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MICROBIAL QUALITY, PHYSICOCHEMICAL CHARACTERISTICS AND FATTY ACID COMPOSITION OF A TRADITIONAL BUTTER MADE FROM GOAT MILK

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Abstract
This is the first report describing microbiological, physicochemical properties and fatty acid composition of a traditional butter produced from goat's milk in East of Algeria. Our results show the presence of lactic acid bacteria ($3.51 \times 10^5 \pm 2.44$ cfu/ g), psychotrophic bacteria ($1.11 \times 10^5 \pm 1.31$ cfu/ g), moulds and yeasts (39.08×10^2 cfu/ g), lipolytic bacteria ($4.41 \times 10^3 \pm 5.91$ cfu/ g) and the absence of total coliforms except in one sample. The presence of Staphylococcus and Salmonella was not detected in the analyzed butter samples. Variations in values of the physico-chemical parameters were recorded. Thus, the average values of moisture and impurity did not exceed 35.73% and 12.25% respectively. Values of iodine index and saponification index extended between 37.17 – 85.95 mg I/g and 84.15 - 254.87 mg KOH/ g respectively. Recorded values for peroxide index and acid index are on average equal to 1.41 ± 1.12 mg KOH/ g and 67.86 ± 19.13 meq O_2/ kg respectively. The determination of fatty acids composition by GC-MS showed the prevalence of the saturated fatty acids dominated by palmitic acid, with a low rate of unsaturated fatty acids, dominated by oleic acid.

Keywords: Goat butter, quality, GC-MS, fatty acid composition, East Algeria

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1. INTRODUCTION

The fact that milk constitutes the first and single food of the mankind during the first stage of its growth, explains the interest that the man carries all along his existence to milk and these derivatives. In the Mediterranean areas, it is traditionally the milk of the small ruminants, like the goat which is used today. The goat's milk, in the milk, butter or cheese shape, enjoys a "health" image, but it is not easy to know how this image is maintained (Desjeux, 1993).

Like cow butter, goat butter is used for a long time like edible fat and source of energy in the African Northern and Middle Eastern kitchen, especially in the rural areas. Goat butter has a melting point lower than the cow butter, which gives him a soft consistence. As the goat butter does not contain carotene, it has a white color. It plays a significant role in the development of the organoleptic characteristics of food (Peterson and Reineccius, 2003; Ito et al, 2005). To fulfill these functions, goat butter must have satisfactory quality. The quality of butter is closely related to its physico-chemical and microbiological characteristics. Goat butter quality depends on a large number of factors which are related to both the quality of milk used for its manufacture and the conditions of production. Goat Butter can contain all the germs met in milk. Lactic acid bacteria (*Lactococcus lactis* ssp *lactis, Lc. lactis* ssp *cremoris, Lc.lactis* ssp *diacetylactis,* sometimes *Leuconostoc*) take part in the development of organoleptic qualities of butter.

Several types of micro-organisms can be agents of degradation. First of all, the lactic acid bacteria can involve a too strong acidity. The acidity of butter can be former to its manufacture. Coliforms and enterobacteria can involve bad tastes in the cream. Lipolytic bacteria destroy and oxidize the fat content, involving the rancidity of butter. Proteolytic bacteria can degrade the casein of butter and involve a cheese taste. Other bacteria are responsible for colorings or abnormal discolorations and bad tastes in butter.

Germs intervening are generally psychrophilous. Finally the yeasts and moulds can cause deteriorations of taste (mildewed, bitter, malted, caramelized, etc.) and to involve

in butter the appearance of pigmentations and abnormal colorings and swellings (Guiraud, 1998).

