Original Research Article

Production of Soft Bloomy Ring Cheese With Lactic Acid Bacteria Isolated From Cameroon's Cow Milk

ABSTRACT

The changing of the diversity of lactic acid bacteria (LAB) from one locality and another is one of the main reasons of the organoleptic and physicochemical differences encountered in the same class of cheese. The objective of this work was to evaluate the influence of LAB isolated from cow's milk produced locally in Cameroon on the organoleptic and physicochemical quality of soft cheese with bloomy rind. To this effect, 05 LAB (IS1, IS2, IS3, IS4 and IS5) were isolated to fresh milk and selected after macroscopic, microscopic and biochemical characterizations. After tests of acidification, fermentation type, compatibility between LAB and LAB concentration on milk coagulation, the combined IS1-IS4-IS5 and IS2-IS3-IS5 were retained to produce 02 soft cheeses with bloomy rind namely respectively FROCAM 145 and FROCAM 235. When used at concentrations of 3×10^7 CFU/mL, these combined LAB coagulate fresh renneted milk after 3 hours at 26°C. During the production of FROCAM 145, the combined IS1-IS4-IS5 resulted in a firm, moist curd and a pH reduction of 14 and 15% respectively after the milk maturation and ripening of FROCAM 145. This cheese recorded 100% mould recovery, with protein, lipid and calcium content of 6.7, 62.2 and 0.1% respectively. On the other hand, during the production of FROCAM 235, the combined IS2-IS3-IS5 resulted in a dry and crumbly curd. The pH recorded with this combined LAB after maturation of the milk and ripening of FROCAM 235 is 12 and 17% respectively. The FROCAM 235 recorded a low mould recovery estimated at 20% and a protein, lipid and calcium content of 7.8, 65.1 and 0.3% respectively. From a microbiological point of view, FROCAM 145 and FROCAM 235 did not record any contamination by *Listeria monocytogenes*, *Salmonella* and Staphylococci. The sensory analysis shows that FROCAM 145 was more appreciated than FROCAM 235 with scores of 0.74 and 0.24 respectively. In view of these results, the IS1-IS4-IS5 combinations isolated from Cameroonian milks present a certain technological interest in the transformation of milk into cheese.

Key words: Lactic acid bacteria, cheese, protein, lipid, calcium, sensory analysis

1. INTRODUCTION

According to Codex Alimentarius [1], cheese is defined as a ripened or unripened product of soft or semi-hard, hard or extra-hard consistency that can be coated and in which the whey protein/casein ratio does not exceed that of milk. It is the most widespread dairy product in the food sector. The world cheese production reached in 2014, 21 million tons and continues to increase nowadays. In 2026, this production will reach 106 billion US dollars on the market [2]. Cheese is a caloric food (351 KCal/100g) mainly due to its lipids (28.2g/100g). It is also rich in proteins (23,5g/100g), calcium (689mg/100g), phosphorus (512mg/100g), vitamin A (250µg/100g) and zinc (3.3mg/100g). Its consumption is adequate for children, adolescents and adults [3].

Moreover, according to the production method, the water content and the maturing time, several categories of cheese can be distinguished, namely fresh cheeses, hard cheeses and soft cheeses. Soft cheeses are grouped into soft cheeses with washed rind and soft cheeses with bloomy rind [4]. However, although belonging to the same cheese category and sometimes having the same production protocol, soft cheeses with bloomy rind have varying organoleptic and nutritional characteristics. The difference between these cheeses may be due to the origin and quality of milk and environmental conditions such as temperature and humidity [5-7], which all contribute to the microbial diversity responsible for the varied organoleptic and physicochemical characteristics of cheeses. Indeed, the development of the characteristics specific to bloomy rind cheeses depends greatly on the coexistence of microorganisms such as bacteria, yeasts and molds [8]. During production, these microorganisms cooperate and compete with each other depending on their metabolic activity within the cheese matrix inducing biological processes such as glycolysis, lipolysis, proteolysis that are responsible for the physicochemical and sensory characteristics of the cheeses [9]. The work conducted by Vázquez-Velázquez et al. [10] showed the variability of the characteristics of soft cheese with bloomy rind depending on the nature, origin of microorganisms. The objective of this study is to evaluate the effect of the association of some LAB isolated from local cow's milk of Yaounde (Cameroon) on the sensory, physicochemical and nutritional characteristics of a soft cheese with bloomy rind.

2. MATERIAL AND METHODS

2.1 Biological Material

For this study, 04 samples (Lv1, Lv2, Lv3 and Lv4) of raw milk samples were collected from one of the main farms supplying the city of Yaounde with fresh milk. This farm is located in the area of *Minka*, a village in the central Region of Cameroon, located in *Makak* and which depends on the Department of Nyong-et-kelle. This fresh cow's milk was used for the isolation of LAB and the production of cheese. The freeze-dried strains of *Penicillium candidum* (DANISCO, CHOOZIT PC NEIGE LYO 2 D) and *Geotrichum candidum* (DANISCO, CHOOZIT GEO15 LYO 2 D) used during the cheese ripening were ordered from the French company AGRODIRECT.

