

## ORIGINAL ARTICLE

# Effect of processing technology on the biodiversity of the Bacterial flora of an Industrial cheese camembert soft type

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### ABSTRACT

Industrial cheese production involves the use of a diversified microbiota composed of a natural endogenous microbial population provided by the milk and the other exogenous by a complementary seeding of ferments selected according to the technology used by the cheesemaker. This paper aims to characterize the bacterial microbiota of two kinds of industrial soft cheese of traditional and stabilized type, and their biodiversity during ripening. Identification of the bacterial flora of refining in selective environments, followed by the phenotypic identification of the milk isolates by API galleries. Changes in levels of cultivable populations of each species are controlled by the enumeration of viable cells expressed in cfu/g of cheese. It has been proven that the dominance of bacterial species varies with the time of ripening. All along the refining; 08 species have been identified phenotypically *Brevibacterium*, *Lactococcus enterococcus*, *Lactobacillus*, the lactococci, the *Leuconostocs*, the micrococci, the *Pediococcus* and *Lactic streptococci*.

### Langue source

Tout au long de l'affinage ;08 espèces ont été identifiées phénotypiquement des *brevibactériums*, des *entérocoques lactiques*, des *lactobacilles*, des *lactocoques*, des *leuconostocs*, des *microcoques*, des *pédiocoques* et des *streptocoques lactiques*

We have observed sequential growth of some bacterial groups, compared to others in which the acidifying flora is minor and to halotolerant flora reported by the majority of milk at the end of ripening. The preservation of this bacterial population that guarantees the richness and sensory diversity of the industrial cow's milk cheeses also depends on the period of lactation and the applied processing technology.

**Keywords.** Microbiota, phenotypic identification, viable cells, sequential growth

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## INTRODUCTION

The understanding and mastery of microbial ecosystems of cheeses today represent a real challenge science, health and economy. The stakes are high because it comes to the survival of a unique food heritage, part of our culture. According to his interest and its consequences in cheese making, the microbial flora, present in milk at the end of treatment, is defined essentially by the methods of breeding, milking and its conduct as well as the thermal treatment, moderated by a simple thermisation or less severe by pasteurization LTST (high temperature short time) that does not completely destroy it. Actually this flora is a necessary parameter for the preservation of the technological potential of milk for its transformation into cheese as it plays an important role in the quality of the cheeses on the organoleptic

plan and, in particular, on the tasting plan [8, 9]. The stakes are high because it comes to the survival of a unique food heritage, part of our culture. The microbial community of cheeses including the relative importance during the ripening process is constantly changing and which affect safety and the sensory characteristics of the cheeses [11,17]. In Algeria, cheese industrial manufacturing of soft cheeses using lactic flora of stem acidifying mesophilic for traditional technology and stem predominantly thermophilic for type technology is stabilized; the proportion of these strains varies during ripening. Thus some strains are present, while others follow one another over time. It is in this context that we'll talk about this study to track the dynamics of the bacterial populations characterizing our samples of cheeses camembert soft type marked by the quantitative importance of the populations of the made lactic bacteria (streptococci), indigenous bacteria (*Lactococci*, *Leuconostocs*) and halotolerant bacteria (*Micrococcus*, *Coryneformebacterias*). Traditionally, the process of ripening of the cheeses and the development of organoleptic qualities were exclusively provided by the natural microbial communities present in milk. Currently and industrially, when milks are purified in microorganisms by heat treatment (pasteurization-thermisation), physical (micro-filtration-bactofugation) and pulsed high-intensity electric currents and exogenous microbial seeding of milk became indispensable. Technological innovation of processing milk, far from tradition, is necessary to facilitate control of microbial populations on the one hand and to ensure uniformity and standardization of the sensory characteristics of the cheeses on the other hand [5,14,21,27,36]. This study is limited to a first characterization of the bacterial flora, dominant of two industrial segments, one of traditional type from thermised milk, stabilized type obtained from a pasteurized milk, at various stages of ripening by the development of appropriate environment of culture for the inventory of representative lactic strains and genres sometimes poorly defined including those halotolerant flora.

## MATERIAL AND METHODS

### Sampling

The samples were taken in two industrial cheese factories; a traditional one carrying the name "Tessala" (SidiBelabbes), and "Safilait" (Constantine) which is of a stabilized type.

Sampling has been established taking into account the periodicity of treatment, i.e. according to the stage of lactation whether low, medium or high.

The samples of soft cheese of camembert type have been taken during the pre-refining stage or unmolding (24 h after production), and the interflow stage (24 hours after salting and variable ripening stages at 04, 09 and 11 days of ripening).

