MICROBIOLOGICAL QUALITY AND SHELF-LIFE OF RAW COW'S MILK PRESERVED BY LACTOPEROXIDASE SYSTEM

By
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THESIS

Submitted in partial fulfilment of the requirement for the degree

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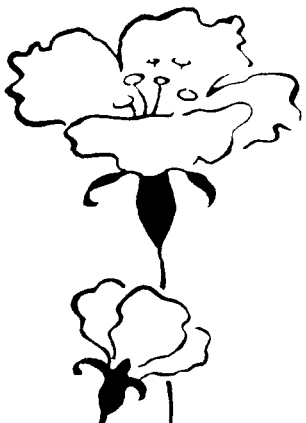

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DECLARATION

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CERTIFICATE

This is to certify that the thesis entitled, 'MICROBIOLOGICAL QUALITY AND SHELF - LIFE OF RAW COW'S MILK PRESERVED BY LACTOPEROXIDASE SYSTEM' submitted in partial fulfilment of the requirements for the degree of MASTER OF VETERINARY SCIENCE IN DAIRY SCIENCE to the Kerala Agricultural University, Vellanikkara, Thrissur, is a record of bonafide research work carried out by Sri. J. SELVIN JOE under my supervision and guidance and no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in scientific or popular journal or magazine.

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Introduction

1. INTRODUCTION

The Dairy industry in India is fast progressing with an annual milk production of 74.3 million tonnes in 1997. Milk is regarded as India's largest agricultural commodity and in 1997, the value of output of Dairy industry exceeded Rs. 105,000 crores, with an annual growth rate of 5.6 percent. During 1997, the per capita availability of milk was 214 gm per day (Gupta, 1997). Milk, one of the highly nutritious foods is easily perishable and likely to get spoiled rapidly by growth of contaminating micro-organisms. Being rich in nutrients, milk provides an excellent medium for bacterial multiplication.

Freshness and microbial quality of raw milk are the key elements in deciding the quality of products and marketing returns. The microbiological quality of the raw milk needs to be improved to ensure that products made from it meet the highest standards.

As per our traditional practice of procurement, milk reaches processing plants in aluminium cans between 4-6 hours after milking. By that time, it's quality might have deteriorated considerably.

Indian council of Agricultural Research (ICAR) has conducted studies during 1986-87 to determine the variations in quality of milk during transportation from the farmers to the dairy plants. The main observations recorded in this study was, that deterioration in the quality of milk was highly significant on its arrival at the dairy plant, and only four percent samples had an MBR time of more than 2 hours.

It is not uncommon for dairy plant to receive raw milk with a standard plate count of 5 to 10 million per ml. It is suggested that milk confirming to the designated standards for a plate count of less than 5,00,000 per ml be initially considered for quality incentive payments (Gupta, 1997).

It may be difficult to achieve these standards in India because milk in small quantities are produced by millions of rural farmers and collected at village societies and from there it is transported to distant dairy plants. So, much attention must be given on village societies and the milk procurement process for improving the milk quality (Aneja, 1997).

In tropical developing countries like India, techno - economic difficulties are faced in chilling of raw milk under rural conditions. This leads to poor microbiological quality of milk that reaches dairy plants for processing.

Even though, milk collected freshly from the milch animals is of good bacteriological quality as it is pooled, stored at ambient temperature and transported to the dairy plants, the bacteria initially present in the milk, those incorporated from atmosphere, utensils, equipment's and handlers etc., will multiply and because of metabolic products and various enzymes produced by the microbes the milk get spoiled. Even if the milk is kept cooled, the psychrotrophs multiply and produce thermostable proteases and lipases which even deteriorate Ultra High Temperature (UHT) processed milks (Mukundan, 1989).

Thus, in general, even though the milk received from the producers at society, immediately after milking is found to be of good quality, there is some deterioration of the quality at the village society level. However,

during transportation to dairy plant at high ambient temperature, and long time lapse between milking and transportation of milk to dairy plants contribute to the significant drop in quality. Hence it is important to adopt suitable measures to preserve the quality of milk during procurement and transportation from village societies to dairy plants.

Preservation of any food material needs assurance of retention of its natural consistency, original flavours and nutritional properties. Raw milk can be preserved with the addition of hydrogen peroxide (H_2O_2) alone. The permissible limit recommended for its use in milk is less than 800 ppm (FAO, 1957). Higher levels of H_2O_2 causes marginal losses in the biological value of protein and the availability of sulphur containing amino acids in milk.

At present, considerable interest has been generated all over the world for harnessing various innate substance present in raw milk for its preservation, under tropical conditions as an alternate to mechanical refrigeration. These antimicrobial factors present in normal milk includes lactoperoxidase, lysozyme, lactoferrin and immunoglobulin.

The three components such as the enzyme lactoperoxidase hydrogen peroxide, and thiocyanate form lactoperoxidase system (LP-system). Of these, the enzyme lactoperoxidase is present in milk in surplus quantities to permit the activation of its natural antibacterial activity. The factors limiting the activation of the lactoperoxidase system (LP-system) in milk are thiocyanate (SCN^-) and hydrogen peroxide.

Bactericidal effect of LP-system is proportional to SCN^- concentration, but the effect is also dependent on level of H_2O_2 supplied (Reiter *et al.*, 1976).

The thiocyanate content observed in Danish Holstein, Danish red and Jersey are 3.22. 3.31 and 4.06 ppm in milk respectively (Jansen and Hansen, 1991). Prasad (1990) reported that thiocyanate level of raw milk is 3.31 $\pm$ 0.73 ppm and the level of hydrogen per oxide is 1.37 $\pm$ 0.33 ppm

Although SCN^- is present in milk, exogenous addition of upto 20 ppm in necessary for formation of oxy acids adequate for preservative effect (Mathur and Ranju, 1995).

Many investigations have been carried out to evaluate the techno-economic feasibility of preserving raw milk, in Kenya (Bjorck *et al.*, 1979), Mexico (Martinez, *et al.*, 1988), Spain (Gaya *et al.*, 1991) and Egypt (Metwally and Moone, 1991).

These studies have demonstrated that a substantial improvement in hygienic quality of raw milk can be achieved during collection and transportation after activation of LP-System at collection centres.

International Dairy Federation (IDF, 1988) also recommended the use of LP-system for temporary preservation of raw milk during collection and transportation of milk to processing plants.

Regarding the toxicological aspects of the LP-system the joint FAO/ WHO Expert committee on food additives has declared that the LP-system is acceptable.

An essential pre-requisite for commercial application of a preservative is that it should be convenient to administer and easy to monitor. Besides this, it should be safe from toxicological point of view. The LP-system

fulfils this criterion and has been endorsed by the International Dairy federation (IDF, Bulletin.No.264, 1991).

However, the information regarding the level at which the SCN^- and H_2O_2 to be added to milk and their effect on the microbiological quality is scanty. Hence the present study was undertaken to determine the different levels of SCN^- and H_2O_2 in activating the LP-system of milk and their effect in controlling the microbial growth at ambient storage temperature.

Review of Literature

2. REVIEW OF LITERATURE

2.1 Bacteriological quality of raw milk

The Collection of milk from villages and its long distance transportation to the chilling centres at ambient temperature, results in the rapid growth of bacteria in milk, leading to its early spoilage. The frequent spoilage of milk result in heavy economic losses.

Singh *et al.*, (1974) while studying the bacteriological quality of milk in Kanpur city observed the average Standard Plate Count(SPC) of aseptically drawn milk, production end, and supply end milk were 2825, 315, 600 and 1,061, 600 per ml, respectively. There was more than hundred fold increase in bacteriological numbers from aseptically drawn milk to the production end milk and three fold increase from production end to supply end.

Higoshi *et al.*, (1977) studied the micro-biological quality of raw milk delivered to a milk processing plant and found that 60 percent of samples supplied in autumn were showing Resazurin disc reading of 1.

Erian and Shaikhli (1980) conducted research on the microbiological quality of raw milk and showed that the total bacterial count of raw milk was very high and 75 per cent had total bacterial count over a million bacteria per ml.

Pandey and Mandal (1980) studied the bacterial flora of raw milk of milk supply scheme in Patna and observed that the individual viable count in 40 pooled raw milk samples varied from 0.25 to 600 million and the average total count was estimated to be 18.4 million per ml.

Desai and Natarajan (1981) examined the milk samples obtained from societies that transport milk to the chilling centres with in 4.5 h after collection and observed that the average Methylene blue reduction time was two hour and ten minutes. The average standard plate count per ml of milk at the societies was 441 x 10^5 and coliform count per ml ranged from 43 x 10^3 to 485 x 10^3.

Siva *et al.*, (1993) carried out an investigation to know the micro-biological quality of raw milk at various stages of its collection. Samples of cow and buffalo milks were collected from individual producers, collection centres and dairy plants and total plate counts were determined. The higher counts reported in the samples collected at the plants was attributed to poor hygiene at the farm and growth of contaminants during in-can transportation.

Eighty samples of pasteurized cow milk from the Tehran region of Iran were analysed to determine their content of microbial flora. (Karim and Mousari, 1994). The 42.5 per cent of samples had counts of 9 x 10^4 to 3 x 10^5.

Yadava *et al.*, (1983) conducted a study to determine the bacteriological quality of milk sold in Ranchi town. Milk samples from three different sources were collected and analysed for standard plate count. The time elapsed between the production and collection of milk samples from one source was nearly one hour and had the count of 4.08 x 10^5 per ml. and the time elapsed between the production and collection of milk samples of other two sources exceeded four hours and had the average count of 74.2 x 10^5 per ml.

The raw milk generally contains a considerable number of spores. Kerala Varma *et al.*,(1950) observed that the spore forming bacteria are regularly present in milk and they constitute about ten percent of the microbial population in milk. Martin(1974) also reported the presence of spore forming bacteria in all raw milk, but usually in small numbers if the milk is produced under sanitary conditions. **Bacillus** species accounted for about 95 percent of the total spore forming bacteria in milk while **Clostridium** species comprised the remainder (Kerala Varma *et al.*,1950)

The predominance of Bacillus subtilis organisms in raw milk among the different species of spore - forming bacilli has been reported by various research workers (Davies, 1975; Mukundan, 1978).

Kaan and Natarajan (1988) examined 40 samples of milk from organised farms, dairy plants, street vendors and villages in India for aerobic spore formers. The aerobic spore former counts reported by them were 3.5 x 10^2, 7.6 x 10^2 ,1.15x 10^4 and 1.1 x 10^3 respectively. Incidence and distribution (148 isolates) of aerobic spore formers isolated by them included Bacillus subtilis 49; B cereus 23; B coagulans 19; B. licheniformis 10; B. sphaericus 9; B. pumilus 8; B. firmis and 2 unidentified species.

Ranganathan *et al.*,(1974) studied the incidence of heat resistant spores in milk and reported that irrespective of the season, the number of spores in milk varied with in the range of 1,000 to 3,000 per ml.

Ethiraj (1976) found that the total spore count of 34 raw milk samples varied from 3,000 to 15,500,000 per ml.

2.2 Preservation of milk by lactoperoxidase system

It has been known for a long time that bovine milk can inhibit the growth of certain bacteria. Hansen (1924) suggested that this could partly be due to an oxidizing enzyme.

Wright and Tramer (1958) found a high correlation between anti streptococcal activity of milk and its lactoperoxidase content.

Reiter *et al.,* (1963) showed that the inhibitory system consisted of lactoperoxidase, hydrogen peroxide and thiocyanate. Of these three components of the lactoperodixase system, lactoperoxidase is the enzyme, thiocyanate (SCN^-) the substrate and hydrogen peroxide (H_2O_2) the promoter. The LP-system fail to get activated in milk and exhibit its antibacterial activity in the absence of any one of these three essential components.

The enzyme lactoperoxidase is synthesized within the mammary gland and is always present in bovine milk, at a concentration of 30 microgram per ml. (Reiter, 1985). The level of lactoperoxidase required for the Lactoperoxidase system (LP - system) to get activated in milk is 0.5 to 1.0 microgram. per ml of milk. Lactoperoxidase is present in milk of various species in surplus quantities to permit the activation of LP-system.

The concentration of lactoperoxidase reported in bovine milk is 3 units per ml . (Kumar and Mathur, 1989 d) ewe's milk 0.14 to 2.30 units per ml. (Medina *et al.,* 1989); and goat milk 0.05 to 3.55 units per ml (Zapico *et al.,* 1991).

The lactoperoxidase activity in milk varies with breed of cow, season, feed and stage of lactation (Kern *et al.,* 1963). The mean

lactoperoxidase activity observed in Danish Holstein, Danish red and Jersey are 20.0, 23.9 and 25.3 enzyme units per ml respectively (Jansen and Hansen, 1991)

Lactoperoxidase level is not significantly correlated with milk yield but differs significantly between individuals, between herds and between week of sampling. (Medina *et al.*, 1989).

Lactoperoxidase, a glycoprotein with molecular weight about 78,000 and one haem group, may be involved in the antibacterial protection of the mammary gland (Bjorck, 1992). Commercial pasteurized milks generally has less than 70 per cent of the lactoperoxidase activity of raw milks (Martin *et al.*, 1990).

The factors limiting the activities of the LP - system in milk are thiocyanate (SCN^-) and hydrogen peroxide. The endogenous level of SCN^- in milk is influenced by various factors namely nature of feed, breed, species and the environmental conditions (Reiter and Harnulv, 1982; Gupta *et al.*, 1986).

The thiocyanate content observed in Danish Holstein, Danish red and Jersey are 3.22, 3.31 and 4.06 mg. per litre of milk respectively (Jansen and Hansen, 1991).

The average level of SCN^- in buffalo milk in summer months is 4.9 ppm and in winter months it is 6.9 ppm. The higher level of SCN in winter season is attributed to the feeding of greens and leguminous fodder (Kumar and Mathur, 1988).

The thiocyanate level shows significant difference between

individuals and between week of sampling and both lactoperoxidase and thiocyanate level are not significantly correlated with milk yield (Medina *et al.*, 1989).

Although SCN^- is present invariably in milk, exogenous addition of upto 20ppm is necessary for the formation of oxyacids adequate for preservative effect (Mathur and Ranju, 1995).

The most important limiting factor for the LP-system to work adequately is hydrogen peroxide (H_2O_2). Lower concentration of H_2O_2 is required to preserve milk by LP-system, in comparison to preservation achieved exclusively by H_2O_2 (Pickering *et al.*, 1962).

Bactericidal effect of LP-system is proportional to the SCN^- concentration, but the effect is also dependent on the level of H_2O_2 supplied (Reiter *et al.*, 1976).

The antibacterial activity of lactoperoxidase occur only in the presence of thiocyanate and hydrogen peroxide. (Sarkar and Misra. 1994 c).

Various researchers recommended different levels of combination of SCN^-: H_2O_2 to preserve the milk at different storage temperature: 15:10ppm (SCN^-: H_2O_2) at 37°C (Chakraborty *et al.*, 1986): 20:20ppm at 23°C (Thaker and Dave, 1986) and 20:10ppm and 20:20ppm at 35°C for 8 h and 12 h storage respectively (Sarkar and Misra, 1994 a)

2.3 LP- System's effect on general micro-biological quality and shelf-life of milk

Bjorck *et al.,* (1979) activated the lactoperoxidase system in raw cows milk with 15:7.5ppm (SCN^- H_2O_2) in Kenya and studied the storage life at various temperatures. Results showed that the preservative effect of lactoperoxidase system is mainly bacteriostatic and inversely related to the storage temperature. At 30°C the effect lasts for 7-8 h, and at lower temperature the effect is prolonged. At 25, 20 and 15°C it lasts 11-12, 15-16 and 24-26 h. respectively. It is concluded that lactoperodixose system improves the keeping quality of raw milk during long periods of transport at high ambient temperature.

The changes in the bacteriological quality of raw milk stabilized by activation of its lactoperoxidase system, stored at different temperature was studied (Zajac *et al.,* 1983). In LP activated milk stored at 4°C for 104 h, the standard plate count (SPC) and count of psychrotrophs and coliforms were unchanged, whereas in untreated milks all the three counts increased after about 48 h. At 17°C, duration of the bactericidal effect was shorter, and after 24 h, there was as increase in SPC, Coliforms and psychrotrophs in the LP-treated milk.

Ewais *et al.,* (1985) reported that at higher storage temperature higher concentration of H_2O_2 was necessary for improving the keeping quality. Preservation of buffalo raw milk at 23,30 and 37°C with LP-system was studied. (Thakar and Dave, 1986) The LP-system was activated by adding 10:10, 20:20, 30:30ppm (SCN^- : H_2O_2) to milk from three village collection centres. Maximum preservative effect was observed with the 30:30ppm addition at all temperatures. At 23°C, titratable acidity of milk increased after 14.2, 16.2 and 19.2 h. in the presence of 10:10, 20:20 and 30:30ppm

levels respectively. In untreated milk the acidity began to increase after 7.5 h at 23°C. Residual amounts of thiocyanate in the milk were low and no H_2O_2 could be detected.