2.2 Isolation and Characterization of LAB

Isolation of LAB was done from decimal dilutions of each sample up to 10^{-6} dilution. Subsequently, 0.1 mL of each dilution was plated by spreading onto Petri dishes containing MRS agar. The seeded plates were incubated at 37°C for 48 hours under anaerobic conditions [11]. The colonies obtained were purified by successive isolation on MRS agar by the streak method and were subjected to a macroscopic characterization (Color, size (mm), relief, shape, surface, aspect and outline), microscopic characterization (Gram type, shape, arrangement and mobility) and biochemical characterization (Catalase).

2.3 Selection of LAB

2.3.1 Fermentation type test

To assess the fermentation type of LAB, a bacterial culture of 24 hours was introduced into 10 ml of MRS broth containing 1% glucose with inverted Durham bells. After expelling the air from the Durham bell, the preparation was incubated at 37°C for 48 hours. The presence of a empty at the upper end ($1/10^{th}$) of the Durham bell reflect the production of gas ($C0_2$), characteristic of heterofermentative LAB. The absence of this gas reflects the homofermentative LAB [12].

2.3.2 Compatibility test between LAB

The solid-state diffusion method was used to evaluate the compatibility between LAB [13]. For this purpose, 04 wells were dug with a Pasteur pipette on MRS agar

previously inoculated with LAB. After 1 hour of incubation at 37°C, 50 µL of supernatant of other LAB obtained by centrifugation at 2000 rpm for 10 min were added to these wells and the resulting preparation was incubated for 48 hours at 37°C.

2.3.4 Influence of the combined LAB on the acidification of milk

In order to select the combined LAB according to their acidification potential, the preparation obtained from the combination of 03 LAB isolates of concentration of 10^8 CFU/mL each, was introduced into a tube containing 10 mL of pasteurized milk and incubated at 37°C. The pH was evaluated after 3, 6, 20, 22, 24, 48 and 72 hours of fermentation [10].

2.3.5 Influence of the concentration of combined LAB on the coagulation of renneted milk

To conduct this test, milk was pasteurized at 90°C for 5 seconds and then dispensed into sterile batch at a rate of 100 mL of milk per batch. After cooling the milk to 32°C, the combined LAB were seeded into the batch to obtain final milk concentrations of 3×10^5, 3×10^6 and 3×10^7 CFU/mL bacterial cultures. After 45 minutes at room temperature (26 ± 2°C), rennet was added (1‰, v/v) to the inoculated milk. Curd formation was evaluated based on the success of the buttonhole or clean break technique. This technique involves dipping the index finger of the hand into the curd and slowly raising it to form a small mound that melts to form a buttonhole.

2.4 Production of Soft Cheese With Bloomy Rind

The production of the soft cheese with bloomy rind was carried out in the following main stages. The first step consisted of pasteurization and curdling of the milk. For this, the milk was pasteurized at 90°C for 5 seconds and then distributed in sterile batch. The cooled milk (32°C) was inoculated with *Penicillium candidum* (0.03 g/L), *Geotrichum candidum* (0.02 g/L) and with combined LAB whose concentration was determined during the coagulation test of renneted milk. The seeded milk was then left to rest for 45 minutes for acidification to optimize the action of the rennet. After this time, rennet was added in the proportion (1‰, v/v) to each batch and the resulting preparation was left to rest on a stable platform at room temperature (26 ± 2°C). The curdling of the milk was evaluated by clean break test.

The second step consisted of cutting, molding and draining the resulting curd. After checking the formation of the curd by the clean break test, the curd was sliced with a knife and left 30 minutes to promote the release of whey. After resting, the curd was then put into cheese strainer (8 cm diameter, 10 cm high) and placed on racks in the maturing cellar at 18°C to continue the release of the remaining whey by draining.

The third step was the removal from the mold, salting and drying of the drained curds. After draining in the cellar, the cheeses were removed from the cheese strainer and salted (5 g of salt for 325 g of cheese) with fine dry salt by sprinkling on all sides. The salted cheeses were then placed in the cellar to dry at 14°C for 24 hours.

Ripening, which is the fourth stage, allowed the cheeses obtained after drying to mature in the cellar at 12°C for 21 days. Figure 1 below shows the production diagram of the soft cheese with bloomy rind.

