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EFFECTS OF MILK PRESERVATION USING THE LACTOPEROXIDASE SYSTEM ON PROCESSED YOGURT AND CHEESE QUALITY

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ABSTRACT

The lactoperoxidase system (LP-system) is an acceptable chemical method for raw milk preservation, especially in rural areas where refrigeration facilities are absent to farmers. Milk production in most African countries is dominated by small-scale traditional production systems using low yielding local breeds. Therefore, processors who operate in such situations must rely on small volumes of milk from many farmers. Application of the LP-system prolongs the shelf life of raw milk and also encourages grouping of farmers hence facilitating milk collection by processors. The application of the LP-system is a recent preservation method for milk in Cameroon whose efficiency has been proven. Therefore, need arose for further studies on the influence of this method on milk processing as well as the quality dairy products.

The LP-system was activated by adding 10 ppm sodium thiocyanate and 8.5 ppm sodium percarbonate to fresh milk. Yoghurt and Bambui cheese were processed separately from treated and untreated (control) milk samples. Yogurt was produced from both the treated and the control milk samples at 2%, 3%, 4% and 5% (v/v) culture levels. Yogurt samples were analysed for acidity, protein content and dry matter content while cheese was analysed for butterfat and moisture content. Statistical tests were conducted by Analysis of Variance using the Fisher's test. Simple organoleptic assessments were conducted to compare yogurt and cheese from the treated and the control milk. Activation of the LP-system delayed lactic acid formation in yogurt during incubation and storage leading to increased energy consumption during processing and an improved keeping quality during storage. LP-system treatment reduced the overall organoleptic quality of yogurt while it improved on that of Bambui cheese. Dry matter content and fat content of yogurt were not significantly affected by LP-system treatment ($P<0.05$). LP-system treatment did not also affect the moisture and fat content of cheese but slightly improved on its yield.

Key words: Cheese, lactoperoxidase system, milk, yogurt

INTRODUCTION

Dairy production in tropical countries is hindered by accelerated milk spoilage due to poor production and transportation facilities as well as high ambient temperatures [1, 2]. Special care needs to be taken in order to keep microbial activity of the milk down to a minimum so as to improve its keeping quality as well as the quality of its products [3]. The lactoperoxidase system (LP-system) has been found useful in prolonging the shelf life of raw milk in countries where refrigeration facilities are absent and its efficiency has been proven [4, 5].

The LP-system is a natural anti-microbial system in milk which results from interaction between three components; the enzyme lactoperoxidase, thoicyanate ion (SCN^-) and hydrogen peroxidase (H_2O_2) [4, 6, 7, 8]. It has been shown to exert bacteriostatic effects on both gram- positive and gram-negative bacteria including psychrotrophes, which decrease shelf life of liquid milk at refrigeration temperatures [9, 10, 11, 12]. It also exhibits antiviral properties and plays a role in degrading carcinogens and in protection of animal cells against peroxidative effects [10].The natural LP-system phenomenon in fresh milk is short-lived and a prolongation of its effects is done by addition of SCN^- and H_2O_2. The LP-system, when properly applied, is harmless to mammalian cells. Instead, oxidation products of SCN^- may protect these cells against toxic effects of H_2O_2 [8]. The LP-system also occurs in saliva, milk and tears [4, 13], where it is involved in the natural host defence system against invading microbes [8], and in the human airway, where it plays a defensive role against some bacteria like *Pseudomonas aeruginosa, Burkholderia cepacia* and *Haemophilus influenzae* [14]. In addition to the LP-system, other non-specific factors like lactoferrin and lysosyme also exist in milk. Lactoferrin plays an anti-microbial role in depriving bacteria from iron ions and may protect the dry udder from infection [10, 15].

Before now, very little work has been done on the LPS in Cameroon compared to other tropical countries like China and Kenya. Some studies were done by Imele and others in 2000 and by Fonteh in 2001 [16, 17]. Research by Fonteh was done using different doses of activators and on individual farmers' milks. With the FAO's activators where a recommended dosage is set for a fixed quantity of bulk milk, further trials on this system were inevitable. Initial trials carried out by Imele and others using the FAO activators were had shortcomings due to financial limitations and shortage of laboratory equipment and chemicals. There was therefore need for a more detailed study first of all on the effectiveness of the lactoperoxidase system as a preservative for raw milk under Cameroonian conditions and secondly, to see how this preservation would affect the dairy industry. A previous study in Cameroon showed that the LP-system did not only improve on milk quality but also encouraged the grouping of farmers and stimulated milk production among them and thereby encouraging collection by processors [5].

