EFFECT OF PRESERVATIVES ON MILK SOLIDS IN COW AND BUFFALO MILK

By

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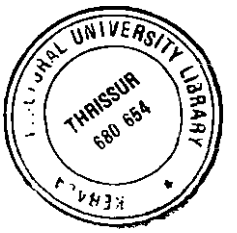

THESIS

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DECLARATION

I hereby declare that this thesis entitled **"EFFECT OF PRESERVATIVES ON MILK SOLIDS IN COW AND BUFFALO MILK"**, is a bonafide record of research work done by me during the course of work and this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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CERTIFICATE

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K.RADHA

Dedicated To
My Father

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LIST OF ABBREVIATIONS

BIS	-	Bureau of Indian Standards
COB	-	Clot on Boiling
EDTA	-	Ethylene Diamine Tetra Acetate
g	-	Gram
ml	-	Millilitre
PFA	-	Prevention of Food Adulteration Act

Introduction

1. INTRODUCTION

India has emerged as the world's largest milk producing country with biggest and fastest growing market for milk and milk products. Dairy industry is now occupying a place of pride in the national economy. India's milk production is expected to exceed 86 million tonnes during the year 2001 with a value output of over ten lakh millions.

Dairy farming is an integral part of our rural households. Nearly 60 million households own either cattle or buffaloes for their livelihood as a source of supplementary income. The last three decades noticed an outstanding improvement in the dairy co-operative sector with over 70000 milk co-operatives across the length and breadth of the country to link even the remotest areas to the National Milk Grid.

Milk production in Kerala showed a phenomenal increase from 2.21 lakh metric tonnes in 1963-64 to 20.73 lakh metric tonnes in 1997-98. This has been achieved by genetic improvement and better marketing facilities provided by dairy co-operatives in the state. While increasing the milk production, emphasis should also be given for assuring the proper quality for milk and milk products.

The existing two axis pricing system of milk based on fat and solids- not- fat content warrants testing each sample of

milk at dairy co-operatives and quality control laboratories in order to make payment. The highly perishable nature of the milk coupled with enormous number of milk samples received for chemical analysis makes it virtually impossible to test all the samples with in a short period. Milk samples will also get spoiled easily, if not tested within a short period. This is much important especially in rural areas, where facilities for transportation or refrigeration of milk samples are limited. Therefore preservation of milk samples using chemical preservatives has become inevitable. Besides being efficient, chemical preservatives are quite cheaper and have longer duration of preservative effect than the refrigeration.

The minimum requirement for a suitable chemical milk preservative is that it must assure testability of the sample. The milk sample must be maintained with its original composition from the time of collection to the time of analysis. An ideal milk sample preservative should have broad-spectrum of activity, high water solubility stability, compatibility and non-toxic.

Various chemical preservatives have been tried for the preservation of milk samples meant for chemical analysis, but their effect on milk constituents are not well established.

In India as per the Prevention of Food Adulteration Act (1954), addition of 0.4 per cent formalin has been legally

permitted for the preservation of milk and milk product samples intended for chemical analysis. Effect of formalin on Gerber fat test has been a controversial subject in the recent past. Several organised dairies in India have also enquired about the effect of formalin on the existing Gerber method, as their standardized milk samples were found substandard under Prevention of Food Adulteration Act (1954). Recently many other chemicals like potassium dichromate, mercuric chloride, sodium azide, sodium sulphate, bronopol and sorbic acid have also been studied by various workers as milk sample preservatives in India and abroad. However reports available on the effect of these chemicals on milk solids are limited under Indian conditions and there is no authentic information in this regard. So the present investigation was undertaken with the following objectives:

1. To assess the effect of chemical preservatives such as formalin, potassium dichromate and bronopol on percentage of fat, total solids and solids not fat in cow and buffalo milk.
2. To determine the impact of these preservatives on physical properties of milk such as pH, titratable acidity, lactometer reading and clot on boiling test.
3. To recommend the most suitable preservative with least effect on the estimation of milk solids.
4. To know the duration of preservation of raw and pasteurized milk on addition of different preservatives.

