Dirasat, Pure Sciences, Volume **34**, No. **2**, 2007

Physicochemical and Microbiological Aspects of "*Smen*", a Traditional Butter Made in Algeria

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ABSTRACT

Aerobic bacteria, coliforms, lactic acid bacteria, psychrotrophs, lipolytic bacteria and yeasts were isolated from 20 samples of smen, a traditional butter made from soured cow milk in Algeria. A total of 141 isolates of lactic acid bacteria were identified as Lactobacillus delbrueckii ssp. bulgaricus (24.8%), Enterococcus faecium (23.4%), Lactobacillus plantarum (14.2%), Lactococcus lactis ssp. lactis biovar diacetylactis (14.2%), Lactococcus lactis ssp. cremoris (9.9%), Lactobacillus paracasei ssp. paracasei (7.2%) and Leuconostoc pseudomesenteroides (6.3%). Forty yeasts were isolated from all samples and were identified as Saccharomyces sp. Physicochemical study of samples showed that the values of pH, titratable acidity, NaCl, total solid, moisture and fat contents ranged between 3.60-4.39, 0.20-0.32%, 1.02-2.86%, 62.03-65.15%, 33.40-34.92% and 48.90-56.05%, respectively.

Keywords: Cow Milk, Traditional Butter, Lactic Acid Bacteria, Yeasts.

1. INTRODUCTION

Indigenous dairy products are traditionally produced and consumed in a majority of African and Arabian countries (Abd-El-Malek, 1987; Ashenafi, 1996; Gonfa et al., 1999; El Gendy, 2001; Abou-Donia, 2002 and Ayad et al., 2004). Among these products, a traditional butter (smen) produced in a Algeria. This product is eaten as butter, used as oil for food preparation or for cooking. It is used also for hairdressing and as a skin cosmetic by both sexes. Furthermore, smen is used for roasting coffe beans in special traditional ceremonies.

In many regions of eastern Algeria (Jijel, Setif, M'sila and Batna), cow milk is treated in a manner similar to that mentioned by El Marrakchi et al. (1988). Raw milk is allowed to ferment spontaneously overnight. Then, sour milk is poured in a churn (closed sheep skin or a cylindrical wooden churn depending on the region) which is properly plugged. If the sour milk is coagulated, and too firm, it is rythmically shaken for several hours until butter grains appear. Buttermilk is drained off and butter grains are washed, with fresh water, to remove most of the buttermilk. Subsequently, butter is worked by hand to give it an homogeneous and even texture without big air or water inclusions. Butter can be packed into bottles or into jars. The product can be preserved for up to one month depending on the moisture, humidity and room temperature of the storage place.

The mirobiological properties of butter made from different milks have been extensively studied by many researchers (Dwiedi and Kushwaha, 1972; Hankin and Hana, 1983; O'Mahony and Bekele, 1985; Ubach, 1986; Tasnim et al., 1993; O'Connor et al., 1993 and Zhao et al., 2000). Some researchers reported that lactococci, lactobacilli, enterococci, streptococci and leuconostocs have been isolated from natural butters (Hukari and Rautavaara, 1972; Maret and Sozzi, 1976; Ozalp et al., 1980 and Karahan, 1992). Yeasts (especially S. cerevisiae) are commonly present in different traditional butter and are considered as a secondary microflora (Benkerroum and Tamime, 2004).

No information exists on indigenous microflora of smen that may be affected by the chemical composition of milk differences in traditional processing methods, packaging material and storage condition. Therefore, the knowledge of bacterial microflora involved in butter

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fermentation is of prime importance in predicting and determining final smen quality.

In this study, we aimed to investigate the biochemical and microbiological characteristics of smen and to isolate and identify lactic acid bacteria and yeasts from this traditional product.

2. MATERIALS AND METHODS

2.1. Samples Collection

Twenty samples of smen produced at the household were obtained from Jijel (four samples), Setif (five samples), Constantine (five samples) and Bejia (six samples) regions, east of Algeria. The samples were aseptically taken, placed into sterilized bottles and transported to the laboratory in ice-containing thermoflasks and analysed on arrival.

2.2. Physicochemical Analyses

The pH of butter was determined with a pH-meter (Micro pH 2002, Crison, Barcelona, Spain). Titratable acidity (as lactic acid) was measured as suggested by James (1995). Moisture and solid content were determined by heating the sample in an oven at 201°C until a constant weight was obtained (Anonymous, 1960). NaCl concentration was obtained using the method of the International Dairy Federation (Anonymous, 1969). Content of fat were determined by the IDF method of (Anonymous, 1977). All analyses were performed in duplicates.

