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# Sensory, microbiological and physico-chemical characterization of *Klila*, a traditional cheese made in the south-west of Algeria

**Benamara R. N.[1,2], Gemelas L.[3], Ibri K.[2], Moussa-Boudjemaa B.[1] and Demarigny Y.[3]***

[1]Laboratory of Microbiology Applied to Agribusiness, Biomedical and Environmental (LAMAABE), Faculty of SNV/STU, University of Tlemcen, 13000 Tlemcen, Algérie.
[2]Departement of Agronomy, Faculty of Natural and Life Sciences, University of Mascara, BP 305 Pole Sidi Said, Mascara 29000, Algérie.
[3]Bioengineering and Microbial Dynamic at Food Interfaces (Associated team n°3733 (BioDyMIA), University of Lyon 1-ISARA Lyon), Isara-Lyon, Agrapole-23 rue Jean Baldassini, F-69364 Lyon Cedex 07, France.

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***Klila,* an Algerian cheese produced in steppe and mountainous areas, is proving increasingly popular with consumers. It is traditionally made with ewe, goat or cow milk, leading to a product with high dry matter content (> 90%). In this work, we have characterized three different *Klila* cheeses made with the three different milks using physico-chemical and microbiological parameters. A triangle test was also performed on naïve consumers, and the three types of *Klila* were clearly distinguished by sensory analysis. They exhibited distinct features, in particular very low Aw (< 0.5) and pH values (< 4.5) preserving them from pathogens. Lactobacilli and enterococci were counted at low levels (< 4 log (cfu)/g) as well as some spore-forming bacteria (< 3 log (cfu)/g). Colonies were picked from MRS and BEA media. They were identified by sequencing and characterized on their ability to produce lactic acid and using REP-PCR. *Lb plantarum* was the main species isolated, followed by *Pediococcus pentosaceus*, *Leuconostoc pseudomesenteroïdes* and *Lactobacillus fermentum*. The *Enterococcus* genus was dominated by *Ec durans*, *Ec faecium* and *Ec hirae*. Among these two main populations, different subgroups were observed by means of the REP-PCR profiles and the lactic acid production of the isolates. Some strains were found in two and even three cheeses. We suppose that these microbes are representative of the environmental context in which *Klila* is produced.**

**Key words:** *Klila*, lactic acid bacteria, Algerian traditional cheese, *Lactobacillus plantarum, Enterococcus.*

## INTRODUCTION

The cheeses were developed to preserve and even improve the biochemical properties of milk over time: vitamins, essential fatty acids, amino acids, minerals (Hill and Kethireddipalli, 2013). Although most cheeses are made with cow milk, in some regions such as Mediterranean countries, ewe, goat and even camel and

buffalo milks are used (Vivar-Qüintana et al., 2009). Today, consumers' appetite for raw milk cheeses seems to be on the increase. The flavour of these products is more intense and varied compared with cheeses made with pasteurised milk (Yohan et al., 2016). Many cheeses produced at the industrial scale derive from traditional farmhouse know-how (Demarigny and Gerber, 2014). In Mediterranean countries, traditional milk products are a major component of the daily diet in many small communities, especially in rural areas. The specificities of the climate (frequently dry and warm) means adapted processes have been developed to store milk over long periods. This is all the more true if we refer to rural areas found in the north of Africa. In these regions, people have developed many fermented milks and cheeses (Christelle et al., 2016).

In Algeria, traditional cheeses are almost always produced at a small, local scale, which explains why they are too frequently unknown to consumers further afield. Consequently, they are less popular than industrial cheeses. At the present time, around 10 traditional cheeses have been identified in this country (Aissaoui et al., 2006), but many others still have to be characterised.

The cheese *Klila* has been consumed by Algerian people for many centuries, probably from as far back as the Antiquity until now (Duval, 1855). This is a traditional handmade North African cheese with good nutritional values. Its high dry matter (more than 80%) allows it to be stored over long periods without any risk of microbial spoilage.

*Klila* is obtained from a fermented churned milk called *Lben*. *Lben* is heated, drained and pressed to obtain *Klila* (Harrati, 1974). This popular cheese is still based on a traditional farmhouse production method which contributes to the pleasant sensory attributes and nutritional properties, It enjoys and partly explains the increasing consumer demand for *Klila* (Lahsaoui, 2012). Unfortunately, *Klila* has never been seriously studied and only few microbiological, biochemical and technological data are available (Benamara and Megaiz, 1998; Leksir and Chemmam, 2015). On the contrary, *Jameed*, a similar Middle-East cheese or *Chhana* a cheese made in India, are well characterized (Mazahreh et al., 2008). These studies laid the groundwork for the development of specific technologies such as atomization or lyophilisation to produce these traditional cheeses at the industrial scale.

