

## Microbial quality, physicochemical characteristics and fatty acid composition of a traditional butter produced from cows' milk in East Algeria

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### RESUMEN

#### Calidad microbiológica, características fisicoquímicas y composición en ácidos grasos de una mantequilla tradicional producida con leche de vaca del Este de Argelia.

Esta es la primera vez que se describen las propiedades microbiológicas y fisicoquímicas y la composición en ácidos grasos de una mantequilla tradicional producida con leche de vaca del Este de Argelia. Cinco muestras de mantequilla fueron preparadas en el laboratorio siguiendo el método tradicional usado por la población del área de Jiels (Este de Argelia). Nuestros resultados muestran la presencia de ácido láctico, bacterias psicrotróficas y levaduras, mientras que stafilococos y bacterias lipolíticas no fueron detectadas. Importantes diferencias fueron encontradas en los valores químicos de las diferentes muestras de mantequilla. Los valores de pH variaron entre 4.64 y 5.53. La humedad e impurezas excedió el 17.5% y 9.19%, respectivamente. Los valores de índice de acidez, índice de peróxidos, índice de saponificación e índice de yodo variaron entre: 23.56-31.35 mg KOH/g, 1.6-4 meq/kg, 140.25-228.60 mg KOH/g and 35.35-53.69 mg/100g, respectivamente. Finalmente la composición de ácidos grasos mostro que el ácido palmítico y el ácido oleico fueron los principales ácidos grasos saturados e insaturados, respectivamente.

**PALABRAS CLAVE:** Ácido graso – Fisicoquímica – Mantequilla tradicional – Microflora.

### SUMMARY

#### Microbial quality, physicochemical characteristics and fatty acid composition of a traditional butter produced from cows' milk in East Algeria.

This is the first report describing microbiological, physicochemical properties and fatty acid composition of a traditional butter produced from cows' milk in East of Algeria. Five butter samples were prepared in the laboratory according to the traditional method used by people in the Jijel areas (Eastern Algeria). Our results show the presence of lactic acid and psychrotrophic bacteria as well as yeasts, while staphylococci or lipolytic bacteria were not detected. Important differences were found in chemical values among butter samples. The pH values ranged from pH4.64 and pH5.53. Moisture and impurities exceeded 17.5% and 9.19% respectively. The values for acid index, peroxide index, saponification index and iodine index ranged from: 23.56-31.35mg KOH/g, 1.6-4 meq/kg, 140.25-228.60 mg KOH/g and 35.35-53.69 mg/100g respectively. Finally, the fatty acid composition showed that palmitic acid and oleic acid were the major saturated and unsaturated fatty acids.

**KEY-WORDS:** Fatty acid – Microflora – Physicochemical – Traditional Butter.

### 1. INTRODUCTION

A wide variety of products are manufactured by processing raw milk into dairy products. Among them, butter is one of the primarily fat sources and an important source of dietary energy. It has been produced since ancient times and was an internationally traded commodity as early as the 14<sup>th</sup> century (Vernam and Sutherland, 1994; Rady and Badr, 2003).

Butter is widely used as a seasoning in cooking, suggesting that it plays an important role in the formation of flavors during heating (Peterson and Reineccius, 2003; Ito *et al.*, 2005). Besides fats, butter contains small percentages of proteins, milk sugar and water which make it a suitable substrate for microorganisms (Catsberg and Kempen-van Dommelen, 1990). Although butter spoilage is most often due to the development of chemical rancidity, microbiological problems do also occur in the form of cheesy, putrid or fruity odors and the rancid flavor produced by hydrolysis (Rady and Badr, 2003). Many psychrotrophic strains of bacteria, yeasts and moulds have been implicated in spoilage and lipolysis of butter at temperatures below 5°C and some even below 0°C. Moreover, some pathogenic bacteria such as *Staphylococcus aureus* and *Listeria monocytogenes* remained recoverable in butter stored in low temperatures (Collins *et al.*, 1989).

Traditional Algerian dairy products, especially the fermented types, have been the pride of culinary tradition for centuries. It is evident that these products have played a major role in the diet of communities in the rural region. Among those, lben, raib, dhan or zebda or sman and jben are the most common and are being increasingly marketed throughout the country in informal ways.