2.3. Microbiological Analyses

Ten grams of butter sample were homogenized with 90 ml of 0.85% (w/v) sterile saline solution at 45°C for microbial analysis. Appropriated dilutions were spread-plated on the culture media indicated below.

Total lactic acid bacteria were enumerated in MRS agar (pH 6.5) (Merck, Darmstadt, Germany) (de Man et al., 1960) after 72 h at 30°C. Lactobacilli were counted in MRS agar adjusted to pH 5.4 with sodium acetate, so that the growth of other organisms could be supressed (Garcia et al., 1987). Plates were incubated under anaerobic conditions (Gas Pak System, Becton Dickinson) at 30°C for 48 to 72 h until growth was observed. Lactococci were counted on M17 agar (Merck, Darmstadt, Germany) (Terzaghi and Sandine, 1975) after incubation for 48 h at 30°C. Total aerobic counts were made on plate count agar (Oxoid Ltd., UK) after incubation at 30°C for 72 h, and coliform counts on violet red bile agar (Oxoid) after incubation at 30°C for 24 h. Leuconostocs were enumerated on Sodium Azide Leuconostoc Agar (SALA) (Merck, Darmstadt, Germany) (Harrigan and McCance, 1966) after incubation at 21°C for 72 h. Enterococci were enumerated on Citrate Azide Agar (CAA) (Merck, Darmstadt, Germany) after incubation at 37°C for 72 h. Yeasts on Potato Dextrose Agar (PDA) (Oxoid) after incubation at 22°C for 5 days. Psychrotrophs on plate count agar incubated at 7°C for 10 days, and lipolytic bacteria on tributyrin agar (Oxoid) after incubation at 30°C for 72 h.

Significance of differences between means was assessed by the Student-Newman-Keul's multiple range tests (Steel and Torrie, 1980) after log transformation for bacterial counts.

2.4. Isolation and Identification of Lactic Acid Bacteria and Yeasts

Lactic Acid Bacteria

After bacterial counts, 10 colonies belonging to different types were randomly picked out from each 30-50 colony count plate of MRS, M17, Sodium Azide Leuconostoc agar or Citrate Azide agar and purified through three cycles of single colony cultures.

Cell shape, cells arrangements, Gram-staining, catalase activity (3% H_2O_2), production of gas from glucose, temperature requirement (15, 40 and 45°C), NaCl tolerance (4, 6.5, 8 and 10% NaCl) and growth at pH 3.9 and 9.6 were performed in M17 or MRS broth, respectively. L-and D-lactic acid were analysed enzymatically by the L-Lactic acid/D-Lactic acid kit (Roche diagnostic, Mannheim, Germany) according to the manufacturer's instructions. Lactobacilli isolates were characterized according to the criteria of Kandler and Weiss (1986). Arginine hydrolysis was tested in MRS broth containing 3 g/l arginine and 2 g/l sodium citrate replacing ammonium citrate. Ammonia was detected using Nessler's reagent (Kacem et al., 2006). Acetoïn production was determined in MRS broth using the Voges-Proskauer test (Schillinger and Lüke, 1989).

Lactobacilli isolates were tested for production of acids from carbohydrates and related compounds by using the API 50 CH strips and API 50 CHL medium (API-System, S.A., La Balme Les Grottes, Montalieu-Vercieu, France). The tests were done according to the manufacturer's instructions and the results were read after incubation at 37°C for 48 h and 72 h. Identification of the

isolates was done by the computerized database program provided by the manufacturer.

Homofermentative cocci which were capable of growing at 15 and 40°C but not at 45°C or at pH 9.6 were considered as lactococci according to the methods and criteria of Mundt (1986). Homofermentative cocci, grouped in pairs or short chains, which grew at 15, 40 and 45°C, survived after heating at 60°C after 30 min, and grew at a pH 9.6 were considered as enterococci (Devriese et al., 1987). Heterofermentative cocci (leuconostoc) were identified according to Garvie (1986).

API 20 STREP (API-System, S.A., La Balme Les Grottes, Montalieu-Vercieu, France) was used for lactococci, enterococci and leuconostocs identification according to the manufacturer's instructions. Readings were done at 30°C after 48 h.