In this study, we compared three different *Klila* cheeses made from ewe, goat and cow milks at the farmhouse scale to establish the interesting sensory, microbiological and physico-chemical properties of these products. In a later work, the nutritional properties will also be characterized. This work aims to provide solid arguments for developing *Klila* production at the industrial scale.

## MATERIALS AND METHODS

### Sampling

Three different *Klila* cheeses made respectively with ewe, goat and cow milk were sampled in February and March 2015 in the Sfissifa daira region from Ain Sefra Wilaya Naama – around 730 km from the capital, Algiers, in the south-west of Algeria. The cheeses were made by the local tribes from this steppe and mountainous area (mean altitude: around 1, 200 m; Figure 1).

### Cheese making

The cheeses were made according to the process summarized on Figure 2, using the three following milks:

i) The ewe milk was obtained from the local breed *El Hamra*,
ii) The cow milk was obtained from the selected breed BLA, a cross between the local breed BLL and the Holstein breed.
iii) The goat milk used to make the cheeses was a mix of two local breeds, *Arabia* and *Kabiles*.

### Sensory analysis

To compare the cheeses, three triangle tests were performed. This methodology was chosen so as to evaluate the ability of untrained panellists to discern differences between similar products. Twenty-four persons were hired to test the cheeses in pairs in a controlled environment (in terms of light, smell and sound). Three sessions were necessary to cover the three possible comparisons: goat vs cow, goat vs ewe and ewe vs cow. Each time, the normalized procedure, as described in (AFNOR, 2007), was followed: sample randomization and coding, use of bread and still water, etc.

On the result sheet, panellists could also express how difficult they found the discrimination test to be (simple, hard, very hard). And they were also asked to qualify the type of sensation which allowed them to differentiate one product from the other two. The descriptors encompassed the following terms: colour, aspect, odour, aroma, rancid, bad taste, “other”.

### Physico-chemical analyses

**pH analysis:** Cheeses were first ground and mixed with distilled water to obtain a diluted slurry (1/10), the pH of which was measured. pH evaluations were made in triplicate with a Hanna instrument pH meter (PHM210 standard, MeterLab®), immediately after grinding, and after a 20 min delay.

**Water availability (Aw):** Aw was measured in triplicate with an Aw-meter apparatus (Sprint TH 500, Novasina) on a 1.5 g cheese sample.

**Dry matter (DM, %):** To determine the dry matter, 5 g of cheese were dried in a halogen desiccator (Mettler). The result was directly expressed in percentages of dry matter.

*Corresponding author. E-mail: ydemarigny@isara.fr.


**Figure 1.** Location of the *Klila* production area, the Sfissifa daira region from Ain Sefra Wilaya Naama. Source: Google map.