Effect of LP-system on percentage titratable acidity with different combinations of SCN^-:H_2O_2 was studied (Gupta *et al.*, 1986). The milk samples showed 0.171, 0.144, 0.144 per cent titratable acidity after 8 h of storage with control, (untreated) 10:10 and 20:20 levels of LP-system.

The lactoperoxidase system of cow milk from a Turkish farm was activated with 10:10 and 15:15ppm SCN^-:H_2O_2 and then the milk was kept for 10 h at 25 or 10°C. Bacterial counts decreased immediately after LP activation and did not return to initial level until 10 h later at 10°C and 4 h later at 25°C. This antibacterial effect was virtually the same with 10:10 as with 15:15 activation. Titratable acidity did not increase significantly in any samples at 10°C but at 25°C it reached the maximum acceptable level of 0.18 per cent (as lactic acid) within 4 h. in control samples without LP activation. This was extended to 6 h. with 10:10 and 8 h. with 15:15 activation. (Oysun and Oztek, 1988).

The enhanced shelf life of milk treated with LP-system depends on the initial microbial quality of milk Dhanalakshmi (1987) studied the shelf life of raw milk samples of different initial micro-biological quality with 30:30ppm (SCN^- : H_2O_2) and observed that shelf life of milk samples with initial MBR time of viz., > 6 h , 5- 6 h, 3-4h and < 2 h had enhanced shelf life of 4 h, 2 h and 56 min , 2 h and 33 min and 1 h and 30 min respectively.

Martinez *et al.*, (1988) studied the effect of LP-system on quality of pasteurized milks. When LP-treated milk was stored raw for 4 days at 4°C and

then pasteurized, it maintained its quality for 12 days at 8°C; pasteurized control milk had a shelf-life of 9 days only. LP treated milk that was stored raw for 6 days retained its quality after pasteurization for 11 days at 8°C and 5 days at 16°C, while shelf-life for control milk of this experiment was 6 days at 8°C and 1 day at 16°C.

Gaya *et al.*, (1991) demonstrated that, with addition of thiocyanate and H_2O_2 to activate the LP-system, raw milk can be satisfactorily preserved for 5 day at 5°C or for 8 day at 3°C without excessive increases in bacterial counts or significant changes in flavour.

The efficacy of lactoperoxidase system for preservation of raw buffalo milk produced under rural conditions during summer and winter months at ambient temperature and efficacy of quality test employed for determining keeping quality of LP-treated milk under field conditions were evaluated (Kumar and Mathur, 1989c). Keeping quality of less than 4 h was shown for untreated raw milk during the summer. When treated with 25:15 LP-system keeping quality was 7 h (on the basis of MBR and alcohol test) and 10 h (on the basis of titratable acidity and clot-on boiling tests). When treated with 70:30 LP-system, keeping quality was 11 h on the basis of MBR test and alcohol tests and 14 h on the basis of titratable acidity and COB tests. In winter, the efficacy of LP-system was further enhanced.

The bacteriological quality of buffalo raw milk, preserved by the activation of lactoperoxidase system was evaluated by Patel and Sannabhadti, (1993). The LP - system was activated in buffalo raw milk samples by addition of (i) 0 (control) (ii) 15:10 and (iii) 45:30ppm thiocyanate and hydrogen peroxide. Activation of the LP-system reduced log standard plate and mesophilic acid producers count respectively, which were (i) 6.14 and 4.13 (ii)

6.04 and 3.72 (iii) 5.95 and 3.62 per ml initially but had recovered to (i) 8.00 and 8.04 (ii) 8.25 and 7.60 (iii) 7.94 and 6.59 per ml after 9 h. Activation of the LP-system had less effect on proteolytic and thermoduric counts, but had a marked effect on log coliform counts. It was concluded that either level of SCN^-: H_2O_2 treatment had primarily a bacteriostatic effect, and that inhibition was best achieved within first 3 h of storage.

The acidity at which milk sours is usually in the range of 0.20 to 0.25 per cent lactic acid and the milk usually shows positive on clot-on-boiling test when the acidity further increases to 0.30 per cent lactic acid (Yadav *et al.*, 1993).

The effect of 13 combinations of thiocyanate (SCN^-) and Hydrogen peroxide (H_2O_2) viz., 10:10, 20:10, 20:20, 20:25, 25:10, 25:20. 25:25, 25:30,30:10, 30:20, 30:25, 30:30 and 30:45ppm were studied to obtain the optimum levels required in preserving cow milk at 15°C and 35°C by LP-system (Sarkar and Misra, 1994 a). LP-system activated at a level of 20:10 and 20:20ppm (SCN^- :H_2O_2) gave maximum preservative effect at 15°C and 35°C in case of raw cow milk.

Harnulv and Kandasamy (1982) evaluated the acceptability of raw milk, preserved by 11.6:8.5ppm (SCN^- : H_2O_2) at ambient storage temperature. After 6 h of activation, 100, 90 and 80 per cent samples showed acceptability based on clot-on-boiling tests, Alcohol stability test and Acidity test respectively and after 10 h of storage 80 per cent samples were with in the acceptability limit according to the clot-on-boiling test and only 50 percent sample were in the acceptable limit based on Acidity and Alcohol test. Sukumar (1996) stated that Alcohol test was more sensitive than clot-on-boiling test.

Chakraborty *et al.*, (1986) studied the storage life of buffalo milk treated with 15:10ppm (SCN^- :H_2O_2) LP-system. The standard plate count, after one hour of activation were 26 x 10^6 and 2.9 x 10^6 in untreated and treated samples respectively. The titratable acidity after 3 h of activation were 0.126 and 0.128 per cent lactic acid respectively. The corresponding values for untreated samples were 0.135 and 0.160 per cent lactic acid.

Sarkar and Misra (1994a) studied the effect of LP-system with various levels of SCN^- :H_2O_2 on the titratable acidity at 15°C. The titratable acidity of milk treated with 20:10 ppm (SCN^- :H_2O_2) were 0.225, 0.225 and 0.225 per cent lactic acid after 4, 8 and 12 h of storage respectively. The corresponding values for samples with 20:20ppm were, 0.216, 0.216,0.216 respectively. The initial titratable acidity of all the milk samples were 0.18 per cent lactic acid.

Sarkar and Misra (1994c) studied the effect of LP-system with different combinations of SCN^-:H_2O_2 on the bacteriological quality of milk at 15°C. The standard plate count (log count cfu per ml) of milk treated with 10:10ppm (SCN^-:H_2O_2) after 4, 8 and 12 h of storage were, 7.16, 7.66 and 7.93 cfu/ml respectively. The corresponding values for milk samples treated with 20:20ppm (SCN^-:H_2O_2) were 6.83, 7.17,and 7.11 respectively. The coliform count of milk treated with 10:10ppm (SCN^- :H_2O_2) after 4,8,12 h of storage at 15°C were 6.84, 7.65 and 8.2 log cfu per ml. The corresponding values of milk samples treated with 20:20ppm (SCN^-:H_2O_2) were 5.30, 6.26and 7.18 log cfu per ml respectively.

Ghosh and Ghatak (1995) studied the efficacy of the LP-system activated at 10:10, 25:10 , 30:15 and 40:20ppm (SCN^-:H_2O_2) level, for the preservation of cow and buffalo milk at 30°C. All those combinations preserved

cow and buffalo milk for 10h and 8h respectively with minimum rise in developed acidity. All these levels preserved cow and buffalo milk effectively up to 12 and 10h respectively on the basis of COB test. The untreated samples of cow milk showed alcohol test to be negative at 6h, whereas treated samples of cow milk showed stability up to 10h in respect of alcohol test.

Kumar and Mathur (1994) analysed the microbial quality of raw buffalo milk preserved by LP-activation and found that a mild bactericidal effect resulted in a small drop in the mesophilic and psychrotrophic counts from 251 x 10^6 cfu per ml to 6.31 x 10^5 cfu per ml (25:15LP-system) and 2.51 x 10^4 cfu per ml (70 : 30 LP-system) and from 3.18 x 10^4 cfu per ml to 1.00 x 10^4 cfu per ml (25:15 LP- system) and 1.00 x 10^4 cfu per ml (75:30 LP-system) respectively.

Lactoperoxidase system affects the heat resistance of spores. Solanky *et al.,* (1994) studied the Thermal death kinetics of Bacillus stearothermophilus sub sp calidolactis in buffalo milk. The decimal reduction times (D-values) at 132,138 and 144°C were 28.0, 6.0 and 1.7 seconds respectively in untreated milk whereas in LP-treated milk the corresponding D-values were 25.5, 5.5and 1.5 seconds.

2.4 Advantages of LP-system over H_2O_2

Nutritional aspect of milk preserved by hydrogen peroxide indicates a marginal loss in the biological value of proteins and the availability of sulphur containing amino acids. (Deodhar and Mehta, 1980) and hydrogen peroxide employed for preservation of milk results in unsatisfactory flavour and body and texture of cheese (Jha, 1984).

Gupta *et al.,* (1986) compared preservative effect of hydrogen peroxide and LP-system in milk. The titratable acidity of milk samples reached 0.153 per cent lactic acid after 8 h of storage, when treated with 30ppm and 50ppm H_2O_2 level., whereas when the milk samples were treated with LP-system the titratable acidity reached only 0.144 per cent after 8 h storage both by 10:10 and 20:20 (SCN^- :H_2O_2) levels. It was concluded that LP-system gave maximum preservative effective with least amount of hydrogen peroxide.

Kamau *et al.,* (1991) studied the extension of shelf life of pasteurized milk, by applying various preservation methods. Milk samples were pasteurized 30 min at 63°C, cooled and then stored at 10°C for 22 days. Observable growth of surviving natural microflora started after 12 days in lactoperoxidase system applied milk compared with 4 days in untreated and in H_2O_2 treated milk. After 22 days, viable counts in untreated and H_2O_2 milk reached 10^6 - 10^7 cells per ml, compared with approximately 10^3 Çells per ml, in LPs treated milk.

2.5 Antimicrobial components of LP-system

The enzymatic oxidation of thiocyanate takes place via a series of oxyacids of SCN^- of which hypothiocyanate in the first member and the higher oxyacids are considered bactericidal agents (Aune and Thomas, 1977; pruitt *et al.,* 1982).

Oram and Reiter (1996) suggested that the inhibitory substance of LP-system may be sulphurdicyanide, while the other investigators suggested it to be cyanosulfurous acid or cyanosulfuric acid (Bjorck and Claesson, (1980).

Some gram-negative, catalase positive micro-organisms are inhibited in milk by lactoperoxidase in the presence of adequate amount of thiocyanate(SCN^-) and hydrogenperoxide. Lactoperoxidase catalyses the reaction

$$SCN^- + H_2O_2 \longrightarrow OSCN + H_2O$$

The antibacterial action is due to an intermediate reaction product, probably hypothiocyanate (OSCN) (Hoogendoorn *et al.*, 1977).

Recent kinetic and polarographic studies suggested that very short lived oxidation products such as O_2SCN and O_3SCN may be formed when H_2O_2 is present at higher concentration. Such higher oxy acids are better oxidizing agents than OSCN and have a bactericidal effect on Escherichia coli whereas the more stable OSCN is only bacteriostatic (Pruitt *et al.*, 1982).

Carlsson (1984) also pointed out that the enzyme-Hypothiocyanate complex formed during the activation of LP-system acted as intermediate in the catalytic activity of lactoperoxidase. Hypothiocyanate which does not the react with bacteria, will be reduced spontaneously to thiocyanate due to the limited stability of OSCN in milk, especially at higher temp (Bjorck *et al.* 1979). It has been shown that heating at 60°C for 15 min completely destroys the antibacterial effect (Bjorck *et al.*,1975)

Oxyacids produced under the catalytic oxidation process of lactoperoxidase instantaneously react with sulfhydryl (SH) group of the protein present on bacterial cell membrane as well as other extra cellular enzymes of bacterial origin, leading to regeneration of SCN^-. The end products of the metabolic oxidation of OSCN, O_2SCN, O_3SCN, have been shown to be CO_2, NH_3 and SO_4 (Reiter,1984)

2.6 Mode of antibacterial action of the LP-system

Hypothiocyanate has the capacity to oxidize some of the bacterial sulphydryl group of proteins and enzymes which yield sulphenic acid and Sulphenyl thiocyanate which inhibit bacterial respiration (Thomas and Aune, 1978)

Hypothiocyanate cause inhibition of bacterial enzymes viz., hexokinase (Adamson and Pruitt, 1981) and glyceraldehyde-3-phosphate dehydrogenase and/or cause depletion of reduced nicotinamide adenine nucleotide (Carlsson *et al.*, 1983). The antibacterial effect is due to an oxidation of the SH groups in various essential proteins in the bacterial cell wall, thus causing the energy metabolism of the bacteria to become destroyed. It is also demonstrated that the SH-independent enzyme D-lactate dehydrogenase is also inhibited by the LP-system (Reiter 1979).

In the case of E. coli it is demonstrated that the oxygen uptake (Bjorck, 1977) and the active transport of glucose and various amino acids is stopped immediately after exposure to the system (Marshall, 1978). Mode of action of LP-system on various micro-organisms may be due to inhibition of transport of glucose and valine and subsequent glycolysis (Mickelson, 1979) destruction of bacterial plasma membrane (Zajac *et al.*, 1981) or inhibition of glucose metabolism (Tenovuo *et al.*, 1982; Purdy *et al.*, 1983; Banks and Board, 1985).

Hypothiocyanate can cause inhibition of uptake of carbohydrates, amino acids etc. and subsequently affecting the synthesis of proteins, DNA and RNA (Marshall and Reiter, 1980).

The bacterial cytoplasmic membrane is obviously structurally damaged (or) changed because, organisms exposed to the LP-system, immediately leaks potassium ions, amino acids, poly peptides into the medium. Subsequently uptake of glucose, purines and pyrimidines and amino acids as well as synthesis of protein, DNA and RNA is also inhibited (Mickelson, 1977).

Mickelson (1981) incorporated the thiocyanate into bacterial protein and observed the reaction with sulphydryls group in the membrane proteins and inhibition of transport of glucose and subsequent glycolysis resulting in the changes in the membrane permeability.

The difference in the behaviour of the gram positive and gram negative bacteria towards LP-system could be attributed to the structure and composition of the cell wall of gram positive and the outer membrane of gram negative organisms, respectively (Reiter, 1985).

The heat resistance of the spore may be marginally reduced under the influence of the LP-system possibly as a result of damage inflicted by antimicrobial metabolites of the system on the spore coat (Solanky, 1994). Activation of LP-system produces a component that reacts with the Thiol group of certain amino acids in bacteria causing either bacteriostatic effect or destruction (Jean-Paul Rennes, 1994).

2.7 Antibacterial effect of lactoperoxidase system on different species of bacteria

Reiter *et al.*,(1963) found that dialysable factor required for the inhibition of some strains of Streptococcus cremoris was thiocyanate. The

inhibition requires thiocyanate, peroxidase and hydrogen peroxide which is produced under aerobic condition by the inhibitory organism. Cell multiplication, lactic acid production in milk and oxygen uptake by resting cells were found to be inhibited .

Steele and Martin(1969) observed that lactoperoxidase system inhibited the growth of Streptococcus cremoris 972 and the inhibition of bacteria required the formation of quarternary complex of cells, thiocyanate, peroxide and enzyme lactoperoxidase.

Bjorck *et al.,* (1975) found that the products of thiocyanate oxidation by lactoperoxidase system inhibit gram positive bacteria that produce peroxide. Oxidation products of thiocyanate are also bactericidal for gram negative bacteria such as **pseudomonads** and Escherichia coli both in synthetic medium and in milk. The remaining bacteria start multiplying when thiocyanate is depleted.

Mickelson (1976) studied the relationship between sulphydryl compound and growth inhibition by lactoperoxidase in skim milk using cultures of Streptococcus agalactiae. It is concluded that when the medium contains 4 to 5 micro gram of L-cystine per ml the growth of culture is inhibited by the addition of lactoperoxidase / thiocyanate / hydrogen peroxide. But at a concentration above 50 micro gram per ml, the inhibitory effect of lactoperoxidase systems is prevented. Prevention of inhibition during the rapid growth of the cultures is due to reduction of cystine to cysteine which competes with thiocyanate as an electron donor.