The traditional stages of the manufacturing of Dhan are the following: Raw milk is left to sour spontaneously at ambient temperature until coagulation occurs which may take up to 24–72 h depending on the temperature during the summer and winter seasons. On gelation, the product is called rayeb, and may be consumed as such; however, by churning the fermentate, the product is separated into lben and raw butter called Dhan

or zebda literally meaning 'butter of the county' (in other Middle Eastern countries the same product is known as zibdeh baladie, samna and in some instances, the mutton fat dripping is also samen or samneh). In the rest of the world the butter-related product is known as butter oil, ghee or anhydrous milk fat (Benkerrouma and Tamime, 2003).

To our knowledge, no information exists on the quality of traditional butter produced in eastern Algeria and especially in the Jijel area. The aim of this study was to describe microbiological, physicochemical properties and fatty acid composition of a traditional butter called "Dhan" produced from cows' milk in the Jijel area (East of Algeria).

## 2. MATERIALS AND METHODS

### 2.1. Samples

Traditional cows' butter was manufactured in our laboratory as described in the introduction. Five fresh cows' milk samples were collected from different locations of Jijel (Eastern Algeria). They were left in the laboratory at ambient temperature for two days. After spontaneous coagulation, Raib was churned, and traditional butter called Dhan was recovered.

### 2.2. Microbiological analysis

For each sample, the liquid phase was separated as described by Idoui and Karam (2008) and Idoui *et al.* (2009). 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.

The media and the conditions for microbial counting were as follows (Leveau and Bourgeois, 1980; Collins *et al.*, 1989; Roberts *et al.*, 1995): Plate count agar incubated at 30°C for 48-72h for total bacteria; violet red bile agar, incubated at 37°C for 24h for total coliforms; Baird-Parker agar base, incubated at 37°C for 48h for staphylococci; MRS agar, incubated at 32°C for 48h to 72h in anaerobiosis for lactic acid bacteria; oxytetracycline glucose agar, incubated at 25°C for 3-7 days for yeasts, plate count agar with 5% of sterilized cream incubated at 25°C for 72h to 5days for total lipolytic bacteria; plate count agar incubated at 6°C for 7 to 10days for psychrotrophic bacteria; plate count agar with 5% of sterilized skim milk incubated at 37°C for 48h to 72days for total caseolytic bacteria.

### 2.3. Physicochemical analysis

Impurities and moisture were determined according to the method described by Lecoq (1965).

Acid index, peroxide index and saponification index were performed according to the methods described by Lecoq (1965).

For the butter acidity, a known weight was dissolved in a mixture of diethyl ether / ethanol. The

mixture was titrated with potassium hydroxide in methanol (0.05M) in the presence of phenolphthalein as indicator. For peroxide index, a known weight was dissolved in a mixture of acetic acid/ chloroform (3:2 v/v), and a saturated solution of KI was then added. The liberation iodine was titrated with a sodium thiosulphate solution in the presence of starch as indicator. For saponification index, a known weight of butter was dissolved in alcoholic potassium hydroxide then evaporated for 30mn. The sample was titrated with chlorhydric acid (0.5N) in the presence of phenolphthalein as indicator.

### 2.4. Analysis of fatty acids

The butter samples were esterified in a methanol solution of 2N KOH for 30minutes at 50°C. The gas chromatographic analysis of fatty acid methyl esters was performed on a Perkin Elmer gas chromatograph, equipped with a flame ionization detector (Shimadzu QP2010): The column was a fused silica capillary SE30 length 25meters, diameter 0.25 µm. Helium was the carrier gas. The column temperature program was: initially isotherm at 140°C for 10min, an initial programmed rate of 1°C/min up to 160°C, then a second rate of 2°C/min up to 220°C and a final isotherm for 15min. Samples were injected into the split mode. The apparatus itself carried out recording and integration.

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. Microbiological characteristics

Table 1 shows the counts of total bacteria, total coliforms, staphylococci, lactic acid bacteria, yeasts, total lipolytic bacteria, total caseolytic bacteria and psychrotrophic bacteria recorded in traditional butter samples.

Butter samples have a relatively high total bacterial count reaching  $60 \times 10^5$  cfu/g. These results are in agreement with those reported by Rady and Badr (2003). Kacem and Karam (2006) reported the high count of aerobic bacteria in traditional butter made from camel milk (mean log counts 2.76 to 3.88). The high count of total bacteria may be attributed to the absence of pasteurization and salt. Adam and Moss (1995) attributed the increase of total bacteria to the effect of both separation and churning processes on the breaking up of bacterial clumps which increases their number.

