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Influence of selected lactic acid bacteria on the sensory characteristics of soft cheese with natural rind

Okamba Elouti, R. B., *Roger, T., Nkoudou Ze, N. and Essia Ngang, J. J.

Department of Microbiology, Faculty of Science, the University of Yaounde 1, P.O. Box 812, Yaoundé, Cameroon

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Abstract

Cheese is obtained by the coagulation of milk, followed by maturation under the action of microorganisms which are responsible for the development of physicochemical and organoleptic characteristics of cheese. Among various microorganisms, lactic acid bacteria (LAB) play an essential role in establishing sensory characteristics of the cheese. In Cameroon, the effect of isolated LAB on the sensory characteristics of cheese produced from local cow milk has not yet been investigated. Therefore, the present work aimed to isolate LAB and evaluate their influence on the physicochemical and sensory characteristics of locally produced soft cheeses. Four strains of LAB were isolated and identified as *Enterococcus* sp.1, *Enterococcus* sp.2, *Pediococcus* sp., and *Leuconostoc* sp. These isolates were combined in batches for production of cheeses, including Frm 741 (*Enterococcus* sp.1 and *Enterococcus* sp.2), Frm 891 (*Enterococcus* sp.2 and *Leuconostoc* sp.), Frm 683 (*Pediococcus* sp. and *Enterococcus* sp.1), Frm 425 (*Pediococcus* sp. and *Leuconostoc* sp.), Frm 503 (*Pediococcus* sp. and *Enterococcus* sp.2), Frm 439 (*Leuconostoc* sp. and *Enterococcus* sp.1), and Frm 625 (*Enterococcus* sp.1, *Enterococcus* sp.2, *Leuconostoc* sp., and *Pediococcus* sp.). Cheese produced from the combination of *Enterococcus* sp.1 and *Enterococcus* sp.2 (Frm 741), unlike the others, did not drain properly throughout the production process. However, all products showed excellent microbiological quality in relation to biological contaminants, such as *Salmonella*, staphylococci, and coliforms. Moreover, cheese Frm 425 was most appreciated by consumers for its sweet taste, yellow colour, pleasant smell, and low acidity (51.7°D). On the other hand, cheese Frm 439 was least appreciated by consumers owing to its bad odour, bitter taste, and high acidity (144.9°D). Taken together, the present work demonstrated that the isolated LAB could be used to produce soft cheese with a natural rind and unique sensory characteristics that are appreciated by Cameroonian consumers.

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Introduction

Cheese is one of the most consumed products, and accounts for 40% of the processed dairy products worldwide. Cheese is a ripened or unripened product obtained by the coagulation of milk *via* the action of rennet, microorganisms, or other coagulating agents, followed by partial draining of the whey resulting from coagulation. There are over 2,000 different varieties of cheese in the world; they can be classified based on different criteria, such as the type of process used (non-pressed cheese, cooked-pressed cheese, blue cheese) and the type of coagulation (lactic and/or acidic, enzymatic, or even mixed). However, the most commonly used classification is based on the water content of the cheese. Fresh cheese has > 70% water content, hard cheese has < 37% water content, and soft cheese has water content between 42 - 55% (Aissaoui and Zidoune, 2017). The difference in sensory characteristics of ripened cheese is due to the metabolic activities of certain bacteria and moulds (Monnet *et al.,* 2015). Additionally, the microbial content and diversity involved in the production, place of production, and season of production also play important roles in the organoleptic characteristics of cheese (Montel *et al.*, 2014; Irlinger *et al.,* 2015; Delbès *et al.,* 2015). The present work aimed to isolate LAB from raw cow milk, and evaluate their effects on the physicochemical and sensory characteristics of locally produced soft cheese with a natural rind.

*Corresponding author.
Email: tchikouar@yahoo.fr

Materials and methods

Sample collection

Raw cow milk samples used for the isolation of LAB were collected from a periodic market in Dang-Bini, a locality in the district of Ngaoundere III (Adamaoua, Cameroon).