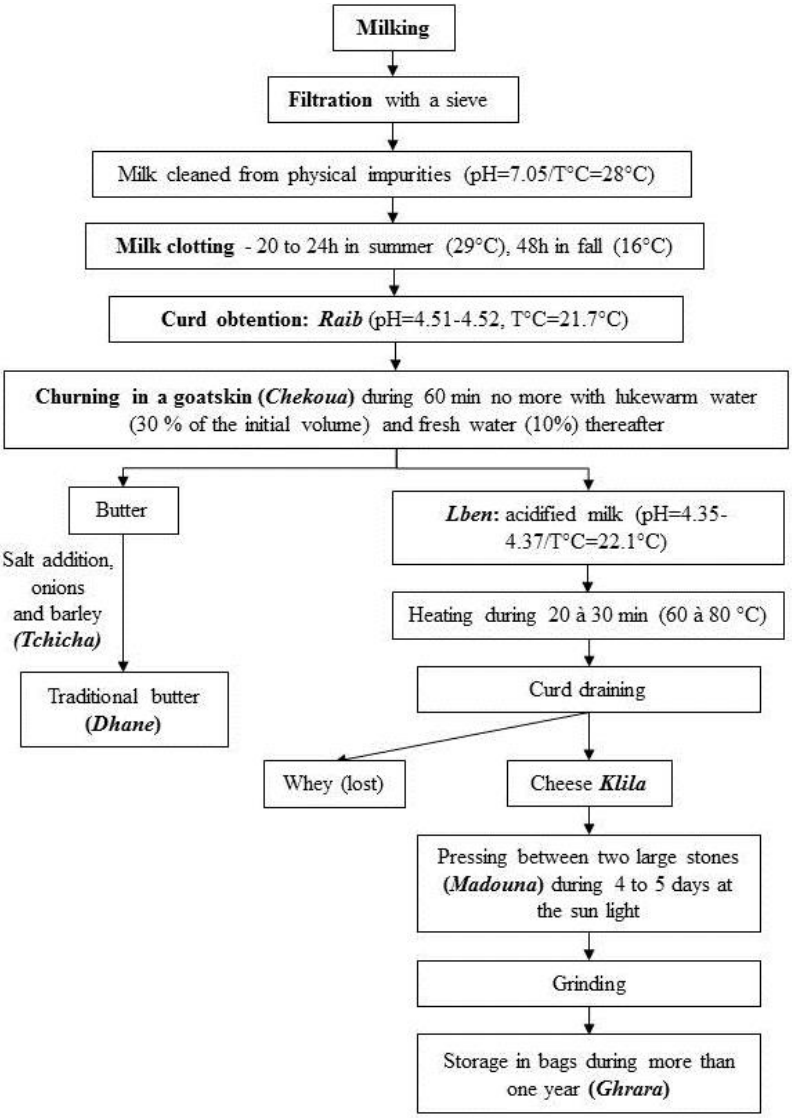


**Figure 2.** *Klila* cheese making.

**Lactic acid concentration:** Ten grams of cheese were first ground with 50 mL of distilled water. After centrifugation (6,000 g, 5 min), the supernatant was sampled for analysis. D- and L-lactic acids were measured with an enzymatic kit (D/L lactic acid Enzytec™ from R-Biopharm).

**Fat rate (g/100 g of cheese):** The evaluation of the fat rate followed the methodology described in the ISO 3433-2002 standard. The fat on dry matter (F/DM) ratio was then calculated.

**Microbiological enumerations**

Ten grams of cheese were diluted in a mix of trisodium citrate (4% w/v, 50 mL) and sterilised distilled water at 50°C (around 40 mL to adjust the final weight to 100 g). The resulting slurry (1/10) was used as the mother solution, and submitted to subsequent dilutions.

The following microbial populations were looked for:

i) Total count was performed on Plate Count Agar (30°C, 48 to 72 h, aerobic conditions, Biokar), according to the NF EN ISO 4833-1:10-2013 standard.

ii) The presumed *Bacillus* population was checked according to the methodology developed by Rosenkvist and Hansen (1995): Aerobic incubation at 30°C during 24 to 48 h.

*iii) Bacillus cereus* was specifically counted on Mossel agar (Biokar): aerobic incubation at 30°C during 24 to 48 h.

iv) Spores of butyric acid bacteria (BAB) were enumerated in the Bryant-Burkey broth modified by Bergère (Biokar) according to the most probable number method: anaerobic incubation during 7 days at 37°C.

v) Mesophilic presumed *Lactobacillus* were searched for on MRS agar (De Man et al*.,* 1960): anaerobic incubation at 30°C during 48 to 72 h.

vi) Positive β-glucuronidase *Escherichia coli* were counted on PTX agar (Biokar): aerobic incubation at 44°C during 18 to 24 h.

*vii) Listeria monocytogenes* was looked for according to the ISO 16140 standard. Twenty-five grams of grated cheese were incubated in semi-Fraser broth (225 mL) during 24 h at 30°C. Suspect colonies were then observed on Compas Listeria agar

(Biokar) after a 24 to 48 h delay at 37°C.
*viii) Salmonella* spp were searched for according to the ISO 6579 standard. This method involves successive cultures and a final check on XLD agar and Hektoen agar.
*ix) Staphylococcus aureus* was counted on Baird-Parker agar (Biokar): Aerobic incubation at 37°C during 24 h. In case of presumed *S. aureus* colonies, the presence of the coagulase was checked by exposing a 24 h culture (BHI, Biokar) to rabbit plasma.
x) Enterococci were enumerated on BEA (Biokar): Aerobic incubation during 24 h at 37°C.
xi) Moulds and yeasts were counted on GGC (Biokar): Aerobic incubation at 25°C for 5 days.

#### Phenotypic identification of presumed *Lactobacillus* and *Enterococcus* colonies

For each cheese, a maximum of 5 colonies of presumed enterococci were sampled from BEA to be cultured in M17 broth (Biokar) during 24 h at 37°C. After purification on M17 agar, isolates were frozen at -80°C in a mixture of glycerol 30% and M17 broth until further analyses. For colonies sampled on MRS, the same operation was performed (10 colonies per cheese) except that purifications, broth cultures and freezing were all performed on MRS. The culture temperature was 30°C.

Identifications relied on microscopic observations (after Gram staining) and catalase tests.

i) Metabolic affiliation: This test allowed the *Lactobacillus* strains to be separated following their preferred metabolic pathway: homofermentative, facultatively or strictly heterofermentative. The procedure described by Demarigny et al. (1997) was applied.
ii) The type of lactic acid produced was checked on a 24 h bacterial culture (M17 or MRS) with an enzymatic kit (D-/L-Lactic acid ENZYTEC$^{TM}$, R-Biopharm).

#### Genotypic characterizations

All the isolates were characterized on the basis of their REP-PCR profile. REP-PCR was performed with primers Rep1R-Dt and Rep2-D according to the methodology followed by Gemelas et al. (2013). The final identification relied on the 16S DNA sequencing. This was carried out by the LGC Genomics Company (Berlin, Germany). Sequences were analysed thanks to the NCBI data base (http://www.ncbi.nlm.nih.gov/BLAST/).