Lipolytic bacteria were not detected in any of the analyzed samples. This result is not in agreement with those reported by Rady and Badr (2003). These authors reported the presence of lipolytic bacteria in all butter samples. The same results were obtained with the count of staphylococci. Also, total coliforms

Table 1  
Counts of microbial populations in traditional butter samples

Sample	01	02	03	04	05
Total bacteria ( $10^4$ cfu/ g)	15	600	20	32	20
Total coliforms ( $10^4$ cfu/ g)	ND	0.2	00	00	00
Staphylococci (10 cfu/ g)	00	00	00	00	00
Lactic acid bacteria ( $10^4$ cfu/ g)	1.04	1.36	1.05	5.55	0.6
Yeasts ( $10^4$ cfu/ g)	0.275	ND	0.01	0.505	0.03
Total lipolytic bacteria ( $10^3$ cfu/g)	00	ND	00	00	00
Total caseolytic bacteria( $10^4$ cfu/g)	0.4	00	0.15	1.45	0.4
Total Psychrotrophic bacteria( $10^4$ cfu/g)	0.38	0.128	1.27	3.6	4.6

ND: Not determined

were present in one out of five samples ( $02 \times 10^3$  cfu/ g). It is not in total agreement with the results reported by some authors (Hassan, 1984; Rady and Badr, 2003; Kacem and Karam, 2006).

The Data given in table 1 indicate that butter samples had a high lactic acid bacteria count ranging from  $0.6 \times 10^4$  cfu /g (sample 05) to  $5.55 \times 10^4$  cfu /g (sample 04). Similar results were reported in earlier studies on butters (Kacem and Karam, 2006; Idoui and Karam, 2008; Idoui *et al.*, 2009). Also, results reveal that samples had a high yeast count, it exceeded  $0.1 \times 10^3$  cfu /g. These results are not surprising because yeasts were routinely isolated from these products. In a previous study, yeasts and moulds have been detected with high counts in butter (Rady and Badr, 2003; Kacem and Karam, 2006). In a study conducted by El Marrakchi *et al.* (1988), a microbial analysis of commercial smen showed that yeast counts, when present, exceeded  $10^6$  cfu/ g. Their high counts and well known high lipolytic activity suggest that yeasts may play an active role in smen ripening.

From these results, it appears that caseolytic bacteria represent an important part of the bacterial microflora of traditional butter, as counts in different samples range from  $1.5 \times 10^3$  cfu /g to  $14.5 \times 10^3$  cfu /g. The presence of this microflora has not been reported in any previous study. Finally, our results reveal that the control butter samples had a count ranging from  $1.28 \times 10^3$  cfu to  $46 \times 10^3$  cfu /g for psychrotrophs bacteria. These results agreed with those reported by Kacem and Karam (2006).

### 3.2. Physicochemical characteristics

The physicochemical characteristics of traditional butter samples are shown in table 2. As shown, important differences were found in chemical values among butter samples. pH values of samples ranged from pH4.64 and pH5.53. Similar results are found by Sagdic *et al.* (2002). In a study conducted by Kacem and Karam (2006), the pH values of smen made from camel milk varied in all samples between pH3.11 and pH4.97. In the same table, moisture and impurity values of butter samples ranged from 17.5% and 26.5%, and 9.19% and 23.5% respectively. These

values are higher than the limits established for industrial butter but they were lower than the values found by Sagdic *et al.* (2002) and Kacem and Karam (2006). In a similar study conducted by Hamama (1997) on the traditional raw butter produced in Morocco, the moisture value was 23%.

As shown in table 2, acid index, peroxide index, saponification index and iodine index of butter samples varied according to the samples of butter and locations. Acid index was in the range of 23.56 mg KOH/g in sample 04 and 31.35 mg KOH/g in the butter of sample 02. These results are not in total agreement with those reported by Rady and Badr (2003). Benkerroum and Tamime (2004) reported that the acid index values in Moroccan raw butter and smen were respectively 2.4 and 52 mg KOH/g. On the other hand, the peroxide index values ranged between 1.6 meq  $O_2$ /kg and 4 meq  $O_2$ /kg of butter. In Moroccan raw butter and smen, Benkerroum and Tamime (2004) reported that peroxide index values were 0.5 and 3.7 meq  $O_2$ /kg of fat respectively. The same authors reported that some proteolysis of the residual milk proteins in the aqueous phase may occur, lipolysis is the primary mechanism which determines the typical flavor of the product and such activity could originate from the microbial cells and/or free lipases. Chemical oxidation also contributes to a lesser degree to the flavor of smen; this is evident as the peroxide value increases to 3.7 meq/kg of fat compared to 0.5 meq/ kg in fresh raw butter. The results obtained also indicated that the saponification index exceed 140.25 mg KOH/g of butter and in all samples the iodine index (35.35 mg I/100g -53.69 mg I/100g of butter) was higher than reported by Rady and Badr (2003).