Interference of the entry of the glucose into the cells by lactoperoxidase complex may lead to the prevention of Streptococcus agalactiae growth.(Mickelson,1977)

Edwin and Thomas(1978) attributed the antimicrobial effect of lactoperoxidase system against Escherichia coli, to the oxidation of bacterial sulphydryls to sulfenic acid and sulfenyl thiocyanate derivatives which inhibits respiration. It was concluded that when the inhibition was reversed the ability to respire would be recovered. Pruitt *et al.*,(1979) noted the inhibitory effect of lactoperoxidase system against Streptococcus mutans.

Valerie *et al.*, (1980) studied the effect of hypothiocyanate anion which played the antibacterial activity on the growth and membranes of Escherichia coli and Streptococcus lactis. Escherichia coli was killed in the presence of anion where as the effect on Streptococcus lactis was only bacteriostatic.

Zajac *et al.*, (1981) observed that after incubation of vegetative cells of Bacillus cereus up to 6 h at 30°C in semi synthetic medium a decrease in viable count was observed in the presence of lactoperoxidase system

Reiter and Harnulv (1982) studied the effect of lactoperoxidase system on psychrotrophs and observed that the lactoperoxidase system had a bactericidal activity initially and subsequent bacteriostatic activity on **pseudomonads** in raw milk at 4°C. They also observed that cheese prepared from the lactopotoxidase treated milk gave a higher yield than untreated milks. This may be due to the suppression of heat resistant proteases of the **pseudomonads**.

Mickelson and Brown (1985) studied the effect of lactoperoxidase system on the growth of Streptococcus dysagalactiae and S uberis and observed that due to the accumulation of hydrogen peroxide by Streptococcus dysagalactiae and since there was no accumulation of hydrogen peroxide by Streptococcus uberis, Streptococcus dysagalactiae was more sensitive to lactoperoxidase system.

Bacillus cereus is inhibited by lactoperoxidase system and the growth inhibition is directly proportional to the amount of hypothiocyanate ion present. (Tenovuo *et al.*, 1985)

Uceda *et al.*, (1994) studied the effect of LP-system in ewe milk on Pseudomonas fluorescens INTA 724. It was concluded that activation of LP-system ensured optimal bacteriological and physico chemical quality of ewe milk where initial bacterial counts exceeded 10^6 cfu per ml when held at 4°C for 4 days and allowed its further storage at 4°C for 1-2 days before a detrimental effect due to Pseudomonas fluorescens proteinases was observed.

Solanky *et al.*, (1994) conducted a study on thermal death kinetics of Bacillus stearothermophilus sub sp. calidolactis in buffalo milk. The decimal reduction times (D - values) at 132, 138 and 144°C were 28.0, 6.0 and 1.7 seconds respectively. When raw buffalo milk inoculated with spore suspension was treated using the lactoperoxidase system for 6h at 37°C the D-values observed were 25.5, 5,5 and 1.5 seconds at 132, 138 and 144°C respectively.

The LP-system has been found to be efficient in inhibiting gram negative, catalase positive organisam, when H_2O_2 was exogenously added at low, non bactericidal concentration (Reiter 1978)

The difference in the behaviour of gram positive and gram negative bacteria towards LP-system could be attributed to the structure and composition of the cell wall of organisms, (Reiter, 1985).

Bjorck *et al.,* (1975) found products of thiocyanate oxidation of lactoperoxidase inhibit gram positive bacteria that produce peroxide. They found these products to be bactericidal for gram negative organisms such as Pseudomonas fluorescens, P. Alcaligenes and E. coli. They also observed that E. coli were killed more efficiently than P. fluorescens.

Marshall and Reiter (1980) compared the antibacterial activity of the hypothiocyanate anion towards Streptococcus lactis and Escherichia coli. and concluded that the cytoplasmic damage was less in Streptococcus lactis than in Escherichia coli.

The cell wall of gram positive organisms may be a more effective barrier than of gram negative organisms. Gram negative catalase-positive organisms, such as **Pseudomonads, coliforms**, **salmonella** and **shigellae** are not only inhibited by the LP- system but depending on the pH of the medium the temperature, the incubation time and the particular electron donor, may be killed provided H_2O_2 is supplied exogenously (Purdy *et al.,* 1983).The inhibition of growth of **Enterococcus** and **Pseudomonas** spp and **Enterobacteriaceae** was observed in a contaminated infant formulae upto 24 h. (Banks and Board, 1985).

2.8 Antibacterial effect of lactoperoxidase system on pathogens

Mickelson (1966) observed that purified lactoperoxidase and thiocyanate completely inhibited the growth of Streptococcus agalactiae and Streptococcus pyogenes due to the peroxidatic conversion of essential enzymic

sulphydryl group to inactive disulphide group, thus interfering with the energy metabolism of Streptococcus pyogenes. Streptococcus agalactiae growth was delayed for 6 h. but not inhibited completely and the effect was neutralized by catalase.

Prudy *et al.*, (1983) showed the effect of lactoperoxidase on Salmonella typhimurium. The lactoperoxidase system was found to be bacteriostatic and bactericidal against different strains of Salmonella typhimurium. The bactericidal activity was dependent upon the permeability of bacterial cell envelope.

Beumer *et al.*, (1985) found that Campylobacter jejuni rapidly reduced in viable numbers when inoculated in raw milk due to antibacterial action of lactoperoxidase system.

Wray and Mclaren (1987) observed that the growth of different **Salmonella** sero types including Salmonella dublin**,** decreased markedly in the acidified milk containing activated lactoerpoxidase system. When calves were fed on raw milk containing lactoperoxidase and then challenged with **Salmonella typhimurium,** clinical findings and **Salmonella** excretion patterns were similar to those of control calves fed on heated milk.

Bibi (1989) observed that Listeria monocytogenes was inhibited by the LP-system for 6, 20 and over 100 h at 30, 20 and 8°C respectively. The LP-system was active against thermophilic starter cultures at 8.7 mg SCN^- per litre. The related proportion of non-acid forming bacteria and **Enterobacteriaceae** in LP-stabilized milk did not shift to any noticeable extent during the first 10 h of preservation.

Gram-negative, catalase-positive organisms, such as **pseudomonads**, **coliforms, salmonellae** and **shigellae** are inhibited by the LP-system. Depending on the medium pH, temperature, incubation time, cell density and the particular donar, these micro organisms may be killed. The LP-system can increase storage life of raw milk by delaying growth of psychotrophs (Woltson and Sumner, 1993)

Prieels *et al.,* (1989) infected a group of 28 calves with Enteropathic E. coli strain with in 24 h of birth. Untreated controls showed severe infection and some died. Calves which were given 4000 units of lactoperoxidase from their first milk substitute feed, remained free of diarrhoea.

Bibi and Bachmann (1990) studied the survival and growth of Listeria monocytogenes and Listeria innocua in the presence of a reactivated LP-system in skim milk at 30 and 20°C when LP-system was found to have an bacteriostastic effect on both organisms. Its duration was dependent on temperature, lasted 6 h at 30°C and above 20 h at 20°C.

LP-system is bactericidal against Salmonella enteritidis and Escherichia coli. at 30°C, the effect being more rapid and persisting for longer time with Escherichea coli. LP-system is more active and recovery is more rapid at the higher temperature. Lactoperoxidase system has a bacteriostatic effect only on Listeria monocytogenes. (Dlamini and Bruce, 1990)

Earnshaw *et al.,* (1990) noted that lactoperoxidase system delayed the onset of exponential growth of Salmonella typhimurium and Escherichia coli. in infant formulae and extended the lag period before the onset of exponential growth of Escherichia coli.

Thakar *et al.*, (1990) Studied the effect of activated Lactoperoxidase system on pathogen in buffalo milk such as Salmonella typhosa, Escherichia coli and Staphylococcus aureus by addition of 10:10 (T1) and 30:30 (T2)ppm SCN^-:H_2O_2. Log pathogen count was initially 2.98-3.24 cfu per ml and increased gradually in non-activated controls to 7.0-9.43 after 16 h. In samples treated with T1 and T2 respectively, log count was 2.62-3.08 and 1.46-1.87 cfu per ml after 16 h. The LP system had a bactericidal effect on all three pathogens for about the first 4 h. of storage.

The lactoperoxidase system enhances thermal destruction of Listeria monocytogenes and Staphylococcus aureus. For Listeria monocytogenes the D 52.2°C values are 30.2 min in untreated milk and 10.7 min in LP-activated milk. For Staphylococcus aureus the D 52.2°C value are 33.3 min in untreated milk and 2.2 min in LP-activated milk. The most rapid killing of Listeria monocytogenes occurs when samples are heated soon after activation of the LP-system. Activation of the LP-system followed by heating can increase the margin of safety with respect to milk borne pathogens. (Kamau *et al.*, 1990)

The activation of LP-system by addition of either H_2O_2 or by addition of H_2O_2 producing lactic acid bacteria inhibit the Escherichia coli O157:H7 and Yersinia enterocolitica. The effect is inversely related to the incubation temperature. At 30°C the effect is limited to 12 h. However this can be of practical importance when raw milk is collected under sub optimal conditions and is then transported and held for a long time at ambient temperature. LP-system provides a simple method to inhibit Escherichia coli O157:H7 and Yersinia enterocolitica (Farrag and Marth, 1992).

Gaya *et al.*, (1991) tested the activity of raw milk LP-system on four strains of Listeria monocytogenes. It was concluded that the LP-system exhibited a bactericidal activity against Listeria monocytogenes at 4 and 8°C. D values at 4°C ranged from 4.1 to 11.2 days and at 8°C it ranged from 4.4 to 9-7 days. The lactoperoxidase level in raw milk declined during a 7-day incubation, the decrease being pronounced at 8°C than at 4°C. The thiocyanate concentration decreases considerably in LP-activated milk at both temperature during at first 8 h. of incubation.

Farrag and Marth (1992) studied the effect of LP-system on the growth of Escherichia coli O157:H7 in raw milk with initial population 10^4 to 10^8 cfu per ml at 30°C. The LP-system caused both initial populations of Escherichia coli in milk to decrease within 4 h, with maximum inactivation occurring after 6 h in the large initial population and 12 h. for the small initial population. The decrease was followed by growth, so that after 24 h, population in treated and control samples were comparable.

The effect of LP-system on the growth of Yersinia enterocolitica strain 2635 NT was studied (Farrag *et al.*, 1992) in raw milk with initial population of 10^4 to 10^8 cfu per ml. At 30°C the LP-system caused both initial population of Y. enterocolitica to decrease with in 2 h with maximum inactivation occurring after 12 h. for large and small initial population. The degree to which Yersinia enterocolitica was inactivated by the LP-system was related to initial population of the pathogen and incubation temperature.

The effect of temperature, on the antibacterial activity of the lactoperoxidase system on Escherichia coli was studied (Dlamini and Bruce, 1992) It was concluded that the death rate of bacteria was positively correlated with temperature, but the persistency of the antibacterial action is inversely

correlated with temperature. Recovery of bacterial cells from inactivation by this system was enhanced at higher temperature.

A significant decrease in the counts of three Listeria monocytogenes strains was observed in raw goat milk, with an activated LP-system stored at refrigeration temperatures for 9 days. (Zapico *et al.*, 1993). The LP-system only retarded the growth of Listeria monocytogenes at 20°C.

Santos *et al.*, (1994) studied the inhibitory effect of LP-system on Aeromonas hydrophila in skim milk and ewe's milk. Activation of LP-system was carried out with 15:15ppm (SCN^-:H_2O_2). In both the LP treated substrates the tested strains were completely inactivated after 2 h at 28°C and after 24 h at 6°C. It was concluded that the use of the LP-system as a preservative against Aeromonas hydrophila could be very useful even when adequate cooling facilities were available.

2.9 Effect of LP-system on milk constituents.

Proteolytic changes in raw buffalo milk preserved by LP-system was studied after the storage period of 17 h at 30°C (Kumar and Mathur,1994a) and observed that there was no proteolytic changes (in terms of tyrosine value) and no statistically significant difference in the total Nitrogen, Casein Nitrogen and Non-Casein Nitrogen levels of LP treated milk. Similar results also have been shown in cow milk preserved by LP-system with 15:10 and 70:30ppm (SCN^-:H_2O_2) levels (Ghosh *et al.*, 1995) and the lactoperoxidase system also inhibited the lipoprotein lipase in milk (Abrne and Bjorck; 1985)

Changes in the fatty acid profile of raw buffalo milk preserved by LP-system when studied, after the storage period of 8,12 and 16h at 30°C

(Kumar and Mathur, 1994 b) showed no significant changes in the proportion of saturated and unsaturated fatty acids. No oxidative changes in fatty acid profile of buffalo milk was also observed. The milk preserved by LP-system did not show any changes in the available methione and lysine (Kumar and Mathur, 1989 b).

An investigation to examine the extent of lipolysis (in forms of free fatty acids content) in raw cow and buffalo milk preserved by LP-system was conducted (Ghosh and Ghatak, 1995). Results indicated that the free fatty acid (FFA) content in LP-treated cow and buffalo milk was considerably lower than the corresponding FFA content in untreated sample of cow and buffalo milk.

Abdou *et al.*, (1994) compared the electrophoretic pattern of protein of yoghurt made from LP treated milk and untreated milk. It was concluded that the electrophoretic patterns of yoghurt protein made from LP treated and untreated milk were identical.

It was found out that hypothiocyanate did not react with lactose (Metwally and Moore, 1991)

2.10 Influence of LP-system on products prepared from LP-treated milk

Kumar and Mathur (1989a) conducted studies on the manufacture of yoghurt and Mozzarella cheese from milk preserved by lactoperoxidase system and concluded that starter cultures produced slower rate of acidity in milk preserved by LP-system and thus setting of curd was delayed by 1 h 30 min in the case of yoghurt. In the case of Mozzarella cheese, curd took about 2 h longer for stretching operation due to lower rate of acid development. However on dry matter basis there were no compositional difference between control cheese and cheese prepared from LP-treated milks.

Equally good quality pizza could be prepared from the Mozzarella cheese from untreated as well as LP-treated milk.

Delayed acid production, and a slightly extended production schedule are readily overcome by minor modification in the manufacturing process by using appropriate starter strains. (Siva *et al.*, 1991). Sarkar and Misra (1994 b) suggested that LP-treated milk produced good quality of fermented milk products but the reduced rate of acid production might lead to extend the production time.

Prasad and Sukumaran (1992) studied the effect of LP-system on the starter growth and sensory qualities of yoghurt. Results showed that good quality yoghurt can be prepared from cow milk stored after LP-activation. Yoghurt prepared from such stored milk had organoleptic qualities comparable with that prepared from LP-non treated milk. Sarkar and Misra (1994) also conducted a study on influence of LP-system on fermented milk products. It was concluded that the fermented milk products viz., Dahi, yoghurt and acidophilus milks, prepared from cow milk with 20:10 and 20:20ppm (SCN^- : H_2O_2) showed no significant difference.

Kumar *et al.*, (1994 b) Studied the quality of products manufactured from milk, treated by LP-system with 70:30 (SCN^- : H_2O_2) and stored at 30°C for 12 h. Ghee prepared by the direct cream method had moisture, free fatty acid contents, iodine, peroxide, and thiobarbituric acid values that were similar to those of control ghee and showed little or no change during storage for 6 months at room temperature. No difference in composition or organoleptic characteristics were detected between paneer manufactured from LP-treated milk and fresh milk. It was concluded that preservation of buffalo milk with 70:30 (SCN^- : H_2O_2) LP-system for 12 h at

30°C did not affect the quality of paneer or the oxidative stability of Ghee during storage for 6 months.

2.11 Public health significance of LP-treated milk

Mammalian cells are not affected by LP-system. Several studies on the various type of mammalian cells have not revealed any toxic effects. (Haenstroem *et al.*, 1983; Tenovuo and Larjava, 1984). The LP-system possess selective damage to the bacterial cytoplasmic membrane but not to mammalian cells.

Milk was thought to have a goitrogenic effect because of its SCN^- content. It was clearly demonstrated that milk containing the highest concentration of SCN achievable by feeding goitrogenic plants to cows failed to prevent accumulation of labelled iodine by the thyroid gland in man.

A reduction of iodide uptake could only be achieved by a dose of at least 200 to 400 mg, which would be equivalent to an intake of 10 to 20 litres of milk containing 20ppm of thiocyanate and if the iodine intake is adequate, ten times the dose of SCN^- would be required. (Vilkki *et al.*, 1962) and the renal clearance of SCN^- also increases in proportion to the quantity of SCN^- ingested (Ermans *et al.*, 1972).