Isolation of LAB

Raw cow milk (25 mL) was aseptically mixed with 225 mL saline water (0.85% NaCl). Decimal dilutions were made up to 10^{-6} dilution. Each dilution (0.1 mL) was inoculated *via* spread-plating onto De Man Rogosa and Sharpe (MRS) culture medium (pH 5.4) enriched with benzemidazole (0.5 g/L). Inoculated plates were incubated at 37°C for 48 h (Misganaw and Teketay, 2016). Following this, isolated colonies were purified by successive subculturing on MRS agar. Purity of the strains was determined *via* microscopic examination after Gram-staining, and catalase test. Gram-positive and catalase-negative bacteria were retained and stored on MRS medium at 4°C (Battah and Bouhamdani, 2017).

Characterisation of LAB

Growth at different NaCl concentrations

Pure strains of LAB isolates were inoculated into MRS broth previously enriched with 2, 4, or 6.5% NaCl, and incubated at 30°C for 24 h (Ghozlane, 2012).

Thermal resistance test

Heat-resistant nature of LAB was evaluated by exposing the LAB strains to MRS broth in a water bath set at 60°C for 30 min. Heated tubes were then rapidly cooled in cold water, and incubated at 30°C for 48 h to ensure bacterial growth (Guiraud and Galzy, 1980).

Type of fermentation

After 48 h, the strains were collected from MRS agar and introduced into tubes containing MRS broth; Durham bell was used to evaluate gas production during the metabolic activity of the bacterial cells. Inoculated tubes were incubated at 30°C for 48 h (Aissaoui and Zidoune, 2017).

Sugar fermentation

Sugar fermentation by LAB was monitored in several tubes containing MRS broth with meat extract and 0.1 g/L glucose being replaced by arabinose, fructose, lactose, sucrose, raffinose, galactose, trehalose, and rhamnose. Phenol red was added to each preparation to demonstrate the metabolism of the different sugars used. Test tubes were then incubated for 24 h at 30°C. Results were compared with those of the control tube, which contained the same MRS broth but without sugar (Leveau *et al.*, 1991).

Catalase test and Gram reaction

During aerobic respiration, microorganisms produce hydrogen peroxide (H_2O_2). The accumulation of this compound destroys bacteria. To protect themselves, the bacteria breakdown this hydrogen peroxide into water and oxygen using an enzyme called catalase. To evaluate the production of catalase, a drop of H_2O_2 was placed on a microscope slide containing a pure colony of LAB. The release of oxygen in the form of gas bubbles reflected the production of catalase by the bacteria (Battah and Bouhamdani, 2017).

Gram reaction was carried out according to Lairini *et al.* (2014).

Motility test

Motility of LAB was determined by observing fresh preparations under a light microscope at 40× magnification.

Production and inoculation of soft cheese with natural rind

Inoculum preparation

Inocula were prepared using combinations of colonies (48 h) of LAB in an Erlenmeyer flask containing 250 mL of milk, and incubated at 37°C for 48 h. For combinations, each bacterial preparation was inoculated into milk at a concentration of 10^6 CFU/mL (Moulay, 2016).

Cheese production

To produce cheese, milk was pasteurised at 80°C for 5 min. Then, pasteurised milk was cooled to 30°C, and renneted (0.5 mL/L) following manufacturer's instructions (Cheagul Ideal, Banatului 52, Chitila, Ilfov, Romania). Milk was simultaneously inoculated with 10^6 CFU/mL of LAB, and then curdling was performed for 4 h. Resulting curd was sliced into a "D" shape to remove whey trapped in the gel. After 15 min, curd curd was introduced into containers, and placed in a cellar set

at 20°C for 24 h to allow further draining and give cheese its final shape. Drained cheeses were salted *via* immersion in brine (18% NaCl) for 15 min. Cheeses separated from brine were dried in a cellar at 15°C for 24 h. Dried cheese was matured in a cell maintained at 12°C for 14 d. Figure 1 summarises the different steps for the production of soft natural rind cheese.

