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Storage Stability of Mixed Raw Milk by the Activation of Lactoperoxidase System at Different Temperature

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Abstract

Quality of raw milk decreases with storage time. Different methods are available for the preservation of raw milk. Among them activation of Lactoperoxidase system (LP-s) is safe and recommended by FAO/WHO. As the dairy industry/milk collection center receive milk of both cow and buffalo so the objective of this study were to know the storage stability of mixed milk by the activation of LP-s at different temperature conditions. Cow and buffalo milk were collected separately and mixed by volume in the ratio of 90:10, 80:20, 70:30 and 60:40 respectively. All the ratios of mixed milk were divided into two portions one as control sample and other as LP-s activated sample. LP-s was activated by the addition of KSCN (14 mg/L) and sodium percarbonate (30 mg/L). Both the control and LP-s activated samples were kept at 35 ± 2°C, refrigeration (5 ± 2°C) and room temperature (25 ± 2°C). Physicochemical analysis (acidity and clot on boiling (COB) test) were done to compare the keeping quality between control and activated samples at these temperature conditions.

At 35 ± 2°C acidity 0.20% and COB +ve test was shown by activated sample of all ratios on average after 6.5h which was already observed for control sample after 3.5h of storage. At 25 ± 2°C acidity 0.20% and COB +ve test was shown by activated sample of all ratio on average after 13 hours which was already observed for control sample after 7 hours of storage. At 5 ± 2°C acidity 0.20% and COB +ve test was shown by activated sample of all ratio on average after 132h which was already observed for control sample after 60 hours of storage.

Keywords: *Acidity; Clot on Boiling Lactoperoxidase; Keeping Quality; Lactoperoxidase System*

Abbreviations

ANOVA: Analysis of Variance; COB: Clot on Boiling; FAO: Food and Agriculture Organization; HSD: Honest Significant Difference; KQ: Keeping Quality; KSCN: Potassium Thiocyanate; LP: Lactoperoxidase; LP-s: Lactoperoxidase System; NDDB: National Dairy Development Board

Background

Milk and milk products have made very significant contributions to human nutrition ever since the earliest civilizations, although there are wide variations in the traditional role of milk in the diet of people in different subcontinents. Because of its constituents, milk could greatly improve people's health in developing countries where a large proportion of the population, especially pregnant and nursing women and children, suffer from severe malnutrition and where people survive on high starch, low protein foods [1].

Dairying provides one of the most cost-effective methods of converting crude animal feed resources into high-quality protein-rich food for human consumption. However, since milk is a very perishable foodstuff special measures and considerations are necessary to ensure that it reaches the market in an acceptable condition. The collection of milk from the farmers and transportation to the dairy is the most critical link in the total handling chain of milk. This problem is recognized worldwide [2].

The most commonly used method to stop or retard the deterioration of milk on its way from the farmer to the dairy is cooling. However, in many parts of the world, this is not possible for various reasons, such as lack of available capital, lack of electricity, less developed road systems, high operational costs, frequent break downs of equipment, lack of spare parts and difficulties in repair of equipment in rural areas. Prevailing high ambient temperature often further compound the problem of milk collection in these areas. This causes a considerable loss of fresh milk, and in many regions only a minor part of the production reaches the dairy industry in an acceptable condition for use as human food. In many regions most of the evening milk is spoiled after storage over-night [3].

To solve this problem, an alternative method to increase the storage stability of milk at high ambient temperatures has been developed. The method makes use of a naturally occurring antibacterial system in milk known as the lactoperoxidase system, which is activated by increasing the concentrations of two components or activators (thiocyanate and hydrogen peroxide) reacting with each other. This reaction is catalysed by the enzyme lactoperoxidase which is naturally present in milk and leads to the formation of antibacterial compounds [4].

Purpose

Since milk is a very perishable foodstuff special measures and considerations are necessary to ensure that it reaches the market in an acceptable condition. The collection of milk from the farmers and transportation to the dairy is the most critical link in the total handling chain of milk. This problem is recognized worldwide [2]. The raw milk quality preservation during transportation is an exceedingly important factor for milk products because some dairy cattle farms are in areas remote from the Milk Collection Centers, especially for those farming small holdings whereby there is a lengthy duration for transportation and mechanical refrigeration is either unavailable or economically prohibitive. Without refrigeration, milk products can only be transported to a very short distance, after which they begin to deteriorate, beginning the process of acidification [4].

The dispersed nature of the production across diverse farm operations, difficulties with collection, poor handling systems and inadequate transportation and refrigeration systems all create considerable challenge to extended raw dairy cow's milk quality during storage in several developing countries [5]. In Nepal, most of the milk producers are farmers of rural areas. In these areas, refrigeration is not economically feasible. On transporting the collected milk up to processing plant, it gets spoiled. This causes a considerable loss of fresh milk, and in many regions only a minor part of the production reaches the dairy industry in an acceptable condition for use as human food. In many regions most of the evening milk is spoiled after storage over-night [3].

Significance of the work

Post-harvest losses are a major issue in dairying in developing countries. Smallholder dairy farmers could increase their participation in worldwide milk production, processing and marketing if they could reduce their losses using any approved milk preservation method. Refrigeration is the preferred means of milk preservation but does require high capital investment and can incur high running and maintenance costs The lactoperoxidase system (LP-s) provides a cost effective method to increase the availability of milk that contributes to the income generation, household security and nutrition in developing countries [4].