In a study (Dahlberg *et al.*, 1984) a group of people were given an extra daily quantity of 8 mg of SCN^- by way of milk. Based on the measurements of levels of Thyroxine, Triiodothyronine and thyrotropic hormone it was concluded that this additional intake of SCN^- did not have any apparent effect on thyroid function.

Thakar and Dave (1986) activated the LP-system in buffalo milk with the levels of 20:20 and 30:30ppm (SCN^-:H_2O_2) at 30°C and they estimated the residual thiocyanate level, when the milk samples gave positive on 'COB' and found that the residual levels of thiocyanate were 5.5, 5.5. and 6.0ppm with untreated, 20:20 and 30:30ppm LP- system . It was concluded that the added SCN^- was completely utilized in the presence of adequate activity of lactoperoxidase, irrespective of the storage temperature.

It is well documented that SCN^- is not concentrated in the Thyroid gland (Meyers *et al.*, 1978) Therefore, it is very unlikely that ingestion of milk containing 25ppm of SCN^- could interfere with the iodination of Thyroxine. Further, if the iodine intake is adequate ten times of the dose of SCN^- is required to cause thyrostatic effect (Gujaral *et al.*, 1985).

It may be easily estimated that dose level of 200 to 400 mg could be equivalent to intake of 8 to 16 litres of milk containing 25ppm of SCN^-. In milk preserved with 25ppm of SCN^-, it is atleast one-fifth to one-eighth dilution of thiocyanate intake at blood serum level. This level will further reduce with the excretion of SCN^- though renal system and gastrointestinal tract. From the farthest stretch of imagination, SCN^- level in the blood plasma could never be expected to reach a point which could interfere with the thyroid function in human (Mathur and Ranju, 1995).

Kumar and Mathur (1989) did not observe any change in growth rate of rats after feeding freeze dried milk powder prepared from milk preserved with 25ppm and 75ppm of SCN^- levels. It was revealed that daily intake of about 5 mg SCN^- per kg body weight was not toxic. (Gujaral *et al.*, 1985).

Shenolikar and Babu (1989) conducted an experiment on monkeys. All the groups of monkeys were fed on milk containing 0.5ppm iodine as KIO3 along with 30ppm of SCN^-. In control groups normal milk was given to monkeys along with iodine deficient diet. The measurement of body weight and blood analysis for level of thyroid hormones (T3 & T4) were continued for one year. It was concluded that decrease in T4 level of serum was due to lower intake of iodine though the diet rather than by SCN^- and daily supplement of 25 mg iodine in diet would rectify the defect.

The possible effect of prolonged intake of milk with enhanced SCN^- level on thyroid function was studied by Dahlberg *et al.,* (1984) on healthy human beings by providing pasteurized milk containing 20ppm of SCN^- and 8.5ppm of H_2O_2. It was observed that the blood plasma level of Thyroxine, Triiodothyronine and Thyrotropic hormone were in normal range and no earlier signs of goiter or thyroid disfunctions were observed. Thus it was concluded that a daily in take of 8 mg SCN^- through 400 ml of milk containing 20ppm of SCN^- did not have any apparent effect on the thyroid function.

Studies carried out at National Institute of Nutrition established that ingesting milk containing SCN^- upto 50ppm for prolonged period did not alter the iodine status or thyroid function of monkeys. Therefore milk with 20ppm was absolutely safe and would not interfere with thyroid function even when the average consumption of milk approached the upper limit of daily intake. (Mathur and Ranju, 1995).

Materials & Methods

3. MATERIALS AND METHODS

Collection of raw milk samples

Fresh pooled cow's milk samples were collected from the University Livestock farm, Mannuthy in properly sanitized stainless steel vessels. The milk samples were immediately transferred to the laboratory for analysis. The time lapse between production of milk and the reception in the laboratory was less than one hour.

Activation of LP-system

In the laboratory, lactoperoxidase system was activated within two hours of production of milk. The collected samples were divided into three equal parts of one litre each. One part was kept as control (C) and in the other two parts activation of lactoperoxidase system was done by using freshly prepared one per cent solution of sodium thiocyanate and one per cent solution of hydrogen peroxide to have the concentrations of chemicals as detailed below:

Treatment Code	Levels of SCN^- : H_2O_2 added (in ppm)	
1. Control (C)	0:0	No exogenous addition
2. Treatment 1 (T1)	20:10	2 ml SCN^- solution plus 1 ml H_2O_2 solution per litre milk
3 Treatment 2 (T2)	20:20	2 ml each of SCN^- and H_2O_2 solutions per litre milk

Preparation of SCN^- solution

One per cent thiocyanate solution was prepared freshly by dissolving one gram of sodium thiocyanate in 100ml of sterile distilled water.

Preparation of H_2O_2 solution

One percent solution of H_2O_2 was prepared freshly by mixing 16.7 ml of six per cent W/V solution of H_2O_2 with 83.3ml sterile distilled water.

Storage of samples

The control and treatment samples were distributed in sterile bottles and kept in the incubator at 30 ± 1^0 C.

3.1 Analysis of milk sample

The physico-chemical and the microbial quality of the milk samples were analysed initially before the application of the treatment (0 h) and after the activation of LP-system at every 3h interval till the samples gave positive on clot-on-boiling test.

The following parameters were observed

3.1.1 Total viable count

Appropriate dilutions of the milk samples were prepared in sterile saline solutions. Plating was done according to the standard procedures. (IS : SP: 18 (Part - I) 1980) using plate count agar having the following composition.

Plate count agar (Pour Plate Technique)

Ingredients	**Gram / Litre**
Casein enzymic hydrolysate	: 5.0
Yeast extract	: 2.5
Dextrose	: 1.0
Agar	: 9.0

Final pH (at 25°C) 7.0 ± 0.2

17.5 gm of medium was suspended in 1000 ml distilled water, boiled to dissolve the medium completely. Sterilization done by autoclaving at 15 lbs pressure for 15 min.

The plates were incubated at 37 ± 1° c for 48 hours and then the total number of colonies were counted.

3.1.2 Coliform count

Appropriate dilutions of milk samples were prepared in sterile saline solution. Plating was done according to the standard procedure (IS: SP: 18 (part-I)-1980. using Violet red bile agar having the following composition.

Ingredients		**Grams / Litre**
Yeast extract	:	3.0
Peptone	:	7.0
Bile salt mixture	:	1.5
Lactose	:	10.0
Sodium chloride	:	5.0
Agar	:	15.0
Neutral red	:	0.03
Crystal violet	:	0.002

Final pH : 7.4 ± 0.2 (at 25 °c)

41.5 gm of medium was suspended in 1000 ml of sterile distilled water and the was boiled to dissolve completely.

The plates were incubated at 37± 1 °c for 18 - 24h. The colonies were counted in accordance with standard procedure.

3.1.3 Titratable Acidity

The titratable acidity of milk samples was determined according to IS: SP 18 (Part - XI) 1981.

Ten millilitre each of thoroughly mixed samples of milk were transferred to 100 millilitre conical flasks. A few drops of phenolphthalin indicator solution were added to the milk sample and titrated against the standard sodium hydroxide solution (0.1 N NaoH) to a pink colour. Titratable acidity was calculated from the titre value as percentage lactic acid.

3.1.4 pH

The pH of the milk samples were measured by using pH scan 2 (Merck) pH meter.

3.1.5 One Hour Resazurin reduction test

One hour Resazurin Reduction test was performed as per the procedure described in IS:SP 18(part - XI) - 1981.

Ten millilitre each of milk samples were taken in sterile test tubes to which one millilitre of sterile standard resazurin solution was added. After inverting test tube twice, the test tubes were placed in a water bath at 37 ^{0}c.

After one hour, the test tubes were removed from the water bath and colour was immediately matched with the standard resazurin disc in the comparator.

3.1.5.1 Preparation of standard Resazurin solution

Stock solution was prepared (0.05 per cent w/v) by dissolving resazurin in glass distilled, sterile water. Bench solution was prepared (0.005 per cent) by diluting with sterile water . This was prepared freshly after every eight hours.

3.1.6 Methylene blue reduction test

Methylene blue reduction time was determined as per the procedure described in IS:SP 18 (part - XI) - 1981.

Ten millilitre each of milk samples were transferred to sterile test tubes, to which one millilitre of methylene blue solution was added. Then the test tubes were inverted slowly twice and incubated in a water bath at 37 ^{0} c. The test tubes were examined and when the whole column of milk was decolourised or completely decolourised upto within 5 mm of the surface., it was considered as the endpoint.

3.1.7 Clot-on-boiling test

Clot-on-boiling test was performed as per the procedure described in IS:SP 18 (part -XI) 1981.

Five millilitre each of the samples were transferred to the test tubes and they were placed in a boiling water bath for five minutes. Then the test tubes were removed and smelled for any acidic flavour, and examined for the presence of precipitated particles.

3.1.8 Alizarin - Alcohol Test

Alizarin - Alcohol test was conducted as per the procedure described in IS:SP 18 (part - XI) 1981.

Five millilitre each of milk samples were transferred to sterile test tubes to which an equal quantity of the alizarin solution (0.2 percent in Ethylalcohol) was added. The contents of test tubes were mixed by inverting the test tubes several times and the mixture was examined for the colour and presence of flakes

Results

4. RESULTS

Milk samples of control group (C) and experimental groups (T1 and T2) after activation of LP-system, were stored at 30 ± 1°C and analysed for the quality after every 3 h of storage, till the samples gave positive results on clot-on-boiling test.

The milk samples were subjected to the following tests viz., standard plate count, coliform count, titratable acidity, pH, Methylne blue reduction time, Resazurin reduction time, Alizarin - Alcohol test and clot-on-boiling test .

The data presented in the case of analysis of control and experimental milk samples represent the average of six trials for the each of the parameters studied. The observations of standard plate count, coliform count, Titratable acidity and pH values were analysed statistically as per Snedecor and Cochran (1989).

The observations of the Methylene blue reduction test, Resazurin - reduction test, Alizarin - Alcohol test presented in the concerned tables are the average of six trials.

The analysis of variance technique was used to compare the different parameters of control and experimental samples.

4.1 Analysis of milk samples

Milk samples of control group (C) and experimental groups (T1 and T2) after activation of LP-system, were stored at 30 ± 1°C and analysed for the

quality after every 3 h of storage, till the samples gave positive results on clot-on-boiling test.

4.1.1 Standard Plate Count

The mean standard plate counts of control and treatment groups milk samples at various periods of storage are given in Table 1.

The mean standard Plate counts (SPC) of milk samples before the application of treatment was 5.280 ± 0.07 log cfu. per ml. The mean SPC of the three groups viz., C, T1 and T2 after 3 h of storage were 5.937 ± 0.21, 5.013 ±0.04 and 4.945 ± 0.02 log cfu per ml respectively. The corresponding values after 6 h of storage were 6.646 ± 0.22, 5.431 ± 0.14 and 5.370 ± 0.15 log cfu per ml respectively.

Analysis of variance of the data pertaining to the mean standard plate count presented in Table 2. shows significant difference ($p < 0.0.1$) between control (C) and treatment groups (T1 and T2) and no significant difference between treatments after 3 and 6 h of storage.

Both treatment groups T1 and T2 showed reduction in the SPC from the initial count as enumerated after 3 h of storage. The mean SPC of C, T1 and T2 after 9 h of storage were 7.349 ± 0.20, 6.277 ± 0.17 and 6.226 ± 0.18 log cfu per ml respectively.

After 12 h of storage the mean SPC of milk samples, viz., C, T1 and T2 were 8.092 ± 0.2, 6.866 ± 0.21, 6.808 ± 0.22 log cfu per ml respectively. The respective values after 15 hours of storage were 9.222 ± 0.18, 7.569 ± 0.21 and 7.523 ± 0.21 log cfu. per ml. There was significant difference

between the SPC of control and Treatment groups T1 and T2 upto 15 h of storage.

As compared to the standards suggested by Bureau of Indian Standards (BIS) the milk samples activated with both levels of LP-system remains 'very good' at 3 h of storage and remains 'Good' quality upto 9 h of storage where as the control samples remains 'Good' quality upto 3 h of storage.

Results presented in the Table 2 reveals no significant difference between treatment groups T1 and T2 during the entire period of storage.

The mean SPC of the control (C) and treatment groups T1 and T2 are pictured in Fig 1 reveals a steady increase in SPC in untreated samples (C) and in the case of T1 and T2 initially there is a reduction in the count after 3 h of storage and after 6 h of storage the SPC of T1 and T2 showed slight increase than the initial count.

The mean SPC, of milk samples C, T1 and T2 when the samples gave COB positive (Table 1a.) were 9.379 $\pm$0.15, 9.399 $\pm$ 0.20 and 9.319 $\pm$ 0.19 log cfu. per ml respectively and showed no significant difference between control (C) and treatments group and between T1 and T2.

4.1.2 Coliform count

The mean coliform counts of control and treatment samples at different successive periods of storage are presented in Table 3.

The mean initial coliform count was 1.587 $\pm$ 0.19 log cfu per ml.

Table.1: Effect of LP-system on standard plate count of raw cow's milk stored at 30° C

Standard plate count (log cfu per ml)			
Period of storage in h	Control	Treatment 1	Treatment 2
0	5.280 ± 0.07[a]	5.280 ± 0.07[a]	5.280 ± 0.07[a]
3	5.937 ± 0.21[a]	5.013 ± 0.04[b]	4.945 ± 0.02[b]
6	6.646 ± 0.22[a]	5.431 ± 0.14[b]	5.370 ± 0.15[b]
9	7.349 ± 0.20[a]	6.277 ± 0.17[b]	6.226 ± 0.18[b]
12	8.092 ± 0.20[a]	6.866 ± 0.21[b]	6.808 ± 0.22[b]
15	9.222 ± 0.18[a]	7.569 ± 0.21[b]	7.523 ± 0.21[b]
18	9.393 ± 0.07[a]	8.441 ± 0.24[a]	8.366 ± 0.26[a]
21	-	8.997 ± 0.24[a]	8.920 ± 0.24[a]
24	-	9.256 ± 0.28[a]	9.190 ± 0.26[a]

Means bearing the same superscript with in the same row do not differ statistically ($P < 0.01$)

Table. 1a: Standard Plate count of milk samples at 'COB' positive stage.

Log cfu per ml			
Sample No.	Control (C)	Treatment 1 (T1)	Treatment 2 (T2)
S1	9.898	9.826	9.792
S2	9.462	9.491	9.431
S3	9.613	9.431	9.255
S4	9.121	9.672	9.612
S5	8.857	8.431	8.462
S6	9.324	9.544	9.362
Mean ± SE	9.379 ± 0.15	9.399 ± 0.20	9.319 ± 0.19

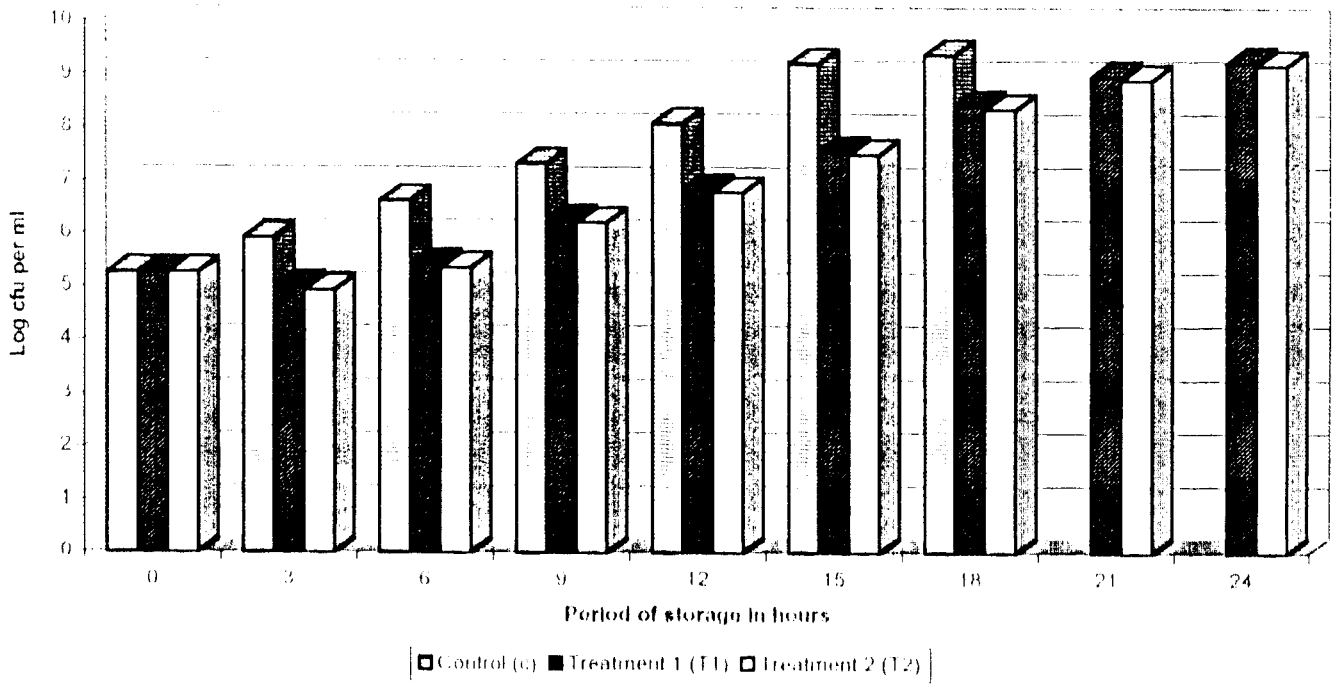
Table 2 Effect of LP-system on standard plate count of cow milk stored at 30°C - ANOVA