Due to the dominance of small-scale traditional production systems coupled with the poor productivity of local breeds, the quantity of milk brought in for sale by each farmer is usually very little [18, 19]. For dairy processing units to operate under such conditions, a well structured dairy system must be built, such that it can collect small quantities of milk from many farmers.

Therefore, need arose to study the quality of dairy products resulting from milk collected from several small producers and preserved using the LP-system. Though the effects of the LP-system has been broadly studied in some countries, very few studies have gone further to show the effect of this preservation on the quality of processed milk. Khalid and Mathur showed that yoghurt processed from milk with smaller concentrations of activators (10 ppm for both) had a higher acceptability than yoghurt from control milk, meanwhile yoghurt resulting from treated milk at higher concentrations (20 ppm and 30ppm for both activators respectively) showed negative results with regards to acceptability [20]. Hirano et al. showed that the gelation pH of milk was increased by the LP-system which probably resulted from an increase in protein hydrophorbicity [21].

This study was aimed at finding out the effects of milk preservation using the lactoperoxidase system on the quality of yogurt and cheese prepared in the Western Highlands of Cameroon.

METHODOLOGY

Milk collection and LP-system activation

Milk samples were collected in Sabga village in the North West Province of Cameroon. This village falls in the Western Highland agro-ecological zone of Cameroon. The Western highlands lie between latitudes $5^{o}20'$ and 7^{o} North and longitude $9^{o}40'$ and $11^{o}10'$ East of the Equator where two main seasons exist: the rainy season (when this study was conducted), which runs from mid March to mid November, and the dry season, which runs from mid November to mid March. Rainfall ranges from 1500 – 2500mm while minimum and maximum temperatures have means of $15.5^{o}C$ and $24.5^{o}C$ [22].

Laboratory analyses were conducted in the Food Technology and Post Harvest laboratory of the Institute of Agricultural Research for Development (IRAD) Bambui, Cameroon.

Milk was obtained from over 60 local cows (white Fulani, red Fulani and Gudali breeds) belonging to 32 herders. Collection was done in the rainy season at a common collection site where all farmers brought their milk. Individual farmers' milks were tested for spoilage and adulteration before bulking. This was done using the specific gravity, clot-on-boiling test, alcohol test, acidity test and sensory tests. Milk which failed to meet the required standards following the above mentioned tests was discarded, while that of acceptable quality was bulked into a sterilised metal churn, after filtering through a sterile cloth. Part of the bulk milk was kept for analysis of

specific gravity, acidity, butterfat, protein and dry matter content. Bulk milk was divided in two parts; one part was treated by activation of the LP-system while the remaining part was kept untreated (control) under ambient temperatures. Activation of the LP-system was done by first of all pouring sodium thiocyanate (10 ppm) solution (supplied as such by distributors), followed by mixing for 30 seconds and then adding sodium percarbonate (8.5 ppm) and mixing again for 2 minutes. Sodium thiocyanate and sodium percabonate used were produced by *BIO SERAE*, France and obtained from the FAO. The treated and the control bulk milks were processed separately into yogurt and cheese.

Analysis of physico-chemical properties of milk

Bulk milk was analysed for butterfat, casein, dry matter, protein and solids-not-fat as described below. All samples were analysed in duplicates.

(i) Specific gravity

A lactometer was used to measure the specific gravity of the bulk milk at room temperature. Milk was poured into the lactometer jar and the lactometer was allowed to slide gently into the milk until it reached equilibrium. The reading was taken directly on the lactometer to the nearest 0.1 on the lactometer [23].

(ii) Butterfat (BF)

Butterfat was determined using the Gerber method [23]..

(iii) Protein and casein

Protein and casein were determined using the Formol Titration method [24].