- Temperature between 11 and 15 °C  
After interflow, i.e. 24 H after salting  
04 days of ripening in hâloir  
09 days of ripening in hâloir  
11 days of ripening to dry before packaging.

### Microbiological analyses

#### Microbial enumeration

**Total Flora:** in the environment of lactate with the following composition (by liter): tryptone (10g), extract of yeast (5g), sodium lactate (5g), sodium chloride (30g), agar (15g) 5 g/l of sodium carbonate added to neutralize a potential acidification. This environment is used to determine the culture of the maximum number of microbial cells present [23, 29].

**Lactic Flora:** research of the lactic flora is important because it is useful in refining. Its quantification allows the monitoring of the maturation of the cheese, particularly of proteolysis. This flora is essentially formed in shells and bacilli gram + and catalase (-).

The principle of this count is based on the use of the specific environment (medium M17) made selective by addition of nalidixic acid. Incubation will take place at 25 °C and 37 °C for 72 hours [4, 10, 28].

And by the use of the MRS (De Man, Rogosa, and Sharp) medium with incubation at 37°C for 48 hours. This environment takes into account acidogenic characters and acidophilic and nutritional requirements of these germs.

#### Halotolerant flora

Its consists mainly of *Micrococcus* and *coryneformes* bacteria. The principle of this count is based on the use of a hypersalt selective medium added to 20 mg/L of amphotericin B (Fungizone) to prevent the growth of yeasts and moulds and 5 g/L of calcium carbonate. This environment was held by [31] because he had achieved a satisfactory in growth of flora with positive Gram (bacteria *Micrococcaceae* and

coryneformes in particular) of the surface and the heart of camembert. The incubation is carried out at 30° C for 48 hours.

**Isolation, purification of the isolates** Isolation and identification of strains will be performed by the application of techniques of classic microbiology, based on research of a number of morphological, physiological and biochemical characters. All isolation and technical purification have been described by the international dairy Federation. Conservation in the long term of the purified isolates is conducted in a medium containing a mixture of 70% of skim milk (enriched by 0.05% yeast extract and 0.05% glucose) and 30% glycerol and stored in tubes eppendorf at a temperature of -20 ° c

**Phenotypic identification of isolates, purified by the API bioMérieux galleries**

The phenotypic identification of isolates, purified by the system API bioMérieux is conducted using 50CHL version 5.2 API and API 20 STREP galleries for lactic bacteria, CORYNE API and API STAPH halotolerant bacteria (bioMérieux, Marcy l'Etoile, France). Seeding and the reading of the Gallery have been made according to the manufacturer's instructions. They are as follows:

-Cultivate pure strain specific medium agar 24 h at 30 ° c and 45 ° c, for lactic bacteria at 25° C for halotolerant bacteria, open a bulb suspension API, pick all colonies of culture using a swab and achieve a dense suspension in the bulb, open an ampoule of the API Gallery and inoculate with a few drops of the homogenized suspension, distribute the API bulb so inoculated in the tubules and cover with sterile paraffin oil, incubate at 25 ° c, 30 ° C and 45 ° c aerobically for 48 h, all the tests are read at 24 and 48 h (we're looking in each tubule acidification produced resulting in the bend of the coloured indicator in the middle. Esculine test, observed a turn from purple to black), save the results, the resulting biochemical profile can be read through an APILAB (bioMérieux, Marcy l'Etoile, France) identification software.

**Determination of viable cells concentration**

The method for counting cheese suspensions is one of successive dilutions described by [2, 22]. Dilutions are prepared with tubes containing 9 ml of sterile physiological water. The range of dilution is 10<sup>-2</sup> to 10<sup>-8</sup>. Dilutions used in this range will vary according to the progress of ripening and counted microorganisms. For each dilution, three boxes of petri dishes will be seeded on the surface by 49.2 µL of suspension. Seeding will be in boxes of 90 mm in diameter, by means of a spiral ensemenceur (Spiral plater, Interscience, France). This method will allow counting colonies on all of the box or by sector. The average concentration in viable cells will be established according to the following relationship:

$$C \text{ (CFU/g)} = (C1 + C2 + C3) \times d / 3 \times V$$

Where C (CFU/g) is the concentration unit forming colony (CFU) by gram, dis the dilution from the cheese, Ci is the number of CFU for the box i (Ci= 1 to 3), and Vis the volume of the seeded solution (ml)