#### Statistical analyses

All the statistical analyses were performed using the XLSTAT software (Microsoft, 2010).

## RESULTS

### Sensory analyses

The three triangle tests led to the same results: 15 panellists out of 24 gave the correct answer. According to the binomial test (1/3), the probability is highly significant (< 1%). From a sensory point of view, the cheeses were dissimilar, even if the differences were scarce. In general, tasters found it difficult to separate goat milk from ewe milk cheeses (hard + very hard: 91%) and goat milk from cow milk cheeses (hard + very hard: 80%). However, 50% of the tasters found it easy to differentiate ewe milk from cow milk cheeses. The reasons for the difference observed between cheeses were not systematically recorded. Generally, colour and odour were given as the main discriminatory factors, and less frequently aroma and aspect. In a few rare cases, the rancid sensations (for goat milk vs ewe milk cheeses) and bad taste were also reported.

### Analytical results

If we refer to Table 1, we can make two observations. Firstly, the cheeses were very dry (DM > 90%) and the available water inside was low (Aw < 0.500). Secondly, dry matter is the sole parameter which remains almost identical in all the cheeses (between 93.18 and 94.97%). The other parameters changed from cheese to cheese.

If we look specifically at the microbial populations, they were quite low. Except for the total count and the level of yeasts and moulds, the other populations were all inferior to 6 log (cfu)/g. No trace of *Samonella*, *E. coli*, *S. aureus* and *L. monocytogenes* was found. Spore forming bacteria (BAB and *B. cereus*, *Bacillus* spp) were also undetectable or at very low levels. Moreover, the lower the level of presumed lactobacilli, the higher the total count. And the same tendency opposed the level of spores of BAB and the presumed *Enterococcus* population.

All the physicochemical and microbial data were pooled together through a Principal Component Analysis (results not shown). Each cheese was clearly separated from the other. For instance, ewe milk cheeses were characterized by a low pH (4.25) and a low lactate rate (694 mg/100 g), but rather high levels of enterococci and lactobacilli (respectively, 4.41 and 5.43 log (cfu)/g).

From a general point of view, we could observe a strong positive correlation between total counts and Aw (r = 0.938) and a negative correlation between presumed lactobacilli, *Enterococcus*, *Bacillus* spp levels (r > 0.980). The incidence of pH on the microbial counts was less evident except with those of enterococci (r = -0.963).

### Microbial characterizations

A total of 41 colonies were isolated from the analysis of the three cheeses, 26 on MRS agar (presumed lactobacilli) and 15 on BEA (presumed enterococci). All the isolates proved to be Gram positive and catalase negative. The isolates from BEA were all round (10) or tapered (5) cocci. Five out of the 26 isolates from the MRS sampling presented a shape between coccus and rods. The aspect of the colonies (the morphotype) was nearly the same whatever the origin of the sampling. Generally, they appeared round, smooth, convex and white.

**Table 1.** Physico-chemical and microbiological characterization of goat, ewe and cow milk *Klila*.

| Characteristics | Ewe milk *Klila* | Goat milk *Klila* | Cow milk *Klila* |
|---|---|---|---|
| Total count | 5.19 | 6.48 | 5.24 |
| Yeasts and moulds | > 5.7 | > 5.7 | > 5.7 |
| *Bacillus* | < 0.30 | < 0.30 | 2.05 |
| *Bacillus cereus* | < 0.70 | < 0.70 | 3.63 |
| Butyric acid bacteria *(Clostridium)* | 1.00 | 2.30 | 2.60 |
| *Lactobacillus* | 5.43 | 3.69 | 6.15 |
| *Enterococcus* | 4.41 | 3.43 | 3.44 |
| pH | 4.25 | 4.46 | 4.40 |
| Aw | 0.368 | 0.467 | 0.320 |
| Dry matter | 94.51 | 94.97 | 93.18 |
| Lactic acid | 0.694 | 1.008 | 1.210 |
| Fat | 29.33 | 20.33 | 25.33 |
| Fat on dry matter | 31.03 | 21.41 | 27.18 |

Data are expressed in log(cfu)/g (bacterial populations), g/100 g of cheese (dry matter, lactic acid, fat) and % (fat on dry matter).

