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Chemical Composition, Microbial Profile and Identification of Lactic Acid Bacteria of Moroccan Fermented Camel Milk "Lfrik"

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Abstract

A total of 15 spontaneously fermented camel milk "Lfrik" samples were collected from 15 traditional dairies in the city of Laayoune and analyzed for their physicochemical composition and microbial profile. Theses samples were made from raw camel milk and kept to ferment spontaneously in a goat skin bag during about 12h at room temperature. The same fermentation process was observed in all the dairies. "Lfrik" samples showed the average respective values of 5.21, 0.42 % and 1.027 for pH, lactic acid content and density. Chemical composition average values were 9.55 %, 0.84 %, 3.41%, 3.80 %, 2.46 % and 0.22 % for total solid, ash, fat, lactose, protein and NaCl contents, respectively. Microbiological analysis revealed the predominance of lactic acid bacteria, the presence of high numbers of coliforms and Enterococci and the absence of *Salmonella* and *S.aureus* in "Lfrik" samples analyzed. A major proportion of the 93 lactic acid bacteria isolated from these samples was identified as *Lactobacilli* (35 %), the other isolates belonged to *Lactococcus* (25 %), *Enterococcus* (17 %), *Leuconostoc* (13 %) and *Streptococcus* (10 %). Among the identified lactic acid bacteria, the most dominant species were: *Lactococcus lactis subsp lactis biovar diacetylactis, Lactobacillus brevis* and *Streptococcus salivarius subsp.thermophilus*.

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Keywords

Fermented camel milk, *Lactobacillus, Lactococcus, Leuconostoc*, Microbiological analyses, Physicochemical characteristic

Introduction

Lactic fermentation of dairy and vegetables products is one of the most ancient practices of man, it's generally defined as a chemical changes that is brought in the base food due to the action of inoculated cultures and the enzymes they produce[1].

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Lactic acid bacteria are one of the microorganisms that dominate fermented food and drinks products which constitute a major portion of people's diet in Africa[2]. They predominated all the indigenous processing of cereals, fruits and root crop[3]. Lactic acid fermentations have survived because of the traditional beliefs, they can enhance nutritional, digestibility, shelf life, safety and sensory attributes of vegetables[4] and milk[3]. The majority of fermented milk is made from cow milk, followed by sheep, goat and camel milk.

Camel milk is an important food source for humans in several drought areas and it is, in most cases, drunk as is or left for souring[5]. Camel milk might be processed into a number of fermented milk products. In many parts of the world, camel milk is traditionally allowed to ferment naturally without prior heat treatment and without addition of starter cultures[6,7]. However, these fermented camel products might be named differently depending on their geographical location. For instance, they are called "Gariss" in East[8,9] and in Sudan[10,11] , "Shubat" in Central Asia[12], "Suusac" in Somalia[13] "Kefir" in the Caucasian area[14], "Dhanaan" in eastern Ethiopia[15], "Chal" in Iran[16]and "Lehban" in Syria and Egypt[17].

In these traditional fermented camel milks, lactic acid bacteria (LAB) play the major role in the fermentation process such as in "Suusac"[18] "Gariss"[8,18] and "Shubat"[18,19]. Fermented camel milk has shown potential health benefits, including antimicrobial effects[20,21]. The manufacture of these value added products also generate a large quantity of revenue with potential source of nutrients which is not being utilized so far in any dairy products[22].

On the other hand, certain fermented camel products such as "Gariss" and "Suusac" made from raw milk and very often under poor hygienic conditions[23,24] were frequently found to contain high levels of spoilage and pathogenic microorganisms[7,8,25,26].

In Morocco, fermented camel milk is produced traditionally from raw camel milk in the south part of the country and referred to as "Lfrik". At our knowledge, there is no published previous study on this product. Therefore, the aim of the present work was to determine the physicochemical characteristics and the overall microbial profile of the fermented camel milk 'Lfrik'.

Materials and Methods

Camel Milk Fermentation Survey

A survey was conducted within 23 traditional dairies called 'Mahlabas' of the city of Laayoune (southern Morocco) in order to assess the process of "Lfrik" manufacturing.

Sampling

Fifteen samples of "Lfrik" were collected from traditional dairy shops among those surveyed and aseptically introduced into sterile glass bottles. Portions of these samples were immediately used to carry out the first physical-chemical analysis (pH, acidity and density) in a camel milk Cooperative laboratory located in Laayoune. The other portions were kept in ice coolers and transported the same day by air to the IAV laboratories in the city of Rabat for further analysis.

Physicochemical Analysis

Measurements of pH and density of "Lfrik" samples were done using a digital pH meter (Model minilab-IQ125) and a digital density meter (Mettler Toledo 30 PX, Greifensee,Switzerland), respectively. Titratable acidity, dry matter, fat, ash, proteins and chlorides contents were determined according to the AOAC methods (1990)[27]. For the lactose content, the method outlined in AOAC (2000)[28] was used.

Microbiological Analysis

Total aerobic mesophilic bacteria and psychrotrophics counts were determined using Plate Count Agar medium (PCA, Biokar Diagnostics, Beauvais, France) incubated respectively at 30 °C/48h and 7 °C/10d[29]. Enumeration of total and fecal coliforms were obtained using Violet Red Bile Agar medium (VRBA, Biokar Diagnostics, Beauvais, France) after incubation of 24h at 30 °C and 44 °C, respectively[30]. For the Enterococci count, Slanetz and Bartley Agar medium was used (Bio-Rad, Marnes-la-Coquette, France) with incubation of 48h at 44 °C[31] . Czapec and Dox medium (Difco laboratories, Detroit, Michigan, USA) was used for the fungi count after incubation of 25 °C for a week. The numbers of Staphylococci were determined after 48h incubation at 37 °C on Baird Parker Agar medium

(Difco laboratories, Detroit, Michigan, USA). The identity of presumptive *S.aureus* colonies on this medium was confirmed by the coagulase test[32] and the presence of thermonuclease activity[33] on Toluidine blue O-DNA agar (Sigma, St louis, USA). For the detection of Salmonella, the method recommended by ISO 6579[34] based on buffer peptone water as pre enrichment medium, Rapport vassiliadis broth as enrichment medium broth and xylose lysine descoxychalate (XLD) agar as identification medium. Triple sugar iron agar (TSI) and API 20E Test System (BioMerieux, Marcy l'Etoile, France) were used as confirmation tests. For lactic acid bacteria (LAB) counts, M17 agar medium (Biokar Diagnostics) was used for Lactococci (37 °C/ 48 h); MRS agar medium (Biokar Diagnostics) for Lactobacilli on anaerobic conditions (30 °C/72h) and Hypersaccharosed Agar medium for Leuconostocs (30 °C for 48h) as recommended by Mayeux *et al.,*[25]. After enumeration, colonies were randomly isolated and purified.

Identification of Lactic Acid Bacteria (LAB)

LAB were identified according to the method described by Sharpe[36]. The isolates were Gram stained and examined for catalase production and morphological characters. Gram positive and catalase negative isolates were subjected to the following biochemical and phenotypic tests: Ability to produce gas from glucose, growth at 37 °C and 45 °C for *Leuconostoc*, 40 °C and 45 °C for *Streptococcus* and *Lactococcus* and at 15 °C and 45 °C for *Lactobacillus*, growth in the presence of 4 and 6,5 % of NaCl and at pH 9.5, capacity to hydrolyze esculin in MRS/M17 broths prepared without glucose and supplemented with 0.2 % esculin and 0.1 % ferric ammonium citrate, ammonia production from arginine hydrolysis after adding Nessler's reagent, citrate hydrolysis, and production of acetone for *Lactococcus* and *Leuconostoc*. Isolates were also tested for fermentation of lactose, maltose, mannitol, raffinose, rhamnose, arabinose, sucrose, ribose, sorbitol, melibiose, melezitose, galactose, amygdaline, xylose, cellobiose, trehalose and dextrin using the API 20 STREP and API 50 CHS micro-identification systems (API-System, La Balme Les Grottes, Montalieu-Vercieu, France). The protocol recommended by the manufacturer for inoculation and incubation of media was followed.

Statistical Analysis

Physicochemical and microbiological data analyses were carried out using Microsoft Excel to calculate averages and standard deviations (S.D.)

Results and Discussion

Camel milk Fermentation Survey

Results of the survey on the traditional preparation of the fermented camel milk "Lfrik" showed that all the traditional dairies surveyed use the same technique. The process involves spontaneous fermentation of raw camel milk in a goat skin bag, called "tassoufra", at room temperature during about 12 h. The fermentation is often carried out at night.

Beside Lfrik, the main fermented camel milk produced, other fermented camel milk products, locally named "Sligh" and "M'tame", are prepared using the same technique and equipment but different incubation periods. The fermentation duration is about 3 h and 24 h for "Sligh" and "M'tame", respectively. These products possess different sensory characteristics, in particular sourness, from "Lfrik".

Physicochemical Characteristics of Lfrik

The results of the physicochemical characterization of traditional "Lfrik" samples are shown in Table 1. The pH ranged from 4.7 to 5.9 which is similar to the pH range (4.0-5.8) reported for "Suusac", fermented camel milk in Somalia[37]. Lower pH ranges (3.7 to 4.1) were reported by Shori[7] for "Shubat" another fermented camel milk incubated at 25 °C/8h. These differences in pH ranges can be explained by the time and the temperature of fermentation for each of these products.

The acidity of the examined "Lfrik" samples ranged from 0.32 to 0.50 % lactic acid, which is very much lower than the 0.82 % reported by Boubekri *et al.,*[38] for the traditional fermented cow milk "Lben". These low acidity values of fermented camel milk by comparison to fermented cow milk are probably due to the presence of inhibitor agents in camel milk and natural protective proteins such us: Lysosyme, lactoferrin, lactoperoxydase and immunoglobulin, which have an effect of delaying the rate of development of acidity[39,40,41].

The density of "Lfrik" samples varied from 1.025 to 1.039 and their total solids content from 7.09 to 12.33 % (w/v). Average values for fat, lactose, proteins and ash contents were 3.41, 3.80, 2.46, 0.21 and 0.84 % (w/v), respectively. The obtained results for density, total solids, fat, proteins, chlorides and ash are similar to those reported for raw camel milk produced in the same area of study by our team[42]. However, the lactose content of "Lfrik" is lower than that of raw camel milk because of its use during the spontaneous fermentation process. These results support the fact that "Lfrik" is prepared from whole camel milk and without addition of water as reported by the participants in the survey.

Table 1: Chemical composition of "Lfrik" samples

Component	Mean (n=15)	Range	S.D.
pH value	5.2	4.7-5.9	0.4
Lactic acid %	0.42	0.32-0.50	0.06
Density (g/cm3)	1.027	1.025-1.039	0.003
Total solids %	9.55	7.09-12.33	1.87
Ash %	0.84	0.59-1.29	0.21
Proteins %	2.46	2.02-3.07	0.38
Fat %	3.41	2.30-4.30	0.72
Chlorides %	0.21	0.16-0.25	0.02
Lactose %	3.80	2.90-4.40	0.37

S.D.: Standard deviation

Microbiological Analyses of Lfrik

Results of the microbial profile of "Lfrik" samples are presented in the Table 2. Total aerobic mesophilic bacteria count of the examined samples was high as expected in fermented product and varied from 1.36 10^6 to 3.02 10^8 cfu.ml^{-1}. The overall hygienic quality of the samples was poor as indicated by the relatively high average values of 5.61 10^6 and 3.57 10^6 cfu.ml^{-1} for total and fecal coliforms counts, respectively. The psychrotrophic flora count of the samples was also high and ranged from 1.50 10^4 to 1.12 10^6 cfu.ml^{-1} probably due to refrigeration of "Lfrik" immediately after the end of the fermentation process. Yeasts were found in large numbers in the examined "Lfrik" (1.0 10^6 to 3.4 10^7 cfu.ml^{-1}) as seen in many similar fermented milks[43,44,45]. However, molds were detected only in 4 of 15 analyzed samples of "Lfrik" with an average value of 1.13 10^1 cfu.ml^{-1}.

Generally, yeasts have been reported to positively interact with LAB[46].

As shown in Table 2, LAB are the dominant microorganisms in "Lfrik" samples. The average counts of *Lactococci, Lactobacilli* and *Leuconostocs* were quite similar: 3.67 10^7, 1.18 10^7 and 1.63 10^7 cfu.ml^{-1} respectively.