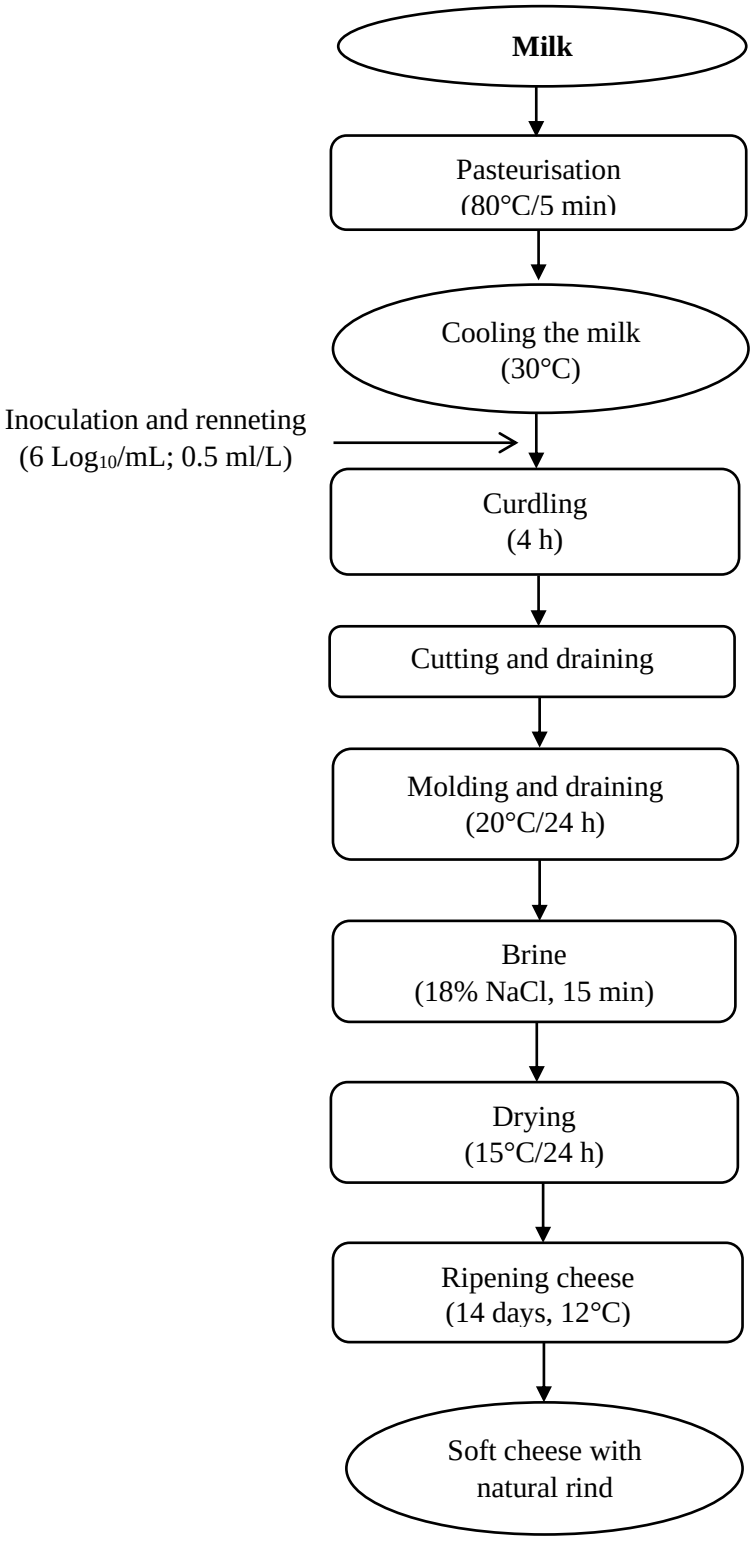

Figure 1. Production diagram of soft cheese with natural rind.

Sensory characteristics and safety profile of cheeses
Health quality assessment

To carry out sensory analysis of different cheeses, microbiological quality of cheeses was determined. To this end, a three-class sampling plan was adopted and leveraged to search for staphylococci, *Salmonella*, and total coliforms. For this purpose, 25 g of each cheese sample was diluted (10^{-3}). To detect staphylococci, each dilution (0.1 mL) was seeded onto the surface of Chapman agar, and incubated at 37°C for 24 h. To enumerate coliforms, 1 mL of each dilution was inoculated in Petri plates and covered with methylene blue eosin agar (EMB). Incubation was performed at 37°C for 24 h. Unlike staphylococci and total coliforms, *Salmonella* first required a pre-fortification of 25 g of cheese in 225 mL of peptone water. The resulting mixture was then incubated at 37°C for 24 h. Following this, 0.1 mL of the enriched broth was then inoculated in 10 mL of a selenite broth, and incubated at 44°C for 24 h. Finally, 0.1 mL of the inoculum was inoculated on the EMB *via* spread-plating, and incubated at 37°C for 24 h (Moulay, 2016).