Period of storage(in h)	Source of error	df	SS	MSS	F Value
3	Between control and treatment groups.	2	3.686	1.843	19.359**
	With in treatment groups.	15	1.428	0.095	
	Total	**17**	**5.114**		
6	Between control and treatment groups.	2	6.220	3.110	17.255**
	Within treatment groups.	15	2.704	0.180	
	Total	**17**	**8.924**		
9	Between control and treatment groups.	2	4.829	2.414	12.183**
	Within treatment groups.	15	2.973	0.198	
	Total	**17**	**7.802**		
12	Between control and treatment groups.	2	6.313	3.156	11.957**
	Within treatment groups.	15	3.960	0.264	
	Total	**17**	**10.272**		
15	Between control and treatment groups.	2	11.250	5.625	23.376**
	Within treatment groups.	15	3.610	0.241	
	Total	**17**	**14.860**		
18	Between control and treatment groups.	2	1.696	0.848	2.538NS
	Within treatment groups.	11	3.677	0.334	
	Total	**13**	**5.373**		
21	Between control and treatment groups.	1	0.018	0.018	0.051 NS
	Within treatment groups.	10	3.495	0.349	
	Total	**11**	**3.513**		
24	Between control and treatment groups.	1	0.009	0.009	0.031 NS
	Within treatment groups.	6	1.710	0.285	
	Total	7	**1.719**		

NS - Not Significant

**** - Significant (P< 0.01)**

Fig. 1 Effect of LP System on standard plate count of raw cow's milk stored at 30°C



The mean coliform count of control (C) and experimental group (T1 and T2) after 3 h of storage were 2.083 ± 0.07 , 1.050 ± 0.05, 1.000 log cfu per ml, respectively. The corresponding values after 6 h of storage were 3.858 ± 0.23, 1.673 ± 0.11, 1.485 ± 0.13 log c,f,u per ml and after 9 h of storage 5.423 ± 0.33, 3.440 ± 0.32 and 3.328 ± 0.33 log cfu per ml respectively.

Analysis of variance of the date presented in Table 4 showed significant difference ($p < 0.01$) between control (C) and treatment groups (T1 and T2) and no significant difference between treatment groups viz., T1 and T2 after 3,6 and 9 h of storage.

After 12 hours of storage the mean coliform count of milk samples viz., C, T1 and T2 were 6.414 ± 0.33, 4.655 ± 0.15 and 4.566 ± 0.16 log cfu. per ml respectively. The corresponding values after 15 hours of storage were 7.134 ± 0.21, 5.156 ± 0.15 and 5.093 ± 0.14 respectively.

Further, the coliform counts of control samples revealed significant difference ($p < 0.01$) from the coliform count of treatment groups (T1 and T2) and no significant difference between treatment groups, during the entire period of storage.

The mean coliform counts of milk samples are pictured in Fig.2 shows, a steady increase in coliform count in control (C) samples and a drop in the colifrom count in T1 and T1 samples, as enumerated after 3 h of storage and the coliform count of T1 and T2 samples just exceeds the initial count after 6 h of storage.

Table.3: Effect of LP-system on coliform count of raw cow's milk stored at 30^0 C

Coliform count (log cfu per ml)			
Period of storage in h	Control	Treatment 1	Treatment 2
0	1.587 ± 0.19^a	1.587 ± 0.19^a	1.587 ± 0.19^a
3	2.083 ± 0.07^a	1.050 ± 0.05^b	1.000 ± 0.000^b
6	3.858 ± 0.23^a	1.673 ± 0.11^b	1.485 ± 0.13^b
9	5.423 ± 0.33^a	3.440 ± 0.32^b	3.328 ± 0.33^b
12	6.414 ± 0.33^a	4.655 ± 0.15^b	4.566 ± 0.16^b
15	7.134 ± 0.21^a	5.156 ± 0.15^b	5.093 ± 0.14^b
18	7.265 ± 0.38^a	5.792 ± 0.17^b	5.727 ± 0.18^b
21	-	6.437 ± 0.23^a	6.377 ± 0.23^a
24	-	7.066 ± 0.26^a	7.012 ± 0.27^a

Means bearing the same superscript with in the same row do not differ statistically ($P < 0.01$)

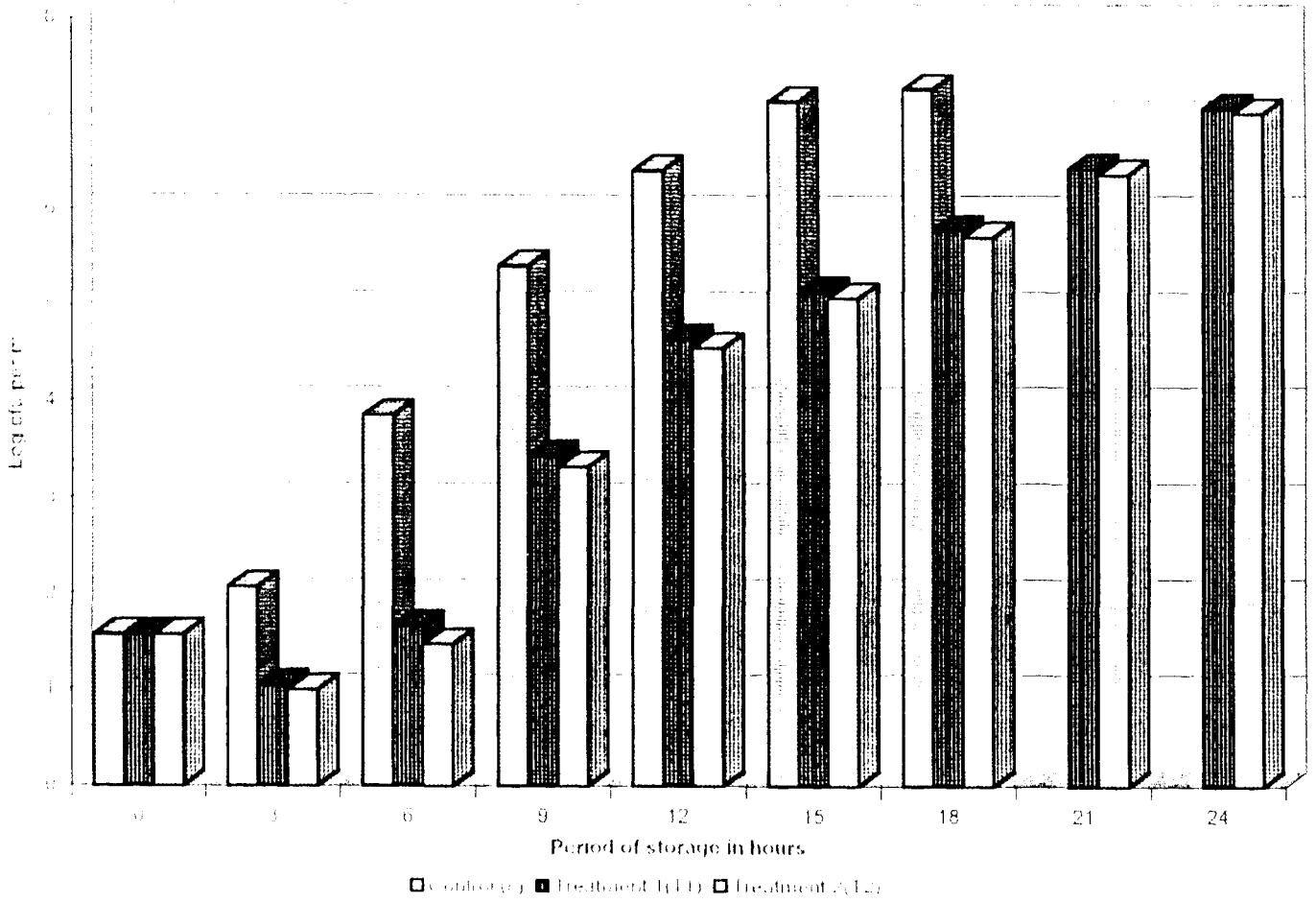
Table.4: Effect of LP-system on coliform count of raw cow's milk stored at 30° C - ANOVA

Period of storage(in h)	Source of error	df	SS	MSS	F Value
3	Between control and treatment groups.	2	4.483	2.241	151.246**
	Within treatment groups.	15	0.222	0.015	
	Total	**17**	**4.705**		
6	treatment groups.	2	20.889	10.445	62.489**
	Within treatment groups.	15	2.507	0.167	
	Total	**17**	**23.396**		
9	Between control and treatment groups.	2	16.734	8.367	13.063**
	Within treatment groups.	15	9.608	0.641	
	Total	**17**	**26.342**		
12	Between control and treatment groups.	2	13.032	6.516	21.003**
	Within treatment groups.	15	4.653	0.310	
	Total	**17**	**17.685**		
15	Between control and treatment groups.	2	16.174	8.087	45.939**
	Within treatment groups.	15	2.640	0.176	
	Total	**17**	**18.814**		
18	Between control and treatment groups.	2	3.897	1.948	10.425**
	Within treatment groups.	11	2.056	0.187	
	Total	**13**	**5.952**		
21	Between control and treatment groups.	1	0.018	0.018	0.051 NS
	Within treatment groups.	10	3.495	0.349	
	Total	**11**	**3.513**		
24	Between control and treatment groups.	1	0.009	0.009	0.031 NS
	Within treatment groups.	6	1.710	0.285	
	Total	7	1.719		

** - Significant ($p<0.01$)

NS - Not significant

Fig. 2 Effect of LP-System on coliform count of raw cow's milk stored at 30°C



4.1.3 Titratable acidity

The mean values of titratable acidity (expressed as per cent lactic acid) in control (C) and treatment samples T1 and T2 at different periods of storage at 30°C are shown in Table 5

The initial mean titratable acidity (0 h) of milk samples was 0.148 ± 0.01 per cent lactic acid. The mean titratable acidity of three groups viz., C, T1 and T2 after the 3 h of storage were 0.162, 0.153 and 0.152 per cent lactic acid respectively. Analysis of variance of the data, with regard to the titratable acidity are presented in Table 6. Results showed no significant difference in the titratable acidity between control and treatment samples and in between treatment samples stored for 3 h.

The titratable acidity of three sample groups viz., C, T1 and T2 after 6 h of storage were 0.178 ± 0.01, 0.155 and 0.155 per cent lactic acid. The corresponding values after 9 h of storage were 0.195, 0.165 and 0.165 per cent lactic acid.

The titratable acidity values of control samples (C) differ significantly ($p < 0.01$) from both treatment samples (T1 and T2) and there is no significant difference between treatment T1 and T2 samples after 6 and 9 h of storage.

After 12 hours of storage the mean titratable acidity of milk samples viz., C, T1 and T2 were 0.220 ±0.01, 0.175 ± 0.01 and 0.175 ± 0.01 per cent lactic acid respectively. After 15 h of storage the samples viz., C, T1 and T2 reached 0.258 ± 0.02, 0.193 ± 0.01 and 0.187 ± 0.01 per cent lactic acid respectively showing significant difference ($p < 0.01$) between control and treatment groups and no significant difference between treatment groups T1 and T2.

Table.5: Effect of LP-system on Titratable acidity of raw cow's milk stored at 30^0 C

Titratable acidity (percent lactic acid)			
Period of storage in h	Control	Treatment 1	Treatment 2
0	0.148 ± 0.01 [a]	0.148 ± 0.01 [a]	0.148 ± 0.01 [a]
3	0.162 ± 0.00 [a]	0.153 ± 0.00 [a]	0.152 ± 0.00 [a]
6	0.178 ± 0.01 [a]	0.155 ± 0.00 [b]	0.155 ± 0.00 [b]
9	0.195 ± 0.00 [a]	0.165 ± 0.00 [b]	0.165 ± 0.00 [b]
12	0.220 ± 0.01 [a]	0.175 ± 0.01 [b]	0.175 ± 0.01 [b]
15	0.258 ± 0.02 [a]	0.193 ± 0.01 [b]	0.187 ± 0.01 [b]
18	0.280 ± 0.01 [a]	0.213 ± 0.01 [b]	0.207 ± 0.01 [b]
21	-	0.252 ± 0.01 [a]	0.247 ± 0.01 [a]
24	-	0.290 ± 0.01 [a]	0.290 ± 0.01 [a]

Means bearing the same superscript within the same row do not differ statistically ($p<0.01$)

Table.5 a: Titratable Acidity (in per cent Lactic acid)

Period of storage in h	Control (C)						Treatment 1 (T1) 20:10 ppm (SCN^-: H_2O_2)						Treatment 2 (T2) 20:20 ppm (SCN^-: H_2O_2)					
	S1	S2	S3	S4	S5	S6	S1	S2	S3	S4	S5	S6	S1	S2	S3	S4	S5	S6
0	0.17	0.14	0.14	0.14	0.15	0.15	0.17	0.14	0.14	0.14	0.15	0.15	0.17	0.14	0.14	0.14	0.15	0.15
3	0.18	0.16	0.16	0.15	0.16	0.16	0.17	0.15	0.15	0.14	0.15	0.15	0.17	0.16	0.15	0.14	0.15	0.15
6	0.21	0.17	0.17	0.17	0.18	0.17	0.17	0.16	0.15	0.14	0.15	0.15	0.17	0.16	0.15	0.15	0.15	0.15
9	0.21	0.18	0.18	0.19	0.21	0.20	0.18	0.16	0.17	0.17	0.16	0.16	0.18	0.16	0.17	0.17	0.16	0.15
12	0.25	0.20	0.20	0.21	0.24	0.22	0.21	0.16	0.17	0.17	0.18	0.17	0.21	0.16	0.17	0.17	0.18	0.18
15	0.32	0.23	0.20	0.26	0.29	0.25	0.21	0.18	0.18	0.17	0.21	0.21	0.21	0.17	0.18	0.17	0.21	0.20
18	-	0.27	0.23	-	-	0.29	0.24	0.20	0.19	0.19	0.21	0.24	0.23	0.19	0.19	0.18	0.22	0.23
21	-	-	-	-	-	-	0.28	0.23	0.23	0.21	0.27	0.28	0.27	0.24	0.23	0.21	0.26	0.27
24	-	-	-	-	-	-	-	0.28	0.28	0.29	0.31	-	-	0.29	0.29	0.29	0.30	-

