JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

POTENTIAL OF LACTIC ACID BACTERIA FROM MOROCCAN GOAT'S MILK FOR STARTER CULTURE DEVELOPMENT

EL GALIOU Ouiam[*1], *BAKRIM Hanane*[1], *LAMHAMDI Mostafa*[2], *HASSANI ZERROUK Mounir*[1], *ZANTAR Said*[3], *ARAKRAK Abdelhay*[1], *BAKKALI Mohammed*[1], *LAGLAOUI Amin*[1]

Address(es):
[1]Abdelmalek Essaâdi University, Tetouan, Morocco, Faculty of Science and Technology, Department of biology, Biotechnology and Biomolecular Engenineering Research Team, B.P.416. Tangier.
[2]Higher School of Technology of El Kelaa Des Sraghna, Cadi Ayyad University, El Kelaa Des Sraghna BP 104, Morocco.
[3]Laboratory of Food Technology and Quality, CRRA, INRA, Tanger, Morocco.

*Corresponding author: ouiamelgaliou@yahoo.fr

https://doi.org/10.55251/jmbfs.9463

ARTICLE INFO

Received 17. 9. 2022
Revised 13. 1. 2023
Accepted 17. 1. 2023
Published 1. 4. 2023

Regular article

OPEN ACCESS

ABSTRACT

Goat milk, a highly nutritious product is widely used in cheese making. In order to properly select suitable milk samples for production, fifty-five samples of raw goat's milk were collected from eight farms in the North-West of Morocco to analyze their counts of various mesophilic bacteria and determining the physicochemical characteristics during different seasons of the year. Lactic acid bacteria (LAB) were isolated, identified, and screened for several technological properties. Overall mean values for mesophilic aerobic flora were above 7.10 log cfu/mL in spring and 5.80 log cfu/mL during winter with a statistically significant difference found between all the microorganisms except for coagulase-positive staphylococci. The physical and chemical composition tests showed higher values for fat (4.9±1.01%), dry matter (13.98±0.95%), ash (1.3±0.12%), lactose (3.1±0.65 g/L), and protein (4.6±1.03 g/L) during the winter season which could be of great interest for cheese production. fatty acids (FA) composition mainly consisted of saturated fatty acids (SFA) in both seasons. Lactococci isolates were the most acidifying (pH_{6h}=4.35–pH_{24h}=4.08) while *Lactobacillus paracasei* produced the highest proteolytic activity (66.93±2.63 mgTyr.L^{-1}) and diacetyl-acetoin (54.83±0.46 mg diacetyl/L). None of the tested isolates were lipolytic, however, several lactobacilli and lactococci exhibited a high salt tolerance and exopolysaccharide (EPS) production. *Lactobacillus plantarum* exhibited the highest lysis rate (67.34±1.76%) while the antibacterial profile ranged between 10.5±0.137 and 22.9±0.23 mm. The obtained results highlight the importance of Moroccan goat milk as a valuable component for cheese making due to its rich fat and protein contents alongside its abundance in LABs with biotechnological properties.

Keywords: goat's milk, microbiological counts, lactic acid bacteria, physical and chemical composition, technological properties

INTRODUCTION

In northern Morocco, goats are presented with great adaptability and can be encountered in the poorest areas, where sheep cannot survive. In these areas, goat herds are mainly used for meat production. Local goat breeds recognized for their adaptation to difficult conditions are worth being exploited. The National Association of Sheep and Goat Farmers' experience in the development of goat farming in mountainous regions, especially in Chefchaouen, is very encouraging **(El Galiou *et al.*, 2015)**. According to the Food and Agriculture Organization of the United Nations **((FAOSTAT), 2021)**, the global goat population increased from 918.189.481 to 1.045.915.764 (13.91% or an average of 1.73% per year) between 2010 and 2018, while in Morocco it increased to 5.731.000 in 2018. Total goat milk production increased from 16.249.163 metric tons to 18.712.088 metric tons between 2010 and 2018 (13.16% or an annual average of 1.64%) (Food and Agriculture Organization of the United Nations **((FAOSTAT), 2021)**, while in Morocco, around 44.618 metric tons of milk were produced in 2018 according to the same reference **((FAOSTAT), 2021)**; However, these statistics are approximate, since goat milk production, especially in developing countries, is likely to be much higher than official statistics, most particularly since statistical data does not include the unknown amounts processed and sold by farmers in organized markets **(Pirisi *et al.*, 2007)**.
For a long time, "Jben" has been the most renowned and consumed fresh cheese in rural and urban areas of Morocco. Recently, as a large number of traditional dairy factories have been set up in cities to prepare 'Jben' from raw milk according to standard manual procedures, the consumption of this product has increased. In addition to this traditional sector, some semi-industrial dairy units are also interested in using raw or pasteurized milk for the preparation of "Jben", as well as applying more or less improved preparation procedures. Therefore, there are many ways to prepare "Jben" today; as a result, several varieties of fresh cheese products are currently available in the Moroccan market under the common popular name "Jben".

Several factors may influence the quality of the goat cheese, especially its raw material, thus it is necessary to be knowledgeable of the composition, the physico-chemical and, the microbiological aspects of the goat milk used in the production of the cheese investigated **(Guo *et al.*, 2004)**. For this reason, our first objective in this study is to carry out a physico-chemical and microbiological investigation of goat milk from 8 zones of the northern region of Morocco during the spring and winter seasons.
For decades, in order to increase goat milk production, the state has encouraged goat farmers to form cheese manufacturing cooperatives to promote cheese production, particularly "Jben" at a semi-industrial scale. Such a goal cannot be achieved without prior knowledge of the milk composition of the local breeds.
Milk composition and quality can be heavily influenced by several factors, including however not limited to seasonal variations **(Chen *et al.*, 2014; Kljajevic *et al.*, 2018)**,therefore the study of raw milk composition and its physicochemical properties throughout the seasons is indispensable in order to understand the effect of seasonal parameters on the quality of dairy products as well as offering a better capability for selecting the most suitable period for milk processing for cheese manufacturing, Thus this study is aimed to identify the FA composition, physicochemical and microbiological properties of raw goat milk obtained from 8 farms situated in North-Moroccan during the spring and winter season, Another objective was to identify and characterize LAB isolated from raw goat's milk in order to select appropriate candidates for use as starter and adjunct cultures. To our knowledge and up to now, no scientific data have been published concerning the microbiological and physicochemical parameters of goat's milk from Northern Morocco.

MATERIALS AND METHODS

Milk sampling and storage conditions

A total of 55 goat milk samples used in this study were taken from 8 dairy farms (areas) classified from A to H, those farms are located in 3 different regions of

northern Morocco: Tangier, Tetouan, and Chefchaouen which are characterized with a typical Mediterranean climate, with mild, wet winters and hot, dry summers. The farms from B to H are traditional dairy goat farms, with extensive livestock farming and widely distributed in the northern region, the estimated average size is 5 heads per farm. These farms are characterized by a total lack of reproduction control, and their diet is based on forests and grasslands. Whereas the milk from Farm A comes exclusively from a goat farming facility located in the National Agricultural Research Institute of Tangier (INRA) with a diet system similar to other the farms.

The average size of the farming zones varies from one area to another. This indicates the presence of some differences in the management of livestock and milk production, and therefore a wider comprehensiveness of the study in the light of the anticipated recommendations.

Milk samples were kept at 4°C until the time of use. Once in the laboratory, the pH and Dornic acidity of the raw milk samples were determined. All samples were analyzed in order to identify their physicochemical and microbiological characteristics.

Sampling and monitoring points were distributed along the following periods:

- Spring (April-May): The average temperature ranges between 18 and 25°C. Most herds are outdoors.
- Winter (January – February): Average temperature ranges between 10°C and 18°C.

In order to only take into account, the factors related to the characteristics and breeding practices, we've chosen to take the samples from one milking procedure to avoid the impact related to low-temperature storage on the milk microbiota.

Microbiological analysis

Milk samples (10 mL) were aseptically pipetted into 250-mL Duran flasks containing 90 mL of sterile 0.1% (w/v) buffered peptone water (BPW; Merck, Darmstadt, Germany) and mixed by shaking for 30 s, and appropriate dilutions in 0.1% BPW were poured in or spread to duplicate agar plates. Samples were analyzed for total mesophilic flora, mesophilic and thermophilic lactic acid bacteria (LAB), enterococci, Total coliforms, Psychrotrophic bacteria, Micrococci, Coagulase-positive staphylococci, Yeast, and Mold. The microbiological media and incubation conditions used were conducted by the method of **Samelis *et al.* (2009)**.

Physicochemical analysis

As soon as raw milk samples arrive at the laboratory, the pH is measured using a HANNA pH-meter. The latter, according to the instructions of the apparatus, was first calibrated in calibration solutions at pH 7 and 4 by dipping into a small volume of milk taken from a beaker.

Dry matter content was determined by the method described by **AOAC. (2000)**. Ash content was determined by a gravimetric method using a muffle furnace at 550 °C as described by **AOAC. (2000)**.

Fat content was determined through three phases by the Rose-Gottlieb method as described by **AOAC. (2000).** Briefly, 10 g of homogenized sample was weighed into a Mojonnier-type fat-extraction flask. Ammonium hydroxide solution (2 mL) was added and the mixture was shaken vigorously. 10 mL of ethanol, 25 mL of diethyl ether, and 25 mL of petroleum ether were subsequently added and mixed gently in an initial extraction step. During the second extraction phase, 5 mL of ethanol, 15 mL of diethyl ether, and 15 mL of petroleum ether was used, while the final extraction phase only required diethyl and petroleum ether (15 mL). After each extraction step, the supernatant was transferred into fat-collecting vessels after a 30 min long separation phase. All solvents were evaporated using a rotary evaporator at room temperature, and the extracted lipids were resolved in 20 mL of diethyl ether: petroleum ether (1:1, vol/vol).

Protein content was determined by method described by **IDF. (1986)**, This reference method is based on the conversion of organic nitrogen to inorganic nitrogen in the ammonia form $(NH4)_2SO_4$ by the oxidative action of boiling sulfuric acid on organics substances in the presence of a mineralization catalyst. Milk samples were placed on mineralization flasks and mineralized in ramp at 420 °C for 3 hours. During the distillation, the ammonium hydroxide (NH_4OH) formed is driven by water vapor and collected in a titration vessel containing boric acid solution in excess. The formed ammonium borate ($(NH_4)_3BO_3$) raises the pH of the solution. The solution is then titrated with sulfuric acid. The amount of sulfuric acid added corresponds to the ammonium content of the initial sample. Total protein nitrogen content is expressed in grams of nitrogen per liter of milk using the following formula:

$$\text{Total nitrogen} = V_1 \times 0.0014 \times 1000 / V_0$$

$\mathbf{V_0}$ is the volume in milliliters of the test sample.
$\mathbf{V_1}$ is the volume in milliliters of the sulphuric acid solution used for ammonia neutralization.