Review of Literature

2. REVIEW OF LITERATURE

The ideal means of preserving milk samples prior to compositional analysis is refrigeration as near the freezing point as possible without freezing the milk. This prevents the growth of bacteria and all the chemical and physical changes following such growth. As sample refrigeration is not always possible/feasible, chemical means of preservation is resorted. The minimum requirement for a chemical milk sample preservative is that it must assure the testability of sample. Chemical preservatives are highly efficient and have longer duration of preservative effect than the refrigeration. Various chemicals have been studied for the preservation of milk samples meant for chemical analysis. Literature concerning the efficiency of various chemical preservatives and their influence on milk solids are critically reviewed below.

2.1 Formalin

Formalin being a strong antiseptic and disinfectant, is used in the preservation of various anatomical specimens and also food samples. Being carcinogenic in nature its use for edible purposes has been prohibited. Under the PFA act (1954) formalin has been permitted as a preservative for milk, channa, cream, dahi

and ice cream samples meant for chemical analysis. Formalin inhibits the enzyme activity and prevents the growth of micro organisms and is capable of controlling the bacteriological spoilage of milk samples for a long period of storage.

2.1.1 Preservation efficiency

Armandola (1967) used formalin with four per cent para formaldehyde for the preservation of raw milk samples and found that it was suitable for the preservation of milk samples though the acidity increased slightly.

Armandola (1969) conducted storage experiments with several preservatives and found that 0.04 per cent formalin with 0.4 per cent trioxymethylene was suitable for preserving milk samples. He also suggested thorough mixing of preservatives with milk samples for better results.

Rakshy and Hassan (1971) studied the effect of formalin on keeping quality of milk samples and reported that 0.8 and 3.2 μg/ml was ideal for preserving milk samples. They also reported complete inhibition of *Streptococcus* strain of bacteria in the formalin preserved milk samples.

Bector and Narayanan (1973) observed complete inhibition of mould growth in milk samples preserved with

0.4 per cent formalin up to six months of storage at room temperature.

Bansal and Singhal (1991) reported that addition of formalin even at low levels completely inhibited the proliferation of bacteria in milk samples.

Karmakar and Ghatak (1995) studied the duration of storage of formalin preserved buffalo milk samples and reported that the samples remained in good condition throughout the entire storage period of 30 days under refrigerated condition. They also reported similar findings in cow milk samples preserved with formalin (Karmakar and Ghatak, 1997).

2.1.2 Effect of formalin on milk analysis

Ashworth *et al.* (1960) reported that the use of formalin lowered the percentage of protein when the milk was stored at room temperature. The extent of lowering depended not only on the amount of formalin added, but also on the length of storage and temperature.

Formalin proved unsatisfactory for fat estimation by Milko-Tester and protein estimation by pro-milk method but

excellent for total solids estimation via total milk solids tester at 0.3 per cent concentration (Kvapilik and Sachanek, 1975).

Hussain *et al.* (1984) studied five categories of preservatives and found no significant difference in fat percentage of formalin preserved samples. They also found no significant difference in solids not fat percentage of samples preserved with formalin-hydrogen peroxide combination

Drastic reduction in protein content in the formalinised milk was reported by Jeyachandran *et al.* (1984).

Sandhu *et al.* (1984) reported that formalin at a concentration of 0.4 per cent as recommended in the Prevention of Food Adulteration Act (1954) was suitable for the effective preservation of milk samples and there was no significant change in fat and solids not fat content of milk samples when stored in glass bottles for twelve months at ambient temperature.

Kroger (1985) found erroneous results for protein analysis when the samples were preserved with formalin.

The Gerber method gave consistently low fat values in milk samples preserved with formalin. The fat values decreased with the increase in formalin concentration and hydrometer

reading of milk samples. The storage of the preserved milk samples further affected the Gerber fat results. Rose -Gottlieb method also gave slightly low fat values in milk samples containing 0.4 per cent formalin (Desraj and Singhal, 1987).

Jandal and Rai (1988) suggested modification in the existing Gerber method by increasing the acid concentration from 91 to 93 per cent for samples treated with preservatives.

Jandal and Rai (1989) reported no change in colour, physical appearance, fat percentage and solids not fat percentage of formalinised samples for the entire storage period of three months. However, they reported increase in titratable acidity. They also recommended a modification in Gerber method by raising the Sulphuric acid concentration from 90 to 93 per cent and incubation temperature from 65 to 70^{0}C for five minutes.