**Table 1.** Morphological identification of gender criteria alleged lactic stem and halotolerants

Macro-morphology	Micro-morphology	Temperature ° c	Groups
White colonies round or lenticular	Coccidiplocoques and in chains	37-45 ° c	<i>Enterococci</i> and lactic streptococci
White colonies round or lenticular	Coccidiplocoques and in chains	25 to 37 ° c	<i>Lactococci</i>
Transparent colonies Very small and round	Oval Coccis in chains	15 to 37 ° c.	<i>Leuconostocs</i>
Small white colonies round or lenticular	Small sticks And in chains	37 to 45 ° c	<i>Lactobacilli</i>
Opaque colony domed and smooth	Short Bacillus	10 to 25 ° c	<i>Brevibacterium</i>
Isolated rounded colony A curved edge smooth and shiny	Grouped into shells Irregular clumps	10 to 25 ° c	<i>Micrococci</i>

**Table 2.** Environments used and conditions of incubation for the isolation of strains

Microorganisms	Community isolation	Temp. ° c	Duration	Incubation
<i>Enterococci</i> and lactic streptococci	M17 pH = 6.5	37 and 45	72 hours	Aerobiosis
<i>Lactococci</i>	M17 pH = 6.5	30 and 37	72 hours	Aerobiosis
<i>Leuconostocs</i>	M17 Hypersalt 6.5% pH = 9.6	30	72-96 hours	Aerobiosis
<i>Lactobacilli</i>	MRS. pH = 6 and pH = 5.5	37 and 45	72 hours	Anaerobiosis

Elamine *et al*

## RESULTS

### Bacterial count

**Table 3.** Average incidence of lactic bacteria isolated in traditional industrial cheese made with thermised milk at 63 ° c cheese Tessala SidiBelabbes during the three stages of lactation (\* unit: g / 100 g)

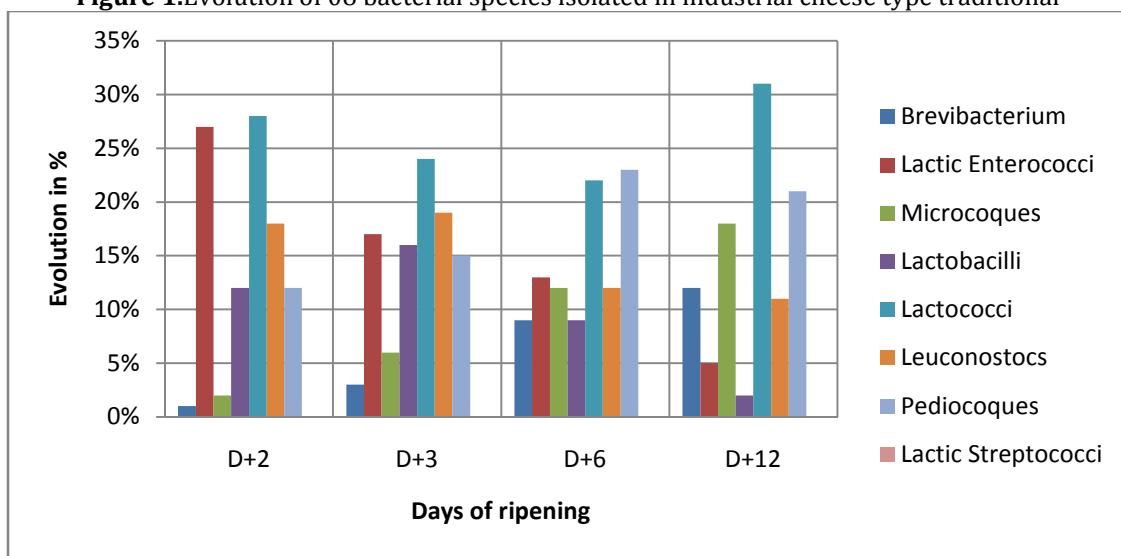
Type of analysis	Camembert in the Release D+ 2	Camembert after salting to the interflow D + 3	Camembert in the 4 <sup>th</sup> day of refining D + 6	Camembert in Late ripening D + 12
1 / Physiological state of the substrate cheese pH NaCl g *. Moisture % Temperature ° c	4.81 1.95 57.15 20	4.74 2.51 51.65 15	5.34 2.1 54.95 12	6.78 1.55 56.2 12
2 / The compound of ripening parameters Temperature ° c HR % Frequency of ventilation in 24 hours	15 85 - -	12 95 1 -	12 95 1 -	12 95 1 -
3 / Microbiological State of the substrate Flora total CFU/g Lactic flora on MRS. CFU/g at pH 6 Lactic flora on M17 CFU/g Refining plant "halotolerant" CFU/g	28 10 <sup>7</sup> 6 10 <sup>6</sup> 14 10 <sup>6</sup> 7-10 <sup>4</sup>	11 10 <sup>7</sup> 10 <sup>3</sup> 6 10 <sup>6</sup> 2 10 <sup>3</sup>	18 10 <sup>7</sup> 8 10 <sup>3</sup> 21 10 <sup>6</sup> 18 10 <sup>3</sup>	21 10 <sup>7</sup> 5 10 <sup>2</sup> 4 10 <sup>7</sup> 4 10 <sup>5</sup>
2 / Related species <i>Brevibacterium</i> <i>Lactic enterococci</i> <i>Microcoques</i> <i>Lactobacilli</i> <i>Lactococci</i> <i>Leuconostocs</i> <i>Pediococques</i> <i>Lactic streptococci</i>	1% 27% 2% 12% 28% 18% 12% 0%	3% 17% 6% 16% 24% 19% 15% 0%	9% 13% 12% 9% 22% 12% 23% 0%	12% 5% 18% 2% 31% 11% 21% 0%