The Staphylococci were found to be coagulase negative and their count varied from less than 30 to 1.90 10^4 with an average of 3.36 10^3 cfu.ml^{-1}. *Salmonella* was not detected in all the samples examined; this absence of *Salmonella* may be due to its inhibition by LAB and/or the produced acidity during the fermentation process. Klaenhammer *et al.,* [47] reported that the acidity caused by the lactic acid fermentation has an important role in inhibiting the growth of pathogenic bacteria.

Identification of Lactic Acid Bacteria

Among the 93 isolates confirmed as LAB, 25 % were identified as *Lactococcus*, 17 % belonged to *Enterococcus*, 13 % to *Leuconostoc*, and 10 % to *Streptococcus*. However, *Lactobacillus* isolates were more frequently encountered (35 %). Like in "Lfrik", *Lactobacillus* was also found in high proportion (60 %) in Sudanese spontaneously fermented camel milk "Gariss"[11].

The species distribution of the LAB isolates is presented in Table 3. *Lactococcus lactis subsp. Lactis biovar diacetylactis* (12 isolates), *Streptococcus salivarius subsp. thermophilus* (9 isolates), *Lactobacillus brevis* (9 isolates) and *Leuconostoc mesenteroides ssp. dextranicum* (8 isolates) were the species most frequently detected among the Lab isolates from "Lfrik". In similar products, *Streptococcus infantarius* subsp. *infantarius* and L. *fermentum* were found to be dominant LAB in "Gariss"[8] while "Suusac" contained predominantly *Streptococcus infantarius subsp. Infantarius, Lactococcus lactis subsp. lactis*, and *Streptococcus thermophilus*[22]. *Lactococcus lactis ssp. Diacetylactis* (24.1%) was found as the dominant specie in raw camel milk obtained from South of Morocco[48].

Table 2: Microbial characteristics of "Lfrik" samples

Flora (cfu.ml^{-1})	Average (n=15)	Min. value	Max. value
Total aerobic mesophilic bacteria	1.42 10^8	1.36 10^6	3.02 10^8
Feacal coliforms	3.57 10^6	7.00 10^4	1.49 10^7
Total coliforms	5.61 10^6	1.85 10^6	2.12 10^7
Psychrotrophic flora	2.33 10^5	1.50 10^4	1.12 10^6
Enterococci	3.76 10^6	4.30 10^4	1.80 10^7
Yeasts	8.28 10^6	1.00 10^6	3.40 10^7
Molds	1.13 10^1	<30	1.00 10^2
Lactococci	3.67 10^7	2.86 10^5	1.50 10^8
Lactobacilli	1.18 10^7	7.50 10^2	5.04 10^7
Leuconostocs	1.63 10^7	2.40 10^6	5.88 10^7
Staphylococci	3.36 10^3	<30	1.90 10^4
Salmonella	ND	ND	ND

Table 3: Species distribution of LAB isolated from "Lfrik"

Genus	Species	Isolates number
Lactococcus	*Lactococcus lactis subsp.cremoris*	3
	Lactococcus lactis subsp lactis .biovar diacetylactis	12
	Lc. lactis subsp.hordnae	2
	Lc.raffinolactis	3
	Lc. Plantarum	3
Streptococcus	*Streptococcus Salivarius subso,thermophilus*	9
Lactobacillus	*Lb.brevis*	9
	Lb.fermentum	5
	Lb. delbrueckii subsp. bulgaricus :	3
	Lb.delbrueckii subsp lactis	4
	Lb. acidophilus	6
	Lb. plantarum,	6
Leuconostoc	*Leuconostoc mesenteroides ssp. Dextranicum*	8
	Leuconostoc lactis	4

Conclusion

"Lfrik" is made by spontaneous fermentation of raw camel milk at room temperature in about 12 hours. Its acidity is significantly lower than the one obtained in the traditional fermented cow milk "Lben".

The absence of pathogenic flora (*Salmonella* and *S. aureus*) in analyzed Lfrik samples may be due to its inhibition by LAB which have the ability to produce active substances and bacteriocins, thus acting as a bactericidal agent in fermented foods.

Species founded with technological interest among the 14 species identified are *Lactococcus lactis ssp lactis biovar diacetylactis* (13 %), *Lactobacillus brevis* (10 %) and *Streptococcus Salivarius subso, thermophilus* (10 %).

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Conflict of Interest

We confirm that there are no known conflicts of interest associated with this publication.

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