Bansal and Singhal (1991) found an increase in titratable acidity of formalin preserved samples with the increase in formalin level and storage period. Initial increase in acidity was due to reaction of formalin with milk proteins liberating hydrogen ions and further increase in acidity during storage was attributed to proteolytic activity of formalin in milk.

Bajaj and Rai (1992) studied the comparative efficiency of various analytical methods for fat, solids not fat and total solids in formalin preserved samples of cow and buffalo milk and concluded that Majonnier method was more suitable in formalin preserved milk samples followed by modified Gerber method for fat determination.

Bajaj and Rai (1993) reported an increase in titratable acidity of fresh milk from 0.14 to 0.21 per cent and 0.15 to 0.23 per cent immediately after addition of formalin in cow and buffalo milk respectively. The acidity continued to increase further with increase in storage period. The author also concluded that the increase in acidity was due to the reaction of formalin with primary amino, amide, and guanidyl groups.

Karmakar and Ghatak (1995) studied the effect of chemical preservatives on chemical quality of buffalo milk stored under refrigeration. They reported no change in fat and protein contents but decrease in lactose content in the formalinised samples during storage. Further they observed that formalin was more effective in controlling the development of titratable acidity and proteolysis during storage than potassium dichromate. In another study they reported similar findings in cow milk preserved with formalin (Karmakar and Ghatak, 1997).

Viveksharma and Desraj (1999) studied the effect of formalin as sample preservative on lactoperoxidase-hydrogen peroxide system activated milk and reported no significant change in lactose and protein values even after the storage of six months.

Formalin preserved samples could not be used for estimation of somatic cell count (SCC) by optical methods like Membrane Filter – DNA method (Sathian, 2001).

2.1.3 Public health aspects

Occupational laryngitis caused by formaldehyde in Finnish dairy foremen was reported by Roto and Sala (1996).

Formaldehyde inhalation may cause sore throat, coughing and shortness of breath. Concentrations of 25 to 30ppm will cause severe respiratory tract injury leading to pulmonary edema and pneumonitis. It may be fatal in high concentrations. Formaldehyde is also a severe skin irritant and sensitizer. Chronic exposure may lead to contact dermatitis. The International Agency for Research on Cancer (IARC) has determined that formaldehyde is probably carcinogenic to humans (Toxicological Profile for Formaldehyde, 1999).

2.2 Potassium dichromate

2.2.1 Preservation efficiency

Rao *et al.* (1950) reported that addition of potassium dichromate was suitable for keeping milk in liquid condition and fit for analysis for about five days.

Ashworth *et al.* (1960) reported that the use of potassium dichromate at a concentration of 0.125mg per 100 ml of milk was effective at room temperature.

Hamdy *et al.* (1961) studied number of preservatives for milk samples and reported that 0.2 per cent potassium dichromate was sufficient to keep the milk fresh for four days either cow or buffalo.

Waite and Taylor (1966) found that 0.025 per cent of potassium dichromate was sufficient to prevent any changes in composition of milk samples stored for 14 days at 20°C.

Kroger (1971) found that potassium dichromate at 0.08 to 0.8 per cent was effective for milk samples stored at 21 to 27°C without changes in fat percentage up to three days of storage.

Kvapilik and Suchanek (1975) studied four preservatives at different concentration and reported that a

saturated solution of potassium dichromate at 0.3 per cent was suitable for the preservation of the milk samples for two to seven days.

Wolfschoon-Pombo (1979) found that the use of potassium dichromate at 0.1 per cent level was effective for preserving milk samples up to three days at 23°C.

Kroger (1985) studied the effect of several preservatives and reported that potassium dichromate at 0.3 per cent was suitable for the estimation of fat (by Milko-Tester), protein (by pro-milk method) and total solids (Via total milks solids tester) and herd milk samples were effectively preserved with potassium dichromate in concentration of 0.3–0.4 per cent for up to 20 days under refrigeration.

Desraj and Singhal (1990) reported that 0.2 per cent potassium dichromate was effective for five to ten days and 0.4 per cent level was effective for one month for preserving the milk samples at room temperature.