Yeasts

Yeasts were enumerated and the isolates distinguished separately according to their different morphological appearances upon growth on potato dextrose agar. Pure cultures of the yeast isolates were identified on the basis of the morphological and physiological criteria described by Kreger-van Rij (1984) as well as the criteria described by Barnett et al. (1990).

Microorganisms were maintained in sterile reconstituted skim milk (10% w/v) at 4°C or at -20°C in MRS broth supplemented with 20% glycerol. Working cultures were also kept on MRS agar, M17 agar or potato dextrose agar slants at 4°C and streaked every four weeks.

3. RESULTS AND DISCUSSION

Table (1) shows the values of pH, Titratable Acidity (TA), NaCl, Total Solids (TS), moisture, and fat obtained from smen. The values of pH varied in all samples (3.60-4.39): samples collected from Setif region had the lowest pH (3.6) values, while smen from M'sila region had the highest (5.1). Similar results were reported in earlier studies on butters (Filkensen, 1987 and Sagdic et al., 2002). In all samples the values of Titratable Acidity (TA) (0.20-0.32%) were higher than values found by Bilgin (1996) and Hayaloglu (1999): samples collected from Jijel region had the highest mean TA value (0.32%) and while the lowest mean TA was from samples collected from Batna (0.20%).

On the other hand, the mean NaCl content (1.02 and 1.86%) in all samples of butter was lower than the values found by El Sadek et al. (1975) and Hayaloglu (1999): mean salt content in smen from Jijel region was the highest (1.86%), the samples from Setif region had the lowest value (1.02%). The total solid, moisture and fat contents of smen samples ranged between 62.03 and 65.15, 33.40 and 34.92, and 48.90 and 56.18 in all regions, respectively. These results agreed with those obtained by Sagdic et al. (2002). Statistically, significant differences ($P<0.01$) were observed in the pH and titratable acidity among the smen samples. However, no significant differences ($P>0.05$) was observed in other aspects (NaCl, total solids, moisture and fat) of smen.

Table (1)
Physicochemical characteristics of butter from cow milk (*smen*)[a] collected in four regions of Algeria.

Region	pH	Titratable acidity (%)	NaCl (%)	Total solid (%)	Moisture (%)	Fat (%)
Jijel	4.4± 0.23	0.3 ± 0.09	1.8 ± 0.03	62.0 ± 0.29	34.4 ± 0.55	56.0 ± 0.09
Setif	3.6 ± 0.26	0.2 ± 0.07	1.0 ± 0.05	65.0 ± 0.22	34.8 ± 0.22	58.1 ± 0.03
Msila	5.1 ± 0 32	0.2 ± 0.08	1.3 ± 0.06	65.1 ± 0.30	34.9 ± 0.33	56.2 ± 0.07
Batna	4.8 ± 0.33	0.2 ± 0.07	1.1 ± 0.07	64.5 ± 0.09	33.4 ± 0.66	48.9 ± 0.11

[a] *Mean ± standard deviation.*

Table (2) shows the counts of aerobic bacteria, coliforms, lactic acid bacteria, lactobacilli, lactococci, enterococci, yeasts, psychrotrophs and lipolytic bacteria recorded in smen samples from the four regions studied.

Aerobic bacteria (mean log counts 2.71 to 3.88), lactic acid bacteria (mean log counts 3.8 to 3.96) and yeasts (mean log counts 2.08 to 3.44) were recorded in all samples. However, low incidences of coliforms (means log counts 0.90 to 1.65) as well as psychrotrophs (mean log counts 1.10 to 1.53) were detected. Lipolytic bacteria were detected in all sample (means log counts 2.10 to 2.56). Statistically, significant differences ($P<0.01$) were observed between microbial count and pH among the smen samples. Difference in microbial count and

titratable acidity was also observed (P<0.01).

Table (2)
Mean log plate counts of different microbial groups of butter from cow milk (*smen*)[a] collected in four regions of Algeria.