(iv) Dry matter (DM)

Each milk sample (3mL) was pipetted into previously dried and weighed steel dishes. The total weight of the dish with sample was taken and the samples dried in an oven at 100^{o}C for five hours. The samples were removed, cooled, weighed and dried again in the oven for one hour. This procedure was repeated until successive recordings differed by less than 0.004g. The dry matter was then calculated as follows:

$$\% \text{ Dry matter} = \frac{\text{Final weight of dish + milk – Weight of empty dish}}{\text{Initial weight of dish + milk – Weight of empty dish}} \times 100$$

(v) Solids-not-fat (SNF)

The value for solids-not-fat was obtained by subtracting the butterfat (BF) value from the dry matter (DM) value as follows:

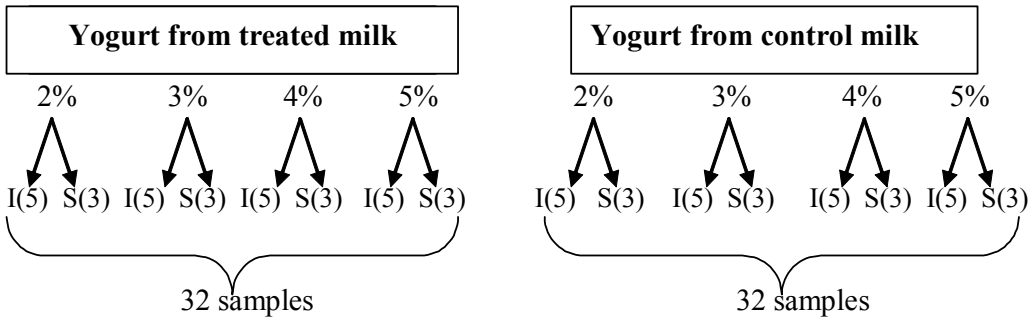
$$SNF = DM - BF$$

Yogurt Manufacture and Analysis

Milk was pasteurised in a steel pot over gas flame at 85^{o}C for 15 seconds and rapidly cooled to 43^{o}C. Dry original yogurt culture obtained from *BIONIC Biotechnologishes, Niebülle- Germany,* containing lactic acid culture of *Lactobacillus yoghurtii, Lactobacillus acidophilus, Lactobacillus bulgaricus and Streptococcus thermophilus*

was used. The mother culture was prepared using 0.5g of powdered culture in 200ml of milk, which was previously sterilised at 100°C for 30 minutes and cooled to 43°C. Milk samples were inoculated at 2, 3, 4 and 5% (v/v), using the mother culture and then incubated at 43°C until gelation occurred (between 4-6 hours) [25]. Extra yogurt was prepared using 3% (v/v) ratios, from both the treated and the control milks, which was used in the organoleptic assessment of yogurt. Both yogurt types were divided into two main sample groups before incubation (Figure 1). One of the groups consisted of five samples at each culture level and intended for analysis during incubation, while the second group consisted of three samples at each culture level and was intended for analysis during storage. During incubation, each sample was only analysed once and then discarded. This was to prevent any effects from the shaking of samples by repeated sampling. The yogurt samples were stored in a refrigerator at 6 - 8°C and analysed weekly for acidity. All analyses were done in duplicates.

Figure 1: Distribution of yogurt samples for analysis



I = Yoghurt used for analysis during incubation.
S = Yoghurt used for analysis during storage.
Figures in brackets represent the number of samples

The Amount of lactic acid in milk and yogurt was estimated using the titratable acidity method [26], while the protein and dry matter content of yogurt were obtained in the same manner as for milk.

Organoleptic assessment of yogurt

A simple organoleptic assessment was done to compare consumers' preference for either yogurt from the treated milk or the untreated milk. Only yogurt inoculated at 3% level was used for both the treated and the control samples. Since commercial yogurt in this area is usually sweetened, 60g of powdered sugar was added per litre of yogurt from both treatments and carefully mixed, prior to the organoleptic assessment. Thirty-two panellists were selected at random and offered both types of yogurt to choose one which they preferred.

Cheese Manufacture and Analysis

Bambui cheese from the treated and the control milk was manufactured (separately) as follows:

Milk was pasteurised by heating to 72-74^{o}C for 15 seconds and cooled rapidly to 30–33^{o}C [27]. Mesophilic culture containing *Streptococcus lactis, Streptococcus cremoris* and *Streptococcus diacetilactis* was obtained from *Laboratoire de ferments bacterie, Ukraine* and used at 1% (v/v) ratio. Powdered rennet obtained from *Hansen laboratory, Denmark* was dissolved in distilled water and used at 3% concentration. Curdling occurred within one hour. The curd was cut manually with a flat metal spoon, cooked at 32^{o}C for 40 minutes and then drained. Pressing was done using local cheese presses and metal moulds, lined with sterilised cheesecloth. After about 18 hours of pressing, salting was done by immersing in 20% brine solution for 6-8 hours/kg of cheese. The cheese was left to dry in a refrigerator at (6-8 ^{o}C) for 4 days and ripened for 40-60 days in cheese shelves under ambient conditions (temperatures of 22-25^{o}C and 85% relative humidity).

