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Microflora and Physical-Chemical Characteristics of Omani Laban

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الخواص الميكروبية والفيزيائية والكيميائية للبن العماني

الملخص: أجريت تحاليل ميكروبية وفيزيائية وكيميائية على خمسة عشر عينة من اللبن المنزلي الصنع في ثلاث مناطق من سلطنة عمان. إحتوى اللبن على نسبة ١,١٦% حموضة و ٣,٩٨ رقم هيدروجيني و ١,١٢% مواد دسمة و٢,١١% بروتينات و ٦,٢٩% مواد صلبة كلية. يتكون الوسط الميكروبي للبن العماني من أنواع اللكتوكوكساي المحبة للحرارة المتوسطة واللكتوبسلاي المتجانسة التخمر. توجد هذه البكتيريا بمعدل ١,٨ × ١٠^٨ لكل مليلتر للكتوكوكساي و ٢,٤× ١٠^٦ لكل مليلتر للكتوبسلاي. احتوت الأنواع الميكروبية الرئيسية لصناعة اللبن على ميكروبات *Lactococcus lactis* ssp. *lactis* و *Lactococcus lactis* ssp. *lactis biov. Diacetylactis* و *Lactococcus lactis* ssp. *Cremoris* و *Lactobacillus plantanun*. أما أنواع *Leuconostoc* فموجودة بأعداد ضئيلة نسبياً بالمقارنة مع بكتيريا حمض اللبن الأخرى. احتوت كل عينات اللبن على أعداد عالية من الخمائر كما أنها كانت ملوثة ببكتيريا Coliforms و تلك المتواجدة في البراز.

ABSTRACT: Fifteen samples of Laban made at home in three Omani regions were subjected to physical-chemical and microbiological analysis. Laban had an average titratable acidity, pH, fat, protein and total solids of 1.12%, 3.98, 1.12%, 2.11% and 6.29%, respectively. The microbial flora of traditional Omani laban was found to be predominantly mesophilic *lactococci*, and homofermentative *lactobacilli*. The mean *Lactococci* and *lactobacilli* counts were 1.3 x 10^8 and 2.4 x 10^6 /ml respectively. The main microbial types involved in the manufacture of Omani laban were *Lactococcus lactis ssp lactis, Lactococcus lactis ssp lactis biov. Diacetylactis, Lactococcus lactis ssp. Cremoris,* and *Lactobacillus plantarum. Leuconostoc species* were present in low proportion compared to other lactic acid bacteria. All Laban samples contained high yeast numbers and were highly contaminated with coliforms, and fecal coliforms.

Fermented milks, with different names and ripened by microorganisms under similar conditions are available in most countries (Kosikowski, 1966; Nickels, 1969). These fermented products have always constituted a major element in the diet of many populations especially those of the Middle East. Since early times, spontaneous and natural fermentation of milk was looked upon by these people as a means of bio-conservation of fresh milk, a very perishable product. In fact, under the action of fermenting microorganisms, acidification of milk serves two purposes: protection against the development of spoilage and pathogenic flora, and the improvement of organoleptic attributes.

Laban is one of the oldest fermented milks known. Apart from being made today in modern dairy plants, it is still prepared by the inhabitants of Oman by the admixture of old laban to milk. Since early times laban has been an important food item of peoples of the Middle East (Abo-elnaga, 1977). It represents a desirable refreshing and nutritive fermented milk beverage, particularly during the hot summer months. Either sheep's, goat's or cow's milk are involved in its manufacture.

The variation in properties of naturally fermented dairy products in Oman is implicitly dependent on the area of production and the environmental conditions the product is subject to during the fermentation process. The role of starter cultures in the manufacture of fermented dairy products is to provide microbiologically safe products with defined organoleptic and structural properties in an efficient

and reproducible way (Weerkamp *et al.*, 1996). Industrially mixed strains of the starter culture used in Oman to produce fermented milk contain unknown numbers and ratios of species and strains of lactic acid bacteria. By obtaining a better understanding of the organisms involved in the fermentation of milk products available in Oman and their ratio, it will be possible to formulate and propose a starter culture that ensures production in an efficient and reproducible way.