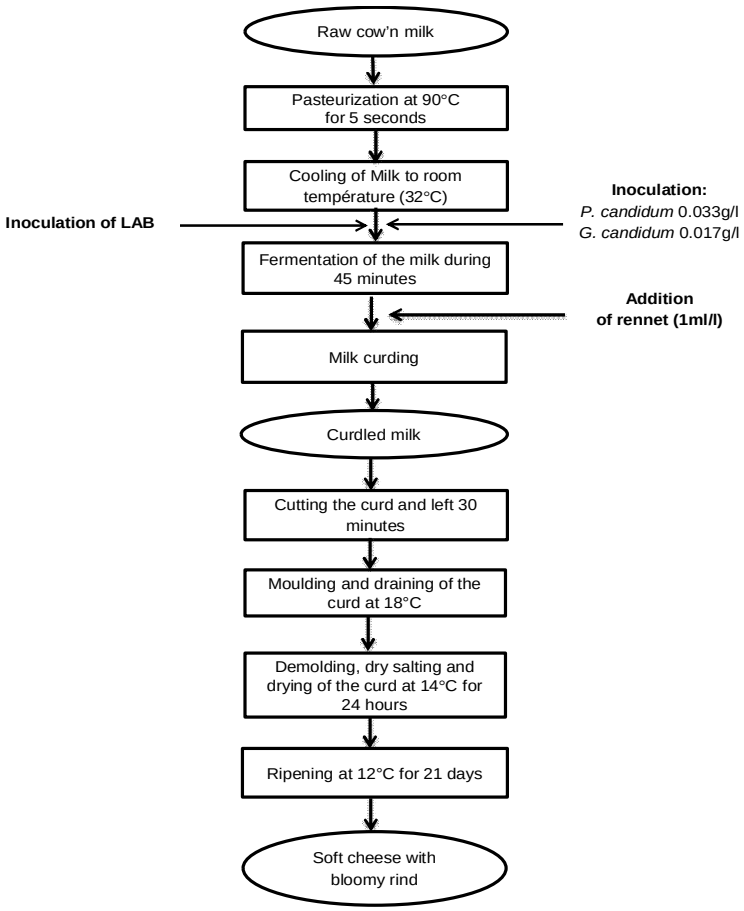

Fig. 1. Production diagram of soft cheese with a bloomy rind

2.4.1 Influence of the combined LAB on the curd of milk

In this part, the time of curdling, firmness and curd color were determined. Curdling time was measured by timing the time from renneting to curd formation determined by the clean break technique. In parallel, the knife test was used to evaluate the firmness of the coagulum. The success of these tests is affirmed when the achieved break is clear, frank and the serum which exudes is limpid and yellowish color without containing casein particles [14].The color of the curd was evaluated by comparing its color with a color chart, which is a palette comprising color samples [15].

2.4.2 Influence of combined LAB on curd draining

The draining time of curd was evaluated by timing the time between the molding of the curds and the end of the whey release. The volume of whey from this draining was measured with a graduated burette (1 L).

2.4.3 Influence of the combined LAB on the formation of the bloomy rind

The rate of mould coverage of the cheeses was observed throughout the ripening process. It was expressed as a percentage by determining the ratio between the surface of the cheese sector covered by mould (S1) in relation to the total surface (S2) of cheese according to the following formula:

$$\mathbf{T} = \frac{\mathbf{S1}(\mathbf{\Pi} * \mathbf{r^2})}{\mathbf{S2}(\mathbf{2} * \mathbf{\Pi} * \mathbf{r^2} + \mathbf{2} * \mathbf{r} * \mathbf{\Pi} * \mathbf{h})} \times 100$$

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Comment [h1]: With ?, T, S, II, h, r...

2.4.4 Determination of pH during cheese production

The pH was measured according to the AOAC [16] method. To determine the pH of milk, the electrode of the pH meter (Yieryi, TPH01139A) was introduced into 10 ml of milk and the displayed value was recorded. The cheese considering like a solid product, a mass of 10g was first finely ground and mixed in 100 ml of distilled water. The resulting solution was then filtered to separate it from the solid particles and the pH of the filtrate was measured.

2.4.5 Determination of the protein content of cheeses

To evaluate total protein content of cheeses, 0.2 mL of the solution obtained after mineralization, 1.2 mL of sodium acetate solution and 1.6 mL of reagent solution (15 mL of formaldehyde + 8 mL of acetylacetone in 77 mL of distilled water) were successively introduced in a test tube. After this, the mixture was incubated in a water bath (97.5°C) for 15 minutes. The product obtained was cooled in cold water and the tube volume was made up to 10 mL by adding 7 mL of distilled water [17]. Using a spectrophotometer, absorbance was read at 412 nm against a blank consisting of sodium acetate solution, reagent solution and distilled water. The amount of nitrogen was determined from the calibration curve obtained from ammonium sulphate. The equation below of the calibration curve allowed to calculate the amount of nitrogen.

$$\mathbf{X} = \mathbf{Y} * \frac{\mathbf{V_t} * \mathbf{100}}{\mathbf{V_p} * \mathbf{m} * \mathbf{a}}$$

With :
Y: Optical density;

Vt (mL): Total volume of mineralization;
Vp (mL): Volume of mineralized sample;
m(g): Mass of mineralized sample;
a: Calibration coefficient (0.006);
X: Amount of nitrogen.

The following formula was used to determine the total protein content in samples expressed in g/100g of dry matter. The conventional coefficient of conversion of nitrogen to protein used is 6.25 [18].

$$\textbf{Protein content} = 6.25 \times X$$

2.4.6 Determination of lipids content

The lipids content were determined by the method described by Bourely [19]. The principle of extraction of lipids contained in a sample is based on the differential solubility of the lipid in an organic solvent. It is done in Soxhlet for 12 hours. After this time, the organic solvent was removed by drying in at 45°C. The lipid content per 100g of sample expressed at 0% moisture was calculated using the following formula.

$$\%\mathbf{lipides} = \frac{(\mathbf{M1} - \mathbf{M2})}{\mathbf{M1} - \mathbf{M0}}\mathbf{x100}$$

With :

-M0: The weight of the empty filter paper bag;

-M1: The weight of the full bag containing the test sample before its treatment;

-M2: The weight of the full bag containing the test sample after extraction of the oil.