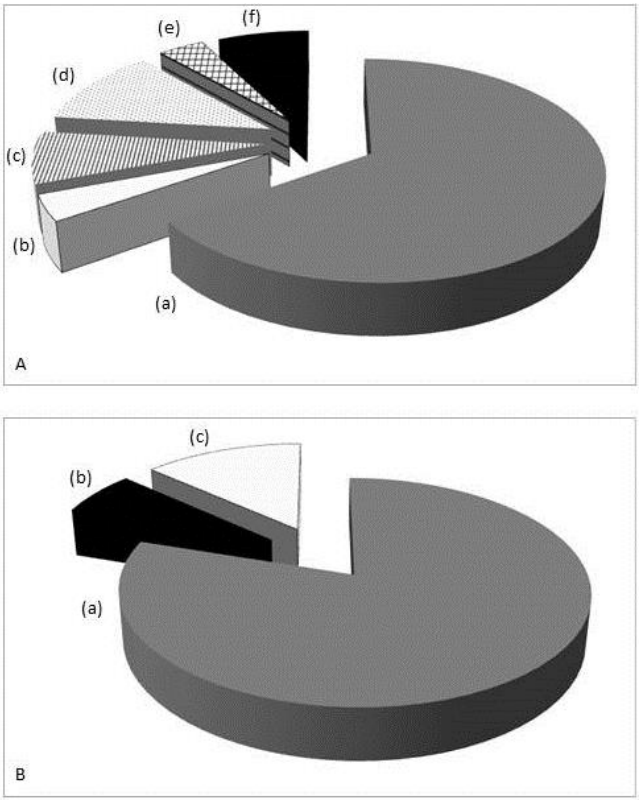


**Figure 3.** Distribution of the isolates according to the DNA 16S sequencing in respect of the culture medium used. (A)-MRS with a) *Lactobacillus plantarum* b) *Enterococcus durans* c) *Leuconostoc pseudomesenteroides* d) *Pediococcus pentosaceus* e) *Lactobacillus fermentum* f) unidentified; (B)-BEA with a) *Enterococcus durans* b) *Enterococcus hirae* c) *Enterococcus faecium*.

The DNA 16S sequencing of the isolates led to the results presented on Figure 3. On MRS, nearly 70% of the isolates belonged to the genus *Lactobacillus*, and more precisely to the species *Lb plantarum*. On BEA,

**Table 2.** Classification of the strains according to their lactate production.

| Group 1 Low lactate production | Group 2 Mid lactate production | Group 3 High lactate production |
|---|---|---|
| *Ec durans* (a) | *Ec hirae* (a) | *Lb plantarum* (a) |
| *Ec durans* (a) | *Ec durans* (a) | *Lb plantarum* (a) |
| *Ec durans* (b) | *Ec faecium* (a) | *Lb plantarum* (a) |
| *Ec durans* (b) | *Ec durans* (b) | *Lb plantarum* (b) |
| *Ec durans* (c) | *Ec durans* (b) | *Lb plantarum* (b) |
| *Ec durans* (c) | *Ec durans* (b) | *Lb plantarum* (b) |
| *Ec durans* (c) | *Lb plantarum* (a) | *Ln pseudomesenteroides* (b) |
| *Ec durans* (c) | *Lb plantarum* (a) | *Lb plantarum* (b) |
| *Ec faecium* (c) | *Lb plantarum* (a) | *Lb plantarum* (b) |
| | *Ec durans* (c) | *Pc pentosaceus* (b) |
| | *Ln pseudomesenteroides* (c) | *Lb plantarum* (b) |
| | | Unidentified |
| | | *Lb plantarum* (b) |
| | | *Lb plantarum* (b) |
| | | *Lb plantarum* (b) |
| | | *Pc pentosaceus* (c) |
| | | *Pc pentosaceus* (c) |
| | | *Lb plantarum* (b) |
| | | *Lb plantarum* (a) |
| | | Unidentified |
| | | *Lb plantarum* (a) |

Ec: *Enterococcus*, Lb: *Lactobacillus*, Ln: *Leuconostoc*. Strains from ewe milk cheeses, goat milk cheeses, and cow milk cheeses are noted respectively (a), (b), and (c).

80% of the isolates belonged to the species *Enterococcus durans*. The other species (*Lb fermentum*, *Pediococcus pentosaceus*, *Leuconostoc pseudomesenteroïdes*, *Ec hirae* and *Ec faecium*) were less frequently observed. No link was detected between the species identified and the type of cheese.

Lactobacilli were separated according to the test proposed by Kandler and Weiss (1986), that is, the production of $CO_2$ in presence of glucose and/ or gluconate. However, this test proved to be inappropriate when we crossed the results with those obtained from the sequencing. Only 6 *Lb plantarum* were identified as facultatively heterofermentative and 2 leuconostocs as strictly heterofermentative. Although the lactate test was pertinent for the lactobacilli, it led to strange results for some isolates of enterococci: some strains produced L- and D-lactate instead of L-lactate solely.

Strains were grouped by hierarchical classification according to the concentration of total lactate produced. Three groups were obtained (Table 2). The first group (low lactate producers; around 630 ppm of lactate) only included *Enterococcus* strains. In the third group (high producers; around 10 000ppm), the only isolates present belonged to the genus *Lactobacillus*, *Pediococcus* and *Leuconostoc*. The second group (around 4 900 ppm) mixed *Lactobacillus*, *Enterococcus* and *Leuconostoc* strains.