Milk collection pattern observed at different dairies in Eastern region of Nepal (Kamdhenu Dairy, NMC Dairy, Dharan Dairy) and DDC had shown a mixed milk (cow and buffalo) collection pattern. Effectiveness of LP-s system on mixed milk of various proportions was therefore selected for the study.

The major benefit of the work is that it elongates the shelf-life of milk. This method is easy to apply and is very much cost effective. Moreover, it reduces the loss of milk caused by spoilage. In overall it increases the availability of milk in poor countries where malnutrition is a customary problem.

Materials

A total of 3 liters milk from cow and buffalo was collected separately every morning from Barahachhetra Municipality ward no.5. Cow (Local variety) and Buffalo (Murrah variety) were given same quality of fodder and water and no any medicines, antibiotics/hormone were used during the collection period to maintain the same quality of milk. Both cow and buffalo were disease free, healthy and at 3rd stage of lactation. After collection both cow and buffalo milk were kept at ice box and taken to the lab for the analysis. All chemicals, glass-ware and equipment required were used from the Central Department of Food Technology, Dharan and Nilgiri College, Itahari.

Methods

Sample preparation

Cow and buffalo milk were mixed as above to make a final volume of 3 liter as shown in the table 1. Each of these four different ratio mixed milk samples were again divided equally into two portion in which one was treated as control and LP-s was activated at other sample. Control and activated sample of different ratio mixed milk were kept at three different temperature conditions (35°C, room temperature (25 ± 2°C) and refrigeration temperature (5 ± 2°C) and physicochemical analysis such as acidity and clot on boiling (COB) test were done to compare the shelf life (Keeping Quality) between control and activated sample of prepared ratio at these three different temperature conditions.

Cow milk	Buffalo milk	Prepared ratio
2.7 liter	0.3 liter	90:10
2.4 liter	0.6 liter	80:20
2.1 liter	0.9 liter	70:30
1.8 liter	1.2 liter	60:40

Table 1: *Preparation of mixed milk of different ratio.*

Analysis of thiocyanate content

Thiocyanate content of milk was determined according to the method given by CAC [6].

Addition of thiocyanate and sodium percarbonate

After determination of thiocyanate content of cow and buffalo milk individually, the additional amount of thiocyanate (as potassium thiocyanate) was added to each ratio to make total content of 14 mg/L. The solution was thoroughly mixed and sodium percarbonate (30 mg/L) added in the form of sodium carbonate peroxyhydrate. The activation was done within 2h of milking as the method given by FAO [4].

Determination of keeping quality (KQ)

Acidity and Clot on boiling test were used to measure the keeping quality. The parameter shows a reliable and consistent estimate. The control milk sample and LP-s activated milk sample were stored at 3 different temperature conditions. At Day 1 physiochemical analysis of 90:10 ratio mixed milk was analyzed at 35 ± 2°C and refrigeration temperature (5 ± 2°C). At Day 2 the same ratio was prepared and physicochemical analysis was carried out at room temperature (25 ± 2°C). At Day 3 mixed milk of 80:20 was prepared and physicochemical analysis was carried out at 35 ± 2°C and refrigeration temperature. At Day 4 physicochemical analysis of same ratio was carried out at room temperature. Likewise the mixed milk of ratio 70:30 and 60:40 was also performed on the similar manner. Acidity and COB test was carried out every hour for sample kept at 35 ± 2°C while for room temperature it was done at every 2 hours and in case of refrigerated sample it was carried out daily until the value of acidity was greater than 0.20% (i.e. end of keeping quality) and COB tests showed positive (milk clot) result. Acidity and COB test were done according to the method given by Nepal Dairy Development Board [7].

Statistical method

All measurements were performed in triplicate. Data on physiochemical and microbiological analysis were tabulated for comparison and graphically represented using Microsoft® Excel-2010. The data were analysed by one-way ANOVA using SPSS (IBM Corporation, Malborough, MA, USA) [8]. In case of significant difference, Tukey's HSD post hoc test was used to separate means at 5% level of significance. All other calculations were performed in Microsoft Office Excel 2010.