**Table 4.** Average incidence of lactic bacteria isolated in the industrial stabilized type manufactured with cow's milk cheese pasteurized to 72 ° c of the dairy Safilait Constantine during the three stages of lactation (\* unit: g / 100 g)

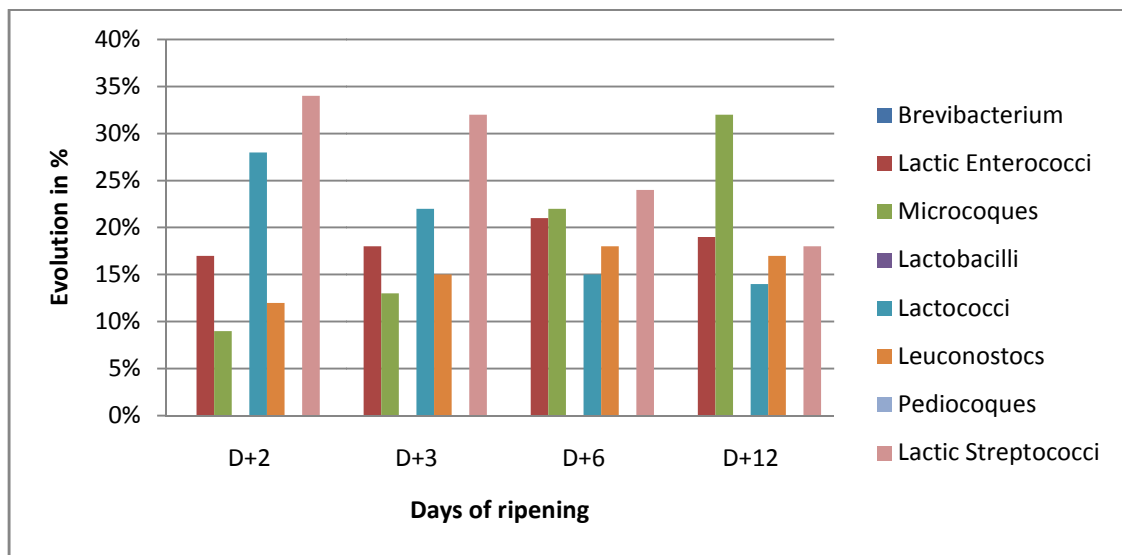
Type of analysis	Camembert in the Release D + 2	Camembert after salting to the interflow D + 3	Camembert in the 4 <sup>th</sup> day of refining D + 6	Camembert in Late ripening D + 12
1 / Physiological state of the substrate cheese pH NaCl g *. Moisture % Temperature ° c	5,1 2.1 55.13 20	4.86 2.62 53.4 15	5.38 2.04 52.4 13	7.1 1.9 53.86 13

2 / The compound of ripening parameters Temperature ° c HR % Frequency of ventilation in 24 hours	15 85 -	13 95 1 <i>Elamine et al</i>	13 95 1	13 95 1
3 / Microbiological State of the substrate Flora total CFU/g Lactic flora on MRS. CFU/g at pH 6 Lactic flora on M17 CFU/g Refining plant "halotolerant" CFU/g	12 10 <sup>7</sup> Abs 4 10 <sup>7</sup> 1,5 10 <sup>2</sup>	4 10 <sup>7</sup> Abs 15 10 <sup>5</sup> 18 10 <sup>2</sup>	11 10 <sup>7</sup> Abs 14 10 <sup>6</sup> 8 10 <sup>3</sup>	23 10 <sup>7</sup> Abs 9 10 <sup>7</sup> 6,5 10 <sup>4</sup>
2 / Related species <i>Brevibacterium</i> <i>Lactic enterococci</i> <i>Microcoques</i> <i>Lactobacilli</i> <i>Lactococci</i> <i>Leuconostocs</i> <i>Pediocoques</i> <i>Lactic streptococci</i>	0% 17% 9% 0% 28% 12% 0% 34%	0% 18% 13% 0% 22% 15% 0% 32%	0% 21% 22% 0% 15% 18% 0% 24%	0% 19% 32% 0% 14% 17% 0% 18%