Sensory analysis

Sensory quality of cheeses was determined using the check-all-that-apply (CATA) method. Three spaces were used in this activity. A space for the preparation of samples, a space comprising five voting booths for tasting, and a space reserved for discussion with panellists. After coding the cheese samples, they were served to panellists on odourless plastic plates. For each sample, panellists were asked to provide an overall assessment of the cheese by assigning a score on a hedonic scale ranging from 1 to 9, with 1 representing extremely unpleasant taste, and 9 extremely pleasant taste. The panellists were also asked to check off from a list of 20 attributes chosen from the literature that best described the product. On each sensory analysis sheet, the order of the appearance of these attributes was randomly arranged to avoid bias. The terms listed as attributes comprised 15 sensory attributes, including friable, firm, rind, salty, soft, milky, fondant, bitter, beige, yellow, acid, white, mouldy aroma, sweet, and astringent, and five emotional attributes, namely nice, unpleasant, bad-smelling, good-smelling, and pleasant.

Physical analysis

During the production of cheese, from the pasteurisation of milk to the ripening of cheese, certain parameters such as the quantity of fluid drained during curdling, time taken for draining, and the presence or absence of alveoli in the cheese were studied.

Determination of available sugars

Sugars were extracted and analysed *via* spectrophotometry according to Dubois *et al.* (1956). The principle is that in an acidic and hot medium, pentoses (C5) and hexoses (C6) undergo cyclisation to give furfural and hydroxymethyl furfural, respectively. The formed compounds react with phenol to form a coloured yellow-orange complex that absorbs at 490 nm. To extract available sugars, cheese (0.5 g) was ground and introduced into a test tube containing 5 mL of 1.5 N sulphuric acid. The mixture was boiled for 45 min in a water bath, and cooled to room temperature (25 ± 2°C). Subsequently, 10 mL of ethanol (70%), 1 mL of zinc acetate (2 g/100 mL), and 1 mL of potassium ferrocyanide (10.6 g/100 mL) were added. The mixture was then filtered into a 50 mL flask, and volume was adjusted with water to the gauge line. The amount of sugar was determined by mixing 0.375 mL of the filtrate with 0.5 mL of phenol (5%), 4.625 mL of distilled water, and 2.5 mL of concentrated sulphuric acid in a test tube. After 10 min incubation at room temperature, the mixture was homogenised and placed in a water bath at 100°C for 20 min. The optical density of the orange colour was measured using a spectrophotometer at 490 nm against a blank prepared under the same conditions. Amount of carbohydrates in samples was determined with reference to a standard range of glucose (1 mg/mL). Available sugar content was expressed as g/100 g of dry matter (DM).

Statistical analysis

All data were processed using the Sigma plot software (version 11.0) to plot the diagrams and curves. XLSTAT software version 2019-3-1 was used to perform principal component analysis (PCA), analysis of variance (ANOVA), and Cochran Q test using data from the CATA.

Results and discussion

Isolation and characterisation of LAB

Based on the morphological characteristics of the colonies, 14 LAB strains were isolated from different cow milk samples. However, based on microscopic and macroscopic observations, only four isolates were retained. These were BL 3, 6, 8, and 10. After characterisation, coccobacilli represented 1/4, whereas cocci represented 3/4 of the isolated LAB. Many studies have shown that cocci are largely dominant over bacilli bacteria in cow milk (Al-Kotami *et al.*, 2015; Misganaw and Teketay, 2016). The characterisation of the isolates reported in Table 1 shows that the LAB BL 3 and 8 belong to the genus *Enterococcus*, BL 6 belongs to the genus *Leuconostoc*, and BL 10 belongs to the genus *Pediococcus*.