2.4.7 Determination of calcium

For this purpose, a volume of 15 mL of Aqua Regia solution was added into 50 mL of a propylene solution. To this mixture, 0.5 g of sample was introduced, preparation obtained was shaken for 10 minutes with a mechanical shaker and then centrifuged at 3000 rpm for 10 minutes. The supernatant obtained was recovered and the determination of calcium was performed by flame atomic absorption spectrophotometry [20]. The standard solutions were prepared by introducing into a

100 mL volumetric flask, 25 mL of Ca^{2+} (1000 ppm) and the volume obtained was made up to the mark with strontium chloride solution.

2.4.8 Microbiological analysis of the cheeses produces

After ripening, the cheeses obtained were subjected to a series of microbiological analyses in order to ensure their compliance with the standards, an important criterion for the health of consumers in general and panelists in particular. The enumeration techniques were performed according to the methodology for the analysis and testing of dairy products established by the FDA [21]. For this purpose, LAB, staphylococci, enterobacteria (*Salmonella* and *Escherichia coli*), *Listeria monocytogenes* and Mould were enumerated on MRS, Chapman (yeast and mould), MacConkey, blood agar and Potatoes Dextrose Agar (PDA) respectively. With the exception of yeasts and moulds, which were incubated at 25°C for 3 to 5 days and *E. coli* which was incubated at 44°C for 48 hours, the rest of the germs tested were incubated at 37°C for 24 hours. At the end of the different incubation periods, the microbial concentrations were calculated using the formula below:

$$\mathbf{C} = \frac{\sum \mathbf{N} * \mathbf{Fd}}{\mathbf{1,1} * \mathbf{V}}$$

With:

-C: Microbial concentration in CFU/g;

-∑N: Sum of colonies of two successive dilutions;

-Fd: Dilution factor of the smaller dilution;

-V: Volume seeded in ml.

2.4.9 Sensory analysis

The sensory analysis of the cheeses produced was based on the descriptive method (ranking test) and the hedonic method (pair wise preference test). During the tests, the samples, which have been divided into small cubes (in proportions of 1 cm^3), identified by 3-digit codes, were served on breakable dishes. The mineral water has been made available for the panelists for mouthwash in plastic cups after tasting each cheese sample. For the descriptive analysis, consumers were asked to give a score on a scale of 0 to 5 on a sensory analysis form and the points awarded for each cheese were added together to obtain the overall score. Attributes that described the product in terms of internal texture (compact, smooth and runny), internal colour (pale yellow and beige), smell (mouldy, acidic and mildewed), feel (soft, hard, supple and crumbly), crust appearance (regular, cracked and bumpy), crust colour (white and beige) and taste (sour, salty and bitter) were selected based on the literature [22, 23].

For the hedonic test, panelists were asked to choose which of the cheeses produced they most preferred and to explain the reasons for this preference.

2.5 Statistical Analysis

All the data collected were processed with Excel (Office 2016) for the calculation of means, standard deviations, plotting of the radar graph and Sigma plot 11.0 software for plotting the diagrams.

3. RESULTS

3.1 Characterization of LAB Isolated From Milk

A total of 14 bacteria were isolated from the milk. After microscopic and macroscopic observations and biochemical test, 09 isolates of LAB were selected. Among these isolates, 02 have been isolated from the Lv1 milk sample (IS1 and IS2), 04 isolates from the Lv2 milk sample (IS6, IS7, IS8 and IS9), 02 isolates from the Lv3 milk sample (IS4 and IS5) and 01 isolate from the Lv4 milk sample (IS3). Macroscopic observations showed that 08 isolates were white colour and 01 isolated are beige. Among these isolates, we distinguish 03 colonies whose diameters are less than 0.5 mm, 04 isolates between 0.5 and 1 mm and 02 isolates more than 1 mm. As for their appearance, 07 isolates were opaque and 02 were transparent. As for their shape, 06 coccus and 03 coccobacilli were present. We observed cells grouped in tetrads (03), clusters (01), chains (03) and others are isolated (02). All 09 isolates had negative mobility. Table 1 present the results of the macroscopic, microscopic and biochemical characterisation of these LAB.

Table 1. Macroscopic, microscopic and biochemical characterization of LAB isolated from milk

Macroscopic, microscopic and biochemical characterization of LAB isolated from local milk

		IS1	IS2	IS3	IS6	IS7	IS8	IS9	IS4	IS5
Characterization Macroscopic	**Color**	White	Beige	White	White	White	White	White	White	White
	Size (mm)	+	++	+++	+	++	+++	++	+	++
	Relief	Flat	Curved	Curved	Flat	Curved	Curved	Curved	Curved	Curved
	Shape	Round	Round	Round	Round	Round	Round	Round	Round	Round
	Surface	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
	Aspect	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Transparent	Transparent
	Contour	Regular	Regular	Regular	Regular	Regular	Regular	Regular	Regular	Regular
Characterization Microscopic	**Gram type**	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
	Form	Coccobacilli	Coccus	Coccus	Coccus	Coccobacilli	Coccus	Coccobacilli	Coccus	Coccus
	Arrangement	Chain	Cluster	Isolated	Isolated	Chain	Tetrad	Chain	Tetrad	Tetrad
	Mobility	Negative	Negativ	Negativ	Negativ	Negative	Negativ	Negative	Negative	Negative

			e	e	e		e			
Characterization **Biochemical**	**Catalase**	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

+++: Diameter ≥ 1 mm; ++: 1 ≤ Diameter ≥ 0.5 mm; + : Diameter ≤ 0.5 mm

3.2 Selection of LAB with Cheese Making Abilities

3.2.1 Fermentation type test

In order to eliminate any possibility of alveoli formation in bloomy rind cheeses, supposed to be totally compact from the inside like Camembert cheese, the choice was made for LAB with homofermentative characteristics. Among the 09 isolates previously selected, 05 LAB (IS1, IS2, IS3, IS4 and IS5) showed their ability to ferment lactose without producing CO_2, responsible for the alveoli in the cheeses. These isolates have been used in the following work for their homofermentative character.

