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ACTIVATION OF THE LACTOPEROXIDASE SYSTEM AS A METHOD OF PRESERVING RAW MILK IN AREAS WITHOUT COOLING FACILITIES

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ABSTRACT

Milk spoilage is a major problem to the dairy sector of tropical countries. High temperatures hasten spoilage, which is worsened by the absence of cooling facilities and adequate transportation means. Some chemicals have been tried as milk preservatives in rural areas, but are now forbidden due to toxicological reasons. The use of the lactoperoxidase system (LPS) might offer a solution to milk preservation in such areas.

The lactoperoxidase system is a natural antibiotic system in milk, which could be activated to boost its effectiveness. This study focused on the effects of the lactoperoxidase system on raw bovine milk produced in the Western Highlands of Cameroon during the rainy season. Milk was collected from 17 - 31 farmers in Santa village, bulked and activated with thiocyanate and peroxide. The LPS was activated by addition of 10 ppm thiocyanate and 8.5 ppm peroxide to milk, followed by thorough mixing. Part of the milk was left untreated (control). Treated and control samples were kept under three storage conditions: ambient temperature (22 – 25^{o}C), water bath (20^{o}C) and refrigeration (6 – 8^{o}C).

Samples were monitored for spoilage at hourly intervals, except for those in the refrigerator, which were monitored after every 6 hours. Microbial population was also estimated at intervals, using the standard plate count method. The average increase in shelf life of treated milk with respect to the control milk was +7.1 (SD 2.4) hours under ambient temperatures, +8.1 (SD 3.0) hours in a water bath and +46.2 (SD 21.2) hours in the refrigerator. The LPS limited the activity of lactic acid bacteria, which cause spoilage in milk. Treatment reduced the lactic acid content in milk by 29% under ambient temperature and 15% in water bath after 16 hours. The LPS reduced the microbial load in milk stored under ambient temperature by more than one log cycle, after 8 hours of storage. Treated evening milk could remain in good condition for the next day's use without refrigeration. Therefore LPS treatment can improve on income generation from dairying as it enables farmers sell milk in far-off markets.

Key Words: Lactoperoxidase system, raw milk, preservation

INTRODUCTION

The Cameroonian dairy system faces particular problems as milk production is concentrated in particular regions, mostly far off from consumers [1]. In addition, due to transportation difficulties, prices at consumer level are usually very high especially as marketing systems are inefficient [2, 3]. Therefore, small-scale dairy production is worth encouraging, first of all as a means of subsistence and better still as a source of income for rural populations.

Microbial activity in fresh milk could be slowed down by refrigeration. Unfortunately, most tropical milk farmers do not have access to refrigeration facilities. Furthermore, high ambient temperatures accelerate milk spoilage and render dairying a risky enterprise [4]. As a result, a great quantity of milk is lost in Africa [5]. Spoilage is aggravated in West Africa by poor collection systems. According to the World Bank, 20% of milk produced in developing countries is lost because of inappropriate dairy systems [6].

Local demand for milk and dairy products in Cameroon is likely to increase over the years. By the year 2020, the population of Cameroon is expected to rise above 27 million against about 16.5 million today. The urban population of Cameroon is also expected to increase from 8.7 million at present to more than 18 million in the year 2020 [7, 8]. It is estimated that both meat and milk production per head of cattle must almost double by the year 2020 in order to meet the demand for animal products in developing countries [7]. Therefore, there is need to check on the possibilities of improving dairy production at all levels of the production chain.

Chemicals such as boric acid, formalin, hypochlorides and hydrogen peroxide have been tested in the dairy industry for raw milk preservation. Due to their toxicity, these chemicals are now illegal additives except when used as preservatives for laboratory samples or as disinfectants [9]. The lactoperoxidase system is a better recommended chemical preservative for milk because it has been proven to be of no harm to humans, at low concentrations [10].