### Strains diversity

Isolates were characterized on the basis of their REP PCR profile (Figure 4). *Lb plantarum* strains were divided into 7 clusters, containing respectively 10, 5, 4, 3, 1, 1, 1 isolates (Figure 4A). It is interesting to note that in each cluster containing more than one isolate, we systematically found strains coming from two cheeses or even all three cheese types. This was also observed for *Enterococcus* strains – 4 clusters (Figure 4B).

## DISCUSSION

*Klila* cheeses are produced in a semi mountainous area of Algeria. People of this region have developed specific know-how to optimize milk storage for long periods in harsh climatic conditions. Whatever the type of milk used, they obtain a cheese with high dry matter, low water availability, and low pH which allows it to be kept for many months in harsh conditions. As such, it appears interesting to characterize *Klila* more precisely than had previously been done in view of considering industrial scale production (Leksir and Chemmam, 2015).

In this work, we studied the physico-chemical and microbiological characteristics of three *Klila* cheeses made either with cow, goat or ewe milk. Data obtained

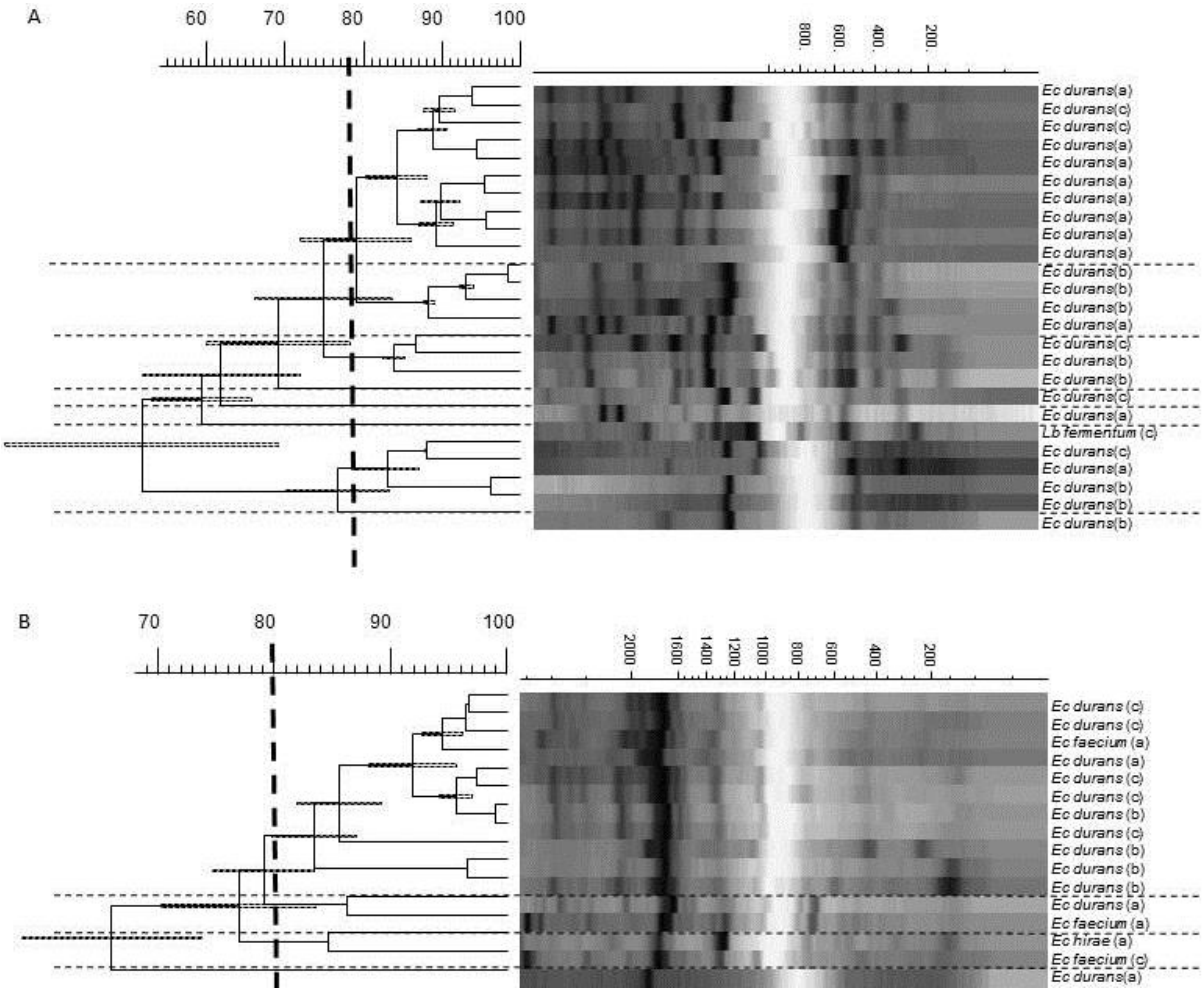


**Figure 4.** Dendrograms drawn by UPGMA of correlation value of normalized REP-PCR patterns from A) *Lactobacillus plantarum* and B) *Enterococcus* spp obtained with primers Rep1R-Dt and Rep2-D. Strains from ewe milk cheeses, goat milk cheeses, and cow milk cheeses are noted in a), b) and c) respectively. Horizontal dotted lines indicate the separations between clusters whereas the vertical dotted lines correspond with the coefficient of similarity (80%).