Traditional Algerian goat butter, called according to the areas Dhan, Sman or Zebda, is manufactured by allowing goat milk to ferment for 24 to 48 hours at room temperature. Churning is carried out in an earthenware jar that a manipulator must shake vigorously with the two hands. This operation lasts 30 to 40 minutes. At the end of churning, butter grains float on the surface of a liquid product called *Lben*. water is generally added to a certain volume (approximately 10% of the volume of milk), heat or cold, according to the temperature of the whole on a suitable level for gathering butter grains, this one is recovered, generally with the hand, but some manufacturers filter *Lben* on a fabric, with an aim of collecting the maximum of butter (Tantaoui-Elarkki et al, 1983).

The physicochemical and microbiological quality of the Algerian traditional cow and camel butters are now recognized (Kacem and Karam, 2006; Idoui et al, 2010). However, no study has led on quality of traditional goat butter produced in Algeria. In this context, the present study is intended to describe the physicochemical and microbiological as well as the fatty acid composition of six traditional goat butters collected from different locations of Jijel (East of Algeria).

2. MATERIAL AND METHODS

2.1. SAMPLES

Traditional goat's butter was elaborated in the laboratory as described by Idoui et al, (2010). Milk samples were collected from the farmers of different locations of Jijel. They were kept in the laboratory at ambient temperature until obtaining the Raib (coagulated milk) who's churned and then traditional butter was collected.

2.2. PHYSICOCHEMICAL ANALYSIS

Impurities and moisture were determinate according to the methods described by Idoui et al, (2010). Acid index, peroxide index, iodine index and saponification index were determinate according to the methods described by Idoui et al, (2010). For the determination of peroxide index, 1 g of butter was dissolved in acitic acid/chloroform (3 v/ 2 v). 0.5 ml of saturated KI was added and the mixture was titrated with a solution of sodium thiosulfate in the presence of starch as indicator. For saponification index, 2 g of butter was dissolved in excess alcoholic KOH and heated for 30 min at 100 °C. The titration of the mixture was carried out with chlorhydric acid (0.5 N) using phenolphthalein as indicator. To determine iodine index, a weight of butter dissolved in hexan was added to 0.1 M iodine monochlorid in acetic acid. After 10 min, a solution of 0.1 M of KI was added and then, the liberated iodine in the mixture was titrated with a solution of 0.1 M sodium thiosulfate in the presence of starch as indicator. And for the acid index, 2 g of butter were dissolved in diethyl ether/ ethanol and the mixture was titrated with KOH in methanol (0.05 M) using the phenolphthalein as indicator.

2.3. MICROBIOLOGICAL ANALYSIS

For each sample, the liquid phase was separated as described by Idoui and Karam (2008) and Idoui et al, (2010). Samples were heated at 45 °C and then centrifuged at 3000 rpm for 15min.The intermediate liquid phase was separated and then decimal dilutions were carried out. We determined aerobic mesophilic bacteria on plate count agar at 30 °C for 72h, total coliforms on violet red bile agar at 30 °C for 24 h, staphylococci on Baird Parker agar base at 37 °C for 48 h, lactic acid bacteria at 32 °C for 48 h to 72 h in anaerobiosis on MRS agar, yeasts and moulds on oxytetracyclin glucose agar at 25 °C for 3-7 days, lipolytic bacteria on plate count agar with 5% of sterilized cream at 25 °C for 72 h to 5 days, psychrophic bacteria on plate count agar at 6 °C for 7 to 10 days and total caseolytic bacteria on plate count agar with 5% of sterilized skim milk at 37 °C for 48 h to 72 h.

2.4. FATTY ACID COMPOSITION

Fatty acid composition was determinate according to the methods described by Idoui et al, (2010): 20 g of each butter sample were dissolved in 0.5 ml of heptan and 0.2 ml of methanolic 2 N KOH was added. The mixture was boiled on a water bath for 2 min and 0.2 ml of 2 N HCl was added. After vigorous shaking and decantation, 100 µl of the superior layer were evaporated and the residue was reconstituted in 50 µl of heptan and then injected into gas chromatograph.