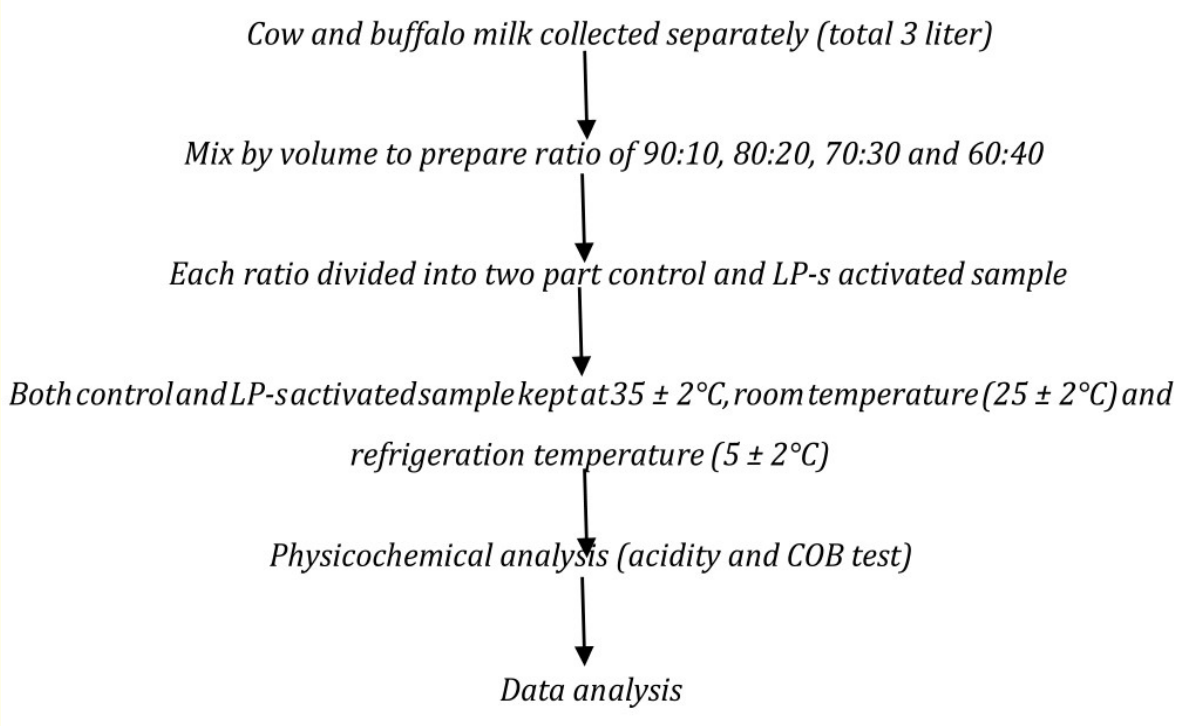

Figure 1: *Methodology of the overall experimental design.*

Result and Discussion

Thiocyanate content

Thiocyanate content of cow and buffalo milk was analyzed separately and the amount was calculated accordingly in mixed milk of various ratios shown in the figure 1. The thiocyanate content of mixed milk of various ratios were found to be significantly different ($p < 0.05$). The highest content was found in the raw milk of cow and buffalo mixed in the ratio of 90:10 followed by mixed milk of ratio 80:20 and 70:30 respectively. Cow and buffalo milk mixed in the proportion of 60:40 has lowest thiocyanate content and was significantly different from the milk of cow and buffalo mixed in the ratio of 90:10.

Levels between 1 and 15 ppm have been reported. Fresh cow milk contains 1 - 10 mg of thiocyanate per litre, which is not always sufficient to activate the LP system. The thiocyanate concentrations in human saliva and in human gastric juices have been reported to vary between 50 - 300 and 40 - 50 ppm, respectively. These values are much higher than the concentration (15 ppm) of SCN^- required for the activation of the LP system [9].

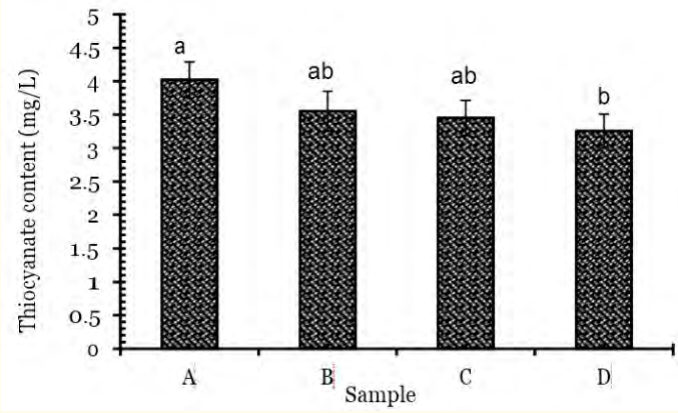

Figure 2: *Thiocyanate content of mixed milk of various proportions.*

Keeping quality of mixed raw milk at 35 ± 2°C

Value of acidity and COB test of control and activated sample of ratio 90:10, 80:20, 70:30 and 60:40 per hour stored at 35 ± 2°C are shown in table 2-5 respectively. LP-s activated sample was significantly different from control sample ($p < 0.05$).

Time (h)	Acidity % (as lactic acid)		COB test	
	Control	LP-s Activated	Control	LP-s Activated
0	$0.1395^{a} \pm 0.0045$	$0.1395^{a} \pm 0.0045$	-ve	-ve
1	$0.153^{a} \pm 0.0045$	$0.144^{a} \pm 0.0045$	-ve	-ve
2	$0.162^{a} \pm 0.0045$	$0.1485^{b} \pm 0.0045$	-ve	-ve
3	$0.189^{a} \pm 0.0045$	$0.156^{b} \pm 0.0026$	-ve	-ve
4	$0.1965^{a} \pm 0.0026$	$0.1665^{b} \pm 0.0045$	-ve	-ve
5	$0.2115^{a} \pm 0.0045$	$0.1725^{b} \pm 0.0069$	+ve	-ve
6	$0.234^{a} \pm 0.0045$	$0.183^{b} \pm 0.0026$	+ve	-ve
7	$0.252^{a} \pm 0.0045$	$0.1965^{b} \pm 0.0026$	+ve	-ve
8	$0.2745^{a} \pm 0.0045$	$0.216^{b} \pm 0.0045$	+ve	+ve

Table 2: *Acidity and COB test of control and activated sample of ratio 90:10 at 35 ± 2^{0}C.*

Values are the means of three determinations ± standard deviation and means with different superscript letters row wise are different ($p < 0.05$).