Microbial group	Jijel	Setif	Msila	Batna
Total aerobic count	3.52 ± 0.12	2.1 ± 0.19	3.4 ± 0.23	3.5 ± 0.19
Coliforms	1.1 ± 0.18	1.0 ± 0.20	1.6 ± 0.11	0.8 ± 0.15
Lactic acid bacteria	3.8 ± 0.13	3.5 ± 0.18	3.9 ± 0.15	3.1 ± 0.12
Lactobacilli	2.3 ± 0.16	2.4 ± 0.12	2.6 ± 0.13	3.8 ± 0.14
Lactococci	1.9 ± 0.15	1.5 ± 0.21	1.9 ± 0.14	1.5 ± 0.11
Enterococci	2.6 ± 0.11	2.3 ± 0.16	1.5 ± 0.17	1.4 ± 0.15
Yeasts	3.8 ± 0.19	3.8 ± 0.18	2.9 ± 0.18	2.0 ± 0.18
Psychrotrophs	1.1 ± 0.16	1.1 ± 0.15	1.5 ± 0.11	1.2 ± 0.17
Lipolytic	2.1 ± 0.13	2.5 ± 0.23	2.4 ± 0.16	2.1 ± 0.14

a Mean ± standard deviation.

Table (3) shows the species of lactic acid bacteria and number of strains isolated from smen. Lactic acid bacteria from M17 agar were identified as Lactococcus (34 isolates). They grew in 4% NaCl but not in 6.5**%** NaCl or at pH 9.6. All isolates grew at 15°C and 40°C but not at 45°C. They produced L-lactic acid with no gas production in the presence glucose. Of these, 14 isolates have ability to hydrolyze arginine and 20 isolates to produce acetoïn. All of these characteristics, together with the API 20 STREP pattern of carbohydrate fermentation, identified the 20 isolates as L. lactis ssp. lactis biovar diacetylactis and the 14 isolates as L. lactis ssp. *cremoris.*

A total of 33 isolates obtained from Citrate Azide Agar (CAA) were identified as Enterococcus. They did not produce gas from glucose fermentation, produced L-lactic acid, grew at 15, 40 and 45°C, survived at 60°C after 30 min., grew in 6.5% salt and at pH 9.6. Using API 20 STREP pattern of carbohydrate fermentation, the 33 isolates were identified as E. faecium.

Isolates picked from MRS agar were identified as Lactobacillus (65 isolates). They grew at 15°C but not at 45°C or at 10% NaCl. They produce L-Lactic acid with no gas production from glucose and have the ability to hydrolyze arginine. All of these characteristics, together with the API 50 CHL pattern of carbohydrate fermentation, identified the isolates as L. plantarum (20 isolates), L. delbrueckii ssp. bulgaricus (35 isolates) and L. paracasei ssp. paracasei (10 isolates).

Heterofermentative cocci from Sodium Azide Leuconostoc agar were identified as Ln. pseudomesenteroides (9 isolates).

A total of 40 isolates of yeasts were isolated from samples on potato dextrose agar plates and were assigned to Saccharomyces genus because they reproduced by multilateral budding, formed pseudohyphae and asci containing one to four globose ascospores, fermented glucose, galactose and maltose, did not assimilate lactose and nitrate, and their cells were globose to subglobose or ellipsoidal to cylindrical.

Results obtained in this study, indicated that L. lactis ssp. cremoris, L. lactis ssp. lactis biovar diacetylactis, E. faecium, L. plantarum, L. delbrueckii ssp. bulgaricus, L. paracasei ssp. paracasei and Ln. pseudomesenteroides can be isolated from smen. Yeasts (Saccharomyces sp.) were also routinely isolated but not identified at species level.

Among lactic acid bacteria, L. delbrueckii ssp. bulgaricus (24.8%) and E. faecium ((23.4%) were the major species of lactic acid bacteria and yeasts isolated from this product. Results on species composition of lactic microflora of smen were similar to those reported by Sagdic et al. (2002) who isolated lactic acid bacteria from traditional butter represented essencially by strains of *L. delbrueckii* ssp. *bulgaricus* and *E. faecium. L. plantarum; L. lactis* ssp. *cremoris* and *L. lactis* ssp. *lactis* biovar *diacetylactis* were also isolated from smen. This type of bacteria have been frequently reported to be the dominant microorganisms among lactic acid bacteria in long ripening butters, due to their unique ability to grow in highly hostile environments and to the possessing of a wide range of hydrolytic enzymes, including lipases and proteases (Benkerroum and Tamine 2004 and Wouters et al., 2002).

Table (3)
Species of lactic acid bacteria and number of strains isolated from smen.