**Figure 1.** Evolution of 08 bacterial species isolated in industrial cheese type traditional



**Figure 2.** Evolution of 08 bacterial species isolated in industrial cheese type stabilized



Elamine *et al*

### Isolation purification of the isolates and identification

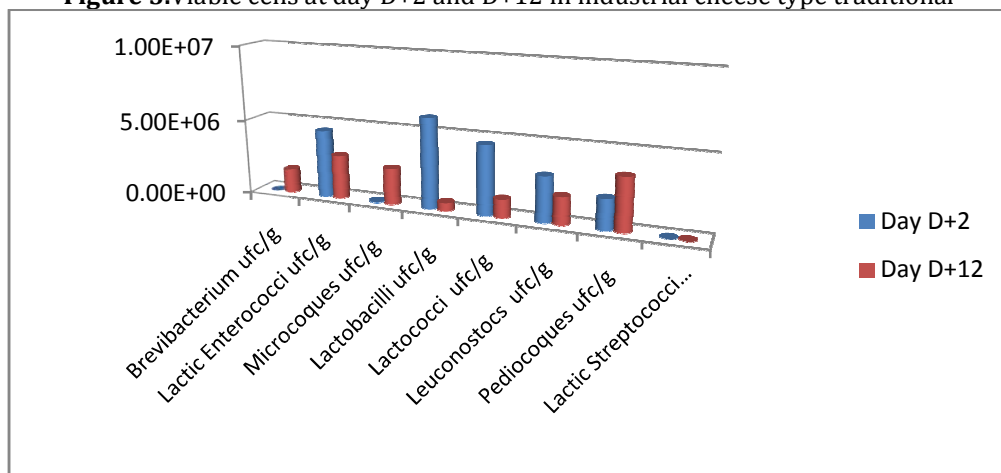
**Table 5.** Number of isolates purified by related species during three periods of lactation on two industrial cheeses of traditional and stabilized type

Related species	Traditional-type cheese			Stabilized type cheese		
	Number of isolates in low lactation	Number of isolates on average lactation	Number of isolates in high lactation	Number of isolates in low lactation	Number of isolates on average lactation	Number of isolates in high lactation
<i>Brevibacterium</i>	2	1	2	0	0	0
<i>Enterococci</i>	4	2	2	3	5	3
<i>Lactobacilli</i>	2	2	7	0	0	0
<i>Lactococci</i>	6	5	7	4	4	4
<i>Leuconostocs</i>	2	3	4	0	2	2
<i>Micrococci</i>	2	3	3	0	1	2
<i>Pediocoques</i>	0	0	5	0	0	0
<i>Streptococci</i>	0	0	0	3	3	4

### Determination of the concentration of viable cells

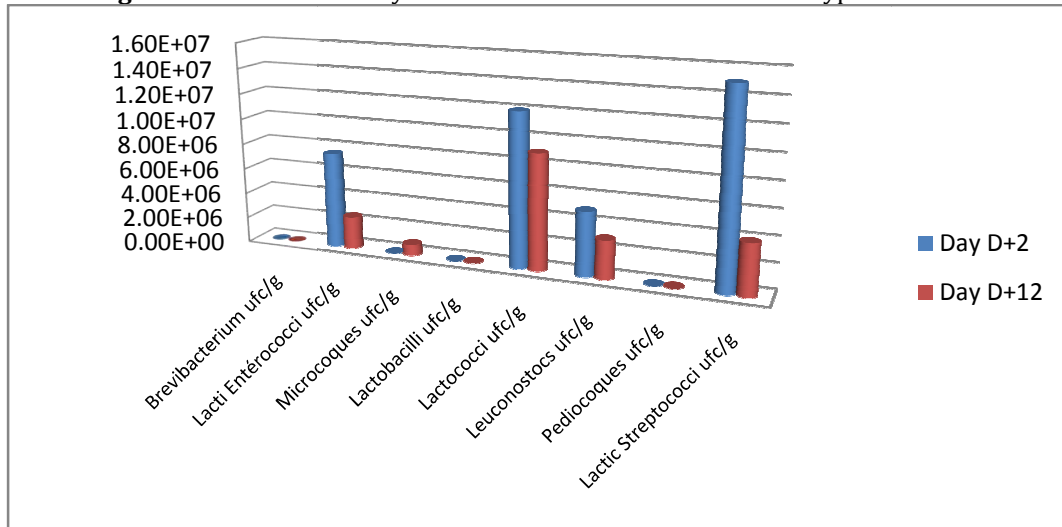
Determination of the concentration average in viable bacterial cells according to the progress of the refining A/industrial cheese of a traditional type "Tessala" of Sidi-Belabbes

