The Effect of Activation of Lactoperoxidase System (LPS) on the Quality and ShelfLife of In-pouch Pasteurised Milk

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Abstract

A study was conducted to investigate the quality of pasteurised milk made from Lactoperoxidase system (LPS) activated milk. Milk LPS was activated by addition of amounts of thiocyanate (SCN) and hydrogen peroxide (H_2O_2) as recommended by the Codex Alimentarius. After LPS activation, the milk was held at ambient temperature (26-27°C) for 0, 3, 6, 9, and 12 h before in-plastic pouch pasteurisation was done at 80°C for 1 min, followed by cooling to 10°C. This milk was then stored under refrigerator (5°C), Air-conditioned room (14°C), charcoal cooler cabinet (19-20°C) and under ambient temperature (26-27°C). The shelf life and quality of the pasteurized milk was monitored and assessed on the basis of Total Plate Count (TPC), Coliform Count (CC), pH and Clot on Boiling (COB) tests. The LPS activation holding time before pasteurization that gave the highest shelf life was 3 h followed by 6 h. Post LPS treatment time above 6 h at ambient temperature (26-27° C) produced milk of more than 40,000 cfu immediately after pasteurisation, resulting into relatively shorter subsequent shelf life under all test storage conditions. Under all storage conditions throughout the experiment, LPS treated pasteurised milk performed significantly ($P < 0.001$) better than the control. It was therefore concluded that LPS treatment was effective in enhancing the shelf life of pasteurised milk if only the milk was pasteurised within 6 h of treatment before storage.

Key words: LPS activation, in-pouch milk pasteurization, shelf life.

Introduction

In developing countries, rural based small-scale dairy producers do not have secured milk marketing outlets due to the absence of the government marketing infrastructures. The situation becomes worse during the rainy season when roads become impassable and when cooling systems break down owing to electricity failures. These circumstances together with high milk perishability, force farmers to accept lower milk prices. These constraints result into two situations in the dairy industry: the presence of surplus milk in the remote rural areas and a deficit of the product in the urban centers where consumers have to pay high price for milk (Kurwijila, 1987). In order to achieve sustainable local dairy development it is therefore imperative that all efforts committed to increasing milk production are accompanied with sound marketing strategies.

There is a need for an appropriate technology which could increase raw milk shelf life at ambient temperature (Kurwijila, 1990). Among the chemical methods of milk preservation, activated LPS is advocated as appropriate for adoption in developing countries (Claesson 1999ab), which has been approved by Codex Alimentarius commission and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). This method is particularly useful for temporary preservation of milk in situations where refrigeration is impractical and/or uneco-

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nomical (FAO. 1999). The activation of LPS has a bacteriostatic effect on raw milk that may last for up 26 hours. depending on the storage temperature (IDF. 1988. Nicholette *et al.*. 1999). This effect extends raw milk shelf life. enabling the milk to be transported over longer distances without refrigeration. Moreover LPS activated milk produces pasteurised milk of better bacteriological and storage quality. due to enhanced thermal destruction of milk spoilage bacteria (Kamau *et al.*, 1990. 1991). The shelf life of pasteurised milk is usually limited by heat resistant bacteria that survive pasteurisation and contaminants during packaging. Plastic-Pouch pasteurisation eliminates the risks of re-contaminants and thus produces a product with a longer shelf life (Dugdill. 1999). The purpose of this study was to investigate the combined effect of activated LPS and in- pouch pasteurisation on the quality and shelf life of fresh milk.

Materials and methods

Milk samples (20 litres were collected from the Sokoine University of Agriculture. Magadu dairy research farm. during morning milking. The samples were divided into two sub-samples of 10 litres each. One sub-sample was subjected to LPS activation and the other was kept as the control sub sample.

Activation of lactoperoxidase system

The activation of LPS in the milk was achieved through addition of Sodium thiocynate (NaSCN) and Sodium percarbonate $NaCO_3$ x3/2 H_2O_2 (Alfa Laval Agri®. Sweden) to give an initial concentration of about 15ppm SCN- and 8.5ppm of H2O2 respectively. In order to achieve the above concentration. 14 mg of NaSCN was added per litre of milk. The milk was then thoroughly mixed to ensure even distribution of SCN. This was followed by addition of 30 mg of Na_2CO_3 x3/2 H_2O_2 per litre of milk. The milk was then stirred for about 3 min to ensure complete dissolution of the Sodium percarbonate and an even distribution of H_2O_2. The milk was then stored at room temperature (RT) (26-270C) in a dark. well-ventilated room.