The objectives of this study are to characterize Omani laban by determining some of its physical, chemical and microbiological (lactic acid flora as well as the contaminating microflora) characteristics, and to identify the major microbial species involved in the elaboration of the product. No research has been conducted, and no published work is available on indigenous Omani products.

Material and Methods

Fifteen samples of laban were collected from local, small and medium scale producers in the region of Muscat, Ibri and Nizwa. Samples were collected in aseptic containers, held at refrigerated temperatures and transported back to Sultan Qaboos University for further analysis. Upon arrival to the university, samples were portioned out into 10 ml test tubes and stored at refrigerated temperatures (4°C). Analysis started the next day.

PHYSICO CHEMICAL ANALYSIS: The fat was determined by the rapid Gerber test, the titratable acidity (% TA) by titrating 10 grams of laban with 0.1 N NaOH using phenolphtalein as an indicator and the pH by a Beckman pH meter. Protein was determined using the Kjeldhal method. For the estimation of total solids, the reference method of the international milk federation was used (F.I.L-IDF, 1962). Analyses were run in duplicates for each sample.

QUANTIFICATION OF LABAN MICROFLORA: Culture media and incubation conditions used for the different microbial groups were: total aerobic plate counts using plate count agar (Harold, 1976), 30°C for 72 h; lactococci using M17 agar (Terzaghi and Sandine., 1975), 30°C for 72 h; lactobacilli using MRS agar (De Man *et al.*, 1960), 30°C for 24-36 h; total coliforms using desoxycholate lactose agar (Harold, 1976), 30°C for 24 h; fecal coliforms using desoxycholate lactose agar (Harold, 1976), 44°C for 48 h; Staphylococci using Baird-Parker medium enriched with egg yolk tellurite, 37°C for 24-48 h (Harold, 1976); yeasts and molds using potato dextrose agar acidified with tartaric acid to pH 3.5 (Harold, 1976), 30°C for 3 days. Plating was performed in duplicates for each dilution.

IDENTIFICATION OF LACTIC ACID BACTERIA: Representative colonies were picked from each plate of a convenient dilution for microscopic examination and subsequent identification. Purified strains of lactic acid bacteria were identified using morphological and cultural characteristics, i.e resistance to 4 and 6.5% NaCl, growth in litmus milk, growth at 10, 15 and 45°C and at pH 9.6.

Isolates were further identified using API 50 CHL for the genus Lactobacilli and API 20 STREP systems for the genus *Lactococci* and *Streptococci* (Biomerieux, France). Other characterization tests included citrate fermentation and the ability to hydrolyze arginine for the genus *Lactococci*.

Results and Discussion

PHYSICO CHEMICAL ANALYSIS: The results of the physico chemical analysis of 15 samples of laban are summarized in Table 1.

The average values for pH and % TA are 3.98 and 1.12 respectively. These values are similar to those reported for a fermented milk called "leben Zabady" in Egypt (Rashed, 1974), to Tunisian lben (Jraidi and Guizani, 1996), to Moroccan lben (Tantaoui-Elaraki *et al*,, 1983) and denote the acid character of laban. Laban is in fact a fermented milk of true lactic acid type similar to yogurt (Abo-Elnaga *et al*; 1977).

The development of acidity, expressed as % TA, with time was also studied. Laban was made in the laboratory by the procedure normally used in Omani homes. The procedure was to inoculate fresh unheated milk with 2.5 to 3.0% starter from a previous batch, adding 1-2% salt, and incubating at room temperature (25°C) for 18 to 20 hours. Results of the % TA are shown in Figure 1. Values reported are the average of 4 observations. It is clear from the curve that following a latent period of about 7-8 hours, the acidity increased rapidly, then this evolution slowed down and after 16-17 hours it was stabilized at an average value of 1.2 %.

The fat content varied considerably from 0.55% to 1.60% with an average value of 1.12% ± 0.12. This large variation may be due to inconsistencies in the amount of extracted butter during the churning process

TABLE 1

Some physical chemical characteristics of laban samples.