Table 1. Characteristics of isolated lactic acid bacteria.

		Lactic acid bacteria			
		BL 3	**BL 6**	**BL 8**	**BL 10**
Microscopic characteristic	Gram type	+	+	+	+
	Form	Cocci	Coccobacilli	Cocci	Cocci
	Grouping	Cluster	Chain	Cluster	Tetrad
	Mobility	-	-	-	-
Biochemical test	Catalase	-	-	-	-
	Glucose	+	+	+	+
	Fructose	-	+	+	-
	Lactose	+	+	-	+
	Galactose	+	+	+	+
	Arabinose	+	+	+	+
	Raffinose	±	-	+	+
	Trehalose	+	+	+	+
	Rhamnose	+	+	+	+
	Sucrose	+	+	+	+
	Respiratory type	Aero-anaerobic	Aero-anaerobic	Aero-anaerobic	Aero-anaerobic
	Fermentation type	Homo-fermenter	Hetero-fermenter	Homo-fermenter	Homo-fermenter
Growth in hostile condition	2% NaCl	+	+	+	+
	4% NaCl	+	+	+	+
	6.5% NaCl	+	+	+	+
	Thermal resistance	+	-	+	-
Identified bacteria		*Enterococcus* sp.1	*Leuconostoc* sp.	*Enterococcus* sp.2	*Pediococcus* sp.

Effects of strains on curd volume, draining time, and alveolus formation

Figure 2 illustrates some cheese products obtained from the combinations of isolated LAB, and highlights seven cheese products. The combination of *Pediococcus* sp. and *Leuconostoc* sp. was used to produce cheese Frm 425; *Leuconostoc* sp. and *Enterococcus* sp.1 to produce cheese Frm 439; *Pediococcus* sp. and *Enterococcus* sp.2 to produce cheese Frm 503; *Enterococcus* sp.1, *Enterococcus* sp.2, *Leuconostoc* sp., and *Pediococcus* sp. to produce cheese Frm 625; *Enterococcus* sp.1 and *Pediococcus* sp. to produce cheese Frm 683; *Enterococcus* sp.1 and *Enterococcus* sp.2 to produce cheese Frm 741; and *Enterococcus* sp.2 and *Leuconostoc* sp. to produce cheese Frm 891.

During the production of these cheeses, the volume of the curd, draining time, and characteristics and formation of alveoli in the cheese after maturation were influenced by LAB. These variations are listed in Table 2.

The volume of the curds varied between 300 and 450 mL, with the exception of the curd from cheese Frm 425, in which there was a strong elimination of whey during curdling (300 mL), while the curds obtained with other bacteria lost less whey (450 mL).