Wright and Tramer were the first to discover that LPS leads to inhibition of bacteria in raw milk in 1958 [11]. Progress was slow on the adoption of this method as the idea was rejected by FAO until 1991, when the joint FAO/WHO expert committee on food additives approved this method for field implementations, following toxicological tests.

The lactoperoxidase system is a natural antibiotic system in milk, saliva and gastric juice and functions in protecting the new-born and grown-ups from bacterial attack. The system naturally lasts in fresh milk for a short period of sometimes less than two hours, due to limitation of substrates [12]. Activation of the system is done by adding hydrogen peroxide and thiocyanate to the milk in concentrations of 15 ppm and 10

ppm respectively which are far less than those existing in common foods and human tissues [13,14].

This study was intended to monitor and compare the changes in microbial activity in treated (LPS-activated) milk and untreated (control) milk under Cameroonian conditions.

MATERIALS AND METHODS

Experimental Site

Milk samples were collected from Sabga village of Mezam Division in the North-West province of Cameroon. Laboratory analyses were conducted in the Food Technology and Post Harvest laboratory of the Institute of Agricultural Research for Development (IRAD) Bambui - Cameroon. Collection was done in Sabga village where the total potential quantity of milk available was greater than 50 litres per collection. 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, which runs from mid March to mid November, and the dry season, which runs from mid November to mid March.

Rainfall ranges from 1500 – 2500 mm per annum, while minimum and maximum temperatures have means of $15.5^{o}C$ and $24.5^{o}C$ [15] Mean rainfall and temperatures ranges are 1500-2500 mm and 15.0 ^{o}C and 24.5 ^{o}C, respectively**.** During the dry season the fields dry up, pastures become limited and there is a great need for feed supplementation for dairy cattle. A drastic drop in milk production is observed at this time, since most rural farmers can not afford feed supplements.

The soils are mostly ferallitic. The vegetation is of Savanna grassland type, spotted with trees. Predominant forage species include *Pennisetum purpureum, Pennisetum clandestinum, Panicum maximum, Sporobolus africana, Imperata cylindrica and Hyperrhenia rufa.* Improved varieties like *Trypsacum laxum, Brachiaria ruziziensis and Stylosanthes guianensis* are cultivated by some breeders.

Quality Tests and Milk Collection

Prior to milk collection, farmers were sensitised on LPS demonstrations. A common collection point was set where milk was collected from all farmers wishing to supply their milk. At the collection point, each farmer's milk was tested for spoilage and/or adulteration before bulking. The specific gravity, clot-on-boiling test, alcohol test, titratable acidity and sensory tests were conducted for each farmer's milk.

Specific Gravity

This was measured using a lactometer. Each milk sample was poured into a lactometer jar. The lactometer was then allowed to slide gently into the milk until it reached equilibrium. The reading was taken directly on the lactometer to the nearest 0.1 unit on the lactometer [16].

Clot-on-boiling Test
Each milk sample (2ml) was pipetted into a test tube, heated gently over a flame to boiling point and observed for clotting. Clotting signified that the milk was sour and past its shelf life [13].

Alcohol Test
Equal volumes (1ml each) of milk sample and alcohol (68% ethanol) were transferred into test tubes. The test tubes were agitated gently and observed for flocculation. Flocculation occurred with increased acidity (souring) of the milk, indicating spoilage [13].

Titratable Acidity
The Amount of lactic acid in milk was estimated using the titratable acidity method [16]. Each sample (10 ml) of was transferred into a conical flask. Then 1.0 ml of 0.5% phenolphthalein indicator was added and titrated with 0.1N NaOH until a faint pink colour appeared and persisted for at least 5 seconds. The titre reading was noted and calculated as percentage lactic acid .

The percentage lactic acid was used combined with the alcohol and clot-on-boiling tests in determining the time of spoilage in milk.

Sensory Tests
The colour of milk and presence of particles was observed. The odour, taste and flavour were evaluated after the clot-on-boiling test. The presence of off-flavours and absence of a mild sweet taste indicated degradation of lactose.