Parameter	Average
pH	3.98 ± 0.13
[1] %TA	1.12 ± 0.12
Total Solids (%)	6.29 ± 0.21
Protein	2.11 ± 0.17
Fat (%)	1.12 ± 0.35

[1] TA - Percent Titratable Acidity.

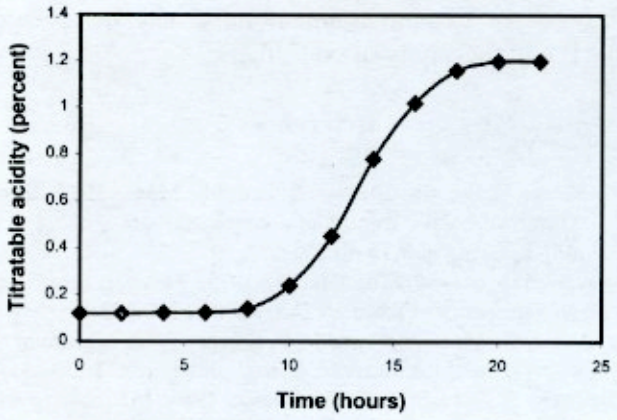

Figure 1. Development of acidity during manufacturing of laban.

and/or to the variation in the quantity of warm water added to induce the coalescence of butter granules during the churning process.

The fat content varied considerably from 0.55% to 1.60% with an average value of 1.12% ± 0.12. This large variation may be due to inconsistencies in the amount of extracted butter during the churning process and/or to the variation in the quantity of warm water added to induce the coalescence of butter granules during the churning process.

Protein and total solids showed also large variations due probably to the quantity of water added to laban that varies from day-to-day production and from manufacturer to manufacturer.

QUANTITATIVE MICROBIOLOGICAL STUDY: Numbers and types of dominating microorganisms on various plating media used are summarized Table 2. It is clear that *Lactococci* are the most important group followed by *Lactobacilli*. The presence of *Lactococcii* was reported in all the studies dealing with laban produced in different countries (Harrati, 1974; Abo-Elnaga *et al.*, 1977; Tantaoui-Elaraki *et al.*, 1983; Jraidi and Guizani, 1996). The presence of this group of microorganisms is not surprising since it represents the normal flora of milk and laban fermented at room temperature. However, the presence of lactobacilli in Omani laban in large numbers is surprising. This group of bacteria were either absent in Tunisian laban (Jraidi and Guizani, 1996), Moroccan laban (Tantaoui-Elaraki, 1983) and Algerian laban (Harrati, 1974) or present in Iraqi and Lebanese laban (Baroudi and Collins, 1975; Abo-elnaga, 1977).

TABLE 2

Microbial flora of laban samples.

Group of Microorganisms	Average
Total Mesophilic Flora	$4.15 \times 10^8 \pm 0.42 \times 10^8$
Lactococi	$1.30 \times 10^8 \pm 0.15 \times 10^8$
LActobacilli	$2.4 \times 10^6 \pm 0.18 \times 10^6$
Yeasts and Molds	$1.67 \times 10^6 \pm 0.25 \times 10^6$
Total Coliforms	$2.50 \times 10^3 \pm 0.87 \times 10^3$
Fecal Coliforms	$1.12 \times 10^3 \pm 0.72 \times 10^3$
Staphylococci	< 10

Molds and yeasts appeared to be clearly present in all laban samples studied. These microorganisms were generally present in relatively small numbers compared with those of lactic acid bacteria. The presence of yeasts in dairy products is not uncommon. Yeasts were reported in many products, such as in yogurt (Suriyarachchi and Fleet, 1981), cottage cheese (Fleet and Milan, 1987), cream (Fleet, 1990), kefir (Engel *et al.*, 1986), quarg (Engel *et al.*, 1980) and laban (Tantaoui-Elaraki *et al.*, 1983; Jraidi and Guizani, 1996). Yeasts seem to have different roles in dairy products. They were found to play a major role as spoilage microorganisms in products such as quarg and yogurt (Engel *et al.*, 1980; Suriyarachchi and Fleet, 1981). They were also reported to play a beneficial role in the production of some fermented dairy products. In fact, Baroudi and Collins (1975) showed that yeasts such as *Kluyveromyces. fragilis* and *Saccharomyces. cerevisiae* were responsible for the production of acetaldehyde and ethanol in Lebanese laban. Tantaoui-Elaraki *et al.* (1983) suggested that yeasts may activate the growth of lactic acid bacteria and contribute to the development of characteristic flavors of laban.