S1 To S6 - Samples of trials 1 to 6

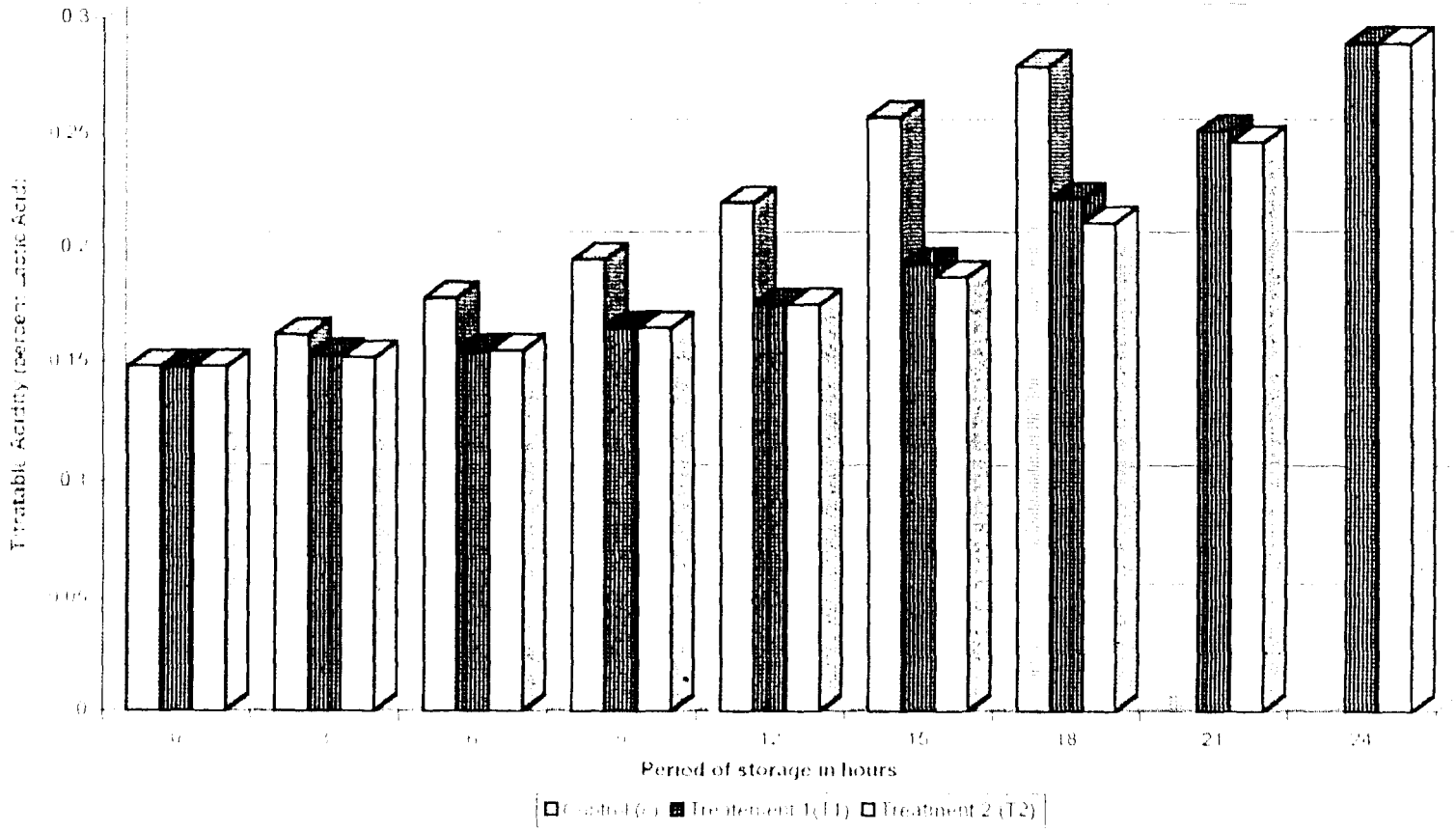
Table.6: Effect of LP-system on Titratable acidity of raw cow's milk at stored at 30^0 C - ANOVA

Period of storage(in h)	Source of error	df	SS	MSS	F Value
3	Between control and treatment groups.	2	0.000	0.000	1.722^{NS}
	Within treatment groups.	15	0.002	0.000133	
	Total	**17**	**0.002**		
6	Between control and treatment groups.	2	0.002	0.001	7.481**
	Within treatment groups.	15	0.002	0.000133	
	Total	**17**	**0.004**		
9	Between control and treatment groups.	2	0.004	0.002	14.595**
	Within treatment groups.	15	0.002	0.000133	
	Total	**17**	**0.006**		
12	Between control and treatment groups.	2	0.008	0.004	10.658**
	Within treatment groups.	15	0.006	0.000133	
	Total	**17**	**0.014**		
15	Between control and treatment groups.	2	0.019	0.009	10.568**
	Within treatment groups.	15	0.013	0.001	
	Total	**17**	**0.032**		
18	Between control and treatment groups.	2	0.009	0.004	9.263**
	Within treatment groups.	11	0.005	0.00047	
	Total	**13**	**0.014**		
21	Between control and treatment groups.	1	0.018	0.018	0.051^{NS}
	Within treatment groups.	10	3.495	0.349	
	Total	**11**	**3.513**		
24	Between control and treatment groups.	1	0.009	0.009	0.031^{NS}
	Within treatment groups.	6	1.710	0.285	
	Total	**7**	**1.719**		

** - Significant ($p<0.01$)

NS - Not significant

Fig 3 Effect of LP-System on Titratable acidity of raw cow's milk stored at 30°C



From the table 5, it can be concluded that the untreated milk samples remains within the acceptable limit (0.18 per cent lactic acid) only upto 6h of storage whereas milk samples of both treatments T1 and T2 remains well within the acceptable limit upto 12 h of storage.

The mean titratable acidity of the milk samples of all three samples are expressed in Fig.3 express that in the control samples (C) the titratable acidity shows a steady increase and in T1 and T2 samples the titratable acidity remains almost unchanged upto 6h storage and then shows a slight increase upto 12 h storage and after that show a steady increase.

4 .1.4 pH

The mean pH values of milk samples stored for different periods are given in Table 7.

The initial mean pH values of the samples was 6.70 $\pm$ 0.06. The mean pH values of samples of group C, T1 and T2 after 3 h of storage are 6.633 $\pm$ 0.02, 6.683 $\pm$ 0.02 and 6.683 $\pm$ 0.02 respectively.

Analysis of data on pH values given in Table 8, does not show significant difference between control (C) and treatment samples T1 and T2.

The mean pH values of samples C, T1 and T2 after 6 h of storage were 6.583 $\pm$ 0.02, 6.667 $\pm$ 0.02 and 6.683 $\pm$ 0.02 respectively. The respective values after 9 h of storage were 6.50, 6.650 $\pm$ 0.02 and 6.650 $\pm$ 0.02. The pH value of sample C differ significantly ($p < 0.01$) from T1 and T2 after 6 and 9 h of storage and there is no significant difference between treatment groups T1 and T2. After 12 hours of storage the pH values of milk samples viz., C, T1 and T2 were 6.433 $\pm$ 0.02, 6.60 $\pm$ 0.02 and 6.6 $\pm$ 0.02 respectively. The corresponding values after 15 hours of storage were 6.383 $\pm$ 0.03, 6.550 $\pm$ 0.02 and 6.550 $\pm$ 0.02.

Table.7: Effect of LP-system on pH of raw cow's milk stored at 30^0 C

pH			
Period of storage in h	Control	Treatment 1	Treatment 2
0	6.700 ± 0.06 [a]	6.700 ± 0.06 [a]	6.700 ± 0.06 [a]
3	6.633 ± 0.02 [a]	6.683 ± 0.02 [a]	6.683 ± 0.02 [a]
6	6.583 ± 0.02 [b]	6.667 ± 0.02 [a]	6.683 ± 0.02 [a]
9	6.500 ± 0.00 [b]	6.650 ± 0.02 [a]	6.650 ± 0.02 [a]
12	6.433 ± 0.02 [b]	6.600 ± 0.02 [a]	6.600 ± 0.02 [a]
15	6.383 ± 0.03 [b]	6.550 ± 0.02 [a]	6.550 ± 0.02 [a]
18	6.350 ± 0.05 [b]	6.483 ± 0.02 [a]	6.467 ± 0.02 [a]
21	-	6.383 ± 0.03 [a]	6.367 ± 0.02 [a]
24	-	6.275 ± 0.03 [a]	6.275 ± 0.03 [a]

Means bearing the same superscript within the same row do not differ significantly ($p < 0.01$)

Table.8: Effect of LP-system on pH of raw cow's milk stored at 30^0 C- ANOVA

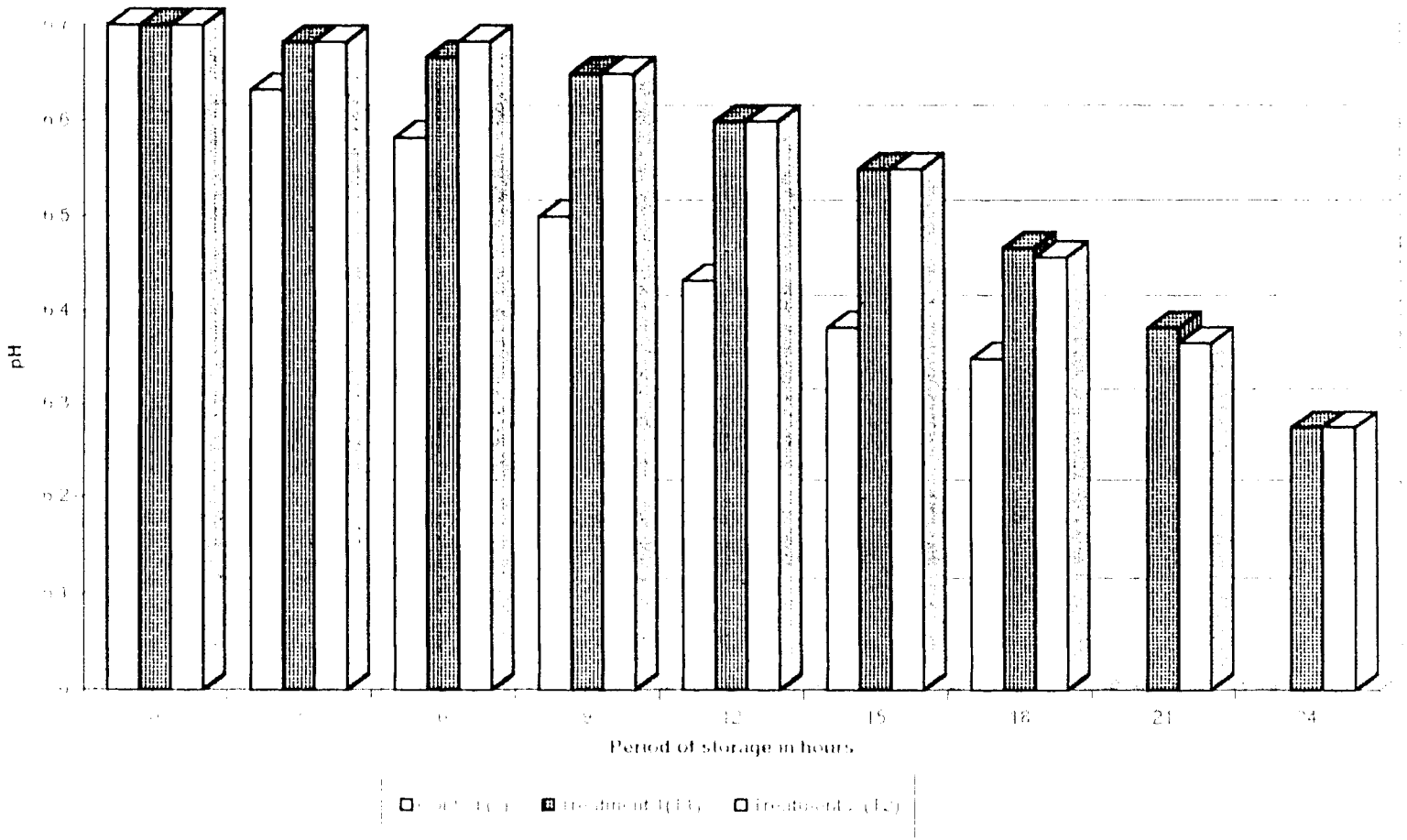
Period of storage (in h)	Source of error	df	SS	MSS	F Value
3	Between control and treatment groups.	2	0.010	0.005	2.500^{NS}
	Within treatment groups.	15	0.030	0.002	
	Total	**17**	**0.040**		
6	Between control and treatment groups.	2	0.034	0.017	8.611**
	Within treatment groups.	15	0.030	0.002	
	Total	**17**	**0.064**		
9	Between control and treatment groups.	2	0.090	0.045	22.500**
	Within treatment groups.	15	0.030	0.002	
	Total	**17**	**0.120**		
12	Between control and treatment groups.	2	0.11	0.056	15.625**
	Within treatment groups.	15	0.053	0.004	
	Total	**17**	**0.164**		
15	Between control and treatment groups.	2	0.111	0.056	14.286**
	Within treatment groups.	15	0.058	0.004	
	Total	**17**	**0.169**		
18	Between control and treatment groups.	2	0.028	0.014	5.696*
	Within treatment groups.	11	0.027	0.002	
	Total	**13**	**0.054**		
21	Between control and treatment groups.	1	0.018	0.018	0.051^{NS}
	Within treatment groups.	10	3.495	0.349	
	Total	**11**	**3.513**		
24	Between control and treatment groups.	1	0.009	0.009	0.031^{NS}
	Within treatment groups.	6	1.710	0.285	
	Total	7	1.719		

*** Significant (p< 0.05)**

**** - Significant (p< 0.01)**

NS - Not significant

Fig. 4 Effect of I P-System on pH of raw cow's milk stored at 30°C



The analysis shows significant difference ($p < 0.01$) between C and treatment groups T1 and T2 and no significant difference between T1 and T2.

After 18 h of storage, the pH value of samples C, T1 and T2 are 6.350 $\pm$ 0.05, 6.483 $\pm$ 0.02 and 6.467 $\pm$ 0.02 respectively. Analysis shows significant difference ($p< 0.05$) between C and treatment groups T1 and T2 and no significant difference between treatment groups.

4.1.5 One Hour Resazurin - reduction test

The mean disc numbers of Resazurin reduction test of milk samples of all three sample groups are presented in Table 9.

The initial mean disc number of milk samples was 4. After 3 h of storage, the disc numbers for the sample groups C, T1 and T2were 2, 3 and 3 respectively. The corresponding values after 6 h of storage were 0, 2 and 2 and after 9 h of storage the milk samples T1 and T2 read 1.

The mean One-Hour Resazurin - reduction test reading (disc number) are depicted Fig5.

4.1.6 Methylene blue reduction test

The mean Methylene blue reduction time of all three group are given Table 10.

The initial mean methylene blue reduction time was 9 h. After 3 h of storage the C, T1 and T2 samples had a MBR Time of 5.0, 9.0 and 9.0 h respectively. There was no decrease in the methylene blue reduction time in samples T1 and T2. The recorded values after 6 h of storage were 1.0, 4.0 and 4.0 h respectively and after 9 h of storage were 0.5, 2.0and 2.0 h respectively. The corresponding vales after 12 h of storage were 0.5, 1.0 and 1.0 h.

Table.9 : One - Hour Resazurin reduction test

Disc Number			
Period of storage in h	Control	Treatment 1	Treatment 2
0	4	4	4
3	2	3	3
6	0	2	2
9	0	1	1
12	0	0	0
15	0	0	0
18	0	0	0
21	-	0	0
24	-	0	0

Table.10 : Methylene blue reduction test

Methylene blue reduction time (in h)			
Period of storage in h	Control	Treatment 1	Treatment 2
0	9.0	9.0	9.0
3	5.0	9.0	9.0
6	1.0	4.0	4.0
9	30 min	2.0	2.0
12	30 min	1.0	1.0
15	30 min	30 min	30 min
18	30 min	30 min	30 min
21	-	30 min	30 min
24	-	30 min	30 min

Fig. 5 Efffect of LP-System on raw cow's milk stored at 30°C as evluated by on hour Resazurin Reduction Test