Time (h)	Acidity % (as lactic acid)		COB test	
	Control	LP-s Activated	Control	LP-s Activated
0	$0.1395^{a} \pm 0.0045$	$0.1395^{a} \pm 0.0045$	-ve	-ve
1	$0.1575^{a} \pm 0.0045$	$0.1485^{a} \pm 0.0045$	-ve	-ve
2	$0.1665^{a} \pm 0.0045$	$0.153^{b} \pm 0.0045$	-ve	-ve
3	$0.1935^{a} \pm 0.0045$	$0.1665^{b} \pm 0.0045$	-ve	-ve
4	$0.1995^{a} \pm 0.0026$	$0.171^{b} \pm 0.0045$	-ve	-ve
5	$0.216^{a} \pm 0.0045$	$0.1755^{b} \pm 0.0045$	+ve	-ve
6	$0.2385^{a} \pm 0.0045$	$0.183^{b} \pm 0.0026$	+ve	-ve
7	$0.2565^{a} \pm 0.0045$	$0.198^{b} \pm 0.0045$	+ve	-ve
8	$0.288^{a} \pm 0.0069$	$0.22^{b} \pm 0.0045$	+ve	+ve

Table 3: *Acidity and COB test of control and activated sample of ratio 80:20 at 35 ± 2^{0}C.*

Values are the means of three determinations ± standard deviation and means with different superscript letter row wise are different ($p < 0.05$).

Time (h)	Acidity % (as lactic acid)		COB test	
	Control	LP-s Activated	Control	LP-s Activated
0	$0.1395^{a} \pm 0.0045$	$0.1395^{a} \pm 0.0045$	-ve	-ve
1	$0.1635^{a} \pm 0.0045$	$0.153^{b} \pm 0.0045$	-ve	-ve
2	$0.1755^{a} \pm 0.0045$	$0.159^{b} \pm 0.0045$	-ve	-ve
3	$0.198^{a} \pm 0.0045$	$0.1755^{b} \pm 0.0045$	-ve	-ve
4	$0.216^{a} \pm 0.0026$	$0.1845^{b} \pm 0.0045$	+ve	-ve
5	$0.243^{a} \pm 0.0045$	$0.1905^{b} \pm 0.0045$	+ve	-ve
6	$0.265^{a} \pm 0.0045$	$0.198^{b} \pm 0.0026$	+ve	-ve
7	$0.2835^{a} \pm 0.0045$	$0.216^{b} \pm 0.0045$	+ve	+ve

Table 4: *Acidity and COB test of control and activated sample of ratio 70:30 at 35 ± 2^{0}C.*

Values are the means of three determinations ± standard deviation and means with different letters row wise are different ($p < 0.05$).

Time (h)	Acidity % (as lactic acid)		COB test	
	Control	LP-s Activated	Control	LP-s Activated
0	$0.1395^{a} \pm 0.0045$	$0.1395^{a} \pm 0.0045$	-ve	-ve
1	$0.165^{a} \pm 0.0045$	$0.153^{b} \pm 0.0045$	-ve	-ve
2	$0.177^{a} \pm 0.0045$	$0.162^{b} \pm 0.0045$	-ve	-ve
3	$0.198^{a} \pm 0.0045$	$0.18^{b} \pm 0.0045$	-ve	-ve
4	$0.225^{a} \pm 0.0026$	$0.186^{b} \pm 0.0045$	+ve	-ve
5	$0.2475^{a} \pm 0.0045$	$0.192^{b} \pm 0.0045$	+ve	-ve
6	$0.27^{a} \pm 0.0045$	$0.1995^{b} \pm 0.0026$	+ve	-ve
7	$0.288^{a} \pm 0.0045$	$0.234^{b} \pm 0.0045$	+ve	+ve

Table 5: *Acidity and COB test of control and activated sample of ratio 60:40 at 35 ± 2^{0}C.*

Values are the means of three determinations ± standard deviation and means with different superscript letters row wise are different ($p < 0.05$).

As the storage period increases there was increase in acidity in both the control and activated sample however increase in acidity was slower in activated sample and fast increment was observed in control sample. Acidity of control sample of ratio 90:10 and 80:20 reach a value of 0.20% after 4h of storage and showed COB positive test whereas it take 8h for activated sample (acidity > 0.20% and COB positive test regarded as end of shelf life). In case of control sample of ratio 70:30 and 60:40 it takes after 3h to reach the same level of acidity while 7h in case of activated sample. There was reduction in keeping quality by one hour for both control and activated sample of this ratio when compared with mixed milk of ratio 90:10 and 80:20.

An extension of 3 hours was observed in case of LP-s activated sample which is similar to the result suggested by Reiter and Harnulv [10]. Slightly higher findings were recorded by Bjorck., *et al.* [11] and Reiter., *et al* [12]. Clot-on-boiling test (COB) apparently indicates spoilage slightly later than the other tests used. This finding was in agreement with that recorded by Bjorck., *et al* [13].

Keeping quality among LP-s activated mixed milk at 35 ± 2°C

Change in value of acidity per hour in case of milk of LP-s activated sample of all four ratio are shown in table6. Value of acidity among mixed milk of various ratios were significantly different ($p < 0.05$). There was no significant difference up to the 1st hour among the activated sample however from 2nd hour significant difference was observed among the samples.