The fat content of cheese was determined using the Gerber method using 3g of cheese [23]. while its dry matter was obtained using 2g of cheese in the same manner as for milk.

Organoleptic assessment of cheese

Panelists were selected for the organoleptic assessment of cheese, based on their familiarity with the product. Eighteen panelists who had the habit of consuming cheese in their homes were selected. This is because cheese consumption was not as common as yoghurt consumption and hence, random sampling was not possible. The test was simpler than standard cheese tests and did not require trained cheese experts. It differed from the tests by Kameni et al, for example where a grading scale of 1-10 was used to assess cheese in the same region [28].

Statistical analysis

Data was submitted to Analysis of Variance using the Fisher's test. Means of cheese yield and composition were compared using Fisher's least significant difference (LSD).

RESULTS

Physico-chemical properties of bulk milk

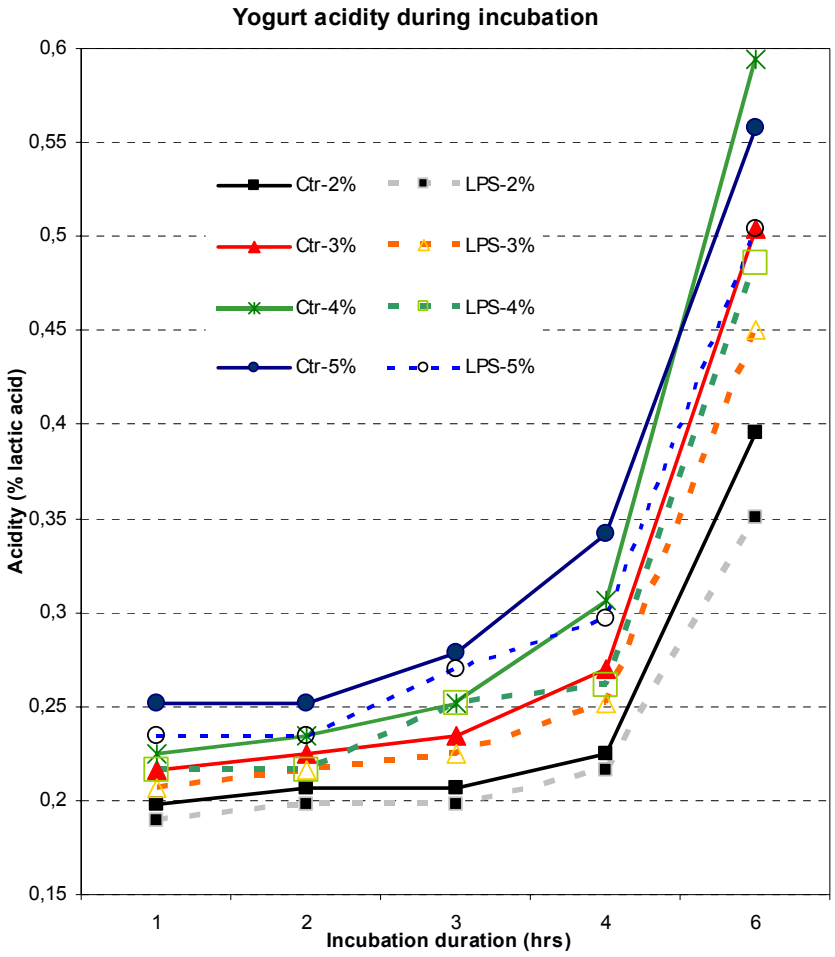
The results of the physico-chemical analysis of bulk milk are shown on Table 1.

Effects of LP-system on acid development in yogurt during incubation

All yogurt samples from the control milk had higher acidity values than those of the treated milk (Figure 2). At the sixth hour of incubation the lactic acid content in

yogurt from the treated milk was 11.36, 10.71, 18.18 and 9.68% lower, as compared to the control yogurt samples at 2, 3, 4 and 5% culture levels, respectively.