Species	Number of isolates	(%)
L. lactis ssp. *cremoris*	14	9.9
L. lactis ssp. *lactis* biovar *diacetylactis*	20	14.2
E. faecium	33	23.4
L. plantarum	20	14.2
L. delbrueckii ssp. *bulgaricus*	35	24.8
L. paracasei ssp. *paracasei*	10	7.2
Ln. pseudomesenteroides	9	6.3
Total	141	100

E. faecium was also isolated in this study and represent an important part of the bacterial microflora of smen: mean log plate counts of enterococci in different samples range from 1.47 to 2.69 (Table 2). This species has been isolated from butter (Hukari and Rautavaara, 1972 and Ozalp et al., 1980) and isolates have been identified, characterized and utilized as starter cultures. This group of bacteria also plays a major role in ripening and aroma development in butter and many type of traditional cheeses (Centeno et al., 1996). In addition, E. faecium was also selected together with other lactic acid bacteria for use in starter culture preparation (Coppola et al., 1988 and Parente et al., 1989). L. delbrueckii ssp. bulgaricus was found in all samples of butters; this microorganism represented (24.8%) of the isolates. Similar result was reported by Dave and Shah (1997). Other bacteria as Ln. pseudomesenteroides and L. lactis ssp. lactis biovar diacetylactis were also found in smen. Generally, conventional method for obtaining butter involves the ripening of cream by addition of starter culture mixture containing L. lactis ssp. lactis biovar diacetylactis and Ln. pseudomesenteroides to produce sufficient quantities of lactic acid to reduce the pH to around 4.6 to 4.4 (Kornacki and Flowers, 1998). Also, L. lactis ssp. lactis biovar diacetylactis strains produce sufficient quantity of diacetyl which is the major flavoring component and antimicrobial property of cultured butter (Schieberle et al., 1993). Probably, the presence of this type of bacteria justified the pleasant taste and odour of smen.

On the other hand, analysis of smen showed that mean log counts of yeast exceeded 3.88. Their high counts and well-known high lipolytic activity suggest that they may play an active role in smen ripening.

4. CONCLUSIONS

The natural Algerian butter from cow milk (smen) revealed a diversity of microflora with low incidences of opportunistic bacteria, including coliforms and psychrotrophs bacteria. In adition, different species of lactic acid bacteria present in this product were isolated and identified. Probably, more emphasis should be orientated in our laboratory towards the role of lactic acid bacteria, which have not been fully investigated in smen so far. We think this type of studies will contribute to standardize the production methods of smen and to improve its safety.

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الخصائص الفسيولوجية والميكروبيولوجية لزبدة السمن التقليدية الجزائرية

قاسم مراد*

ملخـص

تم عزل البكتيريا الهوائية، والبكتيريا الدائرية، وبكتيريا حمض الخل، وبكتيريا حالّة للشحم، والبكتيريا المحللة للدهون والخمائر عن عشرين عينة من السمن، الزبدة التقليدية المصنوعة من حليب البقر المتحمض في الجزائر، فيما مجموعه (141) عينة معزولة من بكتيريا حمض الخل عرفت على أنها:

Lactobacillus delbrueckii ssp. *bulgaricus* (24.8%), *Enterococcus faecium* (23.4%), *Lactobacillus plantarum* (14.2%), *Lactococcus lactis* ssp. *lactis biovar diacetylactis* (14.2%), *Lactococcus lactis* ssp. *cremoris* (9.9%), *Lactobacillus paracasei* ssp. *paracasei* (7.2%) and *Leuconostoc pseudomesenteroides* (6.3%).

تم عزل أربعين عينة من الخمائر عن جميع العينات وعرفت على أنها: .sp *Saccharomyces*. وقد بينت الدراسة الفسيولوجية للعينات أن قيم PH، معايرة الحموضة، نسبة الدهن، الرطوبة، المواد الصلبة، NaCl بما يتراوح بين: 3.60-4.39، 0.20-0.32%، 1.02-2.86%، 62.03-65.15%، 33.40-34.92%، 48.90-56.05% على التوالي.

الكلمـات الدالـة: حليب البقر، زبدة السمن التقليدية، بكتيريا حمض الخلّ، الخمائر.

* مختبر الأحياء الدقيقة والبيوتكنولوجيا، قسم البيوتكنولوجيا، كلية العلوم، جامعة وهران، BP.1524 EL M'Naouar وهران31000، الجزائر. تاريخ استلام البحث 2005/11/23، وتاريخ قبوله 2007/5/6.