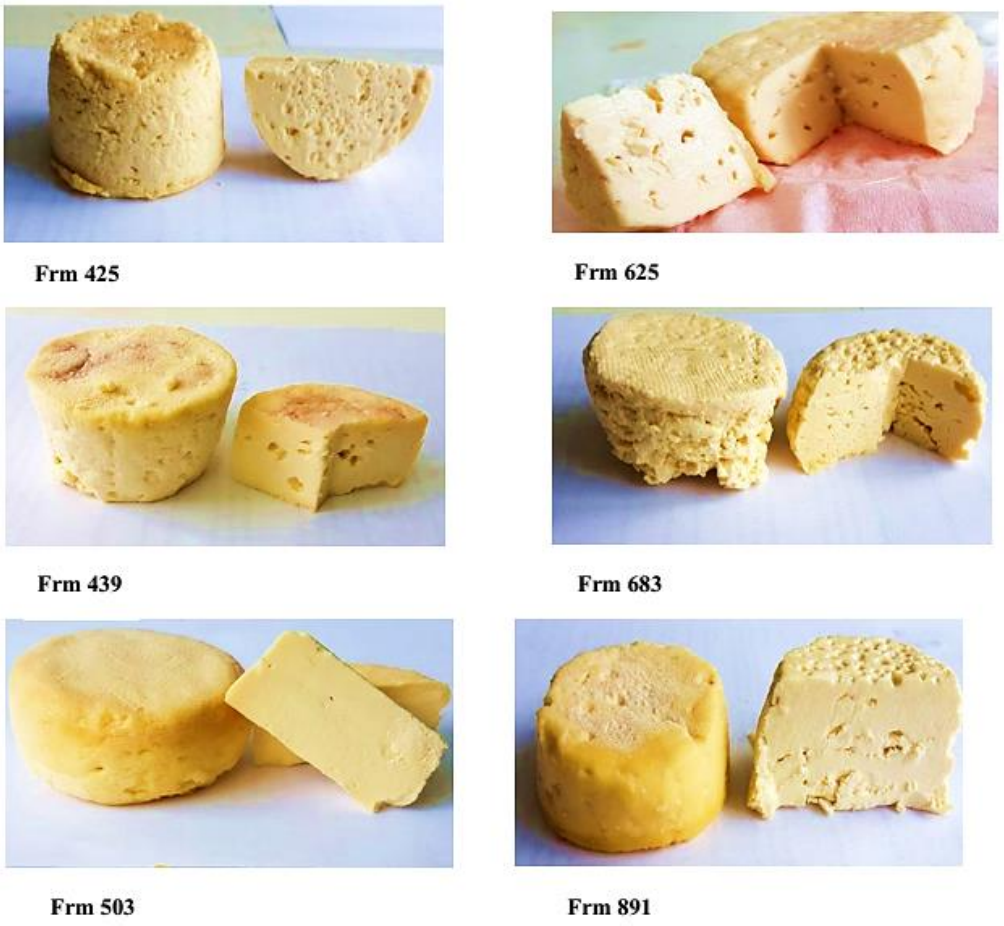

Figure 2. Some cheeses obtained from the combinations of isolated lactic acid bacteria.

Table 2. Influence of lactic acid bacteria on characteristics of cheese during different steps of production.

		Curdling	Dripping	Ripening	
Cheese	**Lactic acid bacteria**	**Volume (mL)**	**Time (h)**	**Alveolus (number per cm^2)**	**Onset time rind (h)**
Frm 425	*Leuco* - *Pedio*	300	48	+++	4
Frm 439	*Entero* 1 - *Leuco*	450	80	++	7
Frm 503	*Entero* 2 - *Pedio*	450	96	-	14
Frm 625	*Entero* 1.2 - *Leuco* - *Pedio*	450	24	++++	5
Frm 683	*Entero* 1 - *Pedio*	450	36	-	6
Frm 741	*Entero* 1 - *Entero* 2	450	T > 288		
Frm 891	*Leuco* - *Entero* 2	450	96	-	9

Leuco: *Leuconostoc* sp.; *Entero*: *Enterococcus* sp.1; and *Pedio*: *Pediococcus*. (-): No alveolus; (++++): at least 5 alveolus per cm^2; (+++): 3 or 4 alveolus per cm^2; and (++): less than 3 alveolus per cm^2.

The draining times of the curds varied between 24 and 288 h. The curd of cheese Frm 625 had the fastest draining time of 24 h. This was followed by the curds of cheeses Frm 683 (36 h) and Frm 425 (48 h). The curds of cheeses Frm 439, 891, and 503 took a longer time to drain (80 h for the first and 96 h for the last two, respectively). However, the curd of cheese Frm 741 was extremely wet, even after 288 h of draining. It is for this reason that this curd was withdrawn from production. From these results, we noted that the curd draining time could be associated with the group of LAB used, and therefore, could be affected by their ability to acidify the milk during curdling. In effect, the rigidity of the curd gel increases with a decrease in pH from 6 (Settani *et al.*, 2013). This explains why the curd of cheese Frm 741, derived exclusively from the genus *Enterococcus*, remained wet throughout the production. Indeed, the genus *Enterococcus* can only lower the pH of milk to 6.1 (Fox *et al.*, 2000; Lucey, 2002).

The presence of alveoli observed in the cheeses Frm 425, 439, 625, and 891 could be attributed to the production of CO_2 by hetero-fermentative bacteria, such as *Leuconostoc*, used during their production (Kindstedt, 2017). Rind appearance was characterised by the formation of a brown and solid film on the surface of the cheese that could only be observed in cheeses Frm 425, 625, and 683 after four, five, and six days of maturation at 12°C, respectively. This difference could be explained by the rate of water elimination in cheeses.