showed that, except for the dry matter, the three types of cheeses were clearly distinct. These observations appeared congruent with those obtained by Leksir and Chemmam (2015) and Guetouache and Guessas (2015) - for instance for the pH which ranged between 4.2 and 4.5 - but slightly higher than those reported by Rhiat et al. (2013). Concerning the dry matter, our measurements were higher than the values reported before, probably as a consequence of differences in the cheese making process (Claps and Morone, 2011). As a result of this high dry matter, the Aw of *Klila* was very low (< 0.467). The decrease of Aw below 0.6 is a guarantee against spoiling; it is explained by the heating, pressing, and drying of the curd (Cuvillier, 2005).

The fat rate of Klila can vary a lot depending on the season, the breed and the feed; but also from one region to another. This explains the differences between our results and those reported by Harrati (1974) (between 20 and 29 g/100 g vs 17 g/100) and Boubekri and Ohta (1996), collected in two Algerian regions (Batna and Sétif). If we consider the fat on dry matter ratio (between 21 and 31 g/100 g), we can argue that *Klila* is a low-fat hard cheese with high protein content (Lahsaoui, 2012).

The sensory tests demonstrated that the three types of cheeses could be differentiated beyond any doubt on the basis of their colour and odour and to a lesser extent of their aroma and aspect. If the colour was mainly due to the cooking of the acidified milk *Lben*, the intense aroma was a further consequence of the high dry matter. Aroma compounds and sapid substances were concentrated in the residual moisture of the cheese, thereby making them more perceptible (Walther et al*.,* 2008). Mallatou et al*.* (2003) compared the degree of lipolysis in three Greek fresh *Teleme* cheeses made either with ewe, cow or goat milks. Although they noticed some differences between the concentrations of short and long chain fatty acids, the

flavour of the three cheeses was not affected. This is congruent with our idea that the high dry matter of *Klila* explains sensory differences rather than other factors.

The physico-chemical characteristics of *Klila* are not favourable for microbial growth. As indicated above, the pH and the Aw are too low. As a consequence, ripening mechanisms are nearly inexistent. The presence of microbes inside the cheeses can, then, be surprising, especially the high total counts (between 5.0 and 6.5 log (cfu)/g). For instance, Leksir and Chemmam (2015) and Guetouache and Guessas (2015) reported lower values: between 3.1 and 3.8 log (cfu)/g. We can suppose with Cuvillier (2005) and Jakob (2011) that hygienic practices, initial contamination of the milk and storage duration explain these variations between studies.

We observed strong correlations between physico-chemical parameters and some microbial populations, especially Aw. A low Aw was associated with high levels of lactobacilli, enterococci, and spore-forming bacteria. These populations were not necessarily favoured by the decrease of Aw, but they were certainly less affected than other microbes. *Bacillus* spp and butyric acid bacteria (*Clostridium butyricum and C. tyrobutyricum*) were dormant, since the technic we used was specifically aimed at the detection of spores. *C. tyrobutyricum* is responsible for late blowing. Its specific metabolism leads to the transformation of lactate into butyric acid, $CO_2$ and $H_2$ (Jakob, 2011).

The numbers of yeasts and moulds detected in our Klila were very high (> 5.7 log (cfu)/g) compared with the results obtained by Guetouache and Guessas (2015): around 2.0 log (cfu)/g. Many explanations can be put forward to explain this discrepancy: hygiene conditions and air contamination for instance (Cuvillier, 2005). We can argue that these spore forming populations explained partially and even totally the high levels of total counts.

*Salmonella*, *E. coli*, *S. aureus* and *L. monocytogenes* were not found. This can be explained by the double effect of low pH and low Aw (Yohan et al*.,* 2016).