As for Lactose, the content was determined according to the Fehling's solution method **(Triebold, 2000)**, This method of determination is based on the reduction of Fehling's solution, a mixture of a solution of copper sulphate and seignette salt in the presence sodium hydroxide, by the sugars present in the milk. Its principle is based on the reduction of the copper oxide CuO into small brick red particles of cuprous oxide CU_2O.

GC analysis of free fatty acid content

The samples were analyzed by gas chromatography in order to determine the composition in free fatty acids following the procedure detailed by **Poveda *et al.* (2006)**. Acidified cheese paste (10g) was used for the extraction of lipids using diethyl ether. Fatty acid methylation was performed using 20% trimethylanilinium hydroxide (TMAH) in methanol. The foam created by TMAH at the lower layer of the solution was neutralized prior to injection and underwent pyrolysis to methyl esters in the chromatograph injector. The identification of Free Fatty Acids was carried out by chromatography with flame ionization detection (FID) using a slightly modified protocol previously cited by **Dela Fuente *et al.* (1993)**. The investigation was performed in a Varian model 3800 instrument (Varian Inc.,Palo Alto, CA, USA) provided with an automatic sampler (CP Wax 52CB, Varian) and a programmable temperature vaporizer (PTV) injector. The separation of the free fatty acids occurred in a fused-silica capillary column with dimensions of 30 m × 0.25 mm i.d. × 0.25 m from DB-FFAP, Agilent Technologies, Wilmington, DE, USA, helium was used as carrier gas (1 mL/min) in split/splitless mode with a ratio of 1:20. The oven was first programmed to 60°C for 20 min before gradually increasing the temperature 5°C per minute until a final temperature of 180 °C is reached, and then maintained for 60 min. The parameters of the PTV program are as follows: 60 °C at the initial phase, 300 °C at 0.05 min, and 25 °C from t=4 minto the end of the analysis. Detector temperature was fixed at 250 °C, cheese samples were supplemented with pentanoic, nonanoic, and heptadecanoic acids as internal standards for FFA quantification. Testing on all samples was performed in duplicates.

Isolation, preservation and phenotypic identification of LAB

Bacterial cultures were streaked on De Man, Rogosa and Sharpe agar (MRS) (pH of 5.7) and plate count milk agar. After incubation (30 °C), bacterial colonies (Four to six colonies) were indiscriminately selected for further purification by sub-culturing on MRS medium using the streaking method. Bacteria with Gram positive and catalase-negative profile were preserved in MRS broth mixed with 20 % glycerol (v/v) at -20 °C. The required cultures were obtained by a double transfer in MRS broth at 30 °C. Isolates were phenotypically attributed to the genus level based upon: (a) Their microscopic aspect in fresh Gram-stained preparations ; (b) Levels of CO_2 generated from glucose contained in MRS broth (Oxoid, Basingstoke, UK); (c) Arginine hydrolysis (d) Facultative hetero-fermentative characteristic of presumed *Lactobacilli* was tested by CO_2 production assay in a glucose/beef-free MRS broth supplemented with potassium gluconate (e) Presumptive *Lactococci* and *Enterococci* were also confirmed by their ability to grow on Slanetz and Bartley agar (Oxoid, Basingstoke, UK) **Garabal *et al*. (2008).**

Table 1 List of primer pairs used for specific PCR amplification, optimized PCR protocol, and expected PCR products.

Genus/Species	(T-t)x[a]	Primers	Sequence 5'-3'	PCR product size(base pairs)	Reference
Lactobacillus plantarum	(58 °C-40 s)35	P16 Lp1	GCTGGATCACCTCCTTTC ATGAGGTATTCAACTTATG	220	**(Berthier *et al*., 1998)**
Lactobacillus paracasei	(60°C-5 s)25	Y2 Para	CCCACTGCTGCCTCCCGTAGGAGT CACCGAGATTCAACATGG	290	**(Young *et al*., 1991)** **(Ward*et al*., 1999)**
Lactococcus lactis	(60 °C–35 s)35	LacreR 1RL	GGGATCATCTTTGAGTGAT TTTGAGAGTTTGATCCTGG	250	**(Pu *et al*., 2002)**
L. lactis subsp. *lactis*	(50°C-40 s)30	Lchis3F Lchis 4R	AAAGAATTTTCAGAGAAA ATTTAGAATTGGTTCAAC	350	**(Beimfohr *et al*., 1997)**
L. lactis subsp. *lactis* var. *diacetylactis*	(50°C-40 s)30	Lchis5F Lchis 6R	CTTCGTTATGATTTTACA AATATCAACAATTCCATG	900/1100	**(Beimfohr *et al*., 1997)**

PCR-identification of LAB isolates

The suspected *Lactococcus* and *Lactobacillus* isolates were attributed to the genus or species by PCR as described by **Garabal *et al*. (2008)** and **El Galiou *et al*. (2015)**, with the aid of specific primers (Table A). The total DNA used for PCR reactions was yielded by a freeze/unfreeze shock method, according to the protocol previously cited by **Garabal *et al*. (2008)**.

Technological characterization of strains

Flavors produced by LAB, acidifying activity and proteolytic activity

Selected isolates of *Lactococcus* spp. and *Lactobacillus* spp. were characterized for the following abilities (all tests were carried out in duplicate): **(a)** flavors produced in (100 mL) pasteurized (100±1°C, 15 min) whole (3.8 g/100 mL fat) milk inoculated at 1mL/100mL with 16 h skim milk cultures and incubated at 30±1°C for 24 and 48 h; the flavors were perceived by smelling, as evaluated by a panel consisting of at least five judges proposed by the PDO Regulating Councils and trained following the IDF Standard 99C **(IDF, 1997a)**; **(b**) acidifying activity in skim milk (Oxoid) after 6 h **(IDF, 1995)** and 24 h incubation at 30±0.1°C; **(c)** proteolytic activity in skim milk (Oxoid), after 24 h incubation at 30±1°C, were detected by a method employing the Folin–Ciocalteau reagent **(IDF, 1997b)**; and **(d)** diacetyl and acetoin production in skim milk (Oxoid) after 48 h incubation at 30±1°C **(IDF, 1997b)**.

Autolytic activity

The autolytic activity was determined in compliance with the method adopted by **Lansgrud *et al*. (1987)**. Cell suspensions were sampled from MRS broth cultures during the exponential growth phase (optical density corresponding to 0.7–0.8 at 650 nm) and collected by centrifugation (10.000g for 10 min at 4 °C). The obtained pellets were washed and resuspended in 20 mM sodium phosphate buffer (pH 6.8). the cells were incubated at 30 °C and their lysis was detected during a 4 h incubation period by monitoring the decrease in optical density (OD650) using a Milton Roy Spectronic 20D spectrophotometer. The autolytic activity was determined by the calculation of the percentage of lysis using the following formula:

Autolytic Activity (%) = 100-(A1/A2 x 100).

A1: The lowest OD650value measured during incubation
A2: The highest OD650value measured during incubation.

Lipolytic activity

Lipolytic activity was determined qualitatively by means of culture streaking on the surface of plates containing cream fat agar prepared by homogenizing nutrient agar with 1% (v/v) milk cream (38% fat). The plates were incubated at 37 °C for 72 h, positive results correspond to colonies surrounded by a clear zone around the inoculation spot **(Buffa *et al*., 2005; Hantsis-Zacharov *et al*., 2007)**. This method is deemed advantageous due to the application of milk fat, which is the substrate used by the LAB in the cheese.

Exopolysaccharide production

Detection of exopolysaccharide (EPS) production was conducted according to **Smitinont *et al*. (2007)**. Isolates were cultivated in modified MRS agar medium supplemented with 2% of glucose (Merck, Darmstadt,Germany), sucrose (Fluka, Buchs, Switzerland), fructose(Merck, Darmstadt, Germany) and lactose (J. T. Baker, Deventer, Netherlands) as carbon sources. The plates were incubated for three to five days at 30°C, strains characterized by the presence of colonies with a slimy texture are considered to be positive for EPS production.

Detection of Antibacterial Activity

L. plantarum strains were screened in order to determine their antibacterial activity against three indicator pathogens (*Listeria monocytogenes* NCTC 10527, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* O157:H7 NCTC 12079) using the well diffusion method **(Papamanoli *et al*., 2003)**. The testing approach consists of supplementing sterile MRS agar (8g/L) sustained at a temperature between 46 and 52 °C with 100 mL of nutrient broth containing one of the indicator strains, the mixture is then introduced to Petri dishes and left to solidify. The solid agar was subsequently perforated with a sterile cork borer to produce wells, in which 50 µl of an overnight culture of each *L. plantarum* strain was added. The plates were left to stand for 2h at 4 °C before incubation (30 °C for at least 24 h to allow the formation of a clear and distinct inhibition zone). Results were expressed by measuring the inhibition zone diameters.

Salt Tolerance

The strains' capacity to withstand and grow in increasing saline conditions was assessed in accordance with the procedure reported by **Ferrari *et al*. (2016)**. The tested strains were incubated for 48 h at 37°C in saline solutions with2, 6, and 10% NaCl (wt/vol). After incubation, a change in color from purple to yellow due to substrate acidification is indicative of bacterial growth.

Statistical analysis

SPSS program (Statistical Package for Social Sciences version 16) was used to perform the statistical analysis of the obtained data. Significant differences between means were determined by one-way ANOVA (Analysis of Variance) using Tukey's range test with 95 % confidence interval ($p < 0.05$).

RESULTS

Microbiological characteristics of milk

Table 2 illustrates the microbiological characteristics of milk samples for both seasons.

Table 2 Microbiological characteristics of goat milk samples (mean values and standard deviations were expressed in $\log_{10}$cfu/mL), for spring (n=55), and winter (n=55). The different letters denote a statistically significant difference at a 5% level of significance (p <0.05).