3.2.1 Compatibility test between the selected LAB

At the end of the compatibility test, no inhibition was observed between the 05 LAB tested. Therefore, these LAB are compatible and can be combined with each other during cheese production.

3.2.2 Influence of combined LAB on milk acidification

From this analysis, it was found that the 10 combinations of 03 isolates obtained from the 05 LAB acidified the milk during the milk fermentation. However, the rate of acidification varied from one combination to another. However, the combined IS2-IS3-IS5 and IS1-IS4-IS5 were selected for their ability to acidify milk at a relatively short time and for the ideal pH values obtained during milk fermentation. The combined IS1-IS4-IS5 lowered the pH of the milk to 5.9 and 4.2 after 6 and 72 hours respectively. Although combined IS2-IS3-IS5 was show a consistent acidification after 6 hours, he lowered the pH of milk to 4.4 after 72 hours. It is for all these reasons that these two combinations were retained for further work. Table 2 below shows the evolution of the pH of milk fermented with combined LAB after 72 hours.

Table 2. Acidification potential of combined LAB

		Fermentation time (hours)							
No.	**Combination of LAB**	**0**	**3**	**6**	**20**	**22**	**24**	**48**	**72**

1	IS1-IS2-IS3	6.8	6.4	6.1	5.4	5.2	5.2	4.8	4.5
2	IS1-IS2-IS4	6.8	6.4	6.1	5.3	5.3	5.1	4.7	4.4
3	IS1-IS2-IS5	6.8	6.4	6.1	5.4	5.3	5.1	4.6	4.4
4	IS1-IS3-IS4	6.8	6.4	6.1	5.4	5.3	5.2	4.8	4.5
5	IS1-IS3-IS5	6.8	6.4	6.4	5.7	5.4	5.4	4.8	4.5
6	IS2-IS3-IS4	6.8	6.4	6.1	6.1	5.9	5.7	5.1	4.9
7	IS2-IS4-IS5	6.8	6.4	6.1	5.4	5.2	5.2	4.7	4.4
8	IS3-IS4-IS5	6.8	6.1	6.1	6.1	6.1	6.1	4.3	3.9
9	IS2-IS3-IS5	6.8	6.1	6.1	5.3	5.2	5.1	4.5	4.4
10	IS1-IS4-IS5	6.8	6.1	5.9	5.2	5.1	5.0	4.4	4.2

3.2.3 Influence of the concentration of combined LAB on the coagulation of renneted milk

This test showed that when rennet was added to fresh milk already inoculated with combined IS1-IS4-IS5 and IS2-IS3-IS5 with a concentration of 3×10^7 CFU/mL, curds were formed after 02 hours. However, it was only after 03 hours that a clear cut in the curds was observed, with a detachment of the curds from the walls of the container. Below this concentration, no effect of the combination IS2-IS3-IS5 on milk coagulation is observed. For this reason, the concentration 3×10^7 CFU/mL, was chosen as the final inoculation concentration for cheese production. Table 3 shows the effect of the concentration of the combined LAB on the coagulation of renneted milk.

Table 3. Effect of the concentration of combined LAB on the coagulation of renneted milk.

	Combined LAB	
Concentration (CFU/ml)	**IS1-IS4-IS5**	**IS2-IS3-IS5**
3×10^5	-	**-**
3×10^6	**+**	**-**
3×10^7	**+**	**+**

+ : Curd formation; - : No curd formation

3.3 Analysis During Cheese Production

3.3.1 Changes in pH during cheese production

The figure 2 shows the evolution of pH from fresh milk to cheese after 21 days of ripening. During the production of FROCAM 145, the pH decreased by 14 and 15% respectively during the renneting of the milk and after ripening. In the case of FROCAM 235, a decrease of 12 and 17% was recorded during renneting and ripening respectively.