Hanus *et al.* (1993) studied several preservatives and reported that potassium dichromate had the best preservative effect for 13 days at 20°C and for 15 days at 4°C. It was

recommended for the preparation of pilot samples for the Milko–Scan apparatus.

Foltys *et al.* (1995) compared the efficiency of Bronopol and potassium dichromate for preserving milk samples prior to infrared analysis. Bulk milk samples were stored for 14 days at 20°C or 17 days at 4°C and analysed daily for fat, protein and lactose contents. Potassium dichromate was found as the best preservative among the two.

2.2.2 Potassium dichromate and milk analysis

Ashworth *et al.* (1960) reported a loss of protein when 125mg of potassium dichromate was used per 100ml of milk. At first higher protein values were found. After one week of storage at room temperature the preserved samples had lower protein values than the controls stored in refrigeration.

Tarassuk and Abe (1963) reported that potassium dichromate interfered with the protein estimation by dye -binding methods and was not a suitable preservative for milk samples meant for analysis by dye -binding method.

Yusa and Tsuchiya (1963) compared several milk preservatives viz. mercuric chloride, potassium dichromate,

potassium chromate, sodium chromate and mixture of mercuric chloride and potassium dichromate for the preservation of milk samples. A mixture of mercuric chloride and potassium dichromate (1:1) was found to be the most effective, while potassium dichromate alone was the least effective.

Several workers studied the effect of potassium dichromate on the estimation of fat by Milko–Tester. No significant effect was observed by many of the workers (Cerna and Pisckey, 1966; Smith *et al.,* 1969; Borsi and Varga, 1971; Sotlar and Arsov, 1971 and Ol'Shevskii and Men'Shikov, 1974).

Preservation of milk samples with 0.5 per cent potassium dichromate for 14 days did not interfere with the fat test by Milko –Tester (Hedrick and Koch, 1967).

Bakke (1968) reported steady decline in fat per cent as determined by Milko-Tester in milk samples preserved with potassium dichromate at low level (0.05 per cent) and stored for four weeks. No effect was observed at higher level (0.3 per cent).

Armandola (1969) conducted storage experiments and found that 0.1 per cent potassium dichromate was the best preservative for analytical milk samples. Density and fat percentage remained unchanged for four months. The author also

suggested thorough mixing of preservatives with milk samples for satisfactory results.

Kroger (1971) observed 0.1 per cent lower fat in samples preserved with 0.08 to 0.8 per cent potassium dichromate for five days at 21 to 27°C. The author also reported that coagulated milk samples and those in which the original yellowish dichromate colour changed could not be accurately analysed for fat by Milko-Tester.

Uzonyi (1977) reported 0.05 per cent depression in fat content after seven days and 0.1 per cent after 14 days of storage of milk preserved with 0.24 per cent potassium dichromate and estimated by Milko –Tester.

Dunham *et al.* (1978) compared the efficiency of potassium dichromate against a new product (Chlorinated quaternized hexamine) and reported that fat percentage was 0.102 per cent lower after two days and 0.103 per cent lower after five days of storage in potassium dichromate preserved samples than the new product.

Wolfschoon-Pombo (1979) reported that the preservation of milk with 0.1 per cent potassium dichromate did not affect the pro-milk results in samples stored for less than three

days at 23^{0}C but after 15 days of storage, protein values dropped by 0.02 per cent. Foss Electric Co., Denmark has also recommended the use of 0.1 per cent potassium dichromate as a milk preservative in case any preservative is to be added, for the estimation of protein using Pro-milk method.

Vaitnus and Kazlauskaite (1982) reported that preservation of milk with potassium dichromate and subsequent storage for zero, three, six and nine days reduced the Milko–Tester fat values by 0.01, 0.04, 0.03 and 0.05 per cent respectively.

Ng-Kwai-Hang and Hayes (1983) reported a lower protein content in milk samples preserved with potassium dichromate when analysed by Milko-scan than the dye binding method. The difference increased linearly with increasing potassium dichromate concentrations.

Hussain *et al.* (1984) preserved the composite milk samples with 30 per cent potassium dichromate solution and analysed the milk samples by Gerber method after seven and ten days of storage. They reported that the mean fat values of preserved samples were lower than the control samples.