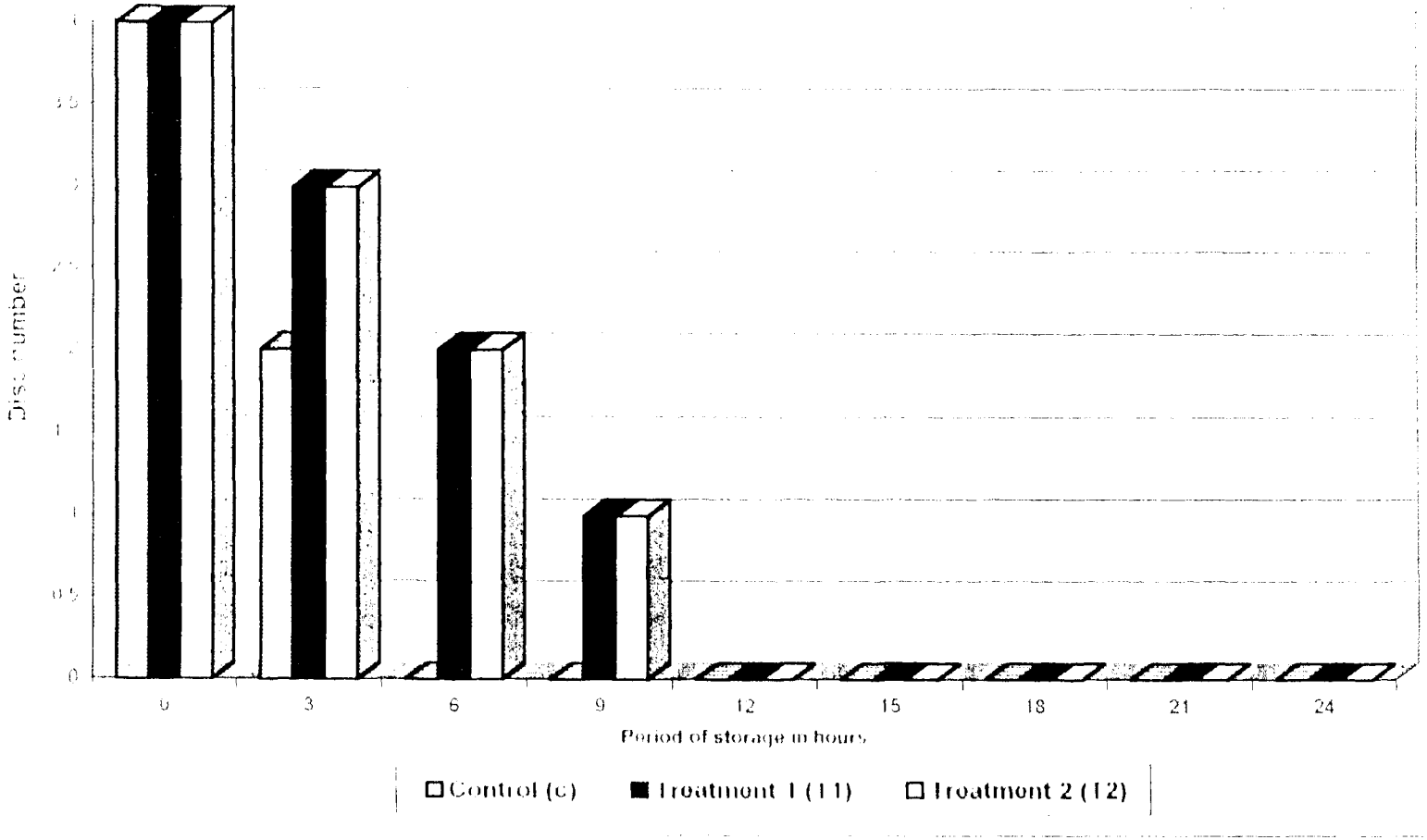
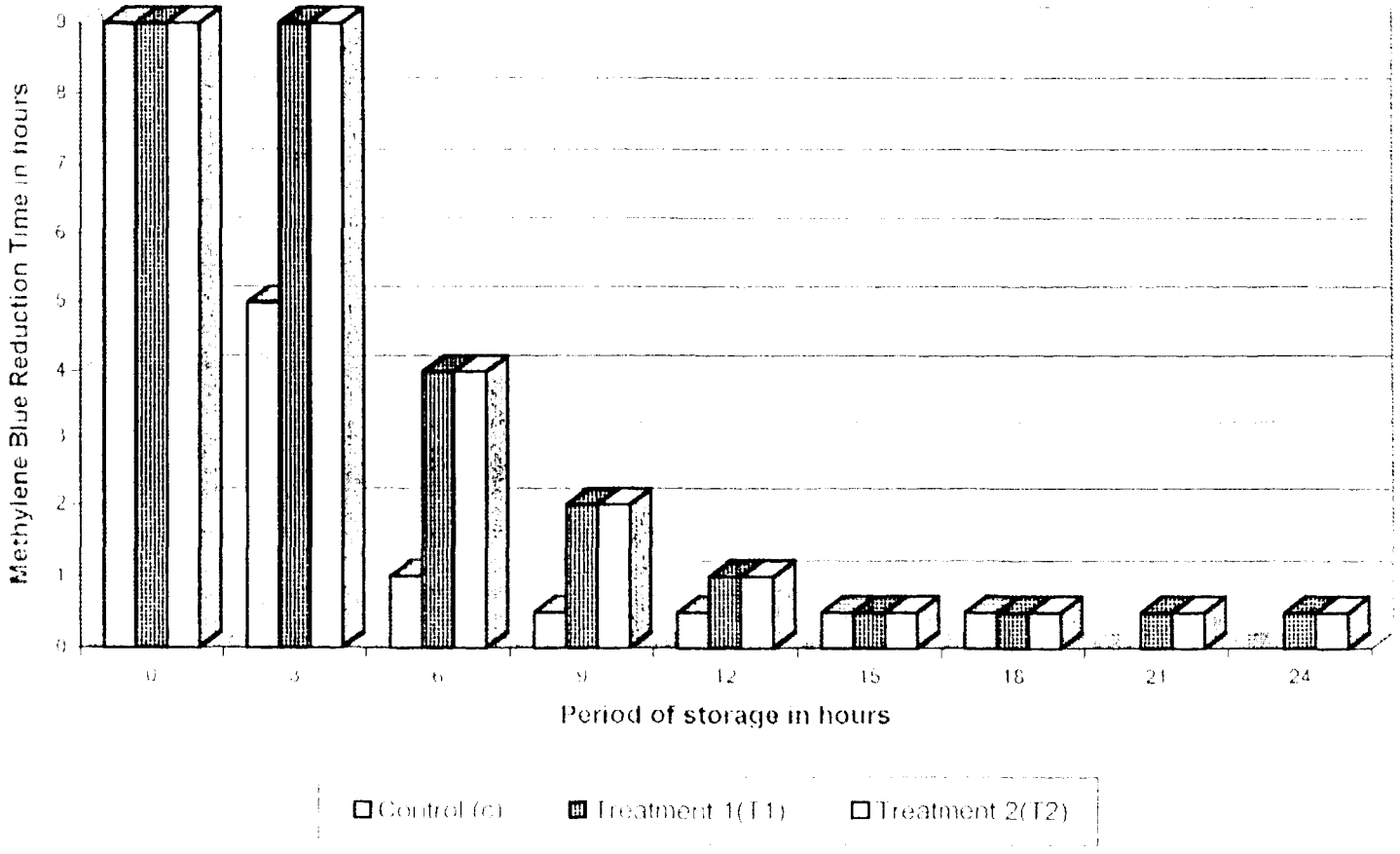


Fig 6 Effect of LP-System on Methylene Blue Reduction Time of raw cow's milk stored at 30°C



According to the standards described by BIS (IS: SP: 18 - Part XI, 1981), the control samples (C) remains 'Very Good' quality for 3 h and remains 'Fair' quality for 6 h of storage, whereas T1 and T2 samples remain 'Very Good' quality for 3 h of storage and remain 'Good' quality for 6 h of storage and they will be of 'Fair ' quality upto 12 h of storage.

Results presented in Table 10, shows that the treated samples T1 and T2 remain Fair quality upto 12 h of storage.

The mean Mthylene blue reduction time of milk samples are shown in Fig.6

4.1.7 Clot-on-boiling test

The result of Clot-on-boiling (COB) test studied, are shown in Table 11

Fifty per cent of the control samples (C) showed positive results on COB after 15h of storage and remaining 50 per cent samples gave positive result after 18 h of storage. These findings indicated that all the control samples had the shelf-life of more than 12 h based on the Clot-on-boiling test.

Regarding the LP-treated samples, all samples both T1 and T2 remained negative for COB test at 18 h of storage and 50 per cent samples showed positive test in 21 h and the remaining 50 per cent took more than 21 h to give a positive result

4.1.8 Alizarin - Alcohol test

The results of Alizarin - Alcohol test are presented in Table 12.

Table 11: Clot - on -boiling test

Period of storage in hr	Control (C)						Treatment 1 (T1) 20:10 ppm (SCN^-: H_2O_2)						Treatment 2 (T2) 20:20 ppm (SCN^-: H_2O_2)					
	S1	S2	S3	S4	S5	S6	S1	S2	S3	S4	S5	S6	S1	S2	S3	S4	S5	S6
0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
18		+	+			+	-	-	-	-	-	-	-	-	-	-	-	-
21							+	-	-	-	-	+	+	-	-	-	-	+
24								+	+	+	+			+	+	+	+	

S1 to S6 - Samples of trials 1 to 6

Approximately 70 per cent of the control samples (C) showed reddish brown to brown colour with flakes after 6h of storage and all the samples showed Reddish Brown to Brown colour with flakes indicating that the control samples showed instability after 6 h of storage.

In the case of experimental samples both T1 and T2, 90 per cent of the samples showed instability only after 12 h of storage. After 15 h of storage cent per cent samples showed instability. This indicated that milk treated with both levels viz., 20 : 10 and 20 : 20 ppm (SCN^- : H_2O_2) had extended period of stability than untreated milk by 6 h.

Table.12: Alzarin - Alcohol Test

Period of storage in h	Control (C)						Treatment 1 (T1) 20:10 ppm (SCN^-: H_2O_2)						Treatment 2 (T2) 20:20 ppm (SCN^-: H_2O_2)					
	S1	S2	S3	S4	S5	S6	S1	S2	S3	S4	S5	S6	S1	S2	S3	S4	S5	S6
0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	2	2	2	1	2	2	1	1	1	1	2	1	1	1	1	1	2	1
6	3	3	2	2	3	3	2	2	2	1	2	1	2	2	2	1	2	1
9	3	3	3	3	3	3	3	2	3	2	2	2	3	2	3	2	2	2
12	4	3	3	4	4	4	3	3	3	3	3	3	3	3	3	3	3	3
15	4	4	4	4	4	4	3	3	3	3	3	3	3	3	3	3	3	3
18	-	4	4	-	-	4	4	3	3	4	4	4	4	3	3	4	4	4
21	-	-	-	-	-	-	4	4	4	4	4	4	4	4	4	4	4	4
24	-	-	-	-	-	-	-	4	4	4	4	-	-	4	4	4	4	-

Direction: colour of mixture and nature of flakes

1 - Lilac red with no flakes
2 - Pale red with no flakes
3 - Reddish brown to brown with flakes
4 - Brownish yellow to yellow with large flakes

Discussion

5. DISCUSSION

Milk, one of the highly nutritious food, is easily perishable and likely to get spoiled rapidly by growth of contaminating micro-organisms. Being rich in nutrients, milk provides an excellent medium for bacterial multiplication.

In India, milk in small quantities are produced by millions of rural farmers and collected at village societies and from there it is transported to distant dairy plants. Techno-economic difficulties are faced in chilling of raw milk under rural conditions, leading to poor micro-biological quality of milk reaching dairy plants for processing.

Hence it is important to adopt suitable measures to preserve the quality of milk during procurement and transportation from village societies to dairy plants. One such feasible measure which can be adopted at village societies is Lactoperoxidase system (LP-system)

In this present study, the effect of LP-system with different levels of thiocyanate and hydrogen peroxide, on the micro-biological quality and shelf-life of milk stored at 30°C was studied. The results obtained in the experimental study are discussed in this chapter.

5.1 Analysis of milk samples

Milk samples of control group (C) and experimental groups (T1 and T2) after activation of LP-system, were stored at 30 $\pm$ 1°C and analysed for the quality after every 3 h of storage, till the samples gave positive results on clot-on-boiling test.

5.1.1 Standard plate count

The data presented in Table 1, on standard plate count (SPC) of milk samples revealed that the initial mean SPC of milk samples was 5.280 ± 0.07 log cfu per ml which falls under the category of 'Very Good' quality as per BIS (IS:SP:18 (Part XI)-1981 standards.

After 3 h of storage at 30°C, the SPC of C, T1 and T2 samples were 5.937 ± 0.21, 5.013 ± 0.04 and 4.945 ± 0.02 log cfu per ml respectively. The SPC of control samples showed a increase while treatment groups T1 and T2 showed a reduction in the count from the initial count.

Analysis of data on standard plate count, presented in Table 2, showed significant difference ($p<0.01$) between control (C) and treatment groups (T1 and T2) and no significant difference between the treatment groups T1 and T2. These findings are in close agreement with the findings of Thakar *et al*, (1990) who reported that LP-system has a bactericidal effect for about the first 4 h of storage.

The mean SPC of milk samples C, T1 and T2, after 6h of storage were 6.646 ± 0.22, 5.431 ± 0.14 and 5.370 ± 0.15 log cfu per ml respectively. The SPC of control samples (C) showed a steady increase whereas the SPC of treated milk samples both of T1 and T2 showed a slight increase than the initial count. As compared with BIS standards (IS:SP:18 (part XI) 1981) the control milk samples (C) fell under 'Fair quality' where as the milk samples of T1 and T2 remained as ' Good quality' even after 6 h of storage at 30°C.

These observations are similar to the findings of Bibi and Bachmann (1990) who reported that bacteriostatic effect of LP-system, depending on the storage temperature lasts for 6 h at 30°C and 20 h at 20°C.

Almost similar observations were made by Bjorck *et al.*, (1979) who observed that the bacteriostatic effect of LP-system lasts for 7-8 h at 30°C. Chakraborty *et al.*, (1986) and Sarkar and Misra (1994 b). also reported the same trend in the LP-treated milk.

Table 1 also revealed that there was a significant difference ($p<0.01$) between control group (C) and treatment groups (T1 and T2) and no significant difference were observed between the treatment groups T1 and T2 during the entire period of storage.

The mean SPC of milk samples C,T1 and T2 after 9 h of storage were 7.349 $\pm$ 0.20, 6.277$\pm$ 0.17 and 6.226$\pm$ 0.18 log cfu per ml and the corresponding values after 12 h of storage were 8.092$\pm$ 0.20, 6.866$\pm$ 0.21 and 6.808$\pm$ 0.22 log cfu per ml respectively. The milk samples C can be categorised as 'Good' quality only upto 3 h of storage while the treatment groups T1 and T2 remained as 'Good' quality even after 9 h of storage at 30°C as per BIS standard (IS:SP:18 (part-XI) 1981).

The mean SPC of milk samples C, T1 and T2 when gave 'COB' positive were 9.379 $\pm$ 0.15, 9.399$\pm$ 0.20 and 9.319$\pm$0.19 log cfu per ml (Table 1a) respectively and showed no significant difference between control (C) and treatment groups and within treatment groups T1 and T2.

The mean SPC of milk samples as pictured in Fig.1 shows a steady increase in SPC in the control (C) samples and T1 and T2 samples shows a drop in the SPC after 3 h of storage. The reduction in the SPC in the treatment samples may be attributed to the bactericidal effect. These findings are comparable with the findings of the Kumar and Mathur (1994) who observed a mild bactericidal effect resulted in a small drop in the mesophilic

count from the initial count when milk was activated with 25:15ppm (SCN-: H_2O_2) LP-system.

5.1.2 Coliform count

Data on the mean coliform count of control (C) and treatment group T1 and T2, at successive period of storage are presented in Table 3.

The mean initial coliform count (CC) was 1.587 ± 0.19 log cfu per ml. The mean coliform count of control (C) and experimental groups T1 and T2 after 3 h of storage were 2.083 ± 0.07, 1.050 ± 0.05 and 1.000 log cfu per ml respectively. In control samples the coliform count showed a steady increase while the CC of T1 and T2 samples showed a reduction from the initial coliform count. These observations agree with the findings of Thakar *et al.*, (1990) who reported that the bactericidal effect of LP-system lasted for about 4 h. These findings also agree with the findings of Bjorck *et al.*, (1979) who observed that the preservative effect of Lactoperoxidase system was mainly bacteriostatic and the effect lasts for 7-8 h at 30°C. Bjorck *et al.*, (1975) reported that LP-system inhibited gram positive bacteria and was bactericidal for gram negative bacteria. The bactericidal effect of LP- system against the gram negative bacteria was also reported by Purdy *et al.*, (1983)

The mean coliform count of milk samples viz., C, T1 and T2 after 6h of storage were 3.858 ± 0.23, 1.673 ± 0.11 and 1.485 ± 0.13 log cfu per ml respectively. The T2 milk samples showed a less coliform count than the initial count whereas T1 milk samples showed a slight increase than the initial count. Table 4, shows no significant difference between treatments T1 and T2.

The mean coliform count of milk samples C, T1 and T2 after 9h of storage were 5.423 ± 0.33, 3.440 ± 0.32 and 3.328 ± 0.33 log cfu per ml respectively. These observations are comparable with the findings of Bjorck *et al.*, (1979) who observed that the preservative effect of lactoperoxidase system was mainly bacteriostatic and the effect lasted for 7-8 h at 30°C.

The results of present work are also in agreement with the work of Patel and Sannabhadti (1993) They reported that LP-system had a marked effect on coliforms. Similar findings were reported by Edwin and Thomes (1978) Farrag and Marth (1992), Farrag *et al.*, (1992) and Sarkar and Misra (1994b).

Analysis of variance of the data pertaining to the mean coliform counts presented in Table 4, express significant difference ($p<0.01$) between control (C) and treatment groups (T1 and T2) and no significant difference between treatment groups viz., T1 and T2 during the entire period of storage.

Fig 2, shows a steady increase in coliform count in the control samples from the initial period where as in the case of treatment samples T1 and T2 shows a steady increase only after 9 h of storage.