Figure 2: Effect of LP-system on acid development in yogurt during incubation

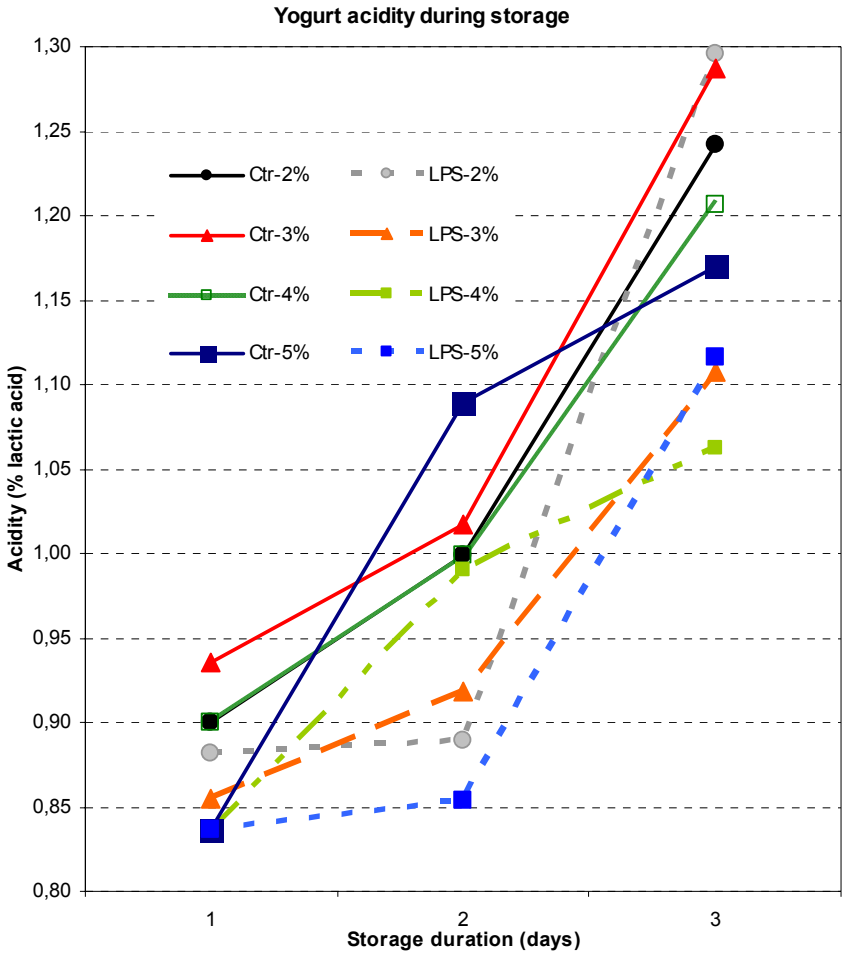


NB: The commas on the Y-axis replace the decimal point.

Effects of LP-system on Acid development in yogurt during storage

There was already a difference in acidity of yogurt during the first hour of incubation, which varied from 0.18% lactic acid in the LPS-2% sample to 0.25% lactic acid in the Ctr-5% sample. During storage, the acidity of individual yogurt samples increased progressively during the first three weeks, as shown in Figure 3. However, there was no significant difference ($P<0.05$) in acidity between the treated and the control samples. At the end of the second week of storage, nine out of twelve (75%) control samples produced off-flavours and gases indicating spoilage. Meanwhile all the treated samples were still good. At the end of the third week of storage, only two of the treated samples were spoilt whereas all the control samples were already spoilt.

Figure 3: Effects of LP-system on Acid development in yogurt during storage



NB: The commas on the Y-axis replace the decimal point.

Effects of LP-system on protein and dry matter of yogurt

Table 2 shows the protein and dry matter content of yogurt samples. The percentage of protein and dry matter were not significantly different (P< 0.05) between yogurt samples from the treated milk and those from the control milk. However, the dry matter content of yogurt from the treated milk samples was slightly higher than that from the control milk samples at each level of culture concentration.

Organoleptic assessment of yogurt

From the simple organoleptic assessment, 18 (56.25%) panellists preferred yogurt from the control milk, 5 (15.63%) didn't find any difference between both types, while the remaining 9 (28.12%) panellists preferred yogurt from the treated milk. This

indicates that the consumer preference was higher for yoghurt from control milk than that from treated milk.