Lee *et al.* (1986) have compared the efficiency of potassium dichromate with bronopol and reported that fat and protein percentage was larger in bronopol preserved samples than

in the potassium dichromate preserved samples. They have also reported that effects of preservative, storage time and storage temperature were smaller for protein percentage than for fat percentage.

Desraj and Singhal (1990) reported that potassium dichromate at 0.4 per cent concentration did not affect the Gerber fat values just after addition. During storage the fat values decreased with the increase of storage period. Both Gerber and Rose–Gottlieb methods gave lower fat values in potassium dichromate preserved samples. The Rose-Gottlieb method gave much lower fat results than the Gerber method in the stored milk samples. He also observed that the decrease in fat values was more in buffalo milk than in cow milk.

Karmakar and Ghatak (1995) reported no change in fat and protein contents either on addition of potassium dichromate or during refrigerated storage for 30 days in buffalo milk. However lactose content decreased during storage and the samples became COB positive after 15 days of storage and the initial titratable acidity increased from 0.16 to 0.18 per cent immediately after the addition of 0.4 per cent potassium dichromate.

Benda (1996) studied the effect of some preservatives on natural micro flora in milk samples. The preservatives used were potassium dichromate, a milko fix preparation, sodium azide

and bronopol. There were highly significant differences between the preservative effects. Bronopol had the best preservative effect for all groups of micro organisms and a lower preservative effect was found for potassium dichromate.

Effect of chemical preservatives on different constituents of cow milk during storage under refrigerated condition was studied by Karmakar and Ghatak (1997). They have reported that potassium dichromate treated cow milk samples exhibited signs of curdling after 15 days of storage. Increase in acidity both immediately on addition of potassium dichromate and during storage was also reported. No significant change in fat and protein content but decrease in lactose content was reported.

Milk samples preserved with potassium dichromate could not be used for estimation of somatic cell count (SCC) by optical methods like Membrane Filter–DNA method (Sathian, 2001).

2.2.3 Public health aspects

Potassium dichromate generally used for the preservation of milk samples is toxic and can cause allergic reactions amongst the laboratory personnel (Kreuzer, 1981).

Molecular structure of potassium dichromate contains "chromium VI" which is a known irritant (Kroger, 1985).

Herzog *et al.* (1988) have opined that milk-testing laboratory workers are at substantial risk of acquiring allergic contact dermatitis from milk preserved with potassium dichromate.

A number of countries discontinued the use of potassium dichromate because of its toxic, allergenic and potentially carcinogenic properties (Gencurova *et al.*, 1995).

2.3 Bronopol (2–Bromo–2– Nitro–1, 3 Propanediol)

2.3.1 Preservation efficiency of bronopol

The effect of bronopol as a milk preservative was studied by Ardo (1979). Bronopol solution with methylene blue indicator was found to be suitable for the analysis of fat and protein contents using Milko –Tester automatic, Pro-milk automatic, Milko –Scan 300 and IRMA up to 9 days of storage at room temperature. However Rose-Gottlieb method gave low fat values after two days of storage. The author concluded that the preservation efficiency of 0.02 per cent bronopol was as good as of 0.1 per cent potassium dichromate during five days of storage at room temperature.

Jeunet and Grappin (1979) used 0.10 ml of 12 per cent bronopol as aqueous solution with 0.012 per cent methylene blue per 60 ml of milk samples. The samples were tested for fat and protein using Milko –Scan, IRMA, Milko –Tester, Amido black and Gerber methods in comparison to samples preserved with potassium dichromate and mercuric chloride. They concluded that bronopol was effective in milk samples having good bacteriological quality and stored below 20° C up to ten days.

Brunt and Higton (1985) suggested a tablet comprising of Bronopol and a water-soluble solid organic carboxylic acids such as citric, tartaric, malic, adipic, succinic and fumaric acids with citric acid being particularly preferred.

Ruttan (1993) recommended bronopol with natamycin as a milk preservative to store the samples for five to ten days. He also recommended mixture of 6-12mg bronopol and 0.3-0.6mg natamycin in the tablet form for the effective preservation of milk samples.

2.3.2 Bronopol and milk analysis

Dunham *et al.* (1978) reported bronopol as a satisfactory preservative for counting somatic cells by the microscopic method.