**Figure 3.** Viable cells at day D+2 and D+12 in industrial cheese type traditional



B / industrial cheese of a stabilized type "Safilait" of Constantine"

**Figure 4.** Viable cells at day D+2 and D+12 in industrial cheese type stabilized



Elamine *et al*

**Table 6.** Results of the identifications by API galleries

Bacterial species	By API 50 CHL	% I.D.	By API 20 STREP	% I.D.	By API STAPH	% I.D.	By API CORYNE	% I.D.
<i>Br linens</i>							+	85
<i>En Faecium</i>			+	92				
<i>En Faecalis</i>			+	88				
<i>En sulfureus</i>			+	90				
<i>Lb acidophilus</i>	+	75						
<i>Lbcasei</i>	+	80						
<i>Lb delbrueckii</i>	+	95						
<i>Lb fermentum</i>	+	85						
<i>Lc subsp. cremoris</i>			+	95				
<i>Lclactis</i>			+	98				
<i>Lc subsp. lactis</i>			+	85				
<i>Diacetylactis Lc</i>			+	70				
<i>Leucremoris</i>	+	72						
<i>Leucmesenteroide</i>	+	75						
<i>Leuclactis</i>	+	82						
<i>Micluteus</i>					+	92		
<i>Micxylosus</i>					+	85		
<i>Pedacidilactici</i>	+	74						
<i>Pedpentosaceus</i>	+	75						
<i>ThermophilicStrept</i>			+	95				

% I.D : % identification, Br : Brevibacterium, Ent : Enterococcus, Lb : Lactobacillus, Lc : Lactococcus, Leuc : Leuconostoc, Mic : Microcococcus, Ped : Pediococcus, Strept : Streptococci

**Table 7.** Assessment of the bacterial species identified phenotypically by API galleries

Bacterial species	To the release At day D + 2		After salting At day D + 3		On the 4 <sup>th</sup> day refining at day D + 6		To the 10 <sup>th</sup> day refining at day D + 12	
	FA	FB	FA	FB	FA	FB	FA	FB
<i>Brevibacterium linens</i>	0	0	1	0	1	0	3	0
<i>Entfaecium</i>	1	1	1	1	1	2	1	2
<i>Entfaecalis</i>	1	1	0	1	0	1	0	2
<i>Entsulfureus</i>	1	0	1	0	1	0	0	0
<i>Lb acidophilus</i>	1	0	1	0	1	0	1	0
<i>Lbcasei</i>	1	0	1	0	0	0	0	0
<i>Lb delbrueckii</i>	2	0	1	0	1	0	0	0

<i>Lbfermentum</i>	1	0	0	0	0	0	0	0
<i>Lc subsp. cremoris</i>	1	2	1	2	1	1	2	1
<i>Llactis</i>	2	1	1	1	1	1	2	1
<i>Lc subsp. lactis</i>	1	0	1	0	0	0	0	0
<i>DiacetylactisLc</i>	1	1	1	1	1	0	2	0
<i>Leuccremoris</i>	1	0	1	0	1	0	1	0
<i>Leucmesenteroide</i>	1	0	1	0	0	0	0	0
<i>Leuclactis</i>	1	1	1	1	1	1	0	1
<i>Micrococculuteus</i>	0	0	1	0	2	0	2	0
<i>Micrococcixylosus</i>	1	0	1	0	1	1	1	2
<i>Pediococciacidilactici</i>	1	0	1	0	1	0	1	0
<i>Pediococcipentosaceus</i>	0	0	0	0	0	0	1	0
<i>Thermophilic Streptococci</i>	0	5	0	2	0	2	0	1

FA : Traditionalcheese , FB : Stabilizedcheese , Ent : Enterococcus , Lb : Lactobacillus , Lc : Lactococcus , Leuc : Leuconostoc