### 3.3. Fatty acid composition

The fatty acid composition of traditional butter is not documented. Nineteen (19) fatty acids are identified (Table 3). As shown, important differences were found in fatty acid compositions among butter samples. Traditional cows' butter contained a high percentage of saturated fatty acids (SFA) and palmitic acid was the major SFA (24.33 % - 36.95%) followed by myristic acid (18.49%- 27.35 %) and

Table 2  
Chemical and physical properties of traditional butter

Sample	01	02	03	04	05
pH	4.64	5.53	ND	ND	ND
Acid Index (mg KOH/g)	24.75	31.35	27.12	23.56	26.36
Peroxide Index (meq / kg)	1.6	2.4	4.0	4.0	3.0
Saponification Index (mg KOH/g)	140.25	168.3	196.35	224.74	228.60
Iodine Index (mg I/100g)	35.35	48.04	53.69	48.33	36.55
Moisture (%)	26	17.5	26.2	18	20
Impurities (%)	16	23.5	11.14	ND	9.19

ND: Not determined

Table 3  
Fatty acid profiles of traditional butter samples (peak area %)

Sample Fatty acids	01	02	03	04	05
Valeric Acid	7.39	-	-	-	-
Lauric Acid	9.90	6.73	-	-	-
Myristic acid	-	18.49	27.35	23.92	24.24
12-Tetradecanoic acid	-	-	-	2.13	-
Pentadecanoic acid	8.87	-	2.55	3.03	2.12
Palmitic Acid	24.33	34.90	31.01	36.95	30.28
14-Hexadecanoic acid	-	-	-	-	0.63
Heptadecanoic acid	-	-	0.48	0.51	0.61
Heneicosanoic acid	-	-	-	1.09	-
Triacontanoic acid	-	-	-	0.98	-
Stearic Acid	13.45	8.19	7.68	11.08	14.05
Unknown	-	-	19.32	-	4.75
9- Hexadecenoic acid	-	-	1.45	-	1.32
2-propenoic acid	11.01	-	-	-	-
8- Octadecenoic acid	-	20.54	-	-	-
Oleic acid	13.99	-	7.41	15.08	14.75
10-Octadecenoic acid	-	-	2.76	3.43	-
Vaccenic acid	4.03	-	-	-	-
6,9-Octadecadienoic acid	-	-	-	1.60	-
Linoleic Acid	7.03	11.15	-	-	-

stearic acid (7.68%-14.05%). These results are not in total agreement with those reported by Rady and Badr (2003). These authors reported that palmitic acid was the major SFA (22.81%) followed by stearic acid (10.21 %). The predominance of palmitic acid was also previously reported with similar trends for other major fatty acids in milk fat (Gunstone, 1996; Dorey *et al.*, 1988) and butter fat (Dorey *et al.*, 1988). In a study conducted by Ito *et al.* (2005) heated butter contained 27.60% palmitic acid. On the other hand, linoleic acid was solely present in samples 1 and 2 while oleic acid was the predominant mono unsaturated fatty acid (7.41%- 15.08%). This result is in agreement with those reported by Rady and Badr (2003) and Ito *et al.* (2005). As shown in table 3, some fatty acids were detected such as valeric

acid, 12-tetradecanoic acid, 14-hexadecanoic acid, vaccenic acid and 6, 9-octadecadienoic acid. These results are not reported in studies conducted on the fatty acid profiles of butter.

#### 4. CONCLUSION

It is generally considered that environmental conditions such as temperature, origin and composition of milk, processing and sanitary conditions, etc., might have a significant influence on the microbial and physicochemical composition of traditionally made dairy products. From these results we can conclude that important differences were found in microbial quality, physicochemical characteristics and fatty acid

composition of a traditional butter produced from cows' milk. Thus, the present study is expected to encourage more research such as microbial diversity and physicochemical composition in traditional Algerian butter.

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