Microbiological analysis

Among all the analysed cheese samples, only four (Frm 683) showed the presence of total coliforms, and contamination varied between 2 and 3 $\log_{10}$ CFU/g. Although some cheese samples were contaminated, the analysed lot remained acceptable when we referred to the 3-class sampling (n = 5, m = 500 CFU/g, M = 1500 CFU/g, and C = 2) of the Canadian standards for cheese made with pasteurised and unpasteurised milk, published by the International Commission on Microbiological Specifications for Foods (ICMSF, 1974). It should be noted that the microbiological quality of cheeses is associated with the efficiency of pasteurisation at the beginning of production, compliance with good production practices, and the acidification of milk during coagulation (Bluma and Ciprovica, 2016; Haddad and Yamani, 2017).

Physicochemical analysis of cheeses

Available sugar content

The quantification of available sugars in cheese products revealed their presence in trace amounts. The cheese produced from *Pediococcus* sp. and *Enterococcus* sp.1 (Frm 683) had the lowest available sugar content, which amounted to 0.22 g/100 g. However, the cheese obtained with *Leuconostoc* sp. and *Enterococcus* sp.1 (Frm 439) had the highest level of available sugars, which was 0.49 g/100 g. The difference in sugar content between the cheeses could be explained by the fact that the rate of carbohydrate breakdown by LAB in food varies from one species to another.

Titratable acidity and water content

The titratable acidity in cheeses ranged between 52 and 212°D, with an average value of 130°D. However, cheese Frm 503 had the highest titratable acidity, while cheese Frm 425 had the lowest.

Apart from cheese Frm 625, all the other cheeses had water contents of less than 50%. The cheeses Frm 439 and 503 had 42% water content. The cheeses Frm 425, 683, and 891 had 43% water content.

Sensory analysis

Appreciation of cheeses

Sensory analysis of the cheeses revealed that not all of them were appreciated by consumers. This difference in appreciation varied significantly ($F = 10.39$, $p < 0.0001$). The most popular cheeses were Frm 425 and 625, with average scores of 6.41 and 5.99, respectively. The least appreciated cheese by consumers was Frm 439, with an average score of 4.86. However, there was no significant difference ($p > 0.05$) in appreciation between some cheeses such as Frm 891, 439, 683, and 503. The average overall acceptability score was between 5.11 and 5.58. This difference in the appreciation of cheeses could be associated with the preference for smell and flavour of one cheese over another, which is based exclusively on subjective criteria (Delahunty and Drake, 2004; Thomas, 2016).

Characterisation and appreciation of cheeses by attributes

After statistical analysis, it was found that 14 attributes highlighted the difference between the six

cheeses produced. These 14 attributes were divided into two groups: sensory and emotional attributes. Among the sensory attributes, beige, salty, and milky were cited 251, 219, and 215 times, respectively. For emotional attributes, a good smell was cited more than 200 times. However, sensory attributes were the most commonly used ones (23.21%) to characterise different cheeses in relation to emotional attributes (22.10%). Cheese Frm 425 and 439 were each cited 48 times as having a rind and beige colour, respectively. Cheese Frm 683 was cited 43 times as being salty, and cheese Frm 891 was cited 39 times as milk cheese. For cheese Frm 503, the astringent attribute was cited 24 times. The yellow attribute was designated to describe the cheese Frm 625. Cheese Frm 683 was found to be acidic and crumbly, with frequencies of 37 and 34. The attributes characterising cheese Frm 891 were tender and bitter, with frequencies of 37 and 50, respectively. Table 3 shows the descriptions and ratings of the cheeses based on each attribute.