Acceptance or rejection of milk from farmers was based on the above tests. Milk of acceptable quality was bulked into a sterilised metal churn after filtering through a sterile cloth and mixed using a metal plunger. Part of the milk (50 litres) was activated, while the remaining part was left untreated (control). Activation of the LPS was done by adding 10 ppm of sodium thiocyanate solution followed by mixing for 30 seconds and then adding 8.5 ppm of sodium percarbonate and mixing again for 2 minutes [13]. The activation time was noted. Sodium thiocyanate and sodium percabonate used were produced by *Bio Serae*, France and obtained from the Food and Agriculture Organisation.

Laboratory Analysis

Spoilage Analysis

Equal volumes of treated (activated) and untreated (control) samples were further divided into three groups and stored under three different conditions. This gave six different treatments as illustrated in Figure 3 bellow:

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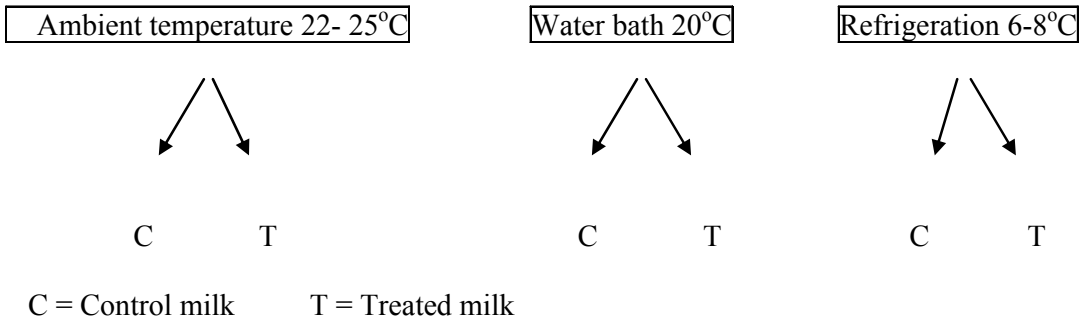

Fig 1: Distribution of milk samples for monitoring spoilage

Spoilage was monitored after every six hours for samples stored in the refrigerator and at hourly intervals for the samples stored in a water bath and those under ambient temperatures. The titratable acidity, clot-on-boiling test, alcohol test and microbial counts were used to monitor spoilage.

Microbiological Analysis

Total microbial count for milk was estimated using the standard plate count (SPC) method [17]. Each milk sample (1 ml) was transferred into sterilised tubes containing 9 ml of Ringer's reagent and properly mixed, forming a 10^{-1} dilution. Serial dilutions were made from 10^{-1} to 10^{-5} by taking 1 ml of solution from one tube and then mixing it with 9 ml of Ringer's reagent in the next tube. Then, 1 ml of solution was plated in petri dishes. About 20 ml of molten agar (sterilised and cooled to 45 ^{o}C) was added to each petri dish and mixed thoroughly.

When the agar was set, the petri dishes were placed in an incubator at 35 ^{o}C for 48 hours. Colony forming units (cfu) were counted using a Gallenkamp colony counter.

Participation Survey

Since the LPS was tried for the first time in this region, a survey aiming at evaluating farmers' attendance was solicited. At each collection (trail), the names of all milk farmers who supplied milk were recorded and compared over the LPS demonstration (trial) period.

Statistical Analysis

Data were submitted to one-way Analysis of Variance using the Fisher's test. Means were compared using Fisher's least significant difference (LSD). Significance difference was at $p<0.05$ level.

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RESULTS

Effects of LPS on Shelf Life of Milk

The LPS extended the shelf life of raw milk by an average of 7.1 (± 2.4 SD) hours under ambient temperatures, 8.1 (± 3.0 SD) hours in a water bath and 46.2 (± 21.2 SD) hours in a refrigerator (Table 1). A maximum difference in shelf life of four hours was noticed between treated milk stored in a water bath and treated milk stored under ambient temperature. Refrigeration alone could store fresh milk in good quality for about two days (42 – 60 hours). Meanwhile, a combination of refrigeration and LPS could preserve fresh milk for up to one week (168 hours).

Effects of Activation Time on Shelf Life of Milk

The increase in shelf life of milk at ambient temperature due to LPS activation is shown in Figure 2. Milk which was activated at 10:30 am had a shelf life extension of 9 hours. Milk samples that were activated later in the morning had reduced shelf lives than those activated earlier.

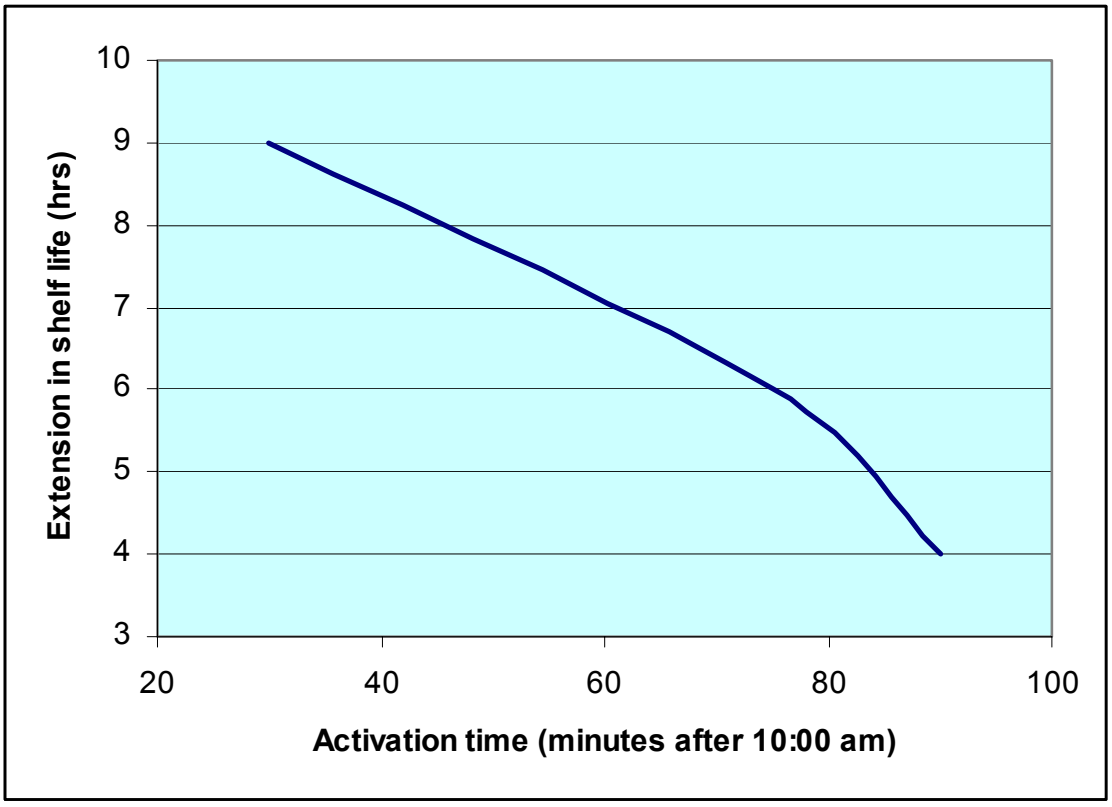

Figure 2: Increase in shelf life of activated milk samples under ambient temperature

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Acid Development in Milk

During storage, the titratrable acidity (which indicates degradation by lactic acid bacteria) of treated milk was always lower than that of control milks, though they had the same initial acidity (Figure 3).

The difference in acidity between treated and control milks increased with storage duration until spoilage was noticed in the milk samples. Increase in lactic acid content of LPS treated milk was only noticed after 16 hours in samples stored both at ambient temperature and those in a water bath.

Meanwhile, an increase in lactic acid content was already noticed after two hours in control milk under ambient temperature and 12 hours in a water bath. After 16 hours of activation, the lactic acid content of treated milk was 29% lower than that of control milk under ambient temperature and 15% lower in a water bath. The acidity curves for all four treatments had a similar trend with a J-shaped pattern.

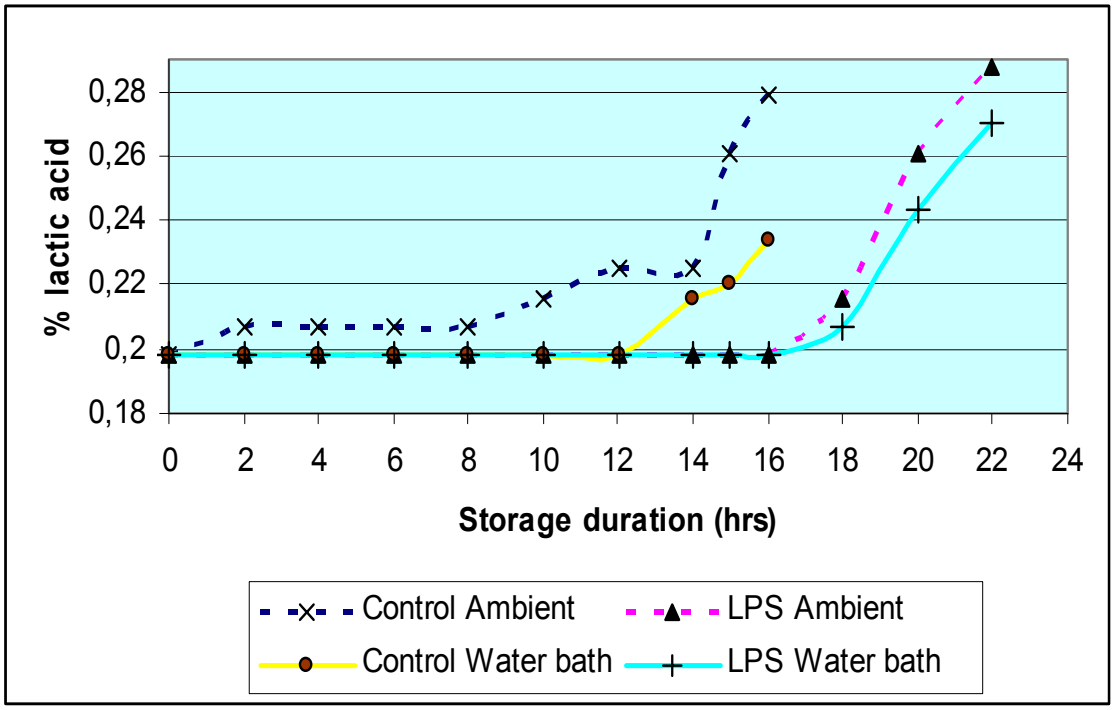

Figure 3: Acid development in milk

Evolution in microbial counts of milk

Lower microbial counts were observed under ambient temperature, for treated samples than for control samples through out the trail period. This difference

increased with time and at the 8th hour after treatment, a microbial load difference of more than one log cycle (1.18) was noticed between treated milk and control milk (Figure 4).

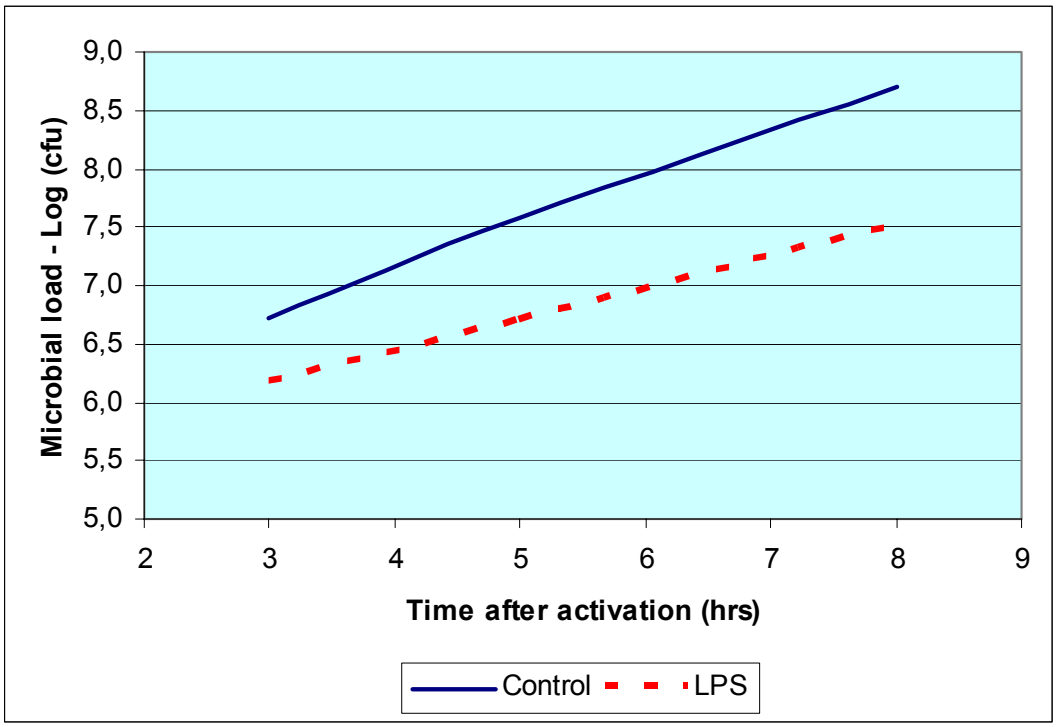

Figure 4: Evolution of total microbial counts in milk stored under ambient temperatures

As is the case under ambient temperature, (Figure 4), the microbial load in treated milk stored under refrigeration remained lower than that for control milk. Evolution of total microbial counts in both treated and control milks showed an S-shaped curve (Figure 5).

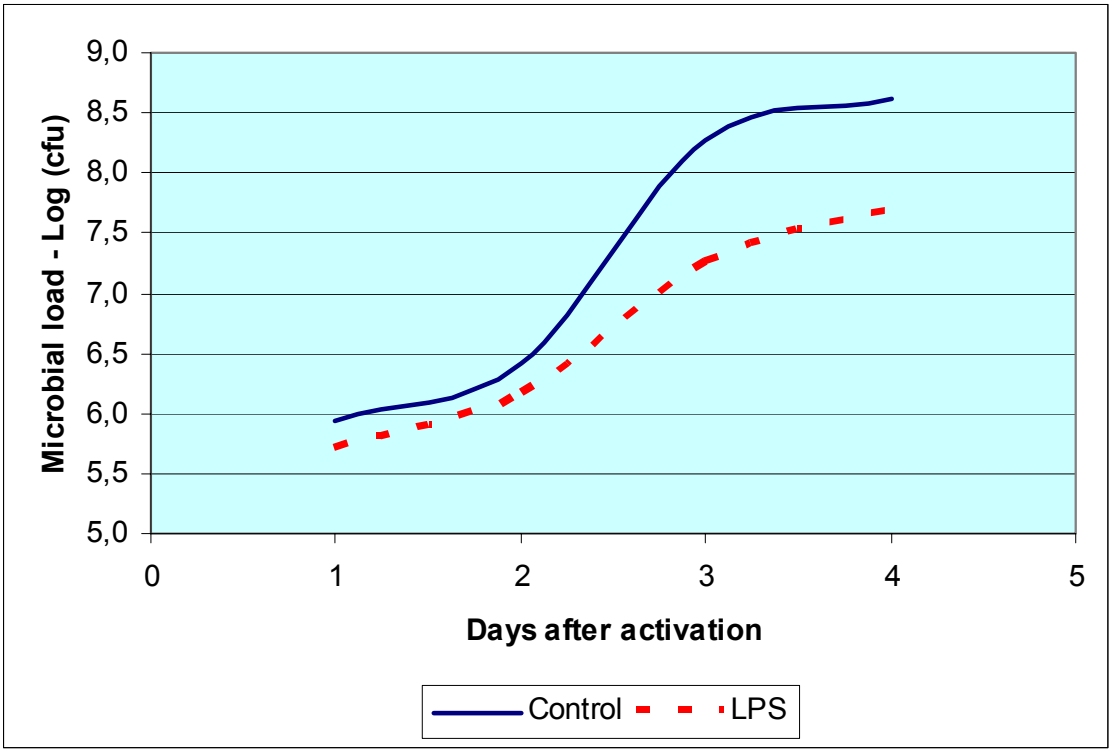

Figure 5: Evolution of total microbial counts in milk stored under refrigeration

Farmer participation in LPS demonstrations

The number of milk suppliers increased within successive collection days (trails) during the LPS demonstrations (Table 2). Almost all milk suppliers were females. There was an increase in the total milk quantity supplied as well as the average individual quantities of milk supplied per farmer over the collection period.

DISCUSSIONS

Differences between shelf lives of treated and control milks at all storage conditions were highly significant ($P<0.01$) showing that the LPS is effective in preserving raw milk (Table 1). The increase in milk shelf life of 7.1 hours under ambient conditions can permit transportation of milk from far-off zones to the processing plant without fear of spoilage.

The average extension in shelf life of 7.1 hours under ambient temperatures is less than values obtained by FAO (11 – 12 hours at 25 $^{\circ}$ C) with milk of good initial quality [18]. Poorer results are probably due to poor hygienic conditions during milking, which gave the milk a higher initial microbial load. This implies that better results would be expected if the hygienic conditions during milk production are improved.

Also, from Table 1, treated samples stored in a water bath could stay longer than those at ambient temperature, (though the difference was not significant $P < 0.05$). The shelf life of up to 26 hours in water bath implies that milk collected in the evenings could be treated and stored in a water bath and sold or consumed up the next morning without risk of spoilage. This is important in such areas where milk collection is technically feasible only once a day (in the mornings), which leaves evening milk most susceptible to spoilage.

There was a shorter increase in shelf life of activated milk, with delayed activation of the LPS (Figure 2), which can be attributed to the fact that the milk had developed a heavier microbial load with delay in activation. Therefore, the LPS effectiveness also depends on the initial microbial quality of milk. The LPS cannot, therefore, be used to disguise the quality of poor milk but can only improve on the keeping quality of milk, depending on its initial quality.

At the time when the titratable acidity of treated milk samples under ambient temperature started increasing (Figure 3), spoilage had already been detected in control milk samples, which had higher acidity levels. This confirms that an increase in acidity of milk is an indication of spoilage [19]. The results also show that, LPS has an inhibitive property on lactic acid bacteria which cause spoilage in milk.

The J-shaped pattern of the acidity curves shows that bacterial activity in milk progresses in different stages. Only two of the four phases of spoilage were noticed in our experiments and the last two phases were not noticed because the experiments did not aim at studying beyond the spoilage level of milk. Milk degradation progressed through an initial (Bacteriostatic) phase where bacteria do not develop during this phase [19]. Next came the acidification phase, where there was fermentation of lactose to lactic acid, which persisted until the milk coagulated. Eventually, two other phases would have been noticed which would give an S-shaped degradation curve; neutralisation phase and putrefaction phase [19].