Time (h)	*Ratio 1	*Ratio 2	*Ratio 3	*Ratio 4
0	$0.1395^{a} \pm 0.0045$	$0.1395^{a} \pm 0.0045$	$0.1395^{a} \pm 0.0045$	$0.1395^{a} \pm 0.0045$
1	$0.144^{a} \pm 0.0045$	$0.1485^{a} \pm 0.0045$	$0.153^{a} \pm 0.0045$	$0.153^{a} \pm 0.0045$
2	$0.1485^{a} \pm 0.0045$	$0.153^{ab} \pm 0.0045$	$0.159^{ab} \pm 0.0045$	$0.16^{b} \pm 0.0045$
3	$0.156^{a} \pm 0.0026$	$0.1665^{ab} \pm 0.0045$	$0.1755^{bc} \pm 0.0045$	$0.18^{c} \pm 0.0045$
4	$0.1665^{a} \pm 0.0045$	$0.171^{a} \pm 0.0045$	$0.1845^{b} \pm 0.0045$	$0.186^{b} \pm 0.0045$
5	$0.1725^{a} \pm 0.0069$	$0.1755^{a} \pm 0.0045$	$0.1905^{b} \pm 0.0045$	$0.192^{b} \pm 0.0045$
6	$0.183^{a} \pm 0.0026$	$0.183^{a} \pm 0.0026$	$0.198^{b} \pm 0.0026$	$0.1995^{b} \pm 0.0026$
7	$0.1965^{a} \pm 0.0026$	$0.198^{b} \pm 0.0045$	$0.216^{c} \pm 0.0045$	$0.234^{d} \pm 0.0045$

***Table 6**: Comparison of acidity among LP-s activated sample of all ratio at 35 ± 2°C.*

**Ratio 1, 2, 3 and 4 = Cow and buffalo milk mixed in the ratio of 90:10, 80:20, 70:30 and 60:40 respectively. Values are the means of three determinations ± standard deviation and means with different superscript letters row wise are different ($p < 0.05$).*

Cow and buffalo milk combined in the ratio of 90:10 (Ratio 1) was found to be significantly different from the Ratio 4 sample (cow: buffalo milk; 60:40) from the 2nd hour of activation to the end of keeping quality (i.e. up to 7th hour) while all the mixed milk ratio (i.e. Ratio 1, 2, 3 and 4) were found to be significantly different among each other at 7th hour of storage. During the 5th and 6th hour of storage Ratio 1 and Ratio 2 was found to be significantly different from Ratio 3 and Ratio 4 sample.

Keeping quality increased for all the LP-s activated mixed milk ratio and among them also as the percentage of cow milk goes on decreasing the keeping quality of mixed milk also goes on decreasing. Similar findings were suggested by Gupta., *et al* [14].

Keeping quality of mixed raw milk at room temperature (25 ± 2°C)

Value of acidity and COB test of control and activated sample of ratio 90:10, 80:20, 70:30 and 60:40 of every two hour stored at 25 ± 2°C are shown in table 7-10 respectively. LP-s activated sample was significantly different ($p < 0.05$).

Time (h)	Acidity % (as lactic acid)		COB test	
	Control	LP-s Activated	Control	LP-s Activated
0	$0.1395^{a} \pm 0.0045$	$0.1395^{a} \pm 0.0045$	-ve	-ve
2	$0.1575^{a} \pm 0.0045$	$0.1485^{a} \pm 0.0045$	-ve	-ve
4	$0.1725^{a} \pm 0.0045$	$0.15^{b} \pm 0.0045$	-ve	-ve
6	$0.192^{a} \pm 0.0069$	$0.153^{b} \pm 0.0026$	-ve	-ve
8	$0.207^{a} \pm 0.0026$	$0.162^{b} \pm 0.0045$	-ve	-ve
10	$0.21^{a} \pm 0.0045$	$0.171^{b} \pm 0.0045$	+ve	-ve
12	$0.225^{a} \pm 0.0026$	$0.18^{b} \pm 0.0052$	+ve	-ve
14	$0.2475^{a} \pm 0.0045$	$0.2025^{b} \pm 0.0069$	+ve	+ve

Table 7: *Acidity and COB test of control and activated sample of ratio 90:10 at 25 ± 2^{0}C.*

Values are the means of three determinations ± standard deviation and means with different superscript letters row wise are different ($p < 0.05$).

Time (h)	Acidity % (as lactic acid)		COB test	
	Control	LP-s Activated	Control	LP-s Activated
0	$0.1395^{a} \pm 0.0045$	$0.1395^{a} \pm 0.0045$	-ve	-ve
2	$0.1605^{a} \pm 0.0045$	$0.1485^{a} \pm 0.0045$	-ve	-ve
4	$0.177^{a} \pm 0.0026$	$0.1515^{b} \pm 0.0026$	-ve	-ve
6	$0.195^{a} \pm 0.0052$	$0.156^{b} \pm 0.0026$	-ve	-ve
8	$0.21^{a} \pm 0.0045$	$0.165^{b} \pm 0.0026$	-ve	-ve
10	$0.2295^{a} \pm 0.0026$	$0.1785^{b} \pm 0.0026$	+ve	-ve
12	$0.2475^{a} \pm 0.0045$	$0.1935^{b} \pm 0.0045$	+ve	-ve
14	$0.252^{a} \pm 0.0045$	$0.2115^{b} \pm 0.0045$	+ve	+ve

Table 8: *Acidity and COB test of control and activated sample of ratio 80:20 at 25 ± 2^{0}C.*

Values are the means of three determinations ± standard deviation and means with different superscript letters row wise are different ($p < 0.05$).