Principal component analysis

To group the cheeses and the different sensory and emotional attributes that made it possible to describe each cheese, principal component analysis was carried out. The graph obtained after the analysis showed that the axes F1 and F2 indicated data obtained about six cheeses at 78.20%. However, the F1 axis alone carried 52.96% of the information on the sensory and emotional attributes of the different cheeses studied. On the same axis, cheeses were grouped into two types. In its positive part, Type 1 included cheeses most appreciated by consumers. Cheeses Frm 425 and 625 were described as soft, sweet, and pleasant. The characteristics noted in these cheeses could be explained by the ability of the bacterial strains (*Leuconostoc* and *Pediococcus*) to produce aromatic compounds such as diacetyl. These aromatic compounds can still intervene in the co-metabolism of citrates, and the formation of fruity and nutty flavours (Beresford *et al.*, 2001; Vasek et *al.*, 2013). Type 2 cheeses, which were the opposite of type 1 cheeses on axis 1, comprised the cheeses least appreciated by the panellists; these were cheeses Frm 439 and 503, which were described as bitter, sour, and unpleasant. This bitterness could be explained by the use of *Enterococcus*, which can release large amounts of bitter peptides and amino acids during metabolism (Nyberg, 2016).

Analysis of penalties based on CATA

The purpose of this analysis was to identify all the major characteristics that contributed to the appreciation and depreciation of the cheeses by the panellists. This analysis revealed that 10ten attributes, of which seven were sensory and three were emotional, significantly influenced ($p < 0.05$), either positively or negatively, appreciation. For the cheeses to be appreciated by the majority of panellists, they must be pleasant to eat and have a good smell, with appreciation scores of 1.97, 1.96, and 1.32, respectively. In addition, they must have a well-formed rind and a firm touch. Cheese should also taste milky and fondant in the mouth. Among the cheeses studied, only cheese Frm 425 and 625 had these characteristics. Cheeses with an unpleasant, bitter, and acidic taste (Frm 439 and 503) were rejected by the majority of panellists, with scores of 1.90, 1.38, and 0.39, respectively.

Conclusion

The present work demonstrated that the LAB isolated from local raw cow milk significantly influenced the sensory characteristics of cheese during ripening. Sensory analysis showed that among the cheeses produced, cheese Frm 425 prepared with *Leuconostoc* sp. and *Pediococcus* sp. was most appreciated by consumers for its sweet taste, yellow colour, pleasant smell, and lower acidity. The cheese Frm 439 obtained from *Leuconostoc* sp. and *Enterococcus* sp.1 was the least appreciated by consumers due to its bad odour, bitter taste, and high acidity. The combination of *Leuconostoc* sp. and *Pediococcus* sp. could be used to produce cheese in Cameroon, a possibility that has not been explored yet.

Acknowledgement

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Table 3. Numbers of citation of each attribute per sample.

Attribute		Frm 425	Frm 439	Frm 503	Frm 625	Frm 683	Frm 891	Citation / attribute	Total Citation	Average number of citation / attribute
Sensory	Astringent*	8	13	24	6	18	12	81	2089	23.21
	Yellow*	18	11	7	22	13	14	85		
	Mouldy aroma*	5	19	8	14	10	12	68		
	Acid***	9	27	30	20	34	21	141		
	Fondant*	17	29	32	29	23	37	167		
	White	13	14	19	8	15	12	81		
	Soft	29	25	26	29	25	27	161		
	Salty	37	37	32	35	43	35	219		
	Milky / Lactic	38	38	32	37	31	39	215		
	Beige	39	48	42	40	39	43	251		
	Rind***	48	25	19	36	21	29	178		
	Friable***	25	12	11	18	37	10	113		
	Bitter***	4	42	32	7	16	50	151		
	Sweet*	7	1	2	3	1	0	14		
	Firm	30	30	28	30	20	26	164		
Emotional	Nice***	31	9	11	17	17	12	97	663	22.10
	Unpleasant***	14	35	26	14	21	31	141		
	Good smell*	47	32	42	45	40	33	239		
	Pleasant***	34	11	12	25	20	10	112		
	Bad smell*	5	19	11	12	10	17	74		
Citation / sample		458	477	446	447	454	470	2752		
Average frequency / sample		22.90	23.85	22.30	22.35	22.70	23.50	137.6		

(*), (**), (***):significant difference between the different samples according to Cochran's Q test at $p \leq 0.05$, $p \leq 0.001$, and $p \leq 0.0001$, respectively.

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