The fatty acid methyl esters were analyzed by GC/MS using a Shimadzu QP2010 GC/MS equipped with a capillary column SE30 (30 m x 0.25 mm i.d., 0.25 µm film thickness) with helium as the carrier gas at a flow rate of 0.76 ml/ min. Samples were injected into the split mode. The column was kept at 140 °C for 10 min and then programmed to increase by 1°C/ min up to 160 °C, then by 2°C/ min up to 220 °C then maintained for 15 min. The gas-chromatographic peaks were identified as corresponding fatty acid methyl esters by checking the elution order on the column and comparing the retention times with those of pure standards.

3. RESULTS AND DISCUSSION

3.1. PHYSICO-CHEMICAL CHARACTERISTICS

Table 01 shows the values of pH, moisture and impurities, acid index, peroxide index, saponification index and iodine index. The results revealed that pH values varied between 3.92 and 4.12 with average of 4.11 ± 0.15. Similar results were reported in earlier studies (Kacem and Karam, 2006). However, these values are lower than the values found by Idoui et al, (2010).

Moisture and impurities values of butter samples ranged from 16 to 35.73% and 9.25 to 12.25% respectively. The moisture level in all butter samples is higher than the international standard (0.05% to 2%). The high level of moisture in traditional butter may have an influence on its microbiological and physicochemical quality since the presence of water in butter can activate lipases, stimulate the growth of micro organisms and cause the hydrolysis of triglycerids (François, 2008). The presence of impurities in butter could be due to a transfer towards these butters of impurities initially presents in the milk used for the manufacture or in the water during washing.

Acid values ranged from 17.39 to 50.48 mg KOH/g of butter. These results are in agreement with those found by Idoui et al, (2010) in a previous study made on the traditional cow butter. The high acid values of analyzed butter samples could be attributable to lipolysis resulting from the lipolytic micro organisms or to lipase naturally present in milk used for the manufacture of butters. In addition, the high number of yeasts and moulds found in the butter samples could increase the enzymatic hydrolysis since some of these micro organisms would have the capacity to secret lipase responsible for enzymatic hydrolysis in lipids (Hultin, 1994).

Table1. Chemical and physical properties of traditional butter

Sample	1	2	3	4	5	6
pH	ND	ND	ND	4.31	3.92	4.12
Acid index (mg KOH/g)	25.24	17.39	29.38	25.94	58.9	50.48
Peroxide index (meq/Kg)	2	3	2,5	0.35	0.21	0.44
Saponification index (mgKOH/g)	254.87	226.19	232.82	154.97	84.15	169.7
Iodine index (mg I/100g)	48	67.8	37.17	82.93	85.95	85.35
Moisture (%)	ND	ND	ND	35.73	16	28.92
Impurities (%)	ND	ND	ND	10.75	12.25	9.25

ND: Not determined

Table 2.Counts of microbial populations in traditional butter samples

Sample	1	2	3	4	5	6
Total bacteria (10^6 cfu/g)	1.3	0.57	1.07	6.76	1.27	1.45
Moulds and yeasts (10^2 cfu/g)	7	3	1	5.5	106	112
Total coliforms (10^4 cfu/g)	0	0	0	3.5	0	0
Lactic acid bacteria (10^5 cfu/g)	1.37	2.35	0.71	3.76	7.96	4.96
Staphylococci (10 cfu/g)	0	0	0	0	0	0
Salmonella	Absence	Absence	Absence	Absence	Absence	Absence
Total caseolytic bacteria (10^3 cfu/g)	4	51.5	1	556	612	298
Total lipolytic bacteria (10^3 cfu/g)	0	0	0	1,5	15	10
Total psychotrophic bacteria (10^6 cfu/g)	25	7	33.2	0.6	0.6	0.3

Table3. Fatty acid profiles of traditional butter samples (peak area %)