Coliform and fecal coliforms were detected in all laban samples in numbers ranging from 6.9×10^3 to 3.6×10^4 and from 0.8×10^3 to 2.56×10^3 per ml, respectively.

Presence of these microorganisms in dairy products is related to poor sanitary conditions and/or the lack of heat treatment to the milk used in the manufacture of laban. These results are in agreement with those reported for the Algerian, Moroccan and Tunisian laban (Harrati, 1974; Tantaoui-Elaraki *et al.*, 1983; Jraidi and Guizani, 1996). However, Abo-Elnaga *et al.* (1977) reported lower values for Iraqi Laban. Boiling of milk used to prepare Iraqi laban could explain these differences. All laban samples examined contained less than 10 staphylococci per ml. The absence of these microorganisms indicates that laban is not a potential source of staphylococcal food poisoning.

QUALITATIVE MICROBIOLOGICAL STUDY: The bacteria isolated on the M17 medium were gram positive, catalase negative and hydrolyzed arginine. These isolates were identified on the basis of their ability to develop at various temperatures (15°C, 30°C and 45°C), at pH 9.6, in the presence of 4 and 6.5% salt, and on their reaction in litmus milk. The identification was completed by using API 20 STREP system.

Among 23 isolates from the M17 medium, 14 were identified as *Lactococcus lactis ssp lactis*, 4 as *Lactococcus lactis ssp cremoris*, 2 as *Leuconostoc ssp*, 1 as *Enterococcus faecium* and 1 as *Enterococcus durans*. Strains of *Lactococcus lactis ssp lactis* were further differentiated based on their ability to metabolize citrate. Strains developing colonies surrounded by clear zones are considered citrate positive and are classified as *Lactococcus lactis ssp. Lactis var diacetylactis*. Among the 17 colonies identified as Lactococcus *lactis* ssp. Lactis, five were citrate positive and belong hence to the *Biovar diacetylactis*.. It is clear that the majority of strains belong to the homofermentative acidifying species (*Lactococcus lactis ssp lactis*) and to the heterofermentative aroma producing species (*Lactococcus lactis ssp cremoris*). These microorganisms play certainly a very important role in the acidification and aroma production in laban

Identification of bacteria with rod-shaped cells isolated from MRS media showed that 9 out of ten colonies were *Lactobacillus plantarum*. This bacteria could have a role in the production of acidity at the end of the fermentation process. The presence of *lactobacilli* was reported in both Iraqi and Lebanese laban (Baroudi and Collins, 1975; Abo-elnaga *et al.*, 1977). However the species involved are not the same. In Lebanese laban, *Lactobacillus acidophilus* was cited to contribute acidity to the product, while *Lactobacillus bulgaricus* was responsible for developing higher acidity in Iraqi laban.

Conclusion

Omani laban is a fermented milk beverage that is appreciated for its refreshing and sensory attributes. However, its nutritive value is not neglected. In fact, it differs from milk only by the slight amount of water added, the fermentation of lactose, and the removal of variable parts of cream.

Manufacture of laban seems to be the result of the development of mainly lactic acid bacteria of the general *Lactococci*, *Lactobacilli*, and to a lesser degree of *Leuconostoc*. Contaminating flora represented mainly by molds, yeasts, coliforms and fecal coliforms may play a secondary role in the production of laban. Their presence is also indicative of poor sanitary conditions. The lack of pasteurization of milk may be a major concern to the safety of consumers.

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