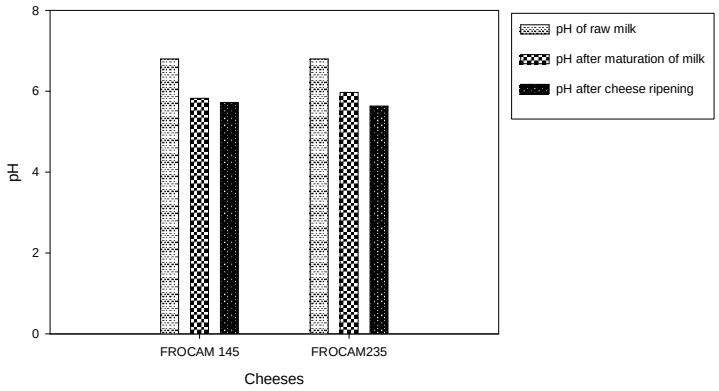

Fig. 2. Evolution of pH during production of FROCAM 145 and FROCAM 235

3.3.2 Influence of combined LAB on milk curdling

During cheese production, the curds obtained from combinations IS1-IS4-IS5 and IS2-IS3-IS5 behaved differently. Contrary to the coagulation test of the renneted milk by the combined cultures where the curd was obtained after 2 hours, it took 20 hours at 26 ± 2°C to obtain a curd of good consistency in the case of FROCAM 145 while it took 22 hours in the case of FROCAM 235 (02 hours more). In addition, the curd of FROCAM 145 had a firmer consistency and expelled whey faster. FROCAM 235 curd had a less firm consistency and was slower to release whey. Another parameter that differentiated the curds was their color. The curds from FROCAM 145 were white while the curds from FROCAM 235 were beige.

3.3.3 Influence of the combined LAB on the draining of the curd

During the draining of the two cheeses in the cheese strainer, it was noticed that the draining time and the volume of whey released were different. Thus, FROCAM 145 released less whey than FROCAM 235 with respective values of 2440 mL and 2730 mL. However, the draining of FROCAM 145 took 68 hours against 96 hours for the draining of FROCAM 235, that is 28 hours more. At the end of draining, FROCAM 235 presented a very crumbly and dry texture compared to FROCAM 145, which was very compact and wet.

3.3.4 Development of the blooming ring of FROCAM 145 and FROCAM 235

For FROCAM 145, the appearance of the bloomy rind began after the 5th day of ripening. It was on the 10th day of ripening that we observed a total coverage (100%) by mould. while, it is from the 8th day of ripening that the bloomy rind appeared on FROCAM 235. Until the 21th days of ripening, the recovery rate did not exceed 20%. Figure 3 shows the rate of covering of the

FROCAM 145 FROCAM 235

cheeses by the bloomy rind after 21th days of ripening.

Fig. 3. Development of the bloomy ring of FROCAM 145 and FROCAM 235 after 21th days of maturing

3.3.5 Microbiological quality of FROCAM 145 and FROCAM 235

The microbiological analysis of these cheeses shows that *Listeria monocytogenes*, *Salmonella* and Staphylococci in FROCAM 145 and FROCAM 235 are absent. But, contrary to FROCAM 235, *Escherichia coli* was recorded in FROCAM 145 with concentration of 4.74 Log_{10}CFU/g. However, in spite of the contamination of FROCAM 145 by *Escherichia coli,* it is still suitable for consumption according to the Directive 92/46 EC on microbiological reference criteria for cheeses made from raw milk and thermized. This Directive sets the minimum contamination (m) of *Escherichia coli* at 4 Log_{10}CFU/g and the maximum (M) at 5 Log_{10}CFU/g. The concentration of LAB recorded in FROCAM 145 and FROCAM 235 is respectively 11 Log_{10}CFU/g for

FROCAM 145 and 10 Log_{10}CFU/g for FROCAM 235. It appears that the Mould concentration of FROCAM 145 is higher than that of FROCAM 235 with respective values of 6,8 and 5,1 Log_{10}CFU/g. The figure 4 shows the microbiological quality of FROCAM 145 and FROCAM 235.

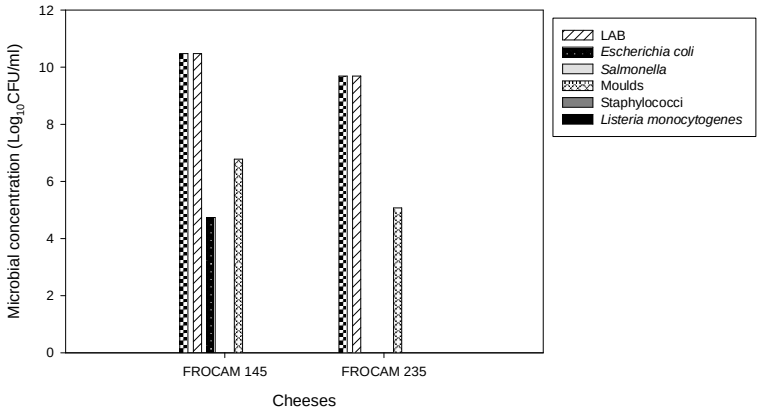

Fig. 4. Microbiological quality of FROCAM 145 and FROCAM 235

3.3.6 Nutritional value of FROCAM 145 and FROCAM 235

Although produced from the same sample of fresh milk, it is noted that the final composition of FROCAM 235 and FROCAM 145 in protein, lipid and calcium varies after ripening. However, FROCAM 235 had 1.15% of protein, 1.04% of lipid and 2.32% of calcium higher than FROCAM 145. The figure 5 below shows the protein, lipid and calcium contents of FROCAM 145 and FROCAM 235 after 21 days of ripening.