Pasteurisation

Duplicate milk samples of ½ litre each were packed in opaque plastic sachets and sealed on an electric sealer (Pronto® Audion Electron. Holland). The plastic sachets were pasteurised in a thermostatically controlled water bath at 80 °C for 1 min followed by cooling to 10 °C in ice water bath. One sachet was used to monitor the temperature rise using a mercury bulb thermometer. The post LPS holding time to pasteurisation was 0. 3. 6. 9 and 12 hours at ambient temperature. After pasteurization. the test milk samples were stored at RT (27 °C). charcoal cooler (19 °C). air-conditioned room (14 °C) or under refrigeration (5 °C).

Milk shelf life

The milk shelf life quality was assessed after 0 h, 6 h. 12 h and every 24 h thereafter. Milk without LPS treatment acted as a control and was subjected to similar pasteurisation intervals. Milk quality was monitored through assessing the titrable acidity (TA). clot-on-boiling (COB). pH. alcohol test. coliform count. and total plate count (TPC).

Clot on boiling (COB)

This was done using a small amount of milk aseptically drawn from the test samples. The milk drawn was boiled under flame in a test-tube. Milk failing the test was taken to have reached an advanced stage of souring (pH 5.8) (TZS120. 1981: DTI and FAO. 1997).

Titratable acidity

Nine ml of milk drawn aseptically from the samples was titrated against 0.1N NaOH solution. using 3-4 drops of 1% alcohol phenolphthalein indicator to monitor lactic acid development. The milk sample together with the indicator in the conical flask was titrated against NaOH under continuous mixing until a faint pink colour appeared (Richardson. 1985. IDF. 1990).

Milk pH

The shelf life of the test milk from different storage conditions was monitored by observing the change in pH by use of a pH meter (Hanna instruments®. HI 8519). This was done in the laboratory daily (every 24 h) until spoilage was confirmed by COB test.

Alcohol test

Initially 2 ml of milk was mixed with equal volume of 68% alcohol in a graduated test tube. The absence of flocculation after addition of another 2 ml of alcohol (i.e. double the volume of milk) was taken to indicate that the milk had acceptable low developed acidity (TZS120. 1981). Good quality milk formed no coagulation, clotting or precipitation.

Coliform count

Milk serial dilutions (10^{-1}. 10^{-2}, and 10^{-3}) in peptone water were transferred into sterile plates, followed by addition of 10 ml of Violet Red Bile agar (VRB) at 45° C, which was thoroughly mixed by tilting and rotating each dish. After solidification (5-10 min), an overlay of about 3-4 ml of VRB was made to cover the surface of the already solidified medium. The plates were inverted and incubated at 32° C for 48 hours. Identification of the coliforms was made from the positive plates with distinct dark red colonies. The total number of coliforms was determined by multiplying the average number of colonies by an appropriate dilution factor (TZS119, 1981; Richardson. 1985).

Total plate counts (TPC)

Total plate count were done according to standard procedure (TZS120. 1981; Richardson 1985.) using standard plate count Agar (DIFCO Laboratories. USA).

Data analysis

The data was analysed using SAS computer software (1990). Both descriptive statistics and general linear model was used to analyse the data. The statistical model used to analyse the data was: -

$Y_{ijkl} = \mu + T_i + H_j + S_k + S_k + e_{ijkl}$

Where as: Y_{ijkl} = Observation from l^{th} sample of milk under i^{th} treatment at k^{th} storage tem perature pasteurised after j^{th} time.

μ = General mean

T_i= The effect of i^{th} LPS treatment

H_j= The effect if j^{th} time taken to pasteurisation

S_k= The effect of k^{th} storage temperature

e_{ijkl} =Random error/residual effect specific to each observation

Table 1: The effect of LPS activation at indicated holding time at room temperature (26° - 27°C) before and after pasteurisation on the test milk initial total plate count (TPC x 10^3)

Treatment	Before Pasteurisation	After Pasteurisation	Pasteurised Milk Upper limit (TBS)
R_0	900[a]	4.67[a]	40
LP_0	800[a]	4.33[a]	40
LP_3	800[a]	12.33[a]	40
LP_6	8333.33[b]	27.33[a]	40
LP_9	22666.67[c]	90[b]	40
LP_{12}	54666.67[d]	290[c]	40

Means within the same column with same superscript are not significantly different (P>0.05)

Key

LP_0: Milk pasteurized immediately after LPS activation

LP^3 Milk Pasteurized 3 hours after LPS activation

LP^6: Milk pasteurize 6 hour after LPS activation

LP^9: Milk pasteurize 9 hours LPS activation

LP^{12}: Milk pasteurized 12 hours after LPS activation

R^0: Raw milk pasteurized immediately without LPS activation

Results

Initial raw milk quality

The bacteriological and chemical characteristics of the milk used was of good quality with a TPC value of 800,000 cfu/ml coliform count of 1900 cfu/ml and pH 6.7 (TBS. 1996). The TPC and pH values of the milk. before and after pasteurisation are shown in Table 1 and in Figure 1 and 2. Table 1 shows the bacteriological quality of the control sample (R0) and the LPS activated samples stored for different lengths of time at ambient temperature before and after pasteurisation.

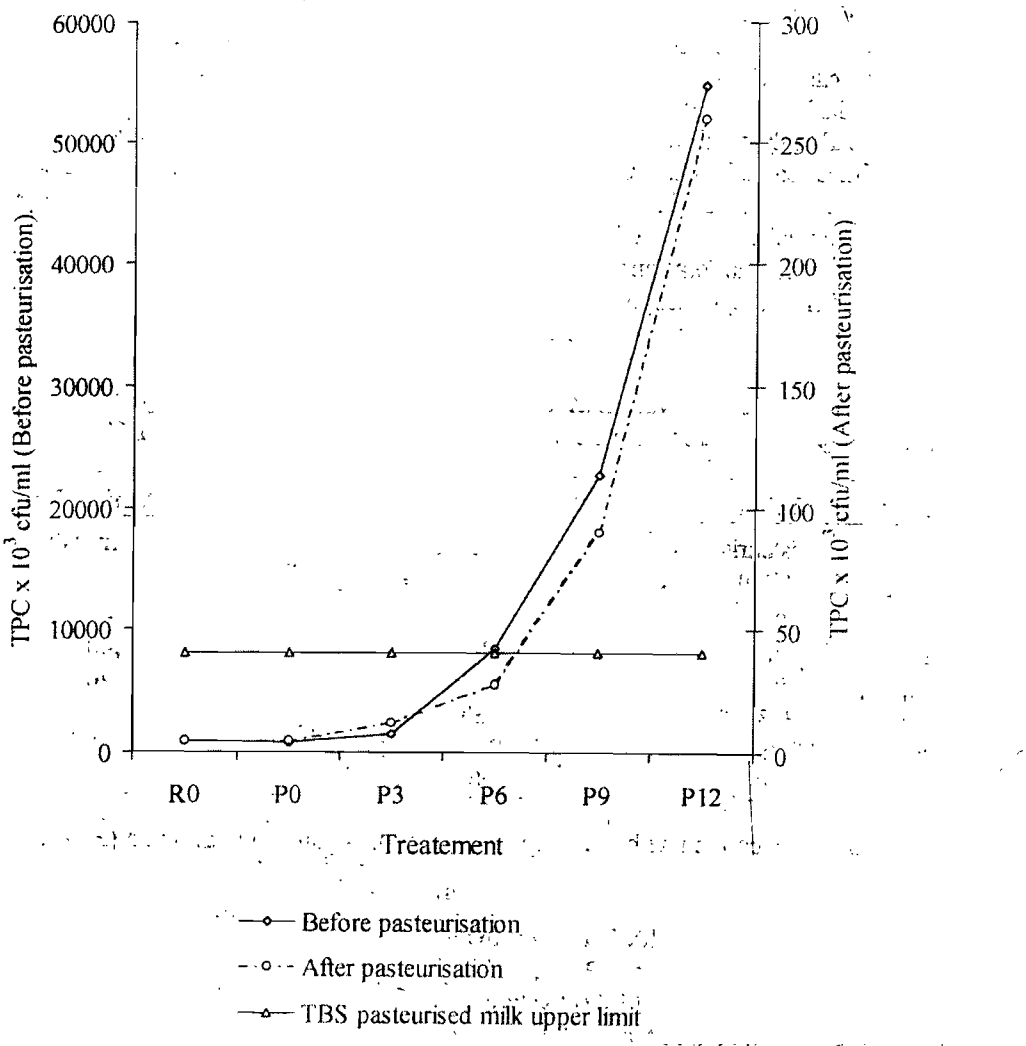

Figure 1: The effect of LPS activation at indicated holding time (h) before and after pasteurisation on the test milk initial total plate count.

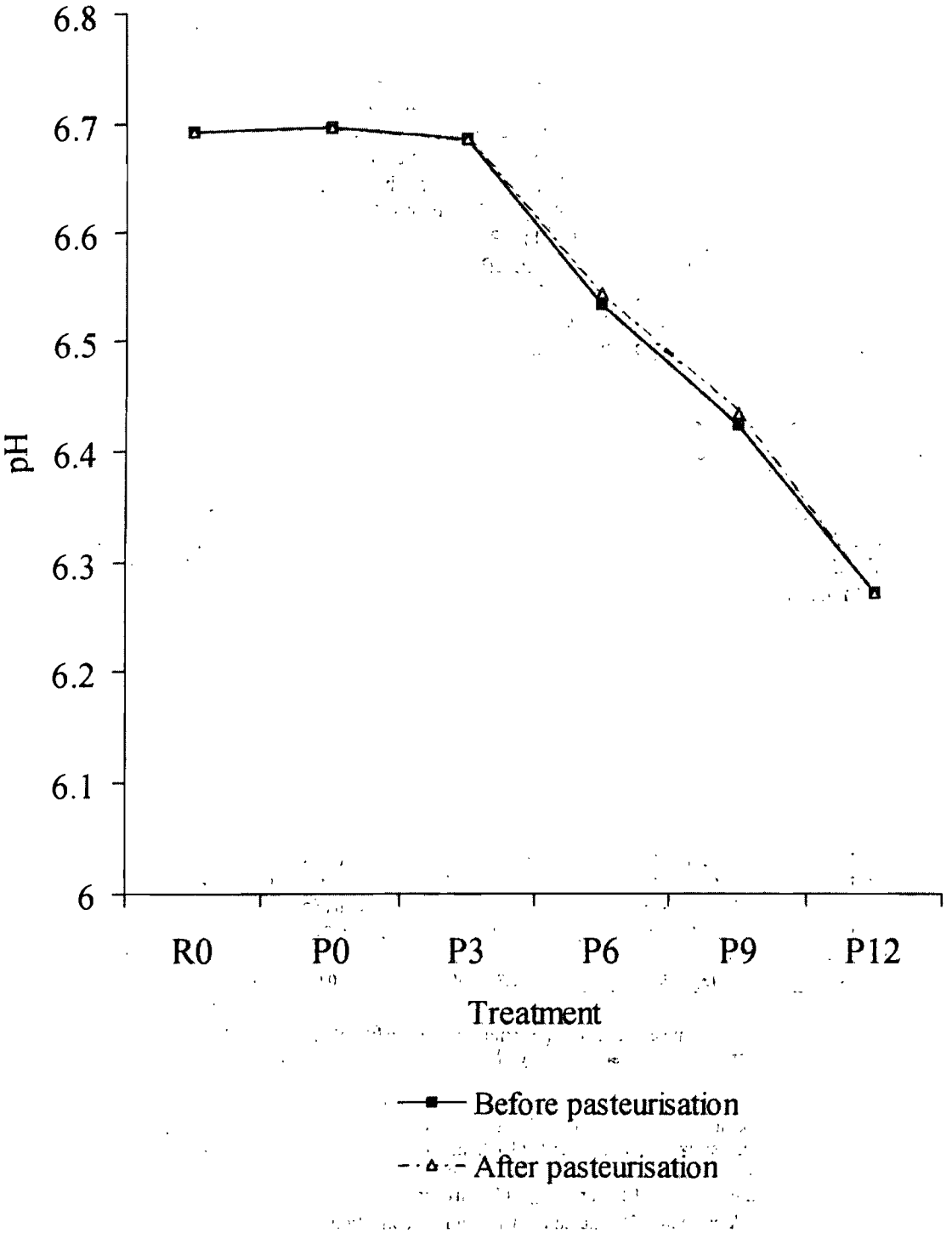

Figure 2: The effect of LPS activation at indicated holding time before and after pasteurisation the test milk pH

There was no significant (P>0.05) difference in the initial TPC between the untreated raw milk (R_0) and the LPS activated milk held for up to 3 h at RT (i.e. LP_0 and LP_3). However, the TPC values of the LPS treated raw milk held for 6 h and above became significantly (P<0.001) higher than those of the control sample (R_0).

After pasteurisation, samples LP_0, LP_3 and LP_6 showed no significant (P>0.05) difference in the initial TPC with the control (R_0). However, (LP-treated milk pasteurised after 9 h (LP_9) and 12 h (LP_{12}) had significantly higher (P<0.001) TPC values than the control and the LP_0, LP_3 and LP_6 samples. It is noteworthy that after 6 h holding period following LP-treatment at RT, the LP_6 treated pasteurised milk sample retained a pH value of 6.6.

The quality of 24 h-old LPS activated pasteurised milk held at different temperatures

TPC values of pasteurised milk stored under different storage conditions for 24 h are presented in Table 2. As expected, refrigerated storage gave milk with the lowest total plate count under each treatment, while the highest TPC was always recorded under the RT storage. Total plate count ranged from $46.0x10^3$ which was the lowest, registered by treatment LP_3 under refrigeration to $25.0x10^6$ (cfu/ml), which was the highest TPC obtained from LP_{12} under RT storage. Refrigeration provided the best storage environment by recording significantly (P<0.001) low TPC values under each treatment. Next to refrigeration was the air-conditioned room, which was significantly (P<0.001) better in TPC than the charcoal cooler box. From the results, LPS effect could not be manifested easily on the quality of pasteurised milk under RT storage.

Within storage condition, results show that a significantly (P<0.001) higher TPC value was always registered under treatment LP_{12} followed by treatment LP_9. Next were treatments R_0 and LP_0 with no significant (P>0.05) difference between them but with significantly (P<0.001) higher TPC than treatment LP_6. The lowest TPC value was registered under LP_3. Further evidence on the superiority of the LP_3 treatment can be visualised in Fig. 3 to 6 depicting pH values.

Table 2: Total plate count (TPC) of LPS activated in -pouch pasteurised (80°C/min) milk after 24 h exposure under various storage conditions (x 10^6 cfu/ml)

[1] Treatment	Storage conditions			
	Charcoal cooler box (18 -20°C)	Air conditioned room (14-15°C)	Refrigerat or (5-8°C)	Room temp (26-27°C)
LP_0	11.0 ± 90^c	6.5 ± 90^b	0.078 ± 90^a	16.4 ± 90^d
LP_3	8.6 ± 90^c	4.1 ± 90^b	0.046 ± 90^a	16.0 ± 90^d
LP_6	9.8 ± 90^c	5.2 ± 90^b	0.062 ± 90^a	17.0 ± 90^d
LP_9	15.0 ± 90^c	12.9 ± 90^b	0.210 ± 90^a	20.0 ± 90^d
LP_{12}	18.2 ± 90^c	14.8 ± 90^b	0.350 ± 90^a	25.0 ± 90^d
R_0	13.8 ± 90^c	6.3 ± 90^b	0.08 ± 90^a	18.2 ± 90^d

Means within the same column (for the same storage condition) and row (LPS treatment) means with the same superscript are not significantly different (P>0.05).

Key

[1]LP_0: Milk pasteurized immediately after LPS activation
LP_3: Milk pasteurized 3 hours after LPS activation
LP_6: Milk pasteurized 6 hours after LPS activation
LP_9: Milk pasteurized 9 hours after LPS activation
LP_{12}: Milk pasteurized 12 hours after LPS activation
R_0: Raw milk pasteurized immediately without LPS activation

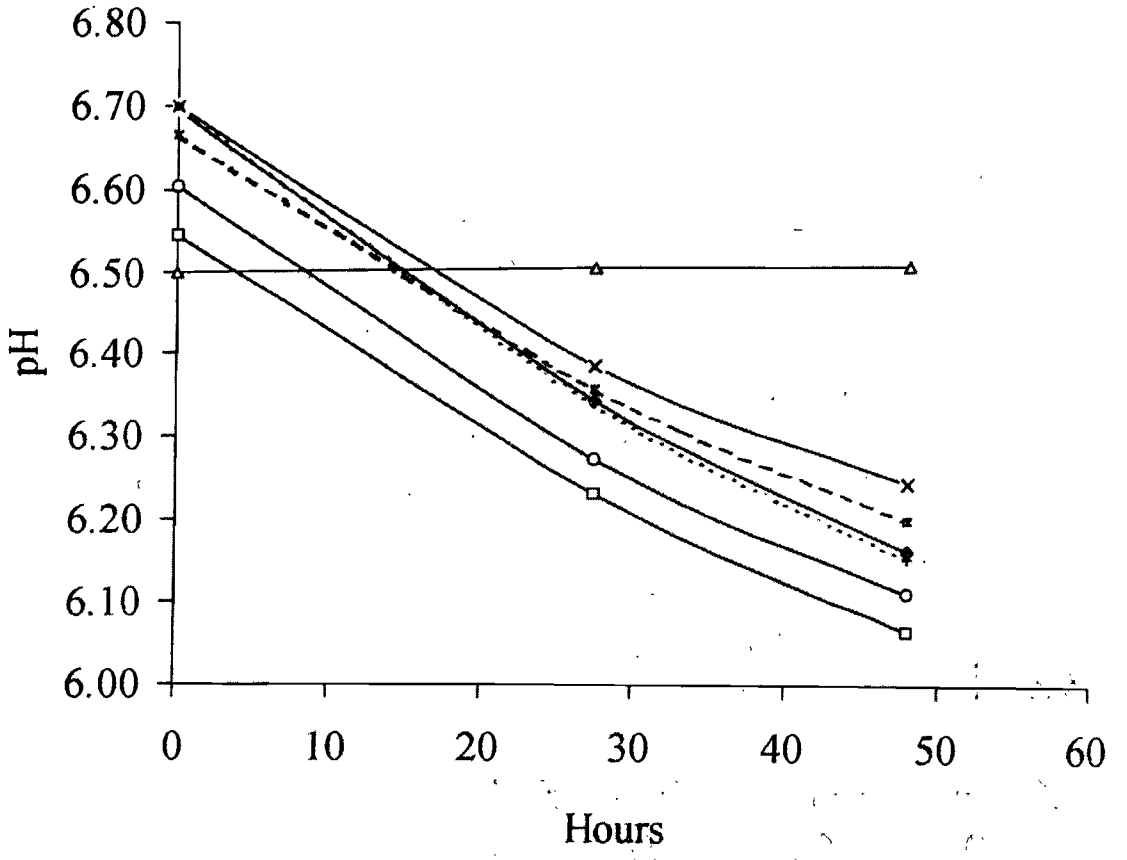

Figure 3: The effect of activated LPS on the pH of inplastic pouch pasteurised (80^0C/1minute)milk stored at room temperature (26^0 - 27^0C)