Effects of LP-system on Bambui cheese

From the Table 3, we see that the average yield of Bambui cheese was 12.77kg/100L of milk and 13.62kg/100L for the control and the treated milk, respectively. After pressing (before ripening), the yield of cheese obtained from the treated milk was significantly higher ($P<0.05$) than that of cheese from the control milk. After ripening, there was no significant difference between the yield of cheese from the treated milk and that from the control milk ($P<0.05$), though the yield of treated milk was higher by 6.39 %.

The moisture and fat content of cheeses from the treated and the control milks were not statistically different ($P< 0.05$). The organoleptic grades of cheeses are illustrated in Figures 4 and 5.

Figure 4: Organoleptic assessment on texture of cheese

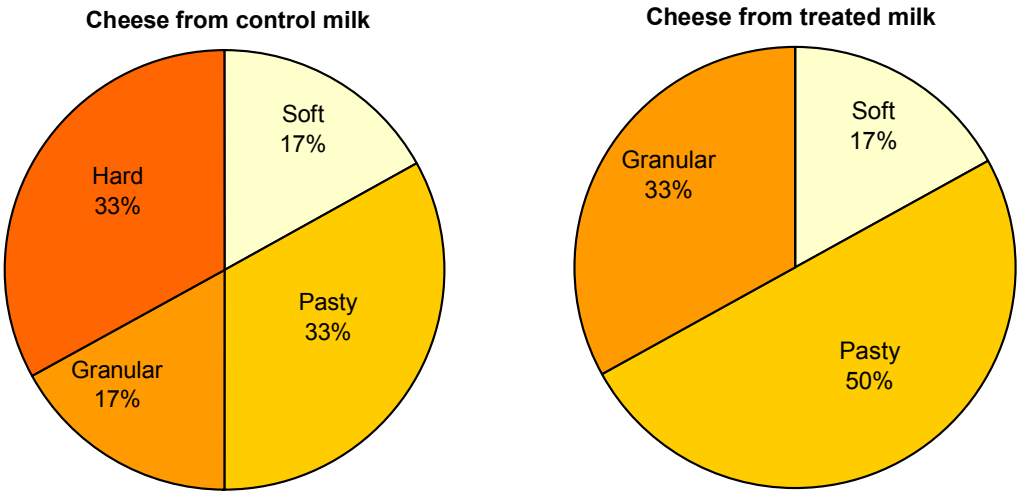


Figure 4 shows that 33 % of panelists (n = 18) graded the texture of cheese from the control milk as pasty, 33 % graded it as “soft”, 17 % graded it “granular” and 17 % graded it “hard”.

Figure 5: Organoleptic assessment on odour of cheese

With respect to odour, 50 % of panellists found cheese from the control milk to have a strong odour (Figure 5), while 33 % found it not pronounced and 17 % found it mild. Generally, the panellists appreciated the milder odour in the treated samples than in the control samples.

Finally, 61% of panellists gave "EXCELLENT" as the overall grade of cheese from the treated milk while only 39% graded the cheese from the control milk as excellent.

DISCUSSIONS

The dry matter and fat content of 13.4% and 4.2% respectively were similar to those of the lower acid development in the treated yogurt samples during incubation shows that LP-system has an inhibitory effect on lactic acid bacteria which retarded acid formation in the treated milk. These results conform to those of Khalid and Musad [30], who also found reduced acidity and prolonged incubation period for yogurt produced from the treated milk. Hirano and others suggested that this effect on gelation was due to protein hydrophobicity [21]. The delayed acid development causes an increase in production time which entails higher use of energy and hence higher cost of production. This effect is undesirable to processing plants especially as energy is becoming limited and more expensive.

The delayed acid development during storage of yogurt from the treated milk was also noticed by Nakada and others [31]. These authors showed treatment of milk by the LP-system resulted to yoghurt retained its acceptable quality for at least two weeks, during storage.

Though there was no significant difference in protein and dry matter content of yogurt from the treated and the control milk, these values were higher for yogurt from the treated milk than for yogurt from the control milk at all culture levels. These results conform to those obtained by Kumar and Mathur [32], who also found a higher dry matter content in the treated yogurt. The higher dry matter content probably results from the inhibitory effects of the LP-system on bacteria that degrade milk solutes.