## DISCUSSION

### Bacterial count

Bacterial counts results show a significant difference of the lactic and halotolerant flora between the two cheeses because of different manufacturing and ~~technology~~ of heat treatment applied to processed milk. The total flora is of the order of  $28 \times 10^7$  cfu at the beginning of ripening for traditional industrial technology and order of  $12 \times 10^7$  cfu for stabilized type; halotolerant florais of the order of  $7 \times 10^4$  cfu for camembert Tessala compared to  $1.5 \times 10^2$  cfu for that of Safilait. These results are similar to those of [11]. Lactic streptococci which come from seeding added to pasteurized milk constitute the essence of the lactic flora in the stabilized-type cheese: their rate is about 34% to the unmolding process and decreases to almost the half by the end of ripening. Some bacterial species not detected in stabilized cheese, lactic such as *Lactobacilli* and the pedicoques, and a halotolerant brevibacteriumare found in on traditional cheese. Of a reduced population at the beginning of ripening, the brevibacteriums and the micrococci develop significantly in halophilic environment, a fter salting. These are proteolytic agents active in refining. The lactic and halotolerant flora represents respectively 80% and 20% at the beginning of ripening so that it is of the order of 60% and 40% at the end of ripening

It also appears that the effect of the intrinsic factors as humidity, salinity, the pH of the substrate or external such as the temperature and the humidity of the enclosure of refining significantly affect characterized bacterial population dynamics [1,3,6,15,32] see tables 3 and 4

### Isolation and purification of the isolates

104 strains of lactic and halotolerant bacteria were isolated from the two types of camembert. they were grown and isolated in specific environment (MRS, M17 and Chapman), and because of the nutritional requirements, these settings have been enhanced especially growth factors, sugars and nitrogen contents [10,18,24,30] . The study of the main morphological, biochemical and physiological characters, as defined on tables 1 and 2, showed a diversity of isolated bacterial species ; These detected species depend essentially on the period of lactation, used processing technology and the stage of maturation of the cheese.

From the results obtained for the three periods of low, medium and high lactation, it turns out that the dominance of the bacterial species varies with the time of ripening. As 08 species identified phenotypically as being the most common and most often dominant at the beginning of ripening distributed by order of importance in lactic streptococci 25%, Lactococci 22%, Leuconostoc 20%, Lactobacilli 15%, Enterococci 8%, Pediococques 5% , Micrococci 3% and Brevibacterium 2%. These results are similar to those obtained by [22,29]

The purpose of refining is to direct the behavior of the microbial flora in the ideal sense [4, 12, 19, 25]. It has also been noticed that the original flora has been highly affected by the heat treatment used in the two processing technology; 08 groups of related species have been isolated in the traditional industrial camembert from of a milk thermized at  $63^\circ \text{C}$  while only two groups of acidifying species related to the lactococci and lactic streptococci with a group of commensal lactic ferments enterococci species have been isolated in the camembert of stabilized type from a milk at  $72^\circ \text{C}$  for different periods of lactation.

Figures 1 and 2 illustrate average changes in two industrial technologies of transformation during the two phases of lactation where it is observed that the acidifying flora, represented by the majority Lactobacilli and Streptococci of early ripening, regresses by late ripening to allow the natural halotolerant florabrought by milk (such as Micrococci, the Brevibacteriums) to take over. However, only *Lactococci*



and enterococci, as well as the *Leuconostocs* remain at a higher level compared to the other species that tend to disappear. These results are consistent with those of [5, 7, 13, 20, 30].

#### **Phenotypic identification of purified isolates**

Knowledge of the diversity of the lactic and halotolerant flora was first acquired by classical methods of isolation and identification based on cultures with specific environments (see table 5), enriched methods in second place by phenotypic analyses in database API galleries by the essential carbohydrates degradation test to identify the bacterial species and even the subspecies. This technique has confirmed the typicality of the species with the purified isolates of the two cheeses traditional and stabilized type (tables 6 and 7). It has also confirmed the studies prepared in this sense [18, 29].

On the isolated species of Lactobacilli, a group either thermophilic homofermentaire, fermenting hexoses, strict produced only lactate but does not ferment the pentose and was assigned, after phenotypic identification to two species *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* subsp. *lactis*. The second group is mesophilic optional heterofermentaire it ferments hexoses in lactate, acetate and ethanol with a species identified from *Lactobacillus casei*. A third group always mesophilic strict heterofermentaire: *Lactobacillus fermentum* ferments hexoses in lactates, acetate and releases CO<sub>2</sub>.

Among the lactic flora of acidification, it has been identified from the *Lactococci*, that cannot stand the temperature of 45 °C and show a positive development in 6.5% NaCl and produce the acetone: *Lactococcus cremoris*, *Lactococcus lactis*, *Lactococcus casei* subsp. *lactis* and *Lactococcus diacetylactis* subsp. and thermoresistant Streptococci among the species *Thermophilic Streptococci*.