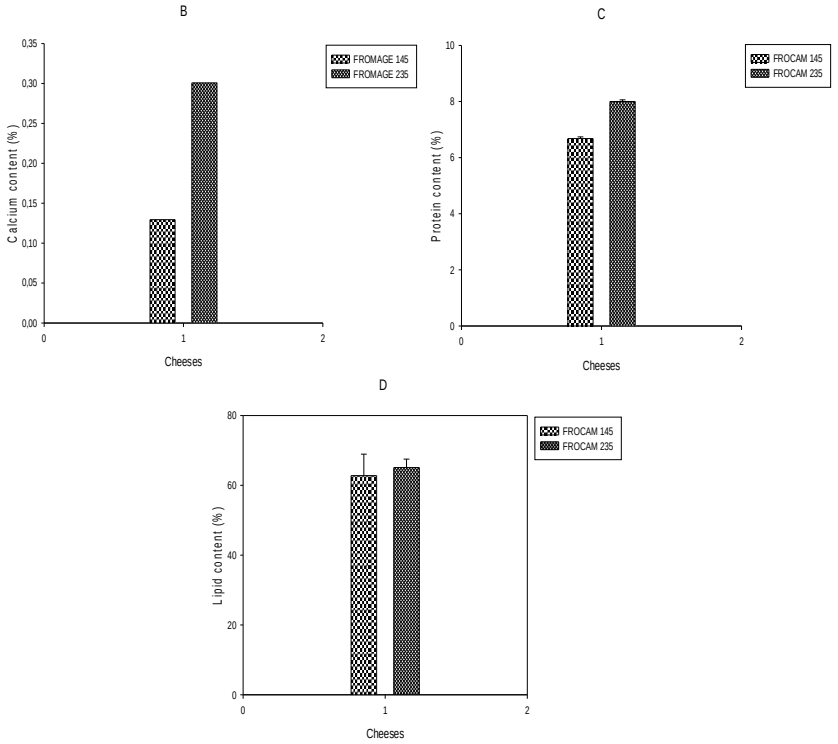

Fig. 5. Calcium (B), Protein (C) and lipid (D) content of

FROCAM 145 and FROCAM 235 after 21 days of ripening

3.3.7 Sensory characteristics and acceptability of FROCAM 145 and FROCAM 235

The figure 6 was shows that, texture of the internal part of FROCAM 145 is more smooth (3.36) and flowing (1.2) compared to the texture of the internal part of FROCAM 235. However, both cheeses have a compact internal texture with a beige color for FROCAM 145 (3.0) and a pale yellow color (2.6) for FROCAM 235. The crust of both cheeses is bumpy, cracked and has a beige color. In terms of odor, FROCAM 235 had a stronger acidic (2.0) and musty (2.1) odor than FROCAM 145. However, FROCAM 145 and FROCAM 235 showed no difference in milky odor (3.0). FROCAM 145 was softer (3.0) and more supple (3.0) than FROCAM 235 which was hard (2.1) and friable (2.0). Finally, in terms of taste analysis, FROCAM 235 was more acidic (3.0) and more bitter (1.8) than FROCAM 145. The attributes studied above allowed panelists to make a choice between FROCAM 145 and FROCAM 235. FROCAM 145 was the more

preferred cheese compared to FROCAM 235, with mean overall acceptability scores of 0.74 and 0.24 respectively.

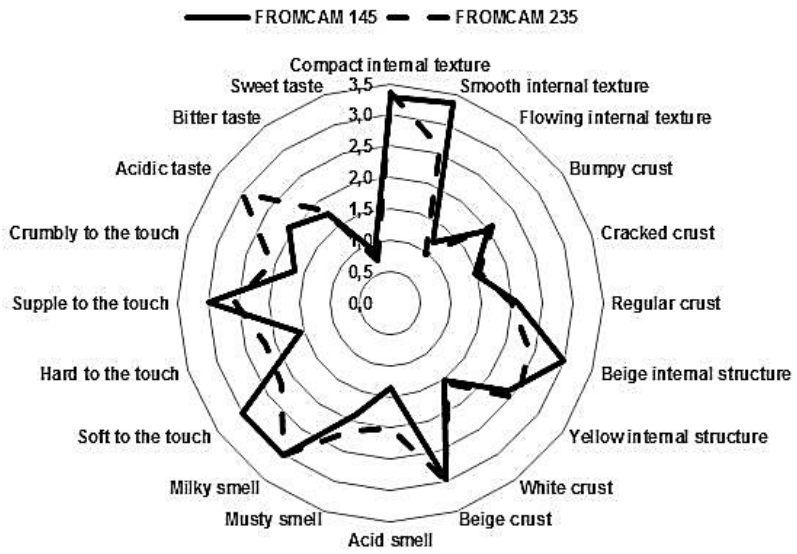

Fig. 6. Sensory characteristics of FROCAM 145 and FROCAM 235

4. DISCUSSION

During the production of the different cheeses, although they all underwent the same treatments, notable differences were observed during the maturation of the milk, the curdling, the draining and the maturing of the different cheeses. It was found that milk inoculated with combined IS2-IS3-IS5 used for the production of FROCAM 235 took 22 hours before curdling, 2 hours longer than the curdling of milk inoculated with the combined IS1-IS4-IS5 during the production of FROCAM 145. Indeed, the time of coagulation of milk would depend on several other factors such as the temperature of milk and the rate of acidification of lactic ferments used. The temperature of the cooled milk (32°C) after pasteurization is very favorable to the multiplication of mesophilic LAB with a short latency time. In these conditions, they will be able to immediately produce lactic acid which will be at the origin of the fall of the pH and consequently, the coagulation of milk in the presence of rennet. Unlike mesophilic bacteria, thermophilic LAB will develop slowly in this environment and take longer to produce the lactic acid necessary for the coagulation of milk in the presence of rennet. Moreover, even when the lactic ferments are all mesophilic, the rate of lactic acid production can vary from one LAB to another [11]. Work by Lairini et al. [22] showed that combined LAB used to produce fermented

milk near to Kefir, a dairy product originating in the Caucasus, all had different acidification profiles.