Time (h)	Acidity % (as lactic acid)		COB test	
	Control	LP-s Activated	Control	LP-s Activated
0	$0.1395^{a} \pm 0.0045$	$0.1395^{a} \pm 0.0045$	-ve	-ve
2	$0.1665^{a} \pm 0.0045$	$0.1485^{a} \pm 0.0026$	-ve	-ve
4	$0.1845^{a} \pm 0.0045$	$0.1545^{b} \pm 0.0026$	-ve	-ve
6	$0.207^{a} \pm 0.0045$	$0.162^{b} \pm 0.0045$	-ve	-ve
8	$0.2295^{a} \pm 0.0045$	$0.1785^{b} \pm 0.0026$	+ve	-ve
10	$0.2475^{a} \pm 0.0045$	$0.1935^{b} \pm 0.0069$	+ve	-ve
12	$0.2565^{a} \pm 0.0045$	$0.207^{b} \pm 0.0045$	+ve	+ve

Table 9: *Acidity and COB test of control and activated sample of ratio 70:30 at 25 ± 2^{0}C.*

Values are the means of three determinations ± standard deviation and means with different superscript letters row wise are different ($p < 0.05$).

Time (h)	Acidity % (as lactic acid)		COB test	
	Control	LP-s Activated	Control	LP-s Activated
0	$0.1395^{a} \pm 0.0045$	$0.1395^{a} \pm 0.0045$	-ve	-ve
2	$0.1695^{a} \pm 0.0045$	$0.1485^{a} \pm 0.0026$	-ve	-ve
4	$0.1875^{a} \pm 0.0026$	$0.165^{a} \pm 0.0026$	-ve	-ve
6	$0.215^{a} \pm 0.0026$	$0.177^{b} \pm 0.0026$	-ve	-ve
8	$0.234^{a} \pm 0.0045$	$0.1845^{b} \pm 0.0069$	+ve	-ve
10	$0.252^{a} \pm 0.0045$	$0.1965^{b} \pm 0.0045$	+ve	-ve
12	$0.2745^{a} \pm 0.0045$	$0.2115^{b} \pm 0.0026$	+ve	+ve

Table 10: *Acidity and COB test of control and activated sample ratio 60:40 at 25 ± 2^{0}C.*

Values are the means of three determinations ± standard deviation and means with different superscript letters row wise are different (p < 0.05).

As the storage period increases there was increase in acidity in both the control and activated sample however increase in acidity was slower in activated sample and fast increment was observed in control sample. Acidity of control sample of ratio 90:10 and 80:20 reach a value of 0.20% after 6h of storage and showed COB positive test whereas it take 14h for activated sample (acidity > 0.20% and COB positive test regarded as end of shelf life). In case of control sample of ratio 70:30 and 60:40 it takes after 4h to reach the same level of acidity while 12h in case of activated sample reduction in keeping quality by two hour for both control and activated sample of this ratio when compared with mixed milk of ratio 90:10 and 80:20 The result showed a pronounced differences between the activated and control samples. This suggests that lower the storage temperature higher will be the shelf life of milk and LP-s have synergistic effect when combine with lower temperature. Similar to our result the shelf life of raw milk for control and stabilized sample at room temperature has been reported by Kumar and Mathur [15] and Chakraborty., *et al* [16].

Keeping quality among LP-s activated mixed milk at 25 ± 2°C

Change in value of acidity at an interval of two hour in case of milk of LP-s activated sample of all four ratio are shown in table 11. Values of acidity among mixed milk of activated sample of various ratios were significantly different ($p < 0.05$). There was no significant difference up to the 4th hour of storage among the activated sample however at 6th hour significant difference was observed among the activated sample. Ratio 1 and Ratio 2 were found to be significantly different from the Ratio 3 and Ratio 4 sample at the 10th hour of storage period. Ratio 1was significantly different from rest of the samples during 12th hour of storage Similar results were suggested by Chakraborty., *et al.* [16], Gupta., *et al.* [14] and Thakar and Dave [17].

Time (h)	*Ratio 1	*Ratio 2	*Ratio 3	*Ratio 4
0	$0.1395^{a} \pm 0.0045$	$0.1395^{a} \pm 0.0045$	$0.1395^{a} \pm 0.0045$	$0.1395^{a} \pm 0.0045$
2	$0.1485^{a} \pm 0.0045$	$0.1485^{a} \pm 0.0045$	$0.1485^{a} \pm 0.0026$	$0.1485^{a} \pm 0.0026$
4	$0.15^{a} \pm 0.0045$	$0.1515^{a} \pm 0.0026$	$0.1545^{a} \pm 0.0026$	$0.165^{a} \pm 0.0026$
6	$0.153^{a} \pm 0.0026$	$0.156^{a} \pm 0.0026$	$0.162^{a} \pm 0.0045$	$0.177^{b} \pm 0.0026$
8	$0.162^{a} \pm 0.0045$	$0.165^{a} \pm 0.0026$	$0.1785^{b} \pm 0.0026$	$0.1845^{b} \pm 0.0069$
10	$0.171^{a} \pm 0.0045$	$0.1785^{a} \pm 0.0026$	$0.1935^{b} \pm 0.0069$	$0.1965^{b} \pm 0.0045$
12	$0.18^{a} \pm 0.0052$	$0.1935^{b} \pm 0.0045$	$0.207^{c} \pm 0.0045$	$0.21155^{c} \pm 0.0026$
14	$0.2025^{a} \pm 0.0069$	$0.2115^{a} \pm 0.0045$		