Ardo (1979) reported low fat values by Rose-Gottlieb method in bronopol preserved samples after two days of storage.

Ardo (1982) investigated the possibility of using the bronopol preserved milk samples to determine cell counts with a Fossomatic instrument. He observed no significant change in cell counts up to five days at room temperature and thereafter cell counts were about 10 per cent higher than in the untreated milk.

Kyla-Siurola (1982) reported that addition of bronopol had no significant effect on fat, protein and lactose determination by Milko –Scan 104. Cell counts of samples preserved with bronopol were 11 per cent higher than the unpreserved samples.

Lee *et al.* (1986) reported a higher fat and protein percentage in bronopol preserved samples than in the potassium dichromate preserved samples. They also reported that freezing of milk samples significantly lowered the fat percentage in bronopol preserved samples.

Foltys *et al.* (1995) compared the effectiveness of bronopol and potassium dichromate for preserving milk prior to infrared analysis and they stored the samples for 14 days at 20^0C or 17 days at 4^0C. They have recommended bronopol as a less toxic

alternative to potassium dichromate for the preservation of the milk samples.

Gencurova *et al.* (1995) reported that samples preserved with 0.04 per cent bronopol lasted eight days and samples with 0.1 per cent bronopol lasted 10 days at 4^0C and five days at 20^0C without significant change in composition. They have also recommended bronopol as a less toxic alternative to potassium dichromate for the preservation of milk samples.

Benda (1996) studied the efficiency of various preservatives like potassium dichromate, a milko fix preparation, sodium azide and bronopol on the micro flora of milk. He concluded that bronopol had the best preservative effect against all naturally occurring groups of micro organisms.

The study carried out by the Quebec dairy herd analysis service compared the performance of three preservatives viz. potassium dichromate, bronopol micro tab and broad-spectrum liquid bronopol. The components studied were milk fat, protein, and somatic cell count. Samples preserved with either of the bronopol preparations gave higher readings for milk fat and protein contents than those preserved with potassium dichromate (Monardes *et al.*, 1996).

2.3.3 Public health aspects

Herzog *et al.* (1988) reported that hand dermatitis is a frequent problem among workers in milk testing laboratories. An epidemiological study was conducted at the Pennsylvania DHIA milk-testing laboratory. Two of 15 subjects who underwent patch testing to milk preserved with potassium dichromate had positive reactions. None reacted to milk alone or bronopol preserved samples.

Many countries discontinued the use of potassium dichromate due to its toxic, allergenic and potentially carcinogenic properties and replaced it with less problematic preservatives such as bronopol or sodium azide (Gencurova *et al.*, 1995).

Materials and Methods

3. MATERIALS AND METHODS

Pooled milk samples were collected from cow and buffaloes maintained at the University Livestock Farm. About 3.5 litre of raw milk was collected at weekly intervals and transferred to 200 ml sample bottles. One sample from each cow and buffalo milk was pasteurised in the laboratory (63°C for 30 minutes) before the addition of preservative to find out the duration of preservation of pasteurised samples.

3.1 Addition of preservatives

Calculated levels of preservatives were added to the milk samples at the following rates.

Formalin	-	0.4 per cent
Potassium dichromate	-	0.4 per cent
Bronopol (2-Bromo- 2-nitro- 1,3 propanediol)	-	0.1 per cent

After the addition of preservatives, bottles were capped airtight and gently shaken for thorough mixing of preservatives with sample. Then the sample bottles were stored at room temperature in a dark place.

Each bottle was labelled with the type of milk (cow or buffalo), name of the preservative and date of collection. The entire procedure was repeated on six occasions.

3.2 Estimation of different parameters

Milk samples were analysed for pH, lactometer reading, clot on boiling test, titratable acidity, fat, total solids and solids- not-fat immediately after the addition of preservatives and thereafter at fortnightly intervals in formalin and potassium dichromate preserved samples. In bronopol preserved samples the analysis was done at four days interval until signs of spoilage appeared. Before each analysis the milk bottles were shaken well and warmed to 40°C for five minutes and cooled down to 27°C.