Enterococci are commensal with many Mediterranean cheeses, in which their level varies in general from 5 to 6 log (cfu)/g. They are known to contribute significantly to the ripening of cheeses, being able to survive in drastic conditions: wide pH range (4.6-9.9), high salt rates (< 6.5%), wide temperature range (5-50°C), bile resistance (Franz et al*.,* 2003; Fisher and Phillips, 2009). They are generally brought by the raw materials but also result from poor hygienic conditions. Among species frequently reported, *Enterococcus faecalis* and *Ec faecium* are the most recurrent, followed by *Ec durans* and seldom *Ec casseliflavus* (Giraffa, 2003). Enterococci are generally involved in the ripening process as a consequence of their proteolytic and lipolytic activities, but also, for some strains, their ability to produce diacetyl (Martín-Platero et al*.,* 2009; Aguilar-Galvez et al*.,* 2012). In our case, we found significant (although low) levels of enterococci (between 3 and 4 log (cfu)/g). And we can suppose with Oliver (2005) that these levels under-estimated the “real” level of enterococci in *Klila* since this bacteria can enter the viable but non cultivable state (VBNC). This is all the more true for *Ec faecium* and *Ec hirae*. The dominant presence of *Ec durans* in our cheeses is difficult to discuss since no preceding work indicated that this species was particularly xerotolerant. The majority of the strains isolated in our work were classified as low acid producers. This is in conformity with previous results reported by Cogan et al*.* (1997) which indicated that the majority of *Enterococcus* strains they isolated were unable to lower the pH bellow 5.3 in milk. We can suppose that the *Enterococcus* population played a part in the cheese making, especially during the acidification step. And they were partly inactivated by the heat treatment.

The levels of lactobacilli varied a lot from one cheese to another, between 3.69 and 6.15 log (cfu)/g. This observation confirms the results obtained by Boubekri and Ohta (1996). These authors noted that the microbial composition of *Klila* cheeses could change from region to region, the levels of *Lactobacillus, Enterococcus, Pediococcus* and *Leuconostoc* varying a lot. Among the strains isolated during our studies, the majority (65%) belonged to the *Lb plantarum* species, followed by *Pediococcus pentosaceus* (12%), *Leuconostoc pseudomesenteroïdes* (8%) and *Lactobacillus fermentum* (4%). The remaining strains were unidentified or identified as *Enterococcus*. The use of the test proposed by Kandler and Weiss (1986) to discriminate lactobacilli on the basis of their fermentative aptitude proved to be less relevant and led to strange results. We also observed on mesophilic lactobacilli originating from other cheeses that this test could fail in some cases (Demarigny, personal communication).

Compared to the *Enterococcus* isolates, the strains isolated on MRS medium were all high lactic acid producers. This is a characteristic feature of *Lb plantarum* (and to a lesser extent of *Lb fermentum*), this species being one of the most acidifying germs among lactic acid bacteria (Vescovo et al*.,* 1993; Todorov and Gombossi de Melo, 2010). *Lb plantarum* is frequently found in spontaneous fermented plant products in relation with other heterofermentative microbes such as *Leuconostoc* (Demarigny et al*.,* 2012). This bacterium is also a major actor in the ripening step during cheese ageing. Our results were therefore as expected. However, in the literature, *Lb plantarum* is not usually referred to as xerotolerant, contrary to *Pediococcus*, a germ frequently found in old hard cheeses (that is, Grana cheeses). Recently Bouton et al*.* (2016) indicated that they found *Lb plantarum* strains on dry hay. It would indicate that this species can contribute to the biopreservation of a fermented food even in harsh conditions (low pH, low Aw, etc.) and can present xerotolerant aptitudes.

Concerning the diversity of the *Lactobacillus* and *Enterococcus* isolates evaluated by REP PCR, we

observed the presence of different clusters: 4 for *Enterococcus* spp and 7 for *Lb plantarum*. It indicates that inside each population different sub-populations could be found and consequently, a relative diversity even in extreme environmental conditions. More interestingly, we observed that the different strains could be found in two or three cheeses simultaneously. Two hypotheses can thus be put forward: 1) these strains are naturally present in the region from which the cheeses were made; 2) and/or, the physico-chemical conditions that prevail in the cheeses tend to select the best adapted strains which consequently exhibit very similar patterns.

## Conclusion

*Klila* is a traditional cheese which has been produced in Algeria for many centuries. The adaptation of the cheese making to the environmental context allowed people of these regions to produce a cheese which can be stored over time without running any sanitary hazard. The type of milk used gives rise to specific products, clearly identified by naïve consumers. The aromatic balance is probably influenced mainly by the different steps of the process, which results in a concentration of the aroma compounds. Indeed, the microbes inside the cheese – especially, *L. plantarum* and *Enterococcus* – probably have no influence on the ageing process. However, they contribute to the preservation of the cheeses over many months (“hurdle concept”) and seem to represent a part of the typicality of the region from where the cheeses are produced. They also possess interesting physiological features (xerotolerance) which could be studied in order, for instance, to develop specific starters dedicated to the making of *Klila*. It could be the first step towards the industrialisation of this cheese. Right now, it would also be interesting to characterise the nutritional virtues of *Klila* more precisely and to correlate them with the technological, physico-chemical and microbiological characteristics of this cheese. This is intended in a future study.

## Conflict of Interests

The authors have not declared any conflict of interest.

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