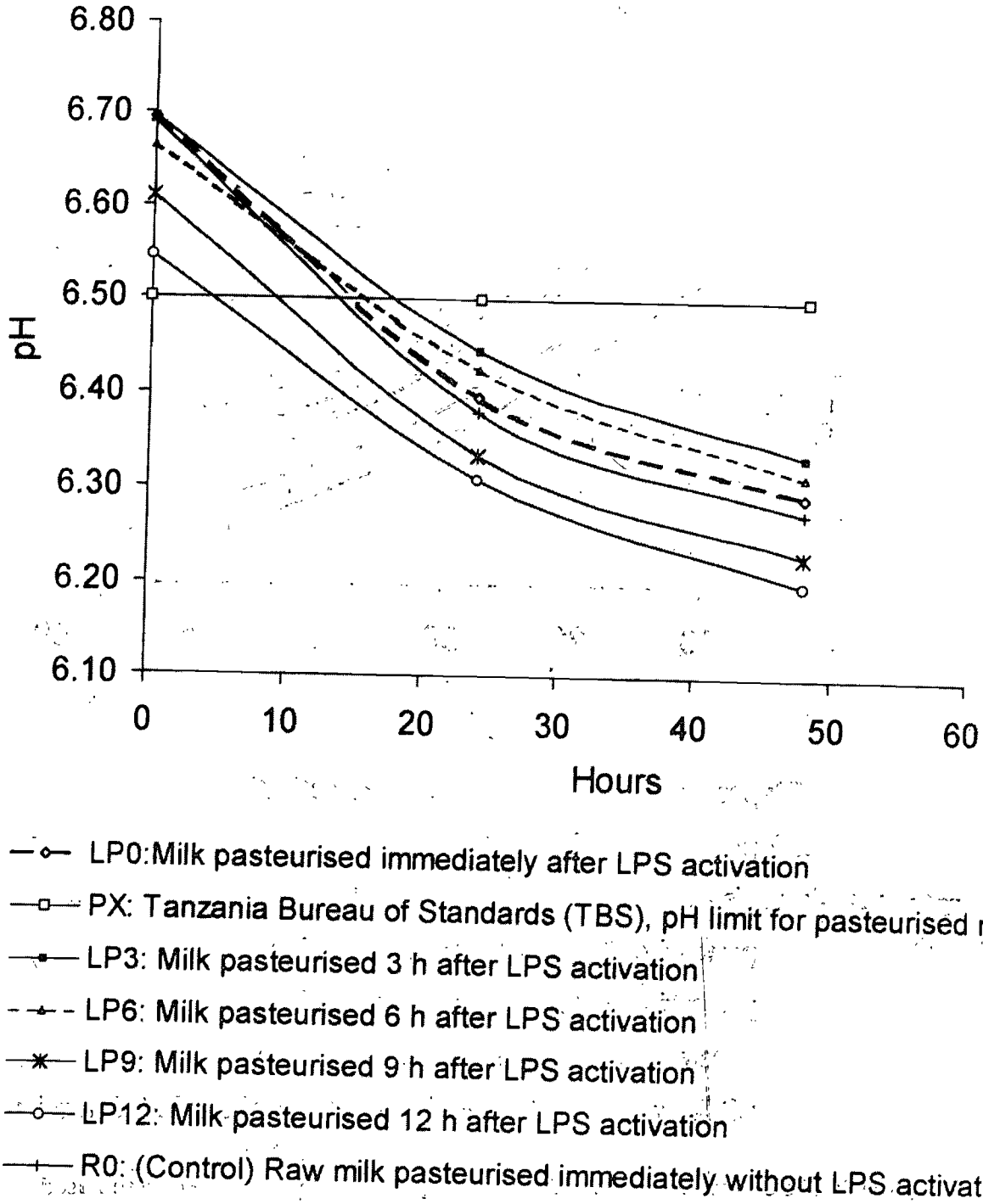

Figure 4: The effect of activated LPS on the pH of inplastic pouch pasteurised (80^{0}C/min.) milk stored in a Charcoal cooler cabinet (19^0 - 20^{0}C)

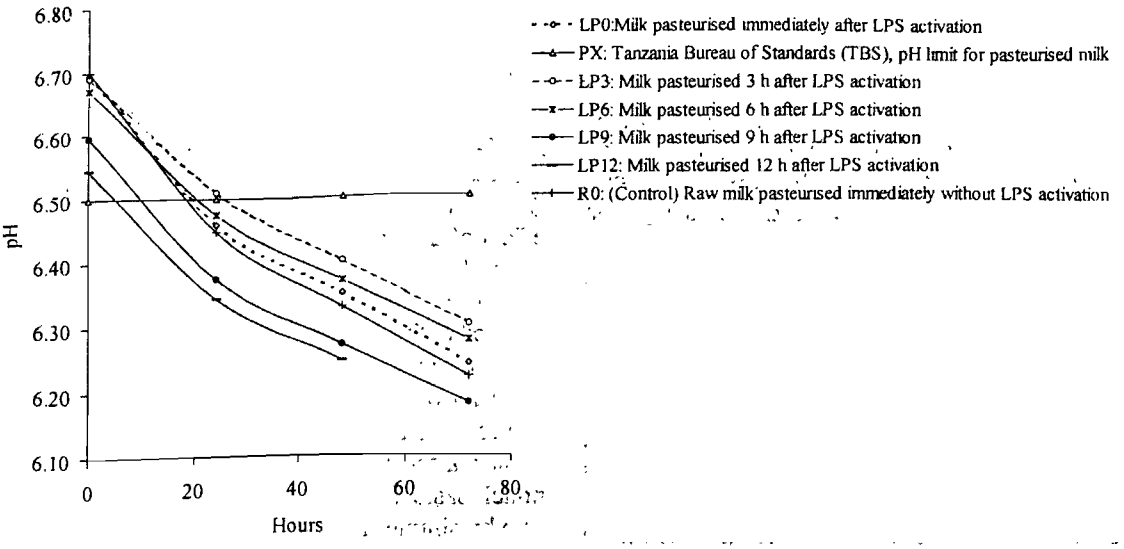

Figure 5: The effect of activated LPS on the pH of pasteurised (80^{0}C/1min) milk stored in an air conditioned room (14^0 - 15^{0}C)

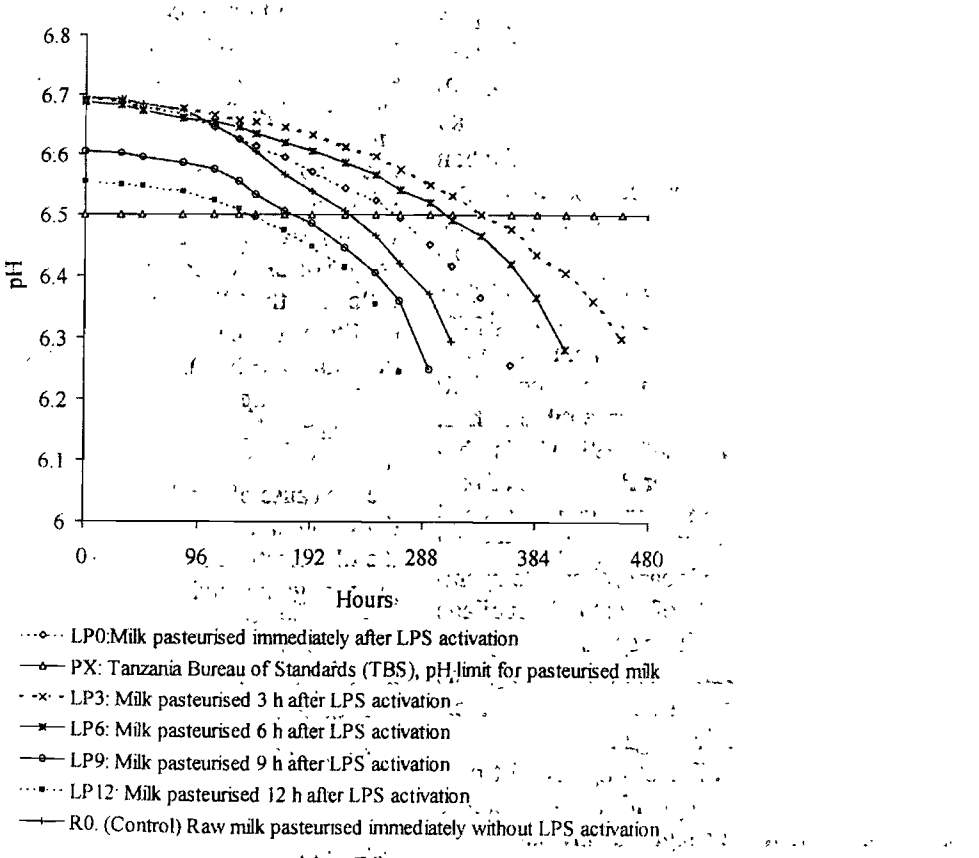

Figure 6: The effect of activated LPS on the pH of in-pouch pasteurised (80^{0}C/min) milk stored under refrigeration (5^{0}C -8^{0}C)

Discussion

Milk shelf life

According to the TBS standards, freshly pasteurized milk should have a TPC of less than 40.000 cfu/ml and pH 6.6. Under tropical conditions the shelf life of pasteurised milk is expected to be 24 h. at RT or seven days under refrigeration. From the Tanzanian consumers' point of view, pasteurized fresh milk should have a sweet taste and be able to withstand re-heating in the kitchen.

This implies that the milk should have pH above 6.4 or acidity of <0.22% lactic acid (O'Connor, 1995). On the basis of these criteria, under RT storage, after 24 h, only LP_3 samples were still of acceptable quality. While under charcoal cooler cabinet (19-20°C) LP_3 and LP_6 were still of acceptable quality after 24 h. Under air conditioned environment (14-15°C). R_0, LP_0, LP_3 and LP_6 were still of acceptable quality at 24 h while LP_9 and LP_{12} were not. Under refrigeration storage (5-7°C), the longest shelf life (400 h≈ 16.5 days (d)) were obtained with the milk under LP_3 treatment followed by LP_6 (370 h ≈ 14.4 d), LP_0 (320 h≈ 13.3d) and the control sample (R_0) (280 h≈ 11.6d). These results show that LPS activation enhances the shelf life of pasteurized milk especially under refrigeration storage since normally pasteurised milk shelf life is less than 24 h at RT and is given a "sale by date" of 7 d under refrigeration. The prolonged shelf life of LP treated pasteurised milk is explained by the fact that certain bacteria become weakened by the effect of LP treatment making them more susceptible to heat treatment (Kamau *et al.*; 1990, 1991) This phenomenon is less evident under RT storage of pasteurised milk where the little time gain of several hours in shelf life of the milk is not of commercial significance. The effect of near optimum temperature conditions for growth of most milk spoilage bacteria including thermoduric spore forming bacteria that survive pasteurisation, far outweigh the beneficial effect of the LP treatment on the thermal destruction of the microorganisms.

Throughout the studied storage conditions, milk treated with LPS and held for 0-6 h before pasteurization performed significantly better than the untreated pasteurised milk. LPS treated milk held for 9-12 h was generally of poorer quality than the control. This shows that the time limit of LPS holding time before pasteurization is between 3 h and 6 h because its influence on shelf life of pasteurised milk diminishes tremendously after LPS lag time of 6 h if not pasteurised. It is recommended, therefore, that in the tropics the proper LPS post-activation resident time before processing should be 3 - 6 h but should not reach 9 h at RT because the bacteriostatic/bactericidal effect of activated LPS declines tremendously after 6 h.

The biggest limitation in preservation of pasteurised milk during marketing is the inadequacy of cold chain. As expected, refrigeration storage maintained the longest shelf life of all other storage conditions. It was the objective of this work to show that a combination of LP treatment, in pouch pasteurisation and lowering of temperature by simpler means (such as use of charcoal cooler cabinet or keeping milk under a low ambient temperature environments, such as might be encountered in highland areas, simulated by an air- conditioned environment) can extend the shelf life of milk to permit the marketing of pasteurized milk over several days without refrigeration. However, the results obtained show that storage temperatures above 10 °C are not sufficiently low to prevent multiplication of the thermoduric bacteria, which had survived in-pouch pasteurization (with or without LP activation). However, it is noteworthy that pasteurization of LP treated milk, when done within 6 h following the LP treatment doubles the keeping quality of the pasteurised milk under refrigeration storage (+5 °C).

It would appear that LPS has the greatest impact on psychrotrophic bacteria and certain heat resistant spore forming bacteria, which normally are the cause of spoilage of pasteurised milk under refrigeration storage. It has been shown that the LPS treatment has bactericidal effect against gram-negative and bacteriostatic effect against gram-positive bacteria (Björck *et al.*: 1978; Claesson 1995; Korhonen, 1999). Since most psychrotrophic bacteria are easily destroyed under standard pasteurization and usually regain entry into pasteurized milk during packaging, they are not expected to contribute considerably to the spoilage of refrigerated, in-pouch pasteurized milk.

It is important to mention here that the pasteurisation temperature (80°C/1 min.) used in this study, completely destroys the lactoperoxidase

enzyme in the milk which is a pre-requisite for pasteurised milk quality test. It is now a requirement by European union that pasteurized milk should show a positive activity under peroxidase test to monitor and limit over processing (Nicholette *et al.*, 2001, Barrett, 1998). However Standard pasteurization temperatures (63 °C for 30 min or 72 °C for 15 s) do not destroy the lactoperoxidase enzyme in milk despite shelf life extension of such pasteurized milk (Barrett, 1998). From this fac,t it would be of practical interest to test whether or not in-pouch pasteurisation temperatures lower than 80° C for LPS activated milk would result in a product of any better keeping quality than has been obtained in the present study.

Conclusion

Results obtained support and demonstrate the suitability of the activated LPS on pasteurized milk keeping quality by recording substantial hygienic quality and shelf life improvement on the treated milk stored under refrigeration. No physical, chemical or organoleptic abnormalities occurred on LP treated milk. LP-treated milk stored/transported under high temperatures 26-27 °C should be pasteurized within 6 h of LP treatment and stored under refrigeration for the best results on shelf life improvement.

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