3.3 Estimation of pH

The pH of milk samples was analysed by Cyber Scan 2500 digital pH meter (Eurotech). After calibration, pH electrode was rinsed using distilled water to remove the impurities. Then measurement mode was selected and the electrode was dipped into the sample until the glass bulb completely immersed into the sample. The reading was directly noted down from the Light Crystal Display.

3.4 Titratable acidity

Titratable acidity of milk was determined by using the procedure described in Hand book of food analysis SP: 18 (Part XI) 1981(BIS).

Ten ml of thoroughly mixed milk sample was taken in two porcelain basins and equal volume of freshly boiled water was added after cooling. Then 1 ml of phenolphthalein indicator solution was added to one basin and one ml of bench solution of rosaniline acetate was added to the other. The contents of the basin containing phenolphthalein indicator was titrated against 0.1 N standard sodium hydroxide solution taken on the burette, until the colour matches the pink tint of the solution in the basin containing rosaniline acetate solution. Then the titratable acidity was calculated by using the following formula:

$$\text{Titratable acidity (as percentage of lactic acid)} = \frac{9V1N}{V2}$$

Where,

V1 - Volume in ml of the Sodium hydroxide required for titration

V2 - Volume in ml of the milk taken for the test

N - Normality of the Sodium hydroxide solution

3.5 Estimation of fat percentage

Milk fat percentage was estimated by Gerber method as per the procedure described in Hand book of food analysis SP: 18 (part X1) 1981 and also by Milko- Tester.

3.5.1 Estimation of fat by Gerber method

Ten ml of Gerber's sulphuric acid was taken in a clean dry butyrometer. To this exactly 10.75 ml. of well-mixed milk sample was added followed by one ml of amyl alcohol. Contents of butyrometer were mixed well and maintained at a temperature of 65^{0}C for five minutes. Then the butyrometer was centrifuged at the maximum speed for four minutes in a Gerber's centrifuge. Again the butyrometer was kept in the water bath at 65^{0}C for three minutes. Then the fat column was read after adjusting it into a main graduation. In formalin preserved milk samples 94 per cent sulphuric acid was used instead of Gerber's sulphuric acid.

3.5.2 Estimation of fat by electronic milko-tester

Milk fat percentage was estimated by using Electronic Milko- Tester as per the procedure given in the Technical Manual

of electronic Milko-Tester provided by Rajasthan Electronics and Instruments Limited (REIL), Jaipur.

3.5.2.1 Preparation of diluent

Diluent was used to dilute the milk samples and dissolve the proteins. Diluent was prepared by using the following chemicals and kept at room temperature.

EDTA	-	5.26g
Triton-X-100 (Emulsifier)	-	0.05ml
Anti foam	-	0.05ml
Distilled water	-	1000 ml

3.5.2.2 Procedure

The instrument was allowed to warm up before starting the estimation. This was done by switching on the instrument half an hour before estimation. Then de airing was done by pressing the milk- in and mix-out buttons alternatively to remove the air bubbles present in the syringes. A clean empty mix beaker was filled with diluent by pressing milk- in and mix- out buttons twice alternatively. Then the beaker was moved to the mix intake tube and the handle was operated up and down for six times. Then the zero knob was turned to adjust the read out to 0.05. After zero setting the repeat button was pressed to get one

decimal read out. Milk sample was mixed gently and placed under the milk intake tube and the milk- in button was pressed then the sample was removed and a clean mix beaker was placed under milk intake tube. The mix- out button was pressed to dispense the milk and diluent into the mix beaker and it was moved to mix intake tube. Then the homogenizer handle was operated up and down for three times and reading was noted down.

3.6 Total solids

Total solids percentage of milk was estimated by the Gravimetric method described in Hand book of food analysis SP: 18 (Part X1) – 1981.

3.6.1 Procedure

Clean dry empty stainless steel dishes with lids were weighed. Five ml of milk was pipetted into the dish and again weighed with the lid. Then the uncovered dish was placed on a boiling water bath. The base of the dish was kept horizontally to promote uniform drying. After 30 minutes the dish was removed and transferred to a well-ventilated hot air oven at 100^0C. After three hours the dish was covered and transferred immediately to the desiccator. The dish was allowed to cool for about 30 minutes and weighed. The dish was again transferred to the oven and

heated for one hour and transferred to the desiccator for cooling and weighed as before. Procedure was repeated until loss of weight between successive weighings did not exceed 0.5 mg. Lowest weight was noted. The percentage of total solids was calculated by using the following formula:

$$\text{Total solids percent by weight} = \frac{100\ w}{W}$$

Where,

w– Weight in g of residue after drying.