Microorganisms	Spring	Winter
	Mean±SD	Mean±SD
Total Mesophilic Flora	7.10±0.50^a	5.80±1.10^b
Mesophilic lactic acid bacteria	5.56±1.12^a	4.30±0.90^b
Thermophilic lactic acid bacteria	4.90±0.95^a	3.94±0,85^b
Enterococci	3.50±0.90^a	2.64±0.93^b
Total coliforms	4.59±0.85^a	2.87± 0,32^b
Fecalcoliforms	2.21±0.95^a	1.07±0.73^b
Psychrotrophic bacteria	6.02±1.23^a	5.01±1.90^b
Micrococci	2.54±0.91^a	1,60±0.56^b
Yeast	4.60±0.90^a	3.58±1.10^b
Mold	4.68±1.30^a	3.82±1.65^b
Coagulase-positive staphylococci	0.54±0.12^a	0.42±0.05^a

The study of the variability of the microbiota in goat milk in northern Morocco highlights a substantial variation between winter and spring. However, despite the large differences between the two seasons, a similarity within the main microbial groups has been observed.

With 6.02 $\log_{10}$cfu/mL in spring and with a slight decrease in winter with 5.01 $\log_{10}$cfu/mL, psychrotrophic bacteria are the most dominant microbial category.

On the other hand, mesophilic and thermophilic lactic acid bacteria were detected with a significant difference (p<0.05) between the seasons, it is equally noted that the hierarchy of these groups of microorganisms is the same for both seasons.

Total coliform levels relayed extensively on the season (4.59 $\log_{10}$cfu/mL in spring and 2.87 $\log_{10}$cfu/mL in winter, during the spring period, the bacterial rates were roughly equal to those of yeast and mold (4.60 $\log_{10}$cfu/mL and 4.68 $\log_{10}$cfu/mL respectively).

For undesirable microorganisms: fecal coliforms, enterococci, micrococci and coagulase positive staphylococci, low survival rates have been detected especially during the winter season (1.07 $\log_{10}$cfu/mL, 2.64 $\log_{10}$cfu/mL, 1.60 $\log_{10}$cfu/mL and 0.42 $\log_{10}$cfu/mL).

Table 3 demonstrates the microbial variability in the different farms studied over the two seasons: spring and winter.

This study proves that seasonal variations influence the microbial composition of the studied goat milk samples.

A Comparative study of the obtained results allowed concluding that the milk samples obtained from Farm A exhibited a higher microbiological quality, most notably during the winter season due to the low level of undesirable flora (Fecal coliforms at an order of 0.21 $\log_{10}$cfu/mL) and a total absence of coagulase-positive staphylococci).

It was equally deducted that milk samples from farms A, E, F, G, and H may be of industrial interest for the production of cheese considering their abundance in microorganisms with technological interest (Mesophilic and thermophilic lactic bacteria). However, coliforms are detected with levels roughly equal to mesophilic lactic acid bacteria in farms E and H.

Overall, the total number of the investigated microorganisms decline during the winter season, with the exception of staphylococci which were unable to survive through winter in farm B despite their presence in the spring season at an order of 0.1 $\log_{10}$cfu/mL.

The aerobic flora was the most prevalent in farm E with a viability level estimated at 8.88 $\log_{10}$cfu/mL in comparison with other farm samples followed by farm H.

Yeasts were found regularly in all samples, the general average was 3.96 log_{10}cfu/mL, and the fluctuation range was from 2.10 log_{10}cfu/mL to 5.95 log_{10}cfu/mL, while the average value of mold detected was estimated at 4.25 log_{10}, the rates varied between 1.99 log_{10}cfu/mL and 5.33 log_{10}cfu/mL.

Bacterial organisms belonging to the *Enterococcus* genus were found in all of the studies zones with a noted significant difference (p<0.05) in comparison with zone A which is characterized by a seasonal average of 1.1 log_{10}cfu/mL.

Regarding psychrotrophic bacteria, the variability in cell count rates presented a significant difference between all of the studied zones (p<0.05).

Micrococci were present in samples obtained from E,F,G, and H zones, however, zone A exhibited a significantly low level of the strains in comparison to other farms (p<0.05).

S. aureus was not detected in Farm A during both seasons, as for Farm B, low levels of the strain were detected in spring (0.1 log_{10}cfu/mL) whereas it was completely absent during winter. While in other farms, levels decreased significantly (p<0.05) from spring to winter with the exception of farm E.

Table 3 Microbiological characteristics of goat milk samples during spring and winter (mean values and standard deviations were expressed in log_{10}cfu/mL) (n=110). Values with different letters denote a statistically significant difference at a 5% level of significance (p <0.05).

Farms		A	B	C	D	E	F	G	H
Number of samples		7	5	6	4	3	15	10	5
Total Mesophilic Flora	Spring	5.5±0.47^{d}	7.25±0.5^{bc}	7.16±0.25^{c}	6.59±0.62^{cd}	8.88±0.24^{a}	6.3±0.55^{cd}	7.15±0.89^{bc}	7.99±0.21^{b}
	Winter	4.69±0.52^{e}	5.25±0.09^{e}	5.61±0.35^{e}	5.55±1.10^{de}	6.95±0.15^{c}	5.,33±0.25^{e}	6.89±0.36^{cd}	6.15±0.73^{de}
Mesophilic lactic acid bacteria	Spring	3.20±0.10^{d}	3.56±0.15^{d}	5.70±0.25^{b}	5.60±0.25^{b}	7.50±0.66^{a}	5.57±0.52^{b}	6.65±0.35^{a}	6.70±0.20^{a}
	Winter	2.55±0.15^{e}	3.02±0.70^{de}	4.65±0.25^{c}	4.25±0.59^{cd}	6.10±0.20^{b}	4.58±0.21^{c}	4.50±0.75^{c}	4.75±0.46^{c}
Thermophilic lactic acid bacteria	Spring	5.20±0.25^{b}	3.69±0.65^{c}	5.60±0.95^{abc}	4.90±1.10^{abc}	3.90±1.02^{bc}	5,68±0.09^{a}	4.98±1.56^{ab}	5.25±0.37^{ab}
	Winter	3.90±0.55^{a}	3.25±1.65^{a}	4.90±1.98^{a}	3.67±1.69^{a}	2.98±1.60^{a}	3.66±1.98^{a}	4.21±0.65^{a}	4.96±0.99^{a}
Enterococci	Spring	1.25±0.05^{f}	2.6±0.12^{d}	3.5±0.08^{b}	3.65±0.16^{b}	4.65±0.25^{a}	4.52±0.36^{a}	3.65±0.17^{b}	4.21±0.31^{a}
	Winter	0.95±0.11^{g}	2.10±0.02^{e}	2.55±0.17^{de}	2.90±0.15^{c}	3.10±0.27^{c}	3.50±0.25^{b}	2.78±0.20^{cd}	3.21±0.28^{bc}
Total coliforms	Spring	2.56±0.25^{f}	4.96±0.30^{b}	4.53±0.15^{b}	4.49±0.10^{b}	5.96±0.22^{a}	3.96±0.15^{c}	4.32±0.22^{bc}	5.9±0.31^{a}
	Winter	2.02±0.10^{g}	2.15±0.16f^{g}	2.55±0.08^{f}	3.50±0.13^{d}	3.50±0.20^{d}	3.02±0.12^{e}	3.15±0.11^{e}	3.08±0.09^{e}
Fecal coliforms	Spring	0.56±0.03^{k}	1.25±0.05^{gh}	2.40±0.16^{c}	2.20±0.02^{d}	3.5±0.05^{a}	1.65±0.03^{f}	2.98±0.16^{b}	3.15±0.02^{b}
	Winter	0.21±0.05^{l}	0.66±0.02^{j}	1.15±0.06^{h}	1.65±0.08^{f}	1.99±0.06^{e}	0.55±0.02^{k}	0.98±0.03^{i}	1.35±0.05^{g}
Psychrotrophic bacteria	Spring	4.98±0.32^{d}	6.15±0.49^{bc}	5.91±0.21^{c}	6.5±0.30^{b}	7.62±0.55^{a}	6.12±0.45^{bc}	5.62±0.42^{cd}	5.23±0.46^{d}
	Winter	3.02±0.25^{f}	5.26±0.32^{d}	4.11±0.20^{e}	5.68±0.33^{cd}	6.55±0.50^{b}	5.66±0.60^{cd}	4.88±0.35^{d}	4.90±0.35^{d}
Micrococci	Spring	0.96±0.05^{h}	1.92±0.05^{f}	2.63±0.06^{c}	2.73±0.08^{c}	3.54±0.04^{a}	2.5±0.06^{d}	2.41±0.07^{d}	3.65±0.07^{a}
	Winter	0.22±0.01^{i}	1.02±0.02^{h}	1.55±0.04^{g}	1.65±0.04^{g}	2.95±0.08^{b}	1.23±0.03^{g}	2.01±0.08^{f}	2.20±0.02^{e}
Yeast	Spring	3.01±0.25^{d}	3.91±0.35^{bc}	4.25±0.58^{bc}	2,16±0.16^{f}	5.95±0.78^{a}	5.42±0.54^{ab}	4.95±0.48^{ab}	5.12±0.69^{ab}
	Winter	2.10±0.15^{f}	2.50±0.13^{e}	3.15±0.35^{cd}	3.90±0.20^{c}	4.25±0.75^{bc}	4.95±0.70^{ab}	3.12±0.31^{d}	4.65±0.55^{ab}
Mold	Spring	2.95±0.12^{d}	4.81±0.26^{ab}	4.15±0.13^{b}	4.65±0,35^{ab}	5.25±0.25^{a}	5.10±0.35^{a}	5.33±0.55^{a}	5.21±0.30^{a}
	Winter	1.99±0.14^{e}	3.64±0.25^{c}	4.01±0.23^{bc}	3.65±0,30^{c}	4.22±0.19^{b}	4.61±0.29^{ab}	4.95±0.51^{a}	3.50±0.22^{c}
Coagulase-positive staphylococci	Spring	0.00±0.00	0.1±0.02^{d}	0.69±0.25^{b}	0.7±0.18^{b}	1.12±0.13^{a}	0.95±0.15^{ab}	0.63±0.14^{bc}	0.12±0.02^{d}
	Winter	0.00±0.00	0.00±0.00	0.1±0.20^{d}	0.25±0.05^{d}	1.05±0.02^{a}	0.65±0.10^{bc}	0.55±0.09^{c}	0.75±0.12^{b}

Physicochemical characteristics

Table 4 demonstrates the results obtained from the screening of goat milk during the spring and winter season.

Table 4 Physicochemical characteristics of raw goat milk according to the season, spring (n=55) and winter (n=55). Different letters indicate a significant difference at a 5% level of significance (p<0.05).