Comparisons were made between storage under ambient temperatures and under refrigeration (Figures 4 and 5). It was observed that control milk stored in the refrigerator for 4 days had a lower microbial load than the same milk stored under ambient temperatures for 8 hours.

Also, treated samples stored under refrigeration for three days had a lower microbial load than control samples stored in ambient temperatures for five hours. These results further confirm the efficiency of refrigeration and the LPS in milk preservation.

These results are in conformity with those obtained by other authors who also noticed bacterial inhibition by the LPS [20, 21, 22]. In one study, it was found that the LPS inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* [20]. The LPS was also found to inhibit *Streptococcus cremoris, Streptococcus agalactiae, Streptococcus pyogenes, Campylobacter jejuni,* and *Campylobacter coli* [21]. The

LPS greatly increased the keeping quality at 37° C, of milk, which was inoculated with *Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus thermophilus* and pasteurised at 72° C [22].

More farmers were informed and got motivated to supply milk for demonstrations on the LPS over time.

With such observations, it was thought that the use of the LPS can stimulate an increase in milk production, particularly because farmers were now sure of a better marketing channel and collection system for milk. Usually, far-off farmers will milk fewer cows for small quantities of milk required for household consumption, unless they have market outlets for their milk. This agrees with the speculations that an organised system for milk collection involving a large number of farmers will promote diary development [23].

CONCLUSIONS AND RECOMMENDATIONS

The LPS is effective in extending the shelf life of raw milk under tropical conditions. Treatment can enable farmers sell milk in far-off markets. Also, treated evening milk could remain in good condition for the next day's use without refrigeration. Enabling storage of milk allows the farmer to increase milk quantity by milking twice a day instead of once while not changing the transport frequency.

In addition, demonstrations on the LPS created a social gathering for dairy farmers and a forum for discussion on common interests between them. For example farmers saw the need and discussed on the creation of a dairy farming groups, which would lead to better-organized dairy activities. Farmer participation in the demonstrations increased with time, showing a positive response. Almost all of the milk suppliers were women, indicating that a continuous use of the LPS could promote income generation by the rural woman.

Dosage of LPS in the field is easy, as FAO provides small-prepared quantities of the activator. Therefore, only little training will be required for the application of this technique under such conditions.

Though the LPS is effective in prolonging the shelf life of raw milk under Cameroonian conditions, its application alone will not be enough to develop the dairy sector. Adequate management schemes at the level of production, processing and marketing should be applied alongside the lactoperoxidase system for a better dairy development in rural areas.

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Table 1: Shelf life of treated and control milk under different storage conditions (n=10)

	Ambient temperature (22 - 25°C)		Water bath (20°C)		Refrigeration (6 - 8°C)	
	Control	**LPS**	**Control**	**LPS**	**Control**	**LPS**
Minimum shelf life (hrs)	9	13	9	13	42	72
Maximum shelf life (hrs)	16	24	17	26	60	168
Average shelf life (hrs)	12.2 ± 2.7^a	19.3 ± 4.2^b	13.8 ± 2.7^a	21.8 ± 4.8^b	54.6 ± 9.1^a	100.8 ± 26.7^b
Extension in shelf life (hrs)	**7.1 ± 2.4**		**8.1 ± 3.0**		**46.2 ± 21.2**	

a, b = Figures bearing different letters for each storage condition are statistically different ($P < 0.01$).

Table 2: Number of milk suppliers and milk supplied at 10 successive demonstrations

	Demonstration number									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Men	2	0	0	2	0	1	2	1	2	1
Women	16	17	18	16	19	19	21	23	26	30
Total suppliers	18	17	18	18	19	20	23	24	28	31
Total milk supplied (litres)	56	54.5	60	63	61.5	65	81	88	103	135
Average supply per farmer (litres)	3.11	3.21	3.33	3.50	3.24	3.25	3.52	3.67	3.68	4.35

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