As for the *Leuconostocs*, all present a positive development at 37°C; They ferment citrate by producing CO<sub>2</sub>, the *Leuconostoc lactis*, *Leuconostoc cremoris* and *Leuconostoc mesenteroide* have been identified.

Two *Pediococcus mesophilus* homofermentative have been detected. Unable to use lactose, they resist salt and also participate in proteolytic maturation of the traditional industrial camembert: a *Pediococcus acidilactici* acidophilic (growth at pH5) and which does not ferment the maltose and a *Pediococcus pentosaceus* which use maltose.

Among the proteolytic and lipolytic species, it has been identified of *Lactic enterococci*: *Enterococcus faecium*, *Enterococcus faecalis* and *Enterococcus sulfureus* which biochemically negative citrate, grow at pH 9.6 in the presence of 6.5% NaCl. Other species lipolytic and proteolytic halotolerant with optimal growth at 15 °C, gram +, + catalase and oxidase - were ranked among the *Micrococcus luteus*, *Micrococcus xylosum* and *Brevibacterium linens*.

#### **Determination of the concentration of viable cells**

The proportions of dominance are different all along the refining because of biochemical reactions and bacterial interactions that take place and which are at the origin of the sequential growth of some groups over others (figures 3 and 4). Among the main factors of induction of decline in growth of some bacteria it is worth noting the presence of the acidifying flora including Lactobacilli and streptococci and the exponential proliferation of others such as the halotolerant flora. You will also notice the influence of physicochemical factors including temperature, pH of the substrate cheese without forgetting the osmotic shock by salting that involves the destruction of some bacterial cells and release of intracellular content of enzymes which play an important role in the development of flavor and texture to the cheese as well as nucleic acids vitamins and minerals that play a role, directly or indirectly, stimulating the growth of other microorganisms [17,24,33,34].

In the traditional cheese dough, Lactobacilli that predominate at the beginning of ripening due to prior growth in milk become almost not cultivable late ripening. The other lactic strains are present cultivable throughout ripening for the two types of cheese. Halotolerant flora represented by the Micrococci and the *Brevibacterium* evolves after salting to become two majority cultivable microflora in late ripening.

According to our results that are consistent with those of the bibliography [16], changing continuous environment of the ecosystem of the cheese with the physiological characteristics of one stage of ripening including temperature, the rate of salt, pH, oxygen and nutrient conditions lead to this difference in assessing quantitatively characterized bacterial populations. On the other hand, the synthesis of the data shows that the analyzed bacterial populations vary from one cheese variety to another, depending on the place of production of the lactation period, treatment made with milk and applied processing technology. Treatment of controlled milk has created an abundance of bacterial species either 06 different species of lactic bacteria, including 17 genera and two species of halotolerant bacteria with three genera.

Finally, the phenotypic intra-species heterogeneity among the lactic and indigenous halotolerant bacteria is notable in comparison to the industrial cheese soft type Camembert cheese from the stabilized type cheese industry where this technology requires the mastery of the endogenous microbiota provided initially by the milk and supplemented by an exogenous brought by the know-how of a cheese maker with

specific biochemical properties highly targeted to achieve a controlled balance and required intrinsic properties.

The understanding of the evolution of the microbial ecosystem during the ripening which influences the organoleptic qualities of the camembert is vitally important to improve its intrinsic value. Technological innovations of processing milk, far from tradition, have emerged to facilitate the control of microbial populations and their dynamics by uniformity and standardization of the characteristics, organoleptic and sensory of cheeses with designation of origin [12,22,26,35,37,38].

According to the interest of each isolated bacterial species and its technological and sensory effects, this work is a contribution which aims to bring the scientific approach that would ensure a better control of the processing technology to industrialists and cheese and that would thus enjoy other strains to the interesting properties in functions of the technological skills and to certify the designation of origin 'A.O.C.' cheese.

## CONCLUSION

The study specified the nature of bacteria of 02 camemberts produced industrially with two different technologies. The relatively recent evolution of the methods of processing could lead to a change of its natural flora, and consequently, that of the cheeses. Moreover, the existence of seasonal variations of one cheese factory to another gave different characteristics and bacterial populations. The work we have carried out is of course not exhaustive, due to the volume of established analyses that it necessitates: it should be completed partly by the highlighting of the fungal flora including yeasts and molds, and partly by the characterization of the caseinolytic activity and electrophoretic splitting of the proteases and the aminopeptidases by intracellular microbial enzymes of technological interest.

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Dynamique en affinage de la flore bactérienne lactique et halotolérante d'un fromage industriel à pâte molle type camembert

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