Sample / Fatty acids	1	2	3	4	5	6
Capric acid	-	-	-	39.55	27	20.26
Caprilic acid	-	-	-	9.98	6.67	5.04
Cerotic acid	-	-	-	0.6	-	-
Lauric acid	-	-	-	16.39	10.17	7.74
Linoleic acid	4.42	2.51	-	1.73	0.64	1.64
Linolenic acid	-	-	-	-	-	1.77
Margaric acid	1.23	-	-	1.83	2.22	1.53
Myristic acid	-	19.93	-	-	15.43	18.37
Oleic acid	19.49	18.42	-	4.35	9.31	16.5
Oxalic acid	-	-	-	0.92	-	0.16
Palmitic acid	50.56	42.03	-	-	20.45	-
Palmitoleic acid	-	-	-	-	0.29	0.34
Pentadecyclic acid	1.46	1.6	-	-	-	-
Propionic acid	-	-	-	1.21	-	-
Stearic acid	20.7	13.44	-	21.61	6.08	19.29

Peroxide values varied from 0.21 to 3 meq/ Kg of butter. These results agreed with those found by Benkerroum and Tamime (2003). The high peroxide values observed in samples 1, 2 and 3 could be attributed to the chemical oxidation of the unsaturated fatty acids (Schreckenberg, 2004). On the other hands, saponification values and iodine values ranged from 84.15 to 254.87 mg KOH/ g of butter and from 37.17 to 85.95 mg I/ 100 g of butter respectively. Values of iodine index, which is a measure for the level of insaturation of oils, was higher than the values reported by Idoui et al, (2010) and by Rady and Badr (2003).

3.2. MICROBIOLOGICAL CHARACTERISTICS

Table 2 shows the count of total bacteria, total coliforms, staphylococci, lactic acid bacteria, yeasts, total lipolytic bacteria, total caseolytic bacteria and psychotrophic bacteria presents in traditional goat butter samples. High counts of total mesophilic bacteria were recorded in all butter samples with an average of $2.07x10^6$ ±

2.11 cfu/ g. These results are in agreement with those reported by Rady and Badr (2003), Karam and Kacem (2006) and Idoui et al, (2010). This can be due to the high microbial load initially present in milk, or to an additional contamination during manufacture.

Except sample 4, coliform bacteria were not found in the traditional goat butter samples. These results did not agree with those obtained by Rady and Badr (2003) and Kacem and Karam (2006). In fact, coliforms are indicators of cleanless of handling, premises and equipment.

Values of lactic acid bacteria were very variables and ranged from 0.71 $x10^5$ to 7.96 $x10^5$ cfu/ g. high counts of lactic acid bacteria (mean log count 3.8 to 3.96) was also found by Kacem and Karam (2006) in studying traditional camel milk butter and by Sakili and Issoual (2005) (2.10 x 10^6 – 6 $x10^6$ cfu/ g) when studying morocco traditional butter called Smen. The high counts of acid lactic bacteria found in all samples of butter are quantitatively coherent with the results of the enumeration of the total mesophilic bacteria. This relation which seems to exist between the degree of contamination by the total mesophilic bacteria and the total number of the lactic acid bacteria was evoked by Cauty and Perreaux (2003). These two authors provide that the fight against the total germs reduces the number of the lactic acid bacteria but also decreases the concentration by the undesirable germs. Among the six samples, lipolytic bacteria were detected in 3 samples of butter. Counts were between 1.5 $x10^3$ - 10 $x10^3$ cfu/ g. In the study carried out by Kacem and Karam (2006), the mean plate count of lipolytic bacteria was between 2.10 and 2.56, also the range was between 1.8 x 10^2 and 7.8 x 10^3 cfu/ g in the study of Rady and Badr (2003). The lipolytic bacteria are endowed with lipolytic activity generally responsible for the appearance in butter of rancid smell. Rancidity is related to the appearance of compounds of unpleasant odors (acids, aldehyds, ketons) resulting from the hydrolysis of the fat content by the means of microbial lipases, the capacity of conservation of butters depends directly on their concentration in lipolytic germs.