Table 11: *Comparison of acidity among LP-s activated sample of all ratio at 25 ± 2^{0}C.*

**Ratio 1, 2, 3 and 4 = Cow and buffalo milk mixed in the ratio of 90:10, 80:20, 70:30 and 60:40 respectively. Values are the means of three determinations ± standard deviation and means with different superscript letters row wise are different (p < 0.05).*

Keeping quality of mixed raw milk at 5 ± 2°C

Value of acidity and COB test of control and activated sample of ratio 90:10, 80:20, 70:30 and 60:40 of each day stored at 5 ± 2°C are shown in table 12-15 respectively. LP-s activated sample was significantly different from control sample ($p < 0.05$).

Time (day)	Acidity % (as lactic acid)		COB test	
	Control	LP-s Activated	Control	LP-s Activated
0	$0.1395^{a} \pm 0.0045$	$0.1395^{a} \pm 0.0045$	-ve	-ve
1	$0.153^{a} \pm 0.0045$	$0.144^{a} \pm 0.0026$	-ve	-ve
2	$0.171^{a} \pm 0.0026$	$0.1485^{b} \pm 0.0026$	-ve	-ve
3	$0.1935^{a} \pm 0.0026$	$0.1575^{b} \pm 0.0026$	-ve	-ve
4	$0.216^{a} \pm 0.0045$	$0.1665^{b} \pm 0.0026$	+ve	-ve
5	$0.2385^{a} \pm 0.0045$	$0.1755^{b} \pm 0.0045$	+ve	-ve
6	$0.252^{a} \pm 0.0045$	$0.1845^{b} \pm 0.0026$	+ve	-ve
7	$0.2745^{a} \pm 0.0045$	$0.1935^{b} \pm 0.0045$	+ve	-ve
8	$0.297^{a} \pm 0.0045$	$0.215^{b} \pm 0.0045$	+ve	+ve

Table 12: *Acidity and COB test of control and activated sample of ratio 90:10 at 5 ± 2^{0}C.*

Values are the means of three determinations ± standard deviation and means with different superscript letters row wise are different ($p < 0.05$).

Time (day)	Acidity % (as lactic acid)		COB test	
	Control	LP-s Activated	Control	LP-s Activated
0	$0.1395^{a} \pm 0.0045$	$0.1395^{a} \pm 0.0045$	-ve	-ve
1	$0.153^{a} \pm 0.0045$	$0.144^{a} \pm 0.0026$	-ve	-ve
2	$0.174^{a} \pm 0.0026$	$0.1515^{b} \pm 0.0026$	-ve	-ve
3	$0.1965^{a} \pm 0.0026$	$0.1605^{b} \pm 0.0026$	-ve	-ve
4	$0.2205^{a} \pm 0.0045$	$0.1695^{b} \pm 0.0026$	+ve	-ve
5	$0.243^{a} \pm 0.0045$	$0.1785^{b} \pm 0.0026$	+ve	-ve
6	$0.2565^{a} \pm 0.0045$	$0.1875^{b} \pm 0.0026$	+ve	-ve
7	$0.2775^{a} \pm 0.0026$	$0.1965^{b} \pm 0.0026$	+ve	-ve
8	$0.3015^{a} \pm 0.0045$	$0.2205^{b} \pm 0.0045$	+ve	+ve

Table 13: *Acidity of control and activated sample of ratio 80:20 at 5 ± 2^{0}C.*

Values are the means of three determinations ± standard deviation and means with different superscript letters row wise are different ($p < 0.05$).

Time (day)	Acidity % (as lactic acid)		COB test	
	Control	LP-s Activated	Control	LP-s Activated
0	$0.1395^{a} \pm 0.0045$	$0.1395^{a} \pm 0.0045$	-ve	-ve
1	$0.153^{a} \pm 0.0045$	$0.147^{a} \pm 0.0026$	-ve	-ve
2	$0.1845^{a} \pm 0.0045$	$0.153^{b} \pm 0.0026$	-ve	-ve
3	$0.207^{a} \pm 0.0045$	$0.162^{b} \pm 0.0045$	+ve	-ve
4	$0.225^{a} \pm 0.0045$	$0.171^{b} \pm 0.0026$	+ve	-ve
5	$0.2475^{a} \pm 0.0045$	$0.189^{b} \pm 0.0069$	+ve	-ve
6	$0.27^{a} \pm 0.0045$	$0.198^{b} \pm 0.0045$	+ve	-ve
7	$0.3015^{a} \pm 0.0069$	$0.216^{b} \pm 0.0045$	+ve	-ve

Table 14: *Acidity of control and activated sample of ratio 70:30 at 5 ± 2^{0}C.*

Values are the means of three determinations ± standard deviation and means with different superscript letters row wise are different ($p < 0.05$).