	Seasons		
	Spring	Winter	Mean±SD
	Mean±SD	Mean±SD	
pH	6.5±1.10^{a}	5.02±0.90^{a}	5.76±1.00
Fat content (%)	4.2±0.65^{a}	4.9±1.01^{b}	4.55±0.83
Dry Matter (%)	12.05±0.61^{a}	13.98±0.95^{b}	13.01±0.78
Ash (%)	0.8±0.05^{a}	1.3±0.12^{b}	1.05±0.08
Lactose g/L	4.5±1.2^{a}	3.1±0.65^{b}	3.80±0.92
Proteins g/L	3.9±0.61^{a}	4.6±1.03^{b}	4.25±0.82

The obtained results show that the chemical composition of goat milk varies over the seasons. The average pH level of raw milk is 6.50 in spring and 5.02 in winter (p>0.05). Meanwhile, Fat, lactose, and protein levels varied significantly across the two seasons (p<0.05). The lactose content decreased significantly with the seasons (p<0.05) reaching a concentration of 4.5g/L in spring and registering a remarkable decrease in winter with a concentration of 3.1g/L.

The average value in dry matter (DM) fluctuates significantly over the seasons (p<0.05), with the highest value obtained over the winter (13.98%) and the lowest value in spring (12.05%).

The range of ash content varied significantly from one season to another (p<0.05), it was noted that this component increases with the season (0.8% in spring and 1.3% in winter).

Fatty acid profile

Goat milk varied in FA composition being mainly higher in saturated fatty acids (SFA) in both seasons with average values of 73.87% and 72.47% for spring and winter respectively, followed by monounsaturated fatty acids, the FA profile varied according to season and nature of the components found in the sample.

Statistically significant differences of goat milk FA profile between seasons were found among 10 of the 23 fatty acids detected (p<0.05), short and medium-chain fatty acids (C4:0 ; C6:0) decreased significantly during the winter recording a decline from 2.75% to 2.22%and 2.5% to 2.1% respectively, the same trend was noticed for long-chain fatty acids C14:1 ; C16:0 and C17:0 yielding a percentages from 0.41 ; 29.03% and 0.78% during the spring to 0.32% ; 26.44% and 0.62% in the winter season. C20:0; C20:2 were equally present in goat milk at significantly higher values during the spring season in comparison to their concentration in the winter (p<0.05). Whereas the following fatty acids increased significantly: C10:0 (10.01% - 12.19%) ; C18:1 (19.03%- 23.12%); C18:3 (0.62%- 0.77%).

Table 5 Fatty acid composition in goat milk fat according to seasonal variations. Spring (n=55) and winter (n=55). Different letters indicate a significant difference at a 5% level of significance (p<0.05).

Free fatty acids (% of total FA)	Seasons	
	Spring	Winter
C4:0	2.75±0.25 ^{a}	2.22±0.21 ^{b}
C6:0	2.50±0.11 ^{a}	2.10±0.22 ^{b}
C8:0	2.18±0.07 ^{a}	2.15±0.13 ^{a}
C10:0	10.01±1.76 ^{a}	12.19±1.81 ^{b}
C11:0	0.09±0.008 ^{a}	0.14±0.005 ^{a}
C12:0	3.79±0.89 ^{a}	3.88±0.91 ^{a}
C13:0	0.12±0.02 ^{a}	0.11±0.02 ^{a}
C14:0	9.12±2.01 ^{a}	9.14±1.96 ^{a}
C14:1	0.41±0.02 ^{a}	0.32±0.01 ^{b}
C15:0	0.76±0.11 ^{a}	0.55±0.06 ^{a}
C15:1	0.04±0.002 ^{a}	0.06±0.001 ^{a}
C16:0	29.03±1.02 ^{a}	26.44±1.51 ^{b}
C16:1	0.80±0.05 ^{a}	0.81±0.07 ^{a}
C17:0	0.78±0.06 ^{a}	0.62±0.08 ^{b}
C17:1	0.21±0.02 ^{a}	0.22±0.01 ^{a}
C18:0	12.43±2.15 ^{a}	12.68±3.01 ^{a}
C18:1	19.03±3.12 ^{a}	23.12±2.86 ^{b}
C18:2 n-6 *trans*	0.34±0.07 ^{a}	0.35±0.06 ^{a}
C18:2 n-6 cis	2.37±0.72 ^{a}	2.17±0.56 ^{a}
C18:3	0.62±0.05 ^{a}	0.77±0.08 ^{b}
C20:0	0.31±0.003 ^{a}	0.25±0.04 ^{b}
C20:1	0.08±0.001 ^{a}	0.08±0.001 ^{a}
C20:2	0.05±0.001 ^{a}	0.04±0.003 ^{b}
SFA	73.87 ^{a}	72.47 ^{b}
MUFA	20.49 ^{a}	24.61 ^{b}
PUFA	3.38 ^{a}	3.33 ^{a}

SFA: Saturated fatty acids; MUFA:Monounsaturated fatty acids; PUFA:Polyunsaturated fatty acid.

The content in polyunsaturated fatty acids was between 3.38% and 3.33% with higher values in spring, however, no significant discrepancies were found in PUFA content between the two seasons.C18:2 n-6 *trans* remained relatively stable across

the seasons, the percentages present in the tested sample were fixed at 0.34% in the spring and 0.35% during the winter, contrary to C18:2 n-6 *cis* which was present at a percentage of 2.37% in the spring only to decline to 2.17%, nevertheless, the values did not vary significantly (p >0.05). Long-chain fatty acids C20:1 and C15:1 along with medium-chain fatty acid C11:0 were present in trace values with percentages not exceeding 0.1%. However, the lowest value recorded in both seasons was detected with C20:2 with an average of 0. 05% in spring and 0. 04% during the winter season.

Identification of LAB isolates

Out of a total of 121 isolates obtained in the present study, 104 strains were identified as *Enterococcus* spp. Which was the predominant genus (85 %). Among the LAB isolates obtained from the North-Morocco raw goat's milk, eleven (9% of the total) were assigned to the genus *Lactococcus*, seven (6%) of these isolates were identified as *L. lactis* subsp. *lactis cit+* (the former diacetylactis variety), which were the predominant lactococci in North-Morocco raw goat's milk.
Six isolates (5 % of the total) have been assigned to the genus *Lactobacillus*, in which four of these isolates were identified as *Lactobacillus plantarum*, one as *Lactobacillus casei* and one as *Lactobacillus paracasei*

Flavors produced by LAB in pasteurized whole milk

The isolates of *Lactococcus* spp.; *Lactobacillus* spp. obtained in the present study were initially subjected to evaluation (by smelling) of the flavors produced in pasteurized whole milk after 24 and 48 h of incubation at 30 °C, with a view of carrying out a preliminary screening of LAB to identify any strains of interest from a technological point of view. Results, reported in terms of the predominant flavor or combination of flavors detected in each strain of the milk cultures, are shown in Table 6.

Properties of the selected LAB related to growth in milk

The results obtained from the tests to characterize the selected isolates, in regards to their behavior in milk (acidifying and proteolytic activities, and diacetyl-acetoin production) are shown in Table 7.

Table 6 Main flavors detected in the cultures of LAB isolates in pasteurized whole milk after 24 and 48 h incubation

Group of LAB	Buttery	Buttery/yoghurt	Buttery/malty	Yoghurt	Malty	Cooked milk/sulfide
Lactococci						
L. lactis subsp. *lactis*	3	1	-	1	-	-
L. lactis subsp. *lactis* var. *diacetylactis*	2	1	1	1	1	-
Lactobacilli						
Lactobacillus plantarum	3	1	-	-	-	-
Lactobacillus casei		1	-	-	-	-
Lactobacillus paracasei	1					

–: not detected.
The lactic flavor most frequently detected was a buttery flavor (ripe butter), which was appreciated in 9of the milk cultures, in 4 cases, this flavor was described together with a yogurt flavor, whereas, it was reported to be accompanied with a malty taste in 1case.

Table 7 Properties related to growth in milk (acidifying activity, proteolytic activity, and diacetyl production) of the selected LAB isolates

		Acidifying activity		Proteolytic activity (mg Tyr L^{-1})	Diacetyl-acetoin Production (mg diacétyle L^{-1})
	Code	pH 6 h	pH 24 h	-	-
Lactococcus lactis spp. *lactis*	LC1	4.84	4.22	42.24±0.87	25.76±1.22
Lactococcus lactis spp. *lactis*	LC2	4.85	4.21	30.75±1.02	30.21±0.34
Lactococcus lactis spp. *lactis*	LC3	4.82	4.20	43.68±0.97	31.65±0.67
Lactococcus lactis spp. *lactis*	LC4	4.87	4.32	31.92±1.25	28.44±1.02
Lactococcus lactis spp. *lactis*	LC5	4.84	4.24	30.81±0.67	27.89±0.56
Lactococcus lactis spp. *lactis* var. *diacetylactis*	LC1D1	4.35	4.08	28.46±0.89	26.30±0.46
Lactococcus lactis spp. *lactis* var. *diacetylactis*	LC1D2	4.56	4.26	32.12±1.12	29.45±1.01
Lactococcus lactis spp. *lactis* var. *diacetylactis*	LC1D3	4.56	4.24	36.52±1.34	32.56±1.07
Lactococcus lactis spp. *lactis* var. *diacetylactis*	LC1D4	4.57	4.17	37.54±1.45	38.67±1.78
Lactococcus lactis spp. *lactis* var. *diacetylactis*	LC1D5	4.52	4.24	29.44±0.67	27.23±1.33
Lactococcus lactis spp. *lactis* var. *diacetylactis*	LC1D6	4.51	4.22	35.13±1.86	35.34±0.67
Lactobacillus plantarum	LBP1	5.87	4.71	38.72±2.12	27.85±0.32
Lactobacillus plantarum	LBP2	5.96	4.66	38.66±2.09	27.89±0.91
Lactobacillus plantarum	LBP3	5.90	4.75	35.63±1.77	22.45±1.05
Lactobacillus plantarum	LBP4	5.92	4.61	37.52±0.83	26.70±1.55
Lactobacillus casei	LBC1	6.04	4.64	17.95±0.12	35.91±1.67
Lactobacillus paracasei	LBPC1	6.11	4.75	66.93±2.63	54.83±0.46

The 11 tested lactococci presented a high acidifying profile with pH values situated between 4.87 and 4.35 after 6h of incubation. The pH value continued to decrease over the course of the incubation period with LC1D1 recording the lowest value of 4.08 after 24h. Meanwhile, *Lactobacillus* strains were capable of inducing an acidifying effect ranging from 5.87 (LBP1) to 6.11 (LBPC1) during the first 6 h of incubation, and 4.61 (LBP4) and 4.75 (LBP3- LBPC1) by the end of the incubation period.
Proteolytic activities of the Lactococcal isolates, as determined by the IDF test were in the range 30.75-43.68 Tyr L^{-1} of *L. lactis* subsp. *lactis* (mean value of 35.88 mg Tyr L^{-1}), and 28.46-37.54 Tyr L^{-1} of *L. lactis* subsp. *lactis* var. *diacetylactis* (mean value of 33.20 mg Tyr L^{-1}).
Proteolytic activities of the selected lactobacilli ranged between 17.95 - 66.93 Tyr L^{-1} (mean value of 39.23 mg Tyr L^{-1}) in the IDF test. The highest proteolytic activity was also observed for strains of *L. paracasei*LBPC1 (Values higher than 50 mg Tyr L^{-1}).
The *Lactobacillus paracasei* was the group that produced the largest amounts of diacetyl-acetoin in milk, with one isolate producing more than 50 mg diacetyl/L.
The tested strains showed a varying autolytic profile, the results presented in table 8 indicate a spectrum of autolytic activity limited between 8.56% and 67.34%, with *Lactobacillus plantarum* (LBP1) exhibiting the highest lysis rate. LBP2 was equally highly autolytic with a rate of 50.44 %, however, strains of the same species revealed a significantly lower effect (26.90 % and 14.51 % respectively for LBP3 and LBP4). Meanwhile, the autolytic extent of *Lactococcus lactis* spp. *lactis* strains (LC1, LC2, LC3,LC4,and LC5) was moderate, with values ranging from 15.50 %; 9.30 %; 24.61 %; 13.43 %; and 25.92 % respectively; however not lower than the lyses potential obtained by *Lactobacillus paracasei* (LBPC1) with percentage value estimated at 8.56%. Regarding *Lactococcus lactis* spp. *lactis* var. *diacetylactis*, some of the highest autolytic potentials were detected within these strains, LC1D6 expressed the second highest percentage with a rate estimated at 57.50 %, followed by LC1D2 (50.50 %), LC1D1 and LC1D5 coming as close seconds with an equally strong ability to auto-aggregate at rates of 46.44 %and 47.23 % respectively.
None of the isolated strains revealed to possess the potential to break down milk fat, however, the investigation of EPS production in the tested LAB isolates showed that some strains are active exopolysaccharide producers, these strains include one *Lactococcus lactis* spp. *lactis* strain (LC1); two strains of *Lactococcus lactis* spp. *lactis* var. *diacetylactis* (LC1D1 and LC1D4); all of the isolated strains of *Lactobacillus plantarum* as well as *Lactobacillus paracasei* (LBPC1).
Table 8 equally illustrates the inhibitory profile of the isolated strains against three pathogenic bacteria. Of the 17 lactic acid bacteria tested. Nine strains produced an inhibition zone against at least one pathogenic strain by the well diffusion assay. *S. aureus* was the most susceptible strain, showing sensitivity in the presence of LC1D2 (12 mm), LC1D5 (14.7 mm), LC1D6 (12.4 mm), LBP1 (13.10 mm), LBP2 (12.2 mm), and LBPC1 (13.1 mm). LC5 was inhibitory towards both *L. monocytogenes* and *E. coli* with inhibition zones reaching 17 mm and 12.1 mm respectively. Meanwhile, LBP3 and LBC1 were only inhibitory towards *L.*

monocytogenes with an antibacterial effect reaching 22.9 mm for LBP3 which is the highest value obtained for all strains, and 13.1 mm for LBC1.

The halotolerance of the goat milk isolates was equally studied by submitting the bacterial strains to increasing concentrations of NaCL in the incubation medium (2-6 and 10%). The results obtained are summarized in table 7. All LAB strains depicted an ability to survive in the presence of 2 % NaCL, this halotolorence was further confirmed whilst increasing the salinity concentration to 6%, where all strains maintained their growth with the exception of LC2 and LBP3, however, no viable cells were recovered from *Lactococcus lactis* spp *lactis* strains after further increasing the concentration to 10% with the exception of LC5 strain, as for *Lactobacillus plantarum*, only LBP1 was able to survive at this concentration, *Lactobacillus casei* and *Lactococcus lactis* spp. *lactis* var. *diacetylactis* strains LC1D1, LC1D2 and LC1D6 similarly reported a bacterial growth in presence of a high NaCL percentage.

Table 8 EPS production, halotolerance, autolytic, lipolytic and antibacterial activities of LAB strains from Moroccan raw goat milk

	Code	Autolysis %	EPS Production	Lipolytic activity	Antibacterial activity (Inhibition zones in mm) a			Growth capacityat different NaCl (%)		
					Staphylococcus aureus	*Listeria monocytogenes*	*Escherichia coli*	2	6	10
Lactococcus lactis spp. *lactis*	LC1	15.50±1.1	+	-	0.0±0.0	0.0±0.0	0.0±0.0	+	+	-
Lactococcus lactis spp. *lactis*	LC2	9.30±0.12	-	-	0.0±0.0	0.0±0.0	0.0±0.0	+	-	-
Lactococcus lactis spp. *lactis*	LC3	24.61±0.55	-	-	0.0±0.0	0.0±0.0	0.0±0.0	+	+	-
Lactococcus lactis spp. *lactis*	LC4	13.43±0.25	-	-	0.0±0.0	0.0±0.0	0.0±0.0	+	+	-
Lactococcus lactis spp. *lactis*	LC5	25.92±0.27	-	-	0.0±0.0	17.00±0.12	12.10±0.35	+	+	+
Lactococcus lactis spp. *lactis* var. *diacetylactis*	LC1D 1	46.44±2.12	+	-	0.0±0.0	0.0±0.0	0.0±0.0	+	+	+
Lactococcus lactis spp. *lactis* var. *diacetylactis*	LC1D 2	50.50±0.89	-	-	12.00±0.67	11.00±0.23	0.0±0.0	+	+	+
Lactococcus lactis spp. *lactis* var. *diacetylactis*	LC1D 3	10.72±0.08	-	-	0.0±0.0	0.0±0.0	0.0±0.0	+	+	-
Lactococcus lactis spp. *lactis* var. *diacetylactis*	LC1D 4	33.33±0.87	+	-	0.0±0.0	0.0±0.0	0.0±0.0	+	+	-
Lactococcus lactis spp. *lactis* var. *diacetylactis*	LC1D 5	47,23±0.12	-	-	14.70±0.37	10.5±0.137	0.0±0.0	+	+	-
Lactococcus lactis spp. *lactis* var. *diacetylactis*	LC1D 6	57.50±1.35	-	-	12.40±0,21	0.0±0.0	17.1±0,31	+	+	+
Lactobacillus plantarum	LBP1	67.34±1.76	+	-	13.10±0.26	0.0±0.0	0.0±0.0	+	+	+
Lactobacillus plantarum	LBP2	50.44±2.12	+	-	12.20±0.45	0.0±0.0	11.20±0.24	+	+	-
Lactobacillus plantarum	LBP3	26.90±1.34	+	-	0.0±0.0	22.9±0.23	0.0±0.0	+	-	-
Lactobacillus plantarum	LBP4	14.51±0.25	+	-	0.0±0.0	0.0±0.0	0.0±0.0	+	+	-
Lactobacillus casei	LBC1	28.21±0.27	-	-	0.0±0.0	13.1±0.36	0.0±0.0	+	+	+
Lactobacillus paracasei	LBPC 1	8.56±0.26	+	-	13.1±0.45	0.0±0.0	0.0±0.0	+	+	-

DISCUSSION

Microbiological content of raw goat milk

The levels of microbial flora are often highly variable within a region, partly due to seasonal effects. This concept superimposes the effect of the duration, along with extrinsic variations of the temperatures and durations of the day, as well as the effect of the lactation stage. However, practice and habits may vary from season to season making it difficult to distinguish between the respective impacts. According to our study, the microbial population observed decreased significantly from spring to winter ($p < 0.05$), except for *S. aureus* which was already detected at trace amounts in both seasons. The results are in accordance with **Tormo *et al.* (2011)**;**Salmeron *et al.* (2002)** and **Bouton *et al*. (2005)** who reported the presence of a seasonal variation within the detected microbial groups, with an overall high prevalence during the spring/summer season. In contrast, **Delgado Pertiñez *et al*. (2003)** recorded the highest levels during January and April. These variations are in part due to the impact of temperature on the growth and maintenance of microbial populations, and in another part by the lactation stage.

However, despite the seasonal differences, the dominant microbiota is actually the same with a predominance of psychrotrophic flora (6.02 $\log_{10}$cfu/mL and 5.01 $\log_{10}$cfu/mL in spring and winter). Similarly to the findings of **Raynaud *et al.* (2005)**.

The aromatic richness of raw cheese depends significantly on the milk microflora **(Bouton, 2005)**. Lactic bacteria are systematically detected in milk with a notable predominance **(Casalta *et al*., 2009;Ercolini *et al*., 2009;Mallet *et al*., 2010)**. Indicating that microbial species depend on farm practices, starting from udder care and sanitary measures to the animal's surrounding environment.

The level of technologically interesting microbiota detected is higher than that of the alteration microbiota, similarly to **Michel *et al*.(2001)** and **Tormo *et al*.(2006)**. The levels of TAMF found in all the farms studied are higher than those reported by **Tormo *et al*.(2011)**, and the existing variations are also correlated to the production area. This variability can be attributed to production and climatic conditions which differ depending on the regions investigated. The levels of micrococci remain low compared to other microbial groups, contrary to other studies that reported their sub-dominance **(Garcia *et al*., 2009;Callon *et al*., 2007)**.Low levels of yeast and mold were detected (rates below 1.0 $\log_{10}$cfu/g), but highly variable depending on the farms and consequently on the production practices, which is in consistence with the findings of **Torkar *et al*.(2008)**.Since intra-mammary milk is sterile, animal surroundings, tests, milking equipment and the environment have been suspected to be reservoirs of microbial species**(Michel *et al*., 2005)**.

Majority of data concerning microorganisms found on the teat surface before preparation has been screened for dairy cows. **Bouton *et al*. (2005)** and **Michel *et al*. (2001, 2005)** revealed multiple microbiota on the teat surface (9 detected out of 11 investigated strains according to **Michel *et al*. (2005)**), and that cheese production microbiota could be 100 times more prevalent than the alteration microflora **(Michel *et*al., 2005)**.

Coliforms are detected in 95% of samples at levels between 10^3 and 10^4 cfu per teat surface unit. Meanwhile, pathogenic microorganisms are part of the minority microflora. **Monsallier *et al*. (2009)** and **Michel*et al*. (2005)** both categorized microflora groups (of interests, alterations and pathogenic), in this study, surface and refining microflora were predominant and lactic microflora is only sub-dominant and consists mainly of enterococci.