Caseolytic bacteria were detected in all samples and ranged from 1 $x10^3$ to 556 $x10^3$ cfu/ g. Some caseolytic germs like *Pseudomonas putrefaciens* are introduced by the washing water and are the cause of development in butter of unpleasant tastes and odors. Psychotrophic bacteria were also present and important count was detected (2.5 x 10^5 - 2.98 $x10^7$ fu/ g). The obtained results are similar when compared to other studies (Kacem and Karam, 2006; Idoui et al, 2010). Psychrotophic bacteria are the single most important group of organisms present in dairy products (Gökçe et al, 2010). In a study conducted by Schultz and Olson (1960) on 586 cultures of psychrotrophic bacteria isolated from milk or dairy products, they reported that 90% of these cultures are either proteolytics or lipolytics and that 66% have both activities. The most characterized are *Pseudomonas*, *Alcaligens*, *Acinetobacter* and *Flavobacterium.* These germs, especially *Pseudomonas*, are carried by the wash water.

Finally, our results reveal that the counts of yeasts and moulds exceeded 10^3 cfu / g in the butter samples. The presence of these microorganisms in butter was reported by some other authors (Kacem and Karam, 2006; Idoui et al, 2010; Rady and Badr, 2003; Gökçe et al, 2010). According to Moreau (1980), the majority of food products during their preparation but also during their storage are likely to be altered by moulds. This deterioration could lead to a modification of the nutritional value of the product and to the appearance of the undesirable flavors.

3.3. FATTY ACID COMPOSITION

The fatty acid profile of the 6 selected samples of traditional goat butters are given in the table 03. Saturated fatty acids (SFAs) were identified and quantified. Among these, palmitic acid was the dominant and ranged between 20.45-50.56% followed by capric acid (20.26 - 39.55%), stearic acid (6.08 - 21.61%) and myristic acid (15.43 - 19.93%), lauric acid

(7.74 -16.39%), caprylic acid (5.04 - 9.98%). These fatty acids have a relatively high melting point (superior with 44 °C) and when they are presents in great quantities in butter, they give him a firmer consistence (Chouinard and Turgeon, 1998). These fatty acids are also suspected to have detrimental effects on human health since they make increase the rate of blood cholesterol (Ney, 1991).

Among the unsaturated fatty acids, the identified fatty acids were oleic acid followed by linoleic acid and palmitoleic acid. The amount of these fatty acids were ranged between 4.35-19.49%; 0.64 - 4.42% and 0.29 – 0.34% respectively.

Poly unsaturated fatty acids depend essentially on the food while the mono unsaturated fatty acids like the oleic acid result in part from their mammary synthesis and in part from the activity of the mammary delta-9 desaturase which converts the saturated acid on monounsaturated acid (Schmidely and Sauvant 2001; Paccard et al, 2006).

Some fatty acids were presents in minor concentrations: cerotic acid (0.6%), margaric acid (1.23 - 2.22%), oxalic acid (0.92 – 0.16%), erucic acid (0.22%) and vaccenic acid (0.9 - 2.69%).

Our results reveal the absence of branched chain fatty acids, characteristics of milk of ruminants, in goat butter. This result is not in agreement with that found by Alonso et al. (1999) who affirm to have detected this family of fatty acid in the goat's milk. Odd chain fatty acids were also detected (margaric acid). These fatty acids are *de novo* synthesized in the mammary gland through the condensation of the propionic acid and malonyl CoA (Massart-Le¨en et al, 1983).

4. CONCLUSIONS

In this study, we evaluated the microbiological and physico-chemical quality as well as the composition in fatty acids of 6 traditional goat butter samples. The results obtained from the microbiological analysis show a significant microbial load without detection of pathogens. Analysis of the fatty acids composition by GC-MS shows the prevalence of saturated fatty acids in goat butter. This study makes it possible to locate the Algerian butter of goat compared to other traditional butters (cow and camel). However, additional studies are needed for the evaluation of the nutritional quality and microbial diversity of this butter.

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