W – Weight in g of prepared sample taken for the test.

3.7 Solids-not-fat

Solids-not-fat content of milk was determined by finding the difference between total solids content and fat content of milk.

3.8 Lactometer reading

Lactometer reading was taken by using a certified Zeal's lactometer. Well-mixed sample of milk was filled in a glass cylinder upto 100 ml mark. The temperature of milk was brought to 29^{0}C. Then the lactometer was immersed into the cylinder without touching the sides of the cylinder. Then the reading

corresponding to the upper margin of the curved meniscus was noted down.

3.9 Statistical analysis

Data were analysed by one- way classified non-orthogonal analysis of variance to study the preservative effect on cow and buffalo milk samples. Mean and standard error were estimated for each parameter. The effects of three preservatives were studied by using analysis of variance (Snedecor and Cochran, 1980).

Results

4. RESULTS

In the present study three milk sample preservatives viz., formalin, potassium dichromate and bronopol were investigated for their effect on major milk components like fat, total solids and solids not fat at room temperature. The other parameters like pH, titratable acidity, clot on boiling test and lactometer reading were also studied. The preservation efficiency of each preservative under room temperature was also recorded in cow and buffalo milk samples. All these parameters were estimated in control and preserved samples. The analysis was done at fortnightly intervals in formalin and potassium dichromate preserved milk samples. In bronopol preserved milk samples the estimations were done at four days interval.

4.1 Formalin

Formalin treated raw and pasteurised milk samples could be preserved for the entire storage period of 90 days without curdling. The samples showed no colour change even after 90 days of storage at room temperature. Cream plug formed within 24 hours of addition of formalin. A white sedimentation appeared at the bottom after one month and it continued to increase in volume with the advancement of storage period. The mean values with

standard errors of different parameters for cow and buffalo milk samples are presented in Table 4.1 and 4.2 respectively.

4.1.1 pH

The average pH values of fresh cow and buffalo milk samples were 6.58 ± 0.02 and 6.76 ± 0.01 respectively. The values decreased to 6.46 ± 0.01 and 6.58 ± 0.03 immediately after addition of formalin. The pH continued to decrease and the values became 6.06 ± 0.02 and 6.21 ± 0.01 at 90 days of storage. The pH values decreased gradually with the advancement of storage period and reached significantly low value at 90 days. Analysis of variance between control and formalin preserved samples showed significant ($P<0.01$) difference.

4.1.2 Titratable acidity

The mean values of titratable acidity in control samples were 0.16 ± 0.01 and 0.13 ± 0.0 in cow and buffalo milk samples respectively. On addition of formalin the values increased to 0.21 ± 0.01 and 0.18 ± 0.01 at zero day and 0.27 ± 0.0 and 0.25 ± 0.01 at 90 days of storage. Titratable acidity increased gradually with the advancement of storage period and showed significantly ($P<0.01$) different values when compared to control samples.

4.1.3 Clot on boiling test

Formalin treated cow and buffalo milk samples remained COB negative throughout the entire storage period of 90 days.

4.1.4 Fat percentage (Gerber Method)

The mean fat percentage of fresh cow and milk samples were 3.40 ± 0.09 and 6.20 ± 0.22. The values became 3.32 ± 0.10 and 6.13 ± 0.22 on zero day of addition of formalin. The values at 90 days were 3.38 ± 0.09 and 6.18 ± 0.22 in cow and buffalo milk respectively. Fat percentage of formalin preserved samples did not vary significantly from the control values.

4.1.5 Fat percentage (Milko-tester)

The average fat per cent in control samples were 3.40 ± 0.09 and 6.20 ± 0.22 in cow and buffalo milk samples respectively. On addition of formalin the values became 3.33 ± 0.10 and 6.13 ± 0.21 on zero day. At 90 days of storage, the values were 3.43 ± 0.27 and 6.32 ± 0.34 in cow and buffalo milk respectively. The readings taken at different stages of storage showed inconