bringing camel milk to an incubator shaker (IKA Shakers, KS 3000 i control). In order to allow the fat to rise to the surface, the milk is stirring (400 stirring/min) at a temperature of 30-35 °C for 20 min, followed by centrifugation at 3500 x g/min during 20 min at 4 °C to have a good mass separation of this fat on the surface.

For the preparation of fermented camel butter (Smen), samples of camels' milk were fermented spontaneously for about 96 hours at room temperature until coagulum formation; the resulting product is called Raib. Raib is then churned in an incubator shaker (500 stirring/min) at room temperature (25°C) for 30 min. After churning, the butter is collected and washed several times by salting cold water (8% of NaCl) in order to remove the remaining milk traces from the butter.

The salt addition was necessary for better preservation and for taste improvement. The traces of water is removed from the salted butter by leaving it to drain for a certain time. Salted butter can then be directly introduced into earthenware pots for further maturation. The butter must be well compacted to remove air; otherwise, undesirable oxidation may occur. The maturation process occurred in a cool, dark and dry place for three months.

Physicochemical characterization

The fat recovery was made indirectly by calculating the ratio between the amount (gram) of fat present in the 100 ml milk taken for preparation of the sample (fresh cream and Smen). Butter yields were calculated as their weight present in 100 ml of milk taken for preparation, expressed as gram per liter (Berhe *et al.*, 2013).

The pH was measured by homogenization of 10 g of sample with 20 ml of distilled water for 5 min at 30°C. The pH of the homogenate was determined using a digital pH meter (HANNA Instrument, Romania model) calibrated with standard buffer solutions (Owusu-Kwarteng *et al.*, 2012).

The acidity value is expressed as the number of milligrams of KOH required to neutralize the acids found in 1 g of samples. 10 g was dissolved in 100 ml of ethanol. The mixture was titrated with potassium hydroxide in ethanol (0.1N) in the presence of phenolphthalein as an indicator (NFT 60 204, 1985).

The total solids content was determined by the loss of mass of samples in an oven heated for 24 hours at 103 ± 2 °C (Robert and Bradley, 2010). Ash content in samples was determined gravimetrically using a dry ashing method, heated at 550 °C for 5 hours in a muffle furnace (Marshall, 2010).

The fat content of samples was determined by the Gerber method (AFNOR NF V 04-287, 2002). Proteins in samples were quantified using the Kjeldahl method (NF EN ISO 8968-1, May 2014) by measuring the nitrogen content and multiplying by a conversion factor of 6.38.

Microbiological analyses

The stock solution and the decimal dilutions were prepared as indicated in JORA No. 74 (2017). 10 g of each sample was weighed into a container. The container was placed and maintained in a water bath set at 45° C until all of the test portions were melted. 90 ml of peptone-salt diluent was added, mixed and brought to 45 °C. Subsequently, the other decimal dilutions are prepared from this solution.

The media and the conditions for microbial counting were as follows (Marchal *et al.*, 1987). Plate Count Agar (PCA) incubated at 30°C for 48-72H for mesophilic aerobic flora (MAF); violet red bile lactose agar (VRBL) incubated at 37°C and 45°C for 24H for respectively total and thermotolerant coliforms. Baird Parker Agar with egg yolk tellurite supplement, incubated at 37°C for 48H for *Staphylococcus aureus*. Enumeration of sulfite-reducing clostridia on meat-liver glucose agar supplemented with iron alum and sodium sulphite, each sample was previously heated for 10 minutes at 80 °C and then cooled. For *Salmonella*, the first enrichment was carried out on Fluid Selenite Cystine Medium (SFB broth) and incubation at 37 °C for 24H followed by isolation on Hecktoen medium and incubation at 37 °C for 24H occurred. Man Rogosa and Sharpe (MRS) agar incubated at 37°C for 48H in a microaerophilic atmosphere for enumeration of lactic acid bacteria. Potato dextrose agar (PDA) was incubated at 22°C for 5 days for the enumeration of yeasts.

Statistical analysis

Statistical analysis was performed by using SPSS 17 for Windows. All the analysis was run in duplicate. The data were presented as Mean \pm Standard error. For the comparison between two variants, the single factor analysis of variance (ANOVA) was used by the Tukey test in order to estimate the significant differences at the 5% probability threshold.

Results and discussion

Physicochemical properties

The yield of butter or Smen depends on the fat content in milk used for the manufacture of this. The fat content of camel milk was an average of 4.1 ± 0.3 %. Butterfat recovery efficiency was 83.3 ± 6.3 and 71.2 ± 4.5 % observed respectively for fresh cream and Smen (Table 1). This result is compared to that reported by Berhe *et al.*, (2013) (80%) and Parmar *et al.*, (2018) (69.43%) and, at the same time, is higher than that of 60% reported by Farah (1996) for camel milk. Similarly, the butter yield was significantly ($p < 0.05$) higher for fresh cream (40 ± 4.0 g/l of milk) than that of Smen (29.7 ± 3.5 g/l of milk) (Table 1).

Both results were significantly lower than that of Berhe *et al.*, (2013) (43.0 ± 3 g/l of milk) for an extended churning time of 120 min.

Therefore, the low-fat yield in Smen was due to the effect of fermentation by the formation of coagulum's protein, which prevents the exit of fat at the time of churning (Omer and Eltinay, 2009). As well as the effect of churning time and its force interferes with

the fat yield, especially in the case of fermented camel milk (Farah, 1996). In addition, the poor skimming efficiency during the separation of cream from the camel milk leads to poor fat recovery (Parmar *et al.*, 2018). This poor efficiency is due to the distribution of fat in camel milk in small micelle-like globules and it is firmly bound to the protein. Moreover, the fat globule membrane of camel milk is thicker than that of the cow (Berhe *et al.*, 2013).

Table 1. Physicochemical properties of fresh cream and Smen made from camel milk.

Parameters	Fresh cream	Smen
Butter yield (g/l of milk)	40 ± 4.0^a	29.7 ± 3.5^b
Fat recovery (%)	83.3 ± 6.3^a	71.2 ± 4.5^b
pH	6.44 ± 0.28^a	4.35 ± 0.56^b
Acid Index (mg KOH/g)	2.08 ± 0.93^a	8.5 ± 1.32^b
Total Solids (%)	68.2 ± 4.57^a	60.8 ± 2.47^b
Ash (%)	0.38 ± 0.03^a	1.16 ± 0.34^b
Fat (%)	63.6 ± 2.35^a	50.5 ± 2.92^b
Protein (%)	1.88 ± 1.53^a	2.18 ± 0.78^a

^{a,b} Means within a row with different uppercase superscripts differ significantly ($P < 0.05$).

The fresh cream samples show a pH value of (6.44 ± 0.28) (Table 1) resembles that of raw milk (6.53 ± 0.14) given by Mosbah *et al.*, (2017) and resembles that of fresh bovine butter (6.88 ± 0.44) given by Ewe and Loo (2016). On the other hand, the Smen samples have an acid pH (4.35 ± 0.56) (Table 1), which is close to the pH of camel Smen given by Kacem and Karam (2006) (4.38 ± 0.33) and slightly lower than that of fermented camel butter (4.90 ± 0.15) given by Berhe *et al.*, (2013). In the same way, the acidity index presents a significant difference ($p < 0.05$) in Smen (8.5 ± 1.32 mg KOH/g) by contribution to fresh cream (2.08 ± 0.93 mg KOH/g) (Table 1). The acidification of Smen is a result of the exponential growth of lactic acid bacteria and the secretion of organic acids during milk fermentation (Mosbah *et al.*, 2021). On the other hand, the activity of lipolytic enzymes resulting from the growth of lactobacilli during fermentation and maturation of Smen leads to the accumulation of free fatty acids released by triglycerides (Ewe and Loo, 2016). Moisture is always present because it cannot be completely removed, especially since no heat treatment was used in this study for the preparation

of Smen. The total solids and fat content of fresh cream (68.2 ± 4.57 %, 63.6 ± 2.35 %) were significantly ($p < 0.05$) higher than that of the Smen (60.8 ± 2.47 %, 50.5 ± 2.92 %) (Table 1). However, the dry matter value of Smen is lower than those given by some authors, such as 65.00 ± 0.22 %, 64.1 ± 5.2 % and 0.75 % in camel Smen was given respectively by Kacem and Karam (2006), Berhe *et al.*, (2013) and Parmar *et al.*, (2018). This difference can be explained by the use of heat treatment during the preparation of Smen in these studies. On the other hand, the significant difference in the dry matter between fresh cream and Smen is probably due to the washing step during the preparation of Smen, which leads to the loss of part of the fat (Parmar *et al.*, 2018), and may also be, due to the remaining of water during the draining phase. Protein shows no significant difference between fresh cream and Smen (Table 1). On the other hand, Ash presents a significant difference between the two products (Table 1). This difference is explained by the addition of salt (NaCl) during the preparation of Smen as a means of maturation and preservation.

Table 2. Microbial counts (cfu/g) of fresh cream and Smen made from camel milk.

Microbial group	Fresh cream	Smen
Mesophilic aerobic flora (MAF)	$3.2 \cdot 10^4 \pm 1.4 \cdot 10^2$ ^a	$6.5 \cdot 10^7 \pm 1.4 \cdot 10^3$ ^b
Lactic acid bacteria	$6.8 \cdot 10^4 \pm 2.5 \cdot 10^2$ ^a	$3.5 \cdot 10^7 \pm 8.1 \cdot 10^3$ ^b
Yeasts	73 ± 15 ^a	$4 \cdot 10^3 \pm 7.2 \cdot 10^2$ ^b
Total Coliforms	$2.6 \cdot 10^2 \pm 80$ ^a	00 ^b
Thermotolerant coliforms	35 ± 32 ^a	00 ^b
<i>Staphylococcus aureus</i>	96 ± 58 ^a	00 ^b
Sulfite-reducing clostridia	52 ± 23 ^a	00 ^b
<i>Salmonella</i>	00 ^a	00 ^a

Microbiological analyses

The level of MAF was considered indicative of poor hygiene practices during production (Yamazaki *et al.*, 2013). For all fresh cream and Smen samples, the MAF was $3.2 \cdot 10^4 \pm 1.4 \cdot 10^2$ cfu/g and $6.5 \cdot 10^7 \pm 1.4 \cdot 10^3$ cfu/g, respectively (Table 2). Ewe and Loo (2016) gave higher values for cream either in the fresh state or after fermentation, 8.26 ± 0.42 and 8.67 ± 0.48 log₁₀ cfu/g, respectively. On the other hand, Kacem and Karam (2006) gave low values for Algerian camel Smen samples (3.88 ± 0.23 log₁₀ cfu/g). In this study, Smen shows a significant difference ($p < 0.05$) in MAF with fresh cream. This increase was explained by the increase in the concentration of lactic flora and yeasts after milk fermentation and Smen maturation.

The results given in table 2 indicate that fresh cream samples had a high lactic acid bacteria count of $6.8 \cdot 10^4 \pm 2.5 \cdot 10^2$ cfu/g. On the other hand, Smen samples present a significant difference ($p < 0.05$) by a very high lactic acid bacteria count $3.5 \cdot 10^7 \pm 8.1 \cdot 10^3$ cfu/g than that of fresh cream samples. Similar results were reported in others studies on butter and fermented butter (Smen) like Ewe and Loo, (2016) (8.67 ± 0.48 log₁₀ cfu/g) in bovine butter; Idoui *et al.*, (2010) ($1.36 \cdot 10^4$ cfu/g) in bovine Smen; Kacem and Karam, (2006) (3.96 ± 0.15 log₁₀ cfu/g) in camel Smen.

The yeasts were routinely isolated from milk products like cheese and butter. They play an active role in Smen ripening by their high lipolytic activity (Idoui *et al.*, 2010). The Smen samples had a high yeasts count ($4 \cdot 10^3 \pm 7.2 \cdot 10^2$ cfu/g) than that of fresh cream (73 ± 15 cfu/g) with a significant difference ($p < 0.05$) (Table

2). Similar results were reported by Idoui *et al.*, (2010) ($0.5 \cdot 10^4$ cfu /g) in cow Smen; Kacem and Karam (2006) (3.84 ± 0.18 log₁₀ cfu/ml) in camel Smen; Galeboe *et al.*, (2018) ($1.4 \cdot 10^4$ cfu /g) in camel milk yoghurt.

Total coliforms and thermotolerant coliforms, respectively, show average values of $2.6 \cdot 10^2 \pm 80$ and 35 ± 32 cfu/g for fresh cream (Table 2). These results resemble the values of contamination of raw camel milk by fecal flora given by several studies such as Elhosseney *et al.*, (2018) ($3.70 \cdot 10^4 \pm 1.20 \cdot 10^4$ cfu/ml); Mosbah *et al.*, (2017) ($1.6 \cdot 10^3 \pm 1.9 \cdot 10^3$ cfu/ml); Adugna *et al.*, (2013) (2.9 ± 2.27 log₁₀ cfu/ml).

Staphylococcus aureus and Sulfite-reducing clostridia give low values, 96 ± 58 and 52 ± 23 cfu/ml respectively, for fresh cream with the total absence of *Salmonella* in all samples (Table 2). On the other hand, the results given in Table 2 show the absence of fecal flora, *Staphylococcus aureus* and Sulfite-reducing clostridia in Smen samples. Similarly, Idoui *et al.*, (2010) noted the absence of these microorganisms in bovine Smen samples. While, Kacem and Karam (2006) gave low values for total coliforms (1.66 ± 0.11 log₁₀ cfu/g) in camel Smen.

During milk fermentation, as an important step in the preparation of Smen, there is an exponential growth of lactic acid bacteria, which hydrolyze lactose as a source of energy necessary for their multiplication (Granier *et al.*, 2013). At the same time, there is synthesis and accumulation of low molecular weight metabolites (hydrogen peroxide (H₂O₂), carbon

dioxide (CO₂), lactic acid, acetic acid, etc.) and high molecular weight compounds such as polypeptides and polysaccharides. This system offers protection against Gram-positive bacteria such as *Staphylococci* and *Clostridium*, especially in the maturation phase of Smen (Granier *et al.*, 2013; Atanasovaa *et al.*, 2014).

Lactic acid bacteria present in fermented dairy products are used as starters in food bio-preservation processes. Their beneficial contributions consist in improving the organoleptic quality of the products. This preservation is conferred by the production of several metabolites with antimicrobial activity (Widyastuti *et al.*, 2014).

Conclusion

This study revealed that the making of fresh cream from camel milk has some physicochemical and microbiological properties similar to those of raw camel milk. At the same time, the making of Smen is a good preservation process of milk fat. This is shown by the increase in acidity, which signifies the presence of different types of acids, such as fatty acids, as a result of lipolysis. However, it was observed the increase of lactic acid bacteria number and absence of contaminant and pathogenic bacteria after fermentation and maturation process of Smen. Consequently, the nutritional and hygienic quality of camel Smen allows it to be used as a remedy by nomadic peoples.

Therefore, further works should be done to isolate and identify the lactic acid bacteria and yeasts that contribute to the fermentation and maturation process of Smen, as well as the identification of fatty acid as bioactive compounds in this traditional product.

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