5.1.3 Titratable acidity

The data pertaining to this parameter are presented in Table 5 and 6. The initial mean titratable acidity of milk samples was 0.148 ± 0.01 per cent lactic acid.

The titratable acidity of samples C, T1 and T2 after 3 h of storage at 30°C were 0.162, 0.153 and 0.152 per cent lactic acid. After 6 h of storage

the titratable acidity of milk samples C, T1 and T2 were 0.178 ± 0.01, 0.155 and 0.155 per cent lactic acid respectively. The corresponding values after 9 h of storage were 0.195, 0.165 and 0.165 per cent lactic acid and after 12 h of storage were 0.220 ± 0.01, 0.175 ± 0.01 and 0.175 ± 0.01 per cent lactic acid.

Since the maximum acceptable level of titratable acidity is 0.18 per cent lactic acid (Oysun and Oztek. 1988) , from the Table 5, it may be inferred that the control (C) milk sample remain within the acceptable limits only upto 6 h of storage while milk samples of T1 and T2 remains well within the acceptable limit upto 12 h of storage.

This slower development in titratable acidity may be attributed to the bacteriostatic effect of LP-system which usually lasts for 7-8 h at 30°C (Bjorck *et al.*, 1979).

This is in close agreement with the findings reported by Gupta *et al.*, (1986) Harnulv and Kandasamy (1982) and Sarkar and Misra (1994 a). The findings of the present work are comparable with the findings of Ghosh and Ghatak (1995) who studied the efficacy of the LP-system activated with 10:10, 25:10, 30:15 and 40:20ppm (SCN^-: H_2O_2) levels, in cow and buffalo milk at 30°C, and observed that the cow milk remained good quality upto 10 h with all levels of SCN^-: H_2O_2.

Oysun and Oztek (1988) also reported that the shelf -life of cow milk can be extended to 6h with 10:10 and 8 h with 15:15ppm (SCN^-: H_2O_2) activation.

Gupta *et al.,* (1986) studied the effect of LP-system on percentage titratable acidity with different combination of SCN^-: H_2O_2.and reported that the milk samples showed 0.171, 0.144 and 0.144 per cent titratable acidity after 8 h of storage with untreated, 10:10 and 20:20ppm (SCN^-:H_2O_2.) levels of LP-system . Thakar and Dave (1986) also reported that at 23°C, titratable acidity of milk increased after 14.2,16.2and 19.2h in the presence of 10:10, 20:20 and 30: 30ppm (SCN^-: H_2O_2.) levels respectively.

Analysis of variance of the data on titratable acidity (Table 6), showed no significant difference in the titratable acidity after 3 h of storage between control and treatment samples as well as between treatment groups T1 and T2. However, significant difference ($P<0.01$) between control (C) and both treatment groups T1 and T2 and no significant difference in between treatment groups T1 and T2 were present after six h of storage till the samples get spoiled.

The result of the present study is comparable with that reported by Bjorck *et al.,* (1979) who reported that the bacteriostatic effect of LP-system lasted for 7- 8 h at 30°C and at 25 and 20°C it lasted for 11-12 and 15-16 h respectively.

5.1.4 pH

The mean pH values of milk samples stored at 30°C for successive periods are presented in Table 7.

The initial mean pH values of the milk samples was 6.700 ± 0.06. The mean pH values of milk samples C, T1 and T2 after 3 h of storage were 6.633 ± 0.02, 6.683 ± 0.02 and 6.683 ± 0.02 respectively and after 6h of storage were 6.583 ± 0.02, 6.667± 0.02 and 6.683 ± 0.02 respectively. The corresponding values after 12 h of storage were 6.433 ± 0.02 , 6.600± 0.02 and 6.600 ± 0.02 respectively.

The mean pH values of milk samples as pictured in Fig.4, shows slower decrease in pH in the T1 and T2 samples upto 12 h of storage and then shows a steady decline in pH value. In the control samples it shows a steady declined from the initial period itself.

The slow decrease observed in pH values upto 12 h of storage may be attributed to the slow rate of development of the acidity which in turn may be due to the bacteriostatic effect of LP-system that usually lasts for 7-8 h at 30°C as reported by Bjorck *et al.*, (1979).

The results are comparable with results observed by Harnulv and Kandasamy (1982), Sarkar and Misra (1994a) and Ghosh and Ghatak (1995).

The results of the present study are in close agreement with the findings of Thakar and Dave (1986). They reported that when the milk was preserved with 30:30ppm at 23°C the titratable acidity of milk increased after 19.2 h whereas in untreated milk, the acidity began to increase after 7.4 h at 23°C.

The data presented in Table 7 revealed that upto 3 h of storage there was no significant difference between control (C) with treatment groups

T1 and T2. After 6 h of storage there was significant difference ($p<0.01$) between control (C) and treatment groups T1 and T2and no significant difference between T1 and T2. This same trend continued till the control samples (C) showed positive on clot-on-boiling test.

Further, no significant difference was observed in the pH values of treatment groups T1 and T2 during the entire period of storage.

5.1.5 One - hour Resazurin reduction test

The results of Resazurin - reduction test presented in Table 9. revealed that the initial mean disc number of milk samples was 4 and when the sample groups were subjected to Resazurin reduction test after 3 h of storage at 30 $\pm$ 1°C the samples C, T1 and T2 read the disc numbers 2,3 and 3 respectively. The corresponding values after 6 h of storage were 0,2 and 2 and after 9 h of storage the milk samples T1 and T2 read 1.

The findings of the work is in close agreement with those of Bjorck *et al.*, (1979) who observed that the bacteriostatic effect lasted for 7-8 h at 30°C.

As compared with the standards suggested by BIS (IS:SP:18 (Part - XI) - 1981), the control samples (C) remained 'Fair' quality only for 3 h at 30°C and the LP-treated samples T1 and T2 remain 'Fair' quality for 9 h at 30°C. The milk samples treated with both levels of LP-system viz., 20:10 and 20:20ppm. (SCN^-:H_2O_2) had equal period of shelf-life.

5.1.6 Methylene blue reduction time

The mean Methylene blue reduction time of milk samples of all three groups C, T1 and T2 are presented in Table 10.

The initial mean Methylene blue reduction time of milk samples was 9 h. After 3 h of storage the C, T1 and T2 samples had an MBR time of 5.0, 9.0 and 9.0 h respectively. The recorded values after 6 h storage were 1.0, 4.0 and 4.0 h respectively and after 9 h of storage were 0.5, 2.0 and 2.0 h respectively. The corresponding values after 12 h of storage were 0.5, 1.0 and 1.0 hour.

As compared with the standard described by BIS (IS:SP:18 (Part - XI) 1981) the untreated control samples remained 'Fair' quality only upto 6 h of storage, whereas the milk samples of T1 and T2 remained 'Good' quality even after 6 h of storage and they would be of 'Fair' quality upto 12 h of storage.

These observation are comparable with the findings of Kumar and Mathur (1989) who observed that milk samples treated with 25:15 and 70:30ppm (SCN^-: H_2O_2) had the keeping quality of 7 h and 11 h respectively on the basis of MBR Test and 10 h and 14 h respectively on the basis of titratable acidity.

These observations are also comparable with the observation of Bjorck *et al.*, (1979) and Bachmann (1990)

5.1.7 Clot-on - boiling test

The results of clot-on-boiling test (COB) as shown in the Table 11, indicated that the untreated control milk samples (C) had the shelf-life of 12 hours whereas milk samples of both treatments T1 and T2 had the shelf - life of 18 hours at 30°C. This is in close agreement with the result obtained by Kumar and Mathur (1989) who showed that when the raw buffalo milk treated with the 70:30 LP-system the shelf - life was 11 h, on the basis of clot-on-boiling test .

These findings are also comparable with the observations of Harnulv and Kandasamy (1982), who evaluated the acceptability of raw milk preserved by 11.6:8.5ppm (SCN^-: H_2O_2) and reported that after 10 h of storage, 80 per cent samples were within the acceptability limit according to the clot-on-boiling test. In the present study, the LP-treated milk samples with both levels of SCN^-:H_2O_2 had a shelf-life of atleast 18 h at the storage temperature 30°C. The milk samples of present study had longer shelf-life as compared with observations made by Kumar and Mathur (1989), Sarkar and Misra (1994a), Ghosh and Ghatak (1995) and Harnulv and Kandasamy (1982). This might be attributed to the initial micro-biological quality of milk samples of present study.

The enhanced shelf-life of milk treated with LP-system depends on the initial microbial quality of milk. Dhanalakshmi (1988) studied the shelf-life of raw milk samples of different initial micro-biological quality with 30:30ppm (SCN^-:H_2O_2) and observed that shelf-life of milk samples with initial MBR time of viz., > 6 h, 5-6 h, 3-4 h and < 2 h had enhanced shelf-life of 4 h, 2 h 56 min, 2 h 33 min and 1 h 30 min respectively.

Since the initial micro-biological quality of milk samples of present study was 'Very Good' they had more enhanced shelf-life.

5.1.8 Alizarin - alcohol test

The results of Alizarin - Alcohol Test presented in Table 12 showed that the untreated milk samples remained stable only for 6 h at 30°C and the milk samples treated with both levels of LP-system viz., 20:10 and 20:20ppm (SCN^-:H_2O_2) remained stable for atleast 12 h based upon Alizarin - Alcohol test.

The shelf-life of milk samples of present study was less based on Alizarin-Alcohol test as compared with clot-on-boiling test. This may be attributed to the higher sensitivity of Alizarin-Alcohol test. (Sukumar, 1996).

The present findings were comparable with the observations of Hanulv and Kandasamy (1982) who evaluated the acceptability of raw milk preserved by 11.6 : 8.5ppm (SCN^-:H_2O_2) and reported that after 10 h of storage, 80 per cent samples were within the acceptable limit according to COB Test and only 50 per cent samples were within the acceptable limit based on the Alcohol test.

Conclusion

The results of the present study shows that the raw cow milk, by the activation of LP-system with the addition of thiocyanate and hydrogen peroxide at the levels of 20:10 and 20:20ppm (SCN^-:H_2O_2) respectively can be preserved for at least 12 h at 30°C. The untreated milk samples remained good quality only upto 3 h of storage whereas the milk samples of both treatment groups remained good quality upto 9 h of storage at 30°C.

Since both T1 and T2 treatments are equally effective on the micro-biological quality and shelf-life of raw milk as observed in the present study treatment T1 can be recommended for the temporary preservation of raw milk under ambient storage temperature.

During the XXII Dairy Industry Conference held at Trivandrum of Kerala, the Chairman of the 'Technology Mission of India' Dr. V. Kurian has stressed the need for preservation of raw milk by LP-system under ambient temperature conditions. Moreover, the joint FAO/WHO Expert Committee on Food Additives has cleared that LP-system is acceptable. In the present Dairy Industry set up in India, being a simple, less expensive and safe method LP-system is recommendable for preservation of raw milk under ambient storage temperature.

Summary

6. SUMMARY

The present study was conducted to evaluate the effect of lactoperoxidase system on the micro-biological quality and shelf-life of raw cow milk stored at 30°C.

The mean value of Standard Plate Count (SPC) of raw milk before the application of treatment was 5.280 log cfu per ml. The mean SPC, after 3 hours of storage, for the untreated control (C) milk samples and milk samples treated with 20:10ppm (SCN^-:H_2H_2) levels (T1) and with 20:20ppm (SCN^-:H_2H_2) levels (T2) were 5.937, 5.013 and 4.945 log cfu per ml respectively. The study revealed significant difference between control and treatment groups ($P<0.01$) and no significant difference between the treatment groups. The mean SPC of both treatment samples remained significantly different from control samples upto the storage period of 15 hours. The control milk samples remains good quality only upto 3 h of storage where as the milk samples of both treatment samples remained good quality upto 9 hours of storage at 30°C . The SPC of control samples showed a steady increase while the SPC of treated milk samples both of T1 and T2 showed a slight increase than the initial count only after 6 h of storage.

The mean coliform count (CC) of raw milk before the application treatment was 1.587 log. cfu per ml. After 3 hours of storage the coliform count for C, T1 and T2 were 2.083, 1.050 and 1.000 log. cfu per ml respectively. There was reduction in the coliform count of both treatment group. The coliform count showed significant difference ($P<0.01$) between the control group. The coliform count showed significant difference ($P<0.01$) between the control group and treatment groups and no significant difference between treatment groups throughout the storage period.

The mean titratable acidity of milk samples before treatment was 0.148 percent lactic acid. The titratable acidity after 6 hours of storage of T1,T2 and T3 milk samples were 0.178,0.155 and 0.155 percent lactic acid respectively. The untreated milk samples were with in the acceptable limit of titratable acidity only for 6 hours whereas the milk samples of both treatment groups were with in the acceptable limit of titratable acidity for 12 hours and throughout the storage period there was no significant difference between T1 and T2.

The mean pH values of milk samples prior to the application of treatment was 6.70. After 3 hours of storage the pH values of milk samples of C, T1 and T2 were 6.633,6.683 and 6.683 respectively showing no significant difference between the groups upto 3 hours of storage. After that, the milk samples of control group showed pH values differed significantly from both the treatment groups.

According to the Alizarin-Alcohol test the milk samples of control group (C) remained stable only upto 3 hours of storage where as the treatment group both T1 and T2 samples remained stable upto 9 hours of storage.

The milk samples of control group (C) had the shelf-life of 12 hours based on the clot-on-boiling test whereas the milk samples treated with both levels of LP-system had the shelf-life of 18 hours based on clot-on-boiling test.

The untreated, control (C) milk samples remained 'Fair' quality only upto 3 hours of storage whereas the milk samples of both treatment group T1 and T2 remained 'Fair' quality upto 9 hours of storage as per the one-hour Resazuin reduction test.

The raw milk samples took 9 hours to reduce methylene blue dye. The control (C) milk samples remained. Good quality upto 3 hours of storage and as a 'Fair' quality upto 6 hours of storage while the samples of both treatment T1 and T2 remained 'Very Good 'quality upto 3 hours of storage and 'Good' quality upto 6 hours of storage and maintained the 'Fair' quality upto 12 hours of storage as evaluated by MBR test.

The present study showed that the micro-biological quality of milk could be controlled and shelf-life can be improved by the activation of LP-system. Since both T1 and T2 treatments are equally effective treatment T1 can be recommended with 20 : 10 ppm (SCN^- : H_2O_2) for the temporary preservation of raw milk.

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MICROBIOLOGICAL QUALITY AND SHELF-LIFE OF RAW COW'S MILK PRESERVED BY LACTOPEROXIDASE SYSTEM

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ABSTRACT OF A THESIS

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ABSTRACT

A detailed study was carried out to determine the micro-biological quality and shelf- life of raw cow's milk preserved by lactoperoxidase (LP) system. Literature related to the LP-system have been reviewed.

A total of 6 trails were conducted to obtain reliable data for statistical analysis. In each trail, three litres of raw cow milk was divided into three equal parts of one litre each. One part was kept as a control (C) and LP-system was activated in the other two parts one part with 20:10ppm (T1) and other with 20:20ppm (T2) (SCN^-:H_2O_2), with in two hours of production of milk. The milk samples were stored at 30 $\pm$ 1°C.

Before the activation of LP- system the micro-biological quality of raw milk, samples were analysed. After the activation of LP-system the micro-biological quality of control and experimental group milk samples were analysed once in every 3 hours, till the samples showed positive on clot-on-boiling (COB) test.

Standard plate count, Coliform count, Titratable acidity, pH, Methylene blue reduction time, one hour Resazurin reduction test, Clot-on boiling test and Alizarin - Alcohol test were the parameters studied.

The mean initial SPC of milk samples was 5.280 log cfu per ml. The control milk samples remained 'Good' quality only for 3 hours storage, while the LP-treated milk samples of both T1 and T2 remained 'Good' quality even after 9 hours of storage.

The standard plate count and coliform count (CC) of control milk samples showed a steady increase from the initial period itself, whereas in the LP-treated milk samples of T1 and T2 the SPC and CC showed a reduction than the initial count after 3 hours of storage and both SPC and CC slightly exceeded the initial count even after 6 hour of storage.

Based on the titratable acidity, the control milk had the acceptable quality (0.18 per cent lactic acid) of only upto 6 hours while the LP-treated milk remained with in the acceptable limit for 12 h.

According to the Alizarin - Alcohol test, the control milk remained stable only for 3 hours and the LP- treated milk remained stable for 9 hours of storage at 30 ± 1^{0}C. The milk samples of control group had the shelf-life of 12 hours where as the LP-treated milk samples of T1 and T2 had the shelf-life of 18 hours based on the clot-on-boiling test.

The micro-biological quality and shelf-life of milk samples, both T1 and T2 did not show significant difference during the entire period of storage.