Evidently, the contact between the bedding and the teat affects the microbial community on the surface of the teat with a notable correlation **(Zdanowicz *et al*., 2004; Bouton *et al*., 2007)**. Previous research suggested that careful hygiene (Washing with detergent, bleach, rinsing and the use of disinfectant) can reduce the udder microbiota by 100 to 1000 factors **(Piton *et al*., 1982)**. For example, **Ménard *et al*. (2004)** showed that twice-daily mulching decreases *E. coli* and enterococcal contamination. This reinforces the assumption that the animal's environment is a source of milk microbiota. However, further research is needed to specify the types of microorganisms involved, their transmissibility and selectivity, and the order of practices that affects this pathway.

Physicochemical analysis of raw goat milk

The average pH (5.76 ±1.00) obtained is lower than that reported by other authors **(Serhan, 2009)**. Conversely, the average protein content (4.25 ±0.82) is higher than that obtained by **Guo *et al*. (2004)**. The content in fatty composition was estimated at 4.55 ±0.83, which is similar to previous reports **(Olarte *et al*., 1999)** and lower than those reported by **Kouniba *et al*.(2007)**. Lactose average (3.8 ±0.92) is lower than previously cited values **(Serhan, 2009)**. While ash (1.05 ±0.08) is higher **(Kouniba *et al*., 2007)**. Dry matter (13.01 ±0.61) is higher than the values reported by **Guo *et al*.(2004)**, in this study the average values were estimated at 0.83 ±0.04 and 12.38 ±0.71.Whereas the mean protein content is similar to the values indicated by **Kouniba *et al*.(2007)**. According to **Park.(2007)**, variation in the chemical composition of goat milk can be linked to several parameters such as season, breed, diet, lactation stage, geographic location, environmental conditions, and herd health status.

Within the tested group, fatty acids displayed a profile distinctive to goat milk and values of components from Butyric (C4:0) to Caprylic acids (C8:0)are in agreement with **Lopez *et al*.(2019)**, while the percentage of: lauric (C12:0); myristic (C14:0) ; pentadecylic (C15:0) and arachidic acids (C20:0) occurred similarly to those reported recently **(Wang *et al*., 2022)**. Our results are in agreement with those mentioned by **Moate *et al*.(2007)** and **Toyes-Vargas *et al*.(2013)** in terms of classification by type of FA. Milk samples of ruminants primarily consist of saturated fatty acids (65–70%) followed by unsaturated fatty acids (30 to 35%). Within saturated fatty acids, around 10–12% are short- and medium-chain fatty acids and 50–55% are long-chain fatty acids. This category consist of stearic (8–10%), myristic (10–12%), and palmitic acids (25–27%). As for unsaturated fatty acids, 25–30% are monounsaturated, with oleic acid being the second most common in milk fat **(Gortzi *et al*., 2022)**. This typical combination is a result of rumen microbial bio-hydrogenation **(Toyes-Vargas *et al*., 2013)**.FA composition can vary depending on different factors including animal breed, the lactation stage, geographical location, season, and feed composition **(Gortzi *et al*., 2022)**, the results obtained reveal that the fatty acid composition is season-dependant with a notable decrease (Saturated fatty acids) during the winter season, similarly to **Delgadillo Puga *et al*. (2009)**.

Overall, the content in fat, dry matter, ash, lactose, and protein is predominantly affected by the seasonal variations (progression in lactation stage). Indeed, the fat content of goat milk increases with the lactation stage while the lactose content decreases **(Haenlein *et al*., 2006)**, which highlights the importance of individual variabilities (lactation stage in particular) in order to control the composition of milk intended for cheese production. However, **Olarte *et al*. (1999)** did not detect any significance between seasons, which was due to the animals' feeding program which remained unchanged during the period of cheese production (April - July).

Technological properties of Lactic acid bacteria isolated from raw milk samples

Diacetyl production from citrated milk is a crucial factor for starter cultures, as it contributes to the aroma of many dairy products **(Mayo *et al*., 2010)**. Similarly, to our work, **Câmara *et al*. (2019)** found that diacetyl production by LAB of dairy origin is strain dependent. Interestingly, most industrial cheese starters rely on a single species, *L. lactis*, due to its acidifying and flavor properties. Many strains of this species are employed in the production of various cheeses which exhibit different characteristics but share several biochemical attributes **(Crow *et al*., 1993)**.

Cogan *et al*. (1997) proposed that a good acidifying Lactococcal strain should reduce the pH value of the milk to below 5.3 after 6 h incubation at 30°C. Therefore, the majority of the selected Lactococci isolated in the present study could be employed as acidifying starters.

EPS production is one of the most important characteristics exhibited by LAB, since they enhance the texture and viscosity of the end product **(Fguiri *et al*., 2016; Meng *et al*., 2018)**. Three *Lactococcus lactis* spp. *lactis*, four *Lactobacillus plantarum*, and one *Lactobacillus paracasei* were EPS producers. Which is in accordance with the findings of **Dertli *et al*. (2016)** and **Islam *et al*.(2021)**. This production depends primarily on the presence of sugar (glucose) in the growth medium (Glucose-based media) **(Cerning *et al*., 1994; Islam *et al*., 2021)**. EPS contribute largely to the smoothness and creaminess of dairy products **(Islam *et al*., 2021)**. Other advantages include the reduction of the products' caloric content, lowering cholesterol levels, as well as immunomodulatory, antitumorigenic, and probiotic properties **(Angelin *et al*., 2020; Castro-Bravo *et al*., 2018; Liu *et al*., 2017)**. Thus, the EPS content is a key factor in selecting LAB strains as starters.

None of the isolated strains revealed lipolytic effect, however, a significant proteolytic and autolytic potentials were registered, the autolytic activity is of great significance since it plays a key role during cheese ripening **(Piraino *et al*., 2008)**, promote the production of flavors, affect proteolysis, and reduce the bitterness of cheese through peptide hydrolysis **(Meng *et al*., 2018; Nieto-Arribas *et al*., 2010)**. Therefore, our isolates are interesting candidates for cheese formulation.

All the strains were able to grow at 2 and 6% NaCL with the exception of LC2 and LBP3 strains whose growth was repressed at 6% NaCL . Similarly, LABs obtained from goat milk in Northeast Brazil also showed high halotolerance **(de Almeida Júnior *et al*., 2015)**. Industrial LABs used as cheese starters require a high tolerance to stress conditions. Different halotorenace mechanisms have been identified, including the uptake or synthesis of a limited number of solutes **(Bremer *et al*., 2000; de Almeida Júnior *et al*., 2015)**.

LABs are frequently screened for their antibacterial effect for a potential application as biopreservatives. The highest inhibition zone was observed against *L. monocytogenes* with *Lb. plantarum* (LBP3) (22.9 mm), the lowest effect belonged to *L. lactis* (LC1D5) (10.5 mm), while LC1D5 inhibited *S. aureus* at a rate of 14.7 mm. Only LC5, LC1D6, and LBP2 inhibited *E. coli.* Similarly to **Kong *et al*.(2020)** and **Mareze *et al*. (2022)**. This potential is mainly exerted through the release of organic acids (Acetic, lactic, succinic acids…) which reduce the pH value and disrupt various metabolic pathways. In addition, LABs can also produce bactericidal molecules (Diacetyl (2,3-butanedione), H_2O_2,and bacteriocines) **(Ağagündüz *et al*., 2022)**.

CONCLUSION

The results obtained in this study show that the microbial composition of raw goat milk is highly variable, with notable seasonal variations. Milk from farms A, E, F, G, and H were found to be dominated by microbiota of technological importance, most particularly during the spring season. Desirable physical and chemical characteristics (Lactose, Protein, Dry matter…) in goat milk were more prominent in the winter. FA recorded a higher presence in the spring season, making this season the most suitable for the collection of milk samples with desired physicochemical and microbiological characteristics for industrial cheese making. The different LAB samples isolated from goat milk exhibited several technological and probiotic properties. The main findings demonstrated in this study in terms of EPS production, autolysis, *in vitro* inhibition of pathogens as well as the strong halotolorence demonstrated by the isolated strains, allowed the distinction of several species as starter cultures for cheese production with a high level of hygienic and organoleptic properties as well as highlighting their potential application as bio-preservatives in the food industry.

Acknowledgements: This study has been financed by the project of the Academy Hassan II of Science and Technique.

REFERENCES

Ağagündüz, D., Şahin, T. Ö., Ayten, Ş., Yılmaz, B., Güneşliol, B. E., Russo, P., Spano, G., & Özogul, F. (2022). Lactic acid bacteria as pro-technological, bioprotective and health-promoting cultures in the dairy food industry. *Food Bioscience*, 47, 101617. http://dx.doi.org/10.1016/j.fbio.2022.101617

Angelin, J., & Kavitha, M. (2020). Exopolysaccharides from probiotic bacteria and their health potential. *International Journal of Biological Macromolecules*, 162, 853-865. http://dx.doi.org/10.1016/j.ijbiomac.2020.06.190

Association of Official Analytical Chemists. (2000). AOAC Official Methods of Analysis International. *17th Edn, Washington DC, USA.*

Beimfohr, C., Ludwig, W., & Schleifer, K. H. (1997). Rapid genotypic differentiation of *Lactococcus lactis* subspecies and biovar. *Systematic and Applied Microbiology*, *20*(2), 216-221. https://doi.org/10.1016/S0723-2020(97)80068-9

Berthier, F., & Ehrlich, S. D. (1998). Rapid species identification within two groups of closely related *lactobacilli* using PCR primers that target the 16S/23S rRNA spacer region. *FEMS Microbiology Letters, 161(1),* 97-106. https://doi.org/10.1111/j.1574-6968.1998.tb12934.x

Bouton, Y., Guyot, P., Vacheyrou, M., Normand, A. C., Piarroux, R., & BEUVIER, E. (2007). Etude des flux bactériens dans les étables de production laitière de Franche-Comté. Exemples des LHF : 15ème colloque du Club des Bactéries Lactiques, 13-15, *Rennes*. hal-02758083

Bouton, Y., Tessier, T., Guyot, T. P., & BEUVIER, E. (2005). Relation entre les pratiques des producteurs et les niveaux de populations microbiennes des laits à Comté : *12ème Rencontres Recherches, Ruminants*(Vol. 12), 403. Paris, France.

Bremer, E. (2000). Coping with osmotic challenges : Osmoregulation through accumulation and release of compatible solutes in bacteria. In: *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*(Vol 126), 17. https://doi.org/10.1016/s1095-6433(00)80031-8

Buffa, M., Morais, J., Jiménez-Belenguer, A., Hernández-Giménez, E., & Guamis, B. (2005). Technological characterization of lactic acid bacteria isolated from raw ewes' milk for cheese making. *Milchwissenschaft*, *61*(4), 404-407.

Callon, C., Duthoit, F., Delbès, C., Ferrand, M., Le Frileux, Y., De Cremoux, R., & Montel, M. C. (2007). Stability of microbial communities in goat milk during a lactation year: Molecular approaches. *Systematic and Applied Microbiology*, *30*(7), 547-560. http://dx.doi.org / 10.1016/j.syapm.2007.05.004

Câmara, S. P., Dapkevicius, A., Riquelme, C., Elias, R. B., Silva, C. C. G., Malcata, F. X., & Dapkevicius, M. L. N. E. (2019). Potential of lactic acid bacteria from Pico cheese for starter culture development. *Food Science and Technology International*, *25*(4), 1-15. http://dx.doi.org/ 10.1177/1082013218823129

Casalta, E., Sorba, J. M., Aigle, M., & Ogier, J. C. (2009). Diversity and dynamics of the microbial community during the manufacture of Calenzana, an artisanal Corsican cheese. *International Journal of Food Microbiology*, *133*(3), 243-251. http://dx.doi.org/10.1016/j.ijfoodmicro.2009.05.022

Castro-Bravo, N., Wells, J. M., Margolles, A., & Ruas-Madiedo, P. (2018). Interactions of Surface Exopolysaccharides From *Bifidobacterium* and *Lactobacillus* Within the Intestinal Environment. *Frontiers in Microbiology*, 9, 24-26. http://dx.doi.org/10.3389/fmicb.2018.02426

Cerning, J., Renard, C. M. G. C., Thibault, J. F., Bouillanne, C., Landon, M., Desmazeaud, M., & Topisirovic, L. (1994). Carbon source requirements for exopolysaccharide production by *Lactobacillus casei* CG11 and partial structure analysis of the polymer. *Applied and Environmental Microbiology*, *60*(11), 3914-3919. http://dx.doi.org/10.1128/aem.60.11.3914-3919.1994

Chen, B., Lewis, M. J., & Grandison, A. S. (2014). Effect of seasonal variation on the composition and properties of raw milk destined for processing in the UK. *Food Chemistry*, 158, 216-223. http://dx.doi.org/10.1016/j.foodchem.2014.02.118

Cogan, T. M., Barbosa, M., Beuvier, E., Bianchi-Salvadore, B., Cocconcelli, P. S., Fernandes, I., Gomez, J., Gomez, R., Kalantzopoulos, G., Ledda, A., Medina, M., Rea, M. C., & Rodriguez, E. (1997). Characterization of lactic acid bacteria in

artisanal dairy products. *Journal of Dairy Research*, *64*(3), 409-421. http://dx.doi.org/10.1017/S0022029997002185
Crow, V. L., Coolbear, T., Gopal, P. K., Martley, F. G., Mckay, L. L., & Riepe, H. (1995). The role of autolysis of lactic acid bacteria in the repening of cheese. *International Dairy Journal*, *5*(8), 855-875. http://dx.doi.org /10.1016/0958-6946(95)00036-4
Da Silva Ferrari, I., de Souza, J. V., Lacerda Ramos, C., Matiuzzi da Costa, M., Freitas Schwan, R., & Silva Dias, F. (2016). Selection of autochthonous lactic acid bacteria from goat dairies and their addition to evaluate the inhibition of *Salmonella typhi* in artisanal cheese. *Food Microbiology*, 60, 29-38. http://dx.doi.org/10.1016/j.fm.2016.06.014
De Almeida Júnior, W. L. G., Ferrari, Í. da S., de Souza, J. V., da Silva, C. D. A., da Costa, M. M., & Dias, F. S. (2015). Characterization and evaluation of lactic acid bacteria isolated from goat milk. *Food Control*, 53, 96-103. http://dx.doi.org/10.1016/j.foodcont.2015.01.013
Delgadillo Puga, C., Cuchillo Hilario, M., & Pérez-G- Romo, M. (2009). Effect of feeding management and seasonal variation on fatty acid composition of Mexican soft raw goats' milk cheese. *Italian Journal of Animal Science*, *8*(2), 402-404. http://dx.doi.org/10.4081/ijas.2009.s2.402
Delgado-Pertinez, M., Alcalde, M. J., Guzman-Guerrero, J. L., Castel, J. M., Mena, Y., & Caravaca, F. (2003). Effect of hygiene-sanitary management on goat milk quality in semi-extensive systems in Spain. *Small Ruminant Research*, *47*(1), 51-61. http://dx.doi.org/10.1016/S0921-4488(02)00239-0
Dertli, E., Mercan, E., Arıcı, M., Yılmaz, M. T., & Sağdıç, O. (2016). Characterisation of lactic acid bacteria from Turkish sourdough and determination of their exopolysaccharide (EPS) production characteristics. *LWT - Food Science and Technology*, 71, 116-124. http://dx.doi.org/10.1016/j.lwt.2016.03.030
El Galiou, O., Zantar, S., Bakkali, M., Laglaoui, A., Centeno, J. A., & Carballo, J. (2015). Chemical and microbiological characteristics of traditional home made fresh goat cheeses from Northern Morocco. *Small Ruminant Research*, 129, 108-113.http://dx.doi.org/10.1016/j.smallrumres.2015.06.005
Ercolini, D., Russo, F., Ferrocino, I., & Villani, F. (2009). Molecular identification of mesophilic and psychrotrophic bacteria from raw cow's milk. *Food Microbiology*. *26*(2), 228-231. http://dx.doi.org / 10.1016/j.fm.2008.09.005
Fguiri, I., Ziadi, M., Atigui, M., Ayeb, N., Arroum, S., Assadi, M., & Khorchani, T. (2016). Isolation and characterisation of lactic acid bacteria strains from raw camel milk for potential use in the production of fermented Tunisian dairy products. *International Journal of Dairy Technology*, *69*(1), 103-113. http://dx.doi.org/10.1111/1471-0307.12226
Food and Agiculture Organization of the United Nations (FAOSTAT) http://www.fao.org/faostat/en/#data.
Garabal, J. I., Rodriguez-Alonso, P., & Centeno, J.A. (2008). Characterization of lactic acid bacteria isolated from raw cows' milk cheeses currently produced in Galicia (NW Spain). *LWT-Food Science and Technology*, 41, 1452-1458. http://dx.doi.org/10.1016/j.lwt.2007.09.004
Garcia, U. A., Rivero, J., Gonzales, P., Valero-Leal, K., Izquierdo, P., Garcia, A., & Colmenares, C. (2009). Bacteriological quality of raw goat milk produced in Faria parish, Miranda Municipality, Zulia state, Venezuela. *Revista De La Facultad De Agronomia De La Universidad Del Zulia*, 26, 59-77. http://ve.scielo.org/scielo.php?pid=S0378-78182009000100004&script=sci_abstract&tlng=en
Gortzi, O., Malissiova, E., Katsoulis, K., Alibade, A., Liappis, D., Lalas, S., & Graikou, K. (2022). Comparative Analysis of Fatty Acid Profile and Fat-Soluble Vitamin Content in Sheep and Goat Milk of Organic and Conventional Origin. *Applied Sciences*, *12*(6), 2809. http://dx.doi.org/10.3390/app12062809
Guo, M., Park, Y. W., Dixon, P.H., Gilmore, J. A., & Kindstedt, P. S. (2004). Relationship between the yield of cheese (Chevre) and chemical composition of goat milk. *Small Ruminant Research*, *52*(1-2), 103-107.http://dx.doi.org/10.1016/S0921-4488(03)00247-5
Haenlein, G. F. W., & Wendorff, W.L. (2006). Sheep milk-production and utilization of sheep milk. In: Park, Y. W., Haenlein, G. F. W. (2017). *Handbook of milk non bovine mammals(Eds).* Oxford, UK and Ames, Iowa, USSA: Blackwell Publishing Professional. https://doi.org/10.1002/9780470999738.ch7
Hantsis-Zacharov, E., & Halpern, M. (2007). Culturable Psychrotrophic Bacterial Communities in Raw Milk and Their Proteolytic and Lipolytic Traits. *Applied and Environmental Microbiology*, *73*(22), 7162-7168.http://dx.doi.org/10.1128/AEM.00866-07
International Dairy Federation .(1997b). Dairy starter cultures of lactic acid bacteria (LAB). Standard of identity. *IDF Standard 149A, Brussels, Belgium.*
International Dairy Federation. (1986). Milk determination of nitrogen content (Kjeldahl method) and calculation of crude protein content. *IDF Standard 20A, Brussels, Belgium.*
International Dairy Federation. (1995). Lait et produits laitiers. Guide de l'échantillonnage.*IDF Standard 50C, Brussels, Belgium.*
International Dairy Federation. (1997a). Sensory evaluation of dairy products by scoring. *IDF Standard 99C, Brussels, Belgium.*
Islam, M., Uddin, M., Rahman, Md. T., Islam, M., & Rashid, M. (2021). Isolation and characterization of dominant lactic acid bacteria from raw goat milk : Assessment of probiotic potential and technological properties. *Small Ruminant Research*, 205, 106532. http://dx.doi.org/10.1016/j.smallrumres.2021.106532
Kljajevic, N. V., Tomasevic, I. B., Miloradovic, Z. N., Nedeljkovic, A., Miocinovic, J. B., & Jovanovic, S. T. (2018). Seasonal variations of Saanen goat milk composition and the impact of climatic conditions. *Journal of Food Science and Technology*, *55*(1), 299-303. http://dx.doi.org/10.1007/s13197-017-2938-4
Kong, Y., Li, M., Li, R., Shan, X., & Wang, G. (2020). Evaluation of cholesterol lowering property and antibacterial activity of two potential lactic acid bacteria isolated from the intestine of snakehead fish (*Channa argus*). *Aquaculture Reports*, 17, 100342. http://dx.doi.org/10.1016/j.aqrep.2020.100342
Kouniba, A., Berrada, M., & El Marakchi, A. (2007). Étude comparative de la composition chimique du lait de chèvre de la race *locale* Marocaine et la race *alpine* et évaluation de leur aptitude fromagère. *Revue de Médecine Vétérinaire*, *158*(3), 152-160.
Langsrud, T., Landaas, A., & Castberg, H. B. (1987). Autolytic properties of different strains of group *N streptococci*. *Milchwissenschaf*, *42*(9), 556-560.
Liu, Z., Zhang, Z., Qiu, L., Zhang, F., Xu, X., Wei, H., & Tao, X. (2017). Characterization and bioactivities of the exopolysaccharide from a probiotic strain of *Lactobacillus plantarum* WLPL04. *Journal of Dairy Science*, *100*(9), 6895-6905. http://dx.doi.org/10.3168/jds.2016-11944
Lopez, A., Vasconi, M., Moretti, V. M., & Bellagamba, F. (2019). Fatty Acid Profile in Goat Milk from High- and Low-Input Conventional and Organic Systems. *Animals : an Open Access Journal from MDPI*, *9*(7), 452. http://dx.doi.org/10.3390/ani9070452
Mallet, A., Guéguen, M., & DESMASURES, N. (2010). Etat des lieux de la diversité microbienne quantitative et qualitative de laits crus normands destinés à la transformation fromagère : 8ème Congrès National de la SFM, *Marseille*.
Mareze, J., Ramos-Pereira, J., Santos, J. A., Beloti, V., & López-Díaz, T. M. (2022). Identification and characterisation of *lactobacilli* isolated from an artisanal cheese with antifungal and antibacterial activity against cheese spoilage and mycotoxigenic *Penicillium* spp. *International Dairy Journal*, 130, 105367. http://dx.doi.org/10.1016/j.idairyj.2022.105367
Mayo, B., Aleksandrzak-Piekarczyk, T., Fernandez, M., Kowalczyk, M. A., lvarez-Martin, P., & Bardowski, J. (2010). Updates in the metabolism of lactic acid bacteria. In: Mozzi, F., Raya, R. R., & Vignolo, G. M. (2015). *Biotechnology of Lactic Acid Bacteria: Novel Applications* (2nd edition). USA: Blackwell Publishing. https://doi.org/10.1002/9780813820866.ch1
Ménard, J.L., Roussel, P., Masselin-Silvin, S., Puthod, R., Hetreau, T., Forêt, A., Houssin, B., Aracil, C., & LE GUENIC, M. (2004). Contamination bactérienne d'une litière de stabulation libre paillée : effet de la fréquence de paillage et proposition d'une méthode pour son évaluation : 11ème Rencontre Recherche Ruminants. *Institut de l'Elevage-INRA, Paris*, 403.
Meng, Z., Zhang, L., Xin, L., Lin, K., Yi, H., & Han, X. (2018). Technological characterization of *Lactobacillus* in semi hard artisanal goat cheeses from different Mediterranean areas for potential use as nonstarter lactic acid bacteria. *Journal of Dairy Science*, *101*(4), 2887-2896. http://dx.doi.org/10.3168/jds.2017-14003
Michel, V., Hauwuy, A., & Chamba, J. F. (2001). Raw cow milk microflora: diversity and influence of conditions of production. *Le Lait*, *81*(5), 575-592.
Michel, V., Hauwuy, A., Montel, M.C., Coulon, J. B., & CHAMBA, J.F. (2005). Pratiques d'élevage et composition microbienne des laits crus. Dans : Territoires et enjeux du développement régional. *Lyon*.
Moate, P. J., Chalupa, W., Boston, R. C., & Lean, I. J. (2007). Milk Fatty Acids. I. Variation in the Concentration of Individual Fatty Acids in Bovine Milk. *Journal of Dairy Science*, *90*(10), 4730-4739. http://dx.doi.org/10.3168/jds.2007-0225
Monsallier, F., Verdier-Metz, I., Chanal, J., Delbès, C., Gagne, G., & MONTEL, M. C. (2009). Le trayon estil une source de diversité microbienne du lait : Journée de Restitution de l'UMT Trefl, *Aurillac*.
Nieto-Arribas, P., Seseña, S., Poveda, J. M., Palop, L., & Cabezas, L. (2010). Genotypic and technological characterization of *Leuconostoc* isolates to be used as adjunct starters in Manchego cheese manufacture. *Food Microbiology*, *27*(1), 85-93. http://dx.doi.org/10.1016/j.fm.2009.08.006
Olarte, C., Sanz, S., Gonzalez-Fandos, E., & Torre, P. (1999). Microbiological and physicochemical characteristics of Cameros cheese. *Food Microbiology*, *16*(6), 615-621. http://dx.doi.org/10.1006/fmic.1999.0276
Papamanoli, E., Tzanetakis, N., Litopoulou-Tzanetaki, E., & Kotzekidou, P. (2003). Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties. *Meat Science*, *65*(2), 859-867. http://dx.doi.org/10.1016/S0309-1740(02)00292-9
Park, Y.W., Juarez, M., Ramos, M., & Haenlein, G. F. W. (2007). Physico-chemical characteristics of goat and sheep milk. *Small Ruminant Research*, *68*(1-2), 88-113. http://dx.doi.org/10.1016/j.smallrumres.2006.09.013
Piraino, P., Zotta, T., Ricciardi, A., McSweeney, P. L. H., & Parente, E. (2008). Acid production, proteolysis, autolytic and inhibitory properties of lactic acid bacteria isolated from pasta filata cheeses : A multivariate screening study. *International Dairy Journal*, *18*(1), 81-92. http://dx.doi.org/10.1016/j.idairyj.2007.06.002
Piton, C., & Richard, J. (1982). Main sources of bacteria in moderately contaminated milk on farms in the Rennes area. *Lait*, *62*(611-612), 67-74.
Poveda, J. M., & Cabezas, L. (2006). Free fatty acid composition of regionally-produced Spanish goat cheese and relationship with sensory characteristics. *Food Chemistry*, 95, 307-311. http://dx.doi.org/10.1016/j.foodchem.2004.12.045

Pirisi, A., Lauret, A., Dubeuf, J.P., 2007. Basic and incentive payments for goat and sheepmilk in relation to quality. *Small Ruminant Research*,*68*(1), 167–178. https://doi.org/10.1016/j.smallrumres.2006.09.009.

Pu, Z. Y., Dobos, M., Limsowtin, G. K. Y, & Powell, I. B. (2002). Integrated polymerase chain reaction-based procedures for the detection and identification of species and subspecies of the Gram-positive bacterial Genus *Lactococcus*. *Journal of Applied Microbiology*, *93*(2), 353-361. http://dx.doi.org/10.1046/j.1365-2672.2002.01688.x

Raynaud, S., Paineau, S., & BRUN, T. (2005). Contamination du lait par différentes flores en Bretagne : 11ème Rencontres Recherches, Ruminants. *Institut de l'Elevage-INRA, Paris*, 406-406.

Salmeron, J., de Vega, C., Perez-Elortondo, F. J., Albisu, M., & Barron, L. J. R. (2002). Effect of pasteurization and seasonal variations in the microflora of ewe's milk for cheesemaking, *Food Microbiology*, *19*(2-3), 167-174. http://dx.doi.org/10.1006/fmic.2001.0475

Samelis, J., Lianou, A., Kakouri, A., Delbès, C., Rogelj, I., Bogovicˇ -Matijasić, B., & Montel, M. C. (2009). Changes in the Microbial Composition of Raw Milk Induced by Thermization Treatments Applied Prior to Traditional Greek Hard Cheese Processing. *Journal of Food Protection*, *72*(4), 783–790. https://doi.org/10.4315/0362-028x-72.4.783

Serhan, M., Cailliez-Grimal, C., Borges, F., Revol-Junelles, A. M., Hosri, C., & Fanni, J. (2009). Bacterial diversity of Darfiyeh, a Lebanese artisanal raw goat's milk cheese. *Food Microbiology*, *26*(6), 645-652. http://dx.doi.org/10.1016/j.fm.2009.04.012

Smitinonta, T., Tansakul, C., Tanasupawat, S., Keeratipibul, S., Navarini, L., Bosco, M., & Cescutti, P. (1999). Exopolysaccharide-producing lactic acid bacteria strains from traditional thai fermented foods: isolation, identification and exopolysaccharide characterization. *International Journal of Food Microbiology*, *51*(2-3), 105-111. http://dx.doi.org/10.1016/s0168-1605(99)00094-x

Tormo, H., Agabriel, C., Lopez, C., Ali Haimoud Lekhal, D., & Roques, C. (2011). Relationship between the production conditions of goat's milk and the microbial profiles of milk. *International Journal of Dairy Science*, *6*(1), 13-28. https://doi.org/10.3923/ijds.2011.13.28

Tormo, H., Ali haimoud – Lekhal, D.,&LAITHIER, C. (2006). Les microflores utiles des laits crus de vache et de chèvre : principaux réservoirs et impact de certaines pratiques d'élevage : 13ème Rencontre Recherche, Ruminants. *Institut de l'Elevage-INRA, Paris*, 305-308.

Toyes-Vargas, E., Cordoba-Matson, M., Espinoza-Villavicencio, J., Palacio-Espinosa, A., & Murillo-Amador, B. (2013). Goat milk fatty acid composition in the Peninsula of Baja California, Mexico. *Revista MVZ Córdoba*, *18*(3), 3843-3850. https://doi.org/10.21897/rmvz.156

Triebold, H. O. (2000). *Quantitative Analysis with Applications to Agricultural and Food Products*. New York: D. van Nostrand Company, Inc.

Wang, F., Chen, M., Luo, R., Huang, G., Wu, X., Zheng, N., Zhang, Y., & Wang, J. (2022). Fatty acid profiles of milk from Holstein cows, Jersey cows, buffalos, yaks, humans, goats, camels, and donkeys based on gas chromatography–mass spectrometry. *Journal of Dairy Science*, *105*(2), 1687-1700. http//dx.doi.org/10.3168/jds.2021-20750

Ward, L. J. H., & Timmins, M. J. (1999). Differentiation of *Lactobacillus casei*, *Lactobacillus paracasei* and *Lactobacillus rhamnosus* by polymerase chain reaction. *Letters in Applied Microbiology*, *29*, 90-92. https://doi.org/10.1046/j.1365-2672.1999.00586.x

Young, J. P., Downer, H. L., & Eardly, B. D. (1991). Phylogeny of the phototrophic *Rhizobium* strain Btai1 by polymerase chain reaction-based sequencing of a 16s ribosomal-RNA gene segment. *Journal of Bacteriology*, 173, 2271-2277. http://dx.doi.org/10.1128/jb.173.7.2271-2277.1991

Zdanowicz, M., Shelford, J. A., Tucker, C. B., Weary, D. M., & von Keyserlingk, M. A. G. (2004). Bacterial populations on teat ends of dairy cows housed in free stalls and bedded with either sand or sawdust. *Journal of Dairy Science*, *87*(6),1694-1701. http://dx.doi.org/10.3168/jds.S0022-0302(04)73322-6