Time (day)	Acidity % (as lactic acid)		COB test	
	Control	LP-s Activated	Control	LP-s Activated
0	$0.1395^{a} \pm 0.0045$	$0.1395^{a} \pm 0.0045$	-ve	-ve
1	$0.1575^{a} \pm 0.0045$	$0.1515^{a} \pm 0.0026$	-ve	-ve
2	$0.189^{a} \pm 0.0045$	$0.1545^{b} \pm 0.0026$	-ve	-ve
3	$0.2205^{a} \pm 0.0045$	$0.1665^{b} \pm 0.0045$	+ve	-ve
4	$0.2385^{a} \pm 0.0045$	$0.1755^{b} \pm 0.0045$	+ve	-ve
5	$0.2565^{a} \pm 0.0045$	$0.192^{b} \pm 0.0026$	+ve	-ve
6	$0.279^{a} \pm 0.0045$	$0.198^{b} \pm 0.0045$	+ve	-ve
7	$0.306^{a} \pm 0.0045$	$0.2205^{b} \pm 0.0045$	+ve	-ve

Table 15: *Acidity of control and activated sample of ratio 60:40 at 5 ± 2^{0}C.*

Values are the means of three determinations ± standard deviation and means with different superscript letters row wise are different ($p < 0.05$).

As the storage period increases there was increase in acidity in both the control and activated sample however increase in acidity was slower in activated sample and fast increment was observed in control sample. Acidity of control sample of ratio 90:10 and 80:20 reach a value of 0.20% at 4th days of storage and showed COB positive test whereas it take 8 day for activated sample (acidity > 0.20% and COB positive test regarded as end of shelf life). In case of control sample of ratio 70:30 and 60:40 it takes 72h to reach the same level of acidity while take 7 day in case of activated sample. There was reduction in keeping quality by one day for both control and activated sample of this ratio when compared with mixed milk of ratio 90:10 and 80:20. which suggest that lower the storage temperature higher will be the shelf life of milk and LP-s have synergistic effect when combine with lower temperature. LP-s system have synergistic effect with low temperature and can increase the shelf life of raw milk up to additional 4 days [18]. Bjorck, Claesson and Shulthes has reported the shelf life of LPs treated milk stored in refrigerator ended after five to six days.

Keeping Quality among LP-s activated mixed milk at 5 ± 2°C

Change in value of acidity per day in case of milk of LP-s activated sample of all ratios (i.e. combination of cow and buffalo milk in the ratio of 90:10, 80:20, 70:30 and 60:40) are shown in table 16. Values of acidity among mixed milk of various ratios were significantly different ($p < 0.05$). There was no significant difference up to the 4th day however significant difference was observed from 6th day of storage among the activated samples.

Time (day)	*Ratio 1	*Ratio 2	*Ratio 3	*Ratio 4
0	$0.1395^{a} \pm 0.0045$	$0.1395^{a} \pm 0.0045$	$0.1395^{a} \pm 0.0045$	$0.1395^{a} \pm 0.0045$
1	$0.144^{a} \pm 0.0026$	$0.144^{a} \pm 0.0026$	$0.147^{a} \pm 0.0026$	$0.1515^{a} \pm 0.0026$
2	$0.1485^{a} \pm 0.0026$	$0.1515^{a} \pm 0.0026$	$0.153^{a} \pm 0.0026$	$0.1545^{a} \pm 0.0026$
3	$0.1575^{a} \pm 0.0026$	$0.1605^{a} \pm 0.0026$	$0.162^{a} \pm 0.0045$	$0.1665^{a} \pm 0.0045$
4	$0.1665^{a} \pm 0.0069$	$0.1695^{a} \pm 0.0026$	$0.171^{a} \pm 0.0026$	$0.1755^{a} \pm 0.0045$
5	$0.1755^{a} \pm 0.0045$	$0.1785^{a} \pm 0.0026$	$0.189^{b} \pm 0.0069$	$0.192^{b} \pm 0.0026$
6	$0.1845^{a} \pm 0.0026$	$0.1875^{ab} \pm 0.0026$	$0.198^{b} \pm 0.0045$	$0.198^{b} \pm 0.0045$
7	$0.1935^{a} \pm 0.0045$	$0.1965^{a} \pm 0.0026$	$0.216^{b} \pm 0.0045$	$0.2205^{b} \pm 0.0045$
8	$0.215^{a} \pm 0.0045$	$0.2205^{a} \pm 0.0045$		

Table 16: *Comparison of acidity among LP-s activated sample of all ratio at 5 ± 2°C.*

**Ratio 1, 2, 3 and 4 = Milk of cow and buffalo mixed in the ratio of 90:10, 80:20, 70:30 and 60:40 respectively. Values are the means of three determinations ± standard deviation and means with different superscript letters row wise are different ($p < 0.05$).*

Ratio 1 and Ratio 2 sample were significantly different from Ratio 3 and Ratio 4 sample at 5th day of storage and after 5th day Ratio1 sample was significantly different with Ratio 3 and Ratio 4 sample till the end of keeping quality. Similar trends were found by Firew., *et al.* [18], Chakraborty., *et al.* [16] and Bjorck, Claesson and Shulthes [11].

Conclusions

Thiocyanate content was found to be higher in cow milk than buffalo milk and its value were 4.02, 3.55, 3.45 and 3.26 mg/L in mixed milk of cow and buffalo of ratio 90:10, 80:20, 70:30 and 60:40 respectively. Increment in acidity and COB +ve test was found faster in control sample of all ratio than LP-s activated sample of similar ratio. Keeping quality of LP-s activated sample was increased by 3h, 6h and 72h at 35°C, room temperature and refrigeration temperature respectively. Lactoperoxidase system have synergistic effect when combined with low temperature and as the percentage of cow milk increases.

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