Beyond the curdling time of the milk, it was also found that the curd of FROCAM 145 had a firmer consistency compared to the curd of FROCAM 235. This firmness of the curd would be due to the lowering of the pH during fermentation. This curd rigidity increases as the pH decreases [24]. Indeed, the decrease in pH leads to a decrease in the negative charges of the micelles as well as a solubilization of minerals (calcium and phosphorus). This leads to a demineralization of casein micelles with reorganization of the matrix, to form a coagulum [25].

Among the 02 cheeses studied, FROCAM 235 showed a longer time and a high amount of whey released during draining. This result could be explained by the degree of micelle aggregation which depends on the level of acidification of the milk. According to Fox et al. [26], casein micelles lose their affinity for the aqueous phase to aggregate with each other due to hydrophobic interactions and electrostatic repulsions during the acidification of milk. During micelle aggregation, which increases with decreasing pH, an increase in whey release is also observed [27].

The bloomy rind of FROCAM 145 developed well (100%) during maturation, unlike that of FROCAM 235 which developed at 20%. This low rate of mould growth in FROCAM 235 could be explained by the antifungal and antimicrobial activity of the combined IS2-IS3-IS5 used for its production. Indeed, LAB can produce metabolites with antimicrobial properties such as organic acids, hydrogen peroxide, carbon dioxide, reuterin, diacetyl and bacteriocins capable of inhibiting or limiting the growth of certain germs. This action is effective on undesirable germs and those essential during ripening process [28]. This antimicrobial activity could also be explained by the results of microbiological analysis obtained in this work. It was observed that FROCAM 235, characterized by low growth of ripening moulds, was not also contaminated by *Listeria monocytogenes*, *Salmonella*, staphylococci and *Escherichia coli*. The presence of *Escherichia coli* in FROCAM 145, which showed a total mould overlay, could be explained by a poor application of hygiene rules during or after production. According to Ariri [29] poor hand hygiene, the production environment, the addition of ferments after pasteurization, defective or contaminated equipment are likely sources of contamination of milk.

It was found that the concentration of proteins, lipids and calcium of FROCAM 145 and FROCAM 235 is not the same at the end of the maturing process, although they were produced from the same milk. The concentration of proteins, lipids and calcium of FROCAM 145 is lower than that of FROCAM 235. This result could be explained by the high concentration of LAB and ripening moulds recorded in FROCAM 145. Indeed, these heterotrophic microorganisms use these different molecules to produce energy and precursor molecules that will be useful for

energy production and for biosynthesis or assimilation reactions necessary for their growth [30]. The more the microbial concentration increases, the more these nutrients are used as it is the case with FROCAM 145.

The sensory analyses carried out after the ripening of the 02 cheeses show that the taste, texture and smell vary. FROCAM 235 and FROCAM 145 both showed a bitter taste. The bitter taste of these cheeses can be explained by proteases secreted by LAB and ripening moulds that cut caseins into longer (bitter) or shorter (less bitter) amino acid chains called peptides. Thus, depending on the length of these peptides, a more or less bitter taste is conferred to the cheese [31]. However, in this work, FROCAM 145 showed a less bitter taste than FROCAM 235. This could be explained by the high growth of LAB (10.47 Log_{10}CFU/g) and ripening molds (100% coverage) which had a more pronounced proteolytic action during the ripening of FROCAM 145. According to Aloun and Hamadache [32], the low bitterness of the cheeses could also be due to *Geotrichum candidum* which is able to reduce the bitter peptides generated by the proteolytic activity of *Penicillium candidum.*

Furthermore, the variations in sensory characteristics between these cheeses could be explained by the different metabolic activities specific to each combined LAB and reinforced by the metabolism of the associated ripening molds during the manufacturing process. Indeed, during the 14-day ripening period, the taste, texture and appearance of cheeses have been transformed by metabolic reactions such as the fermentation of residual lactose, the degradation of fats and the hydrolysis of proteins. Several works have shown the ability of LAB and ripening moulds to convert lactic acid to acetate, amino acids to diacetyl, acetoin, carbonyl, ketoacid, sulfur compounds and fatty acids to volatile compounds (alcohols, branched acids, esters, sulfur compounds and phenols.) [33-35]. Thus, the characteristics of cheese after ripening depending on the properties of each microorganism [28].

5. CONCLUSION

The soft cheeses with bloomy rind produced from LAB isolated from cow's milk produced in Cameroon showed sensory characteristics that were highly appreciated by the panelists. From a nutritional point of view, the calcium, protein and lipid contents of the cheeses obtained were within the range of those of recognized soft and bloomy rind cheeses.

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