The reduced "thickness" in yogurt was also noticed in previous studies, where it was shown that milk treatment with the LP-system resulted to a reduction in hardness and apparent viscousity of yogurt. The same authors suggested that this effect was caused by the action of $OSCN^-$ (hypocyanite ion) on milk proteins [30]. Unfortunately, due to lack of appropriate apparatus, the viscosity of the yogurt could not be determined in this analysis. In order to improve on yogurt "thickness" little amounts of powdered milk could be added to the treated milk during processing, for a better yogurt viscosity. The simultaneous used of reconstituted powdered milk and fresh milk is already practiced by processing units especially in the dry season when fresh milk is limited in supply. Therefore this might not pose a problem with regards to technical feasibility.

The moisture and fat content of Bambui cheese from the treated and the control milk were not significantly different, showing that LP-system treatment has no influence on the chemical composition of cheese. Other authors also found out that LP-system treatment did not affect cheese composition [33]. Organoleptic assessment of cheese showed that the milder odour (which was also found in other studies) and pastier texture of cheese from the treated milk improved its quality and hence consumer

preference [33]. This milder odour of cheese was of much importance and could encourage consumption in such areas where cheese consumption is less common because people do not appreciate its strong odour. Unlike in Cameroon and probably many other African countries where locally produced cheeses are not standardised, grading is a crucial issue in USA and standards are set for different cheeses. The grading process is more complicated and takes several aspects into consideration. Chedder cheese for example, is graded in Wisconsin following four major criteria; flavour, texture, colour and appearance. The flavour is graded using 17 different characteristics: feed, acid, flat, bitter, fruity, metallic, sour, whey-taint, yeasty, malty, old milk, onion, weedy, sulphide, barny, and rancid. The texture of cheddar cheese is graded on 13 characters, the colour on eight and the appearance on 18 characters. Grading is done by filling tables where each of the above characters is judged using the following grade attributes: (a) "Definite" meaning that the trait is not intense but is detectable in the cheese being graded. (b) "Pronounced" means the trait is sufficiently intense as to be easily identified. (c) "Slight" means the trait is detected only upon critical examination. (d) "Very slight" means the trait is detected only upon very critical examination [34]. In Ireland, the maximum scores for flavour, texture and appearance of cheddar cheese are 45, 40 and 5 respectively. Meanwhile, for cheese to be acceptable for commercial purpose, it must meet a minimum grade of 38 and 31 for flavour and texture respectively [35].

CONCLUSION

Activation of the LP-system system delayed lactic acid formation in yogurt during incubation and storage leading to longer incubation duration during processing and a longer yogurt shelf life during storage. LP-system treatment reduced the organoleptic quality of yogurt while it improved on that of Bambui cheese. The moisture content and fat content of cheese were not affected by LP-system treatment. Application of the LP-system could be beneficial to the processor since his products will last longer. However the delayed acid development during yogurt incubation might increase his processing cost.

Table 1: Physico-chemical properties of bulk milk

Parameter	Value
Specific gravity	31.8 (SD 0.2)
Butterfat (%)	4.20 (SD 0.06)
Protein (%)	4.42 (SD 0.05)
Casein (%)	3.51 (SD 0.03)
Dry matter (%)	13.40 (SD 0.91)
Solids-not-fat (SNF) (%)	9.20 (SD 0.50)

Table 2: Effects of LP-system on protein and dry matter of yogurt

	Percentage Culture							
	2%		3%		4%		5%	
	Control	LP-S	Control	LP-S	Control	LP-S	Control	LP-S
Protein (%)	4.61 (SD 0.02)	5.18 (SD 0.07)	4.99 (SD 0.05)	4.80 (SD 0.03)	4.80 (SD 0.08)	4.42 (SD 0.04)	4.03 (SD 0.02)	4.80 (SD 0.03)
Dry matter (%)	11.89 (SD 1.25)	12.25 (SD 1.54)	12.28 (SD 1.46)	13.00 (SD 1.67)	12.59 (SD 1.55)	12.66 (SD 1.32)	12.30 (SD 1.39)	12.58 (SD 1.34)

Table 3: Effects of LP-system on yield, moisture content and fat content of cheese

Parameter	Control	Treated
Yield before ripening (kg cheese/100L milk)	12.77[a] (SD 1.72)	13.62[b] (SD 2.04)
Yield at consumption (kg cheese/100L milk)	10.80 [a] (SD 1.02)	11.49 [a] (SD 1.43)
Moisture content (%)	33.67 [a] (SD 2.12)	33.59 [a] (SD 2.15)
Fat content (%)	20.25 [a] (SD 1.24)	20.38 [a] (SD 1.32)

a,b = Letter superscripts bearing different letters are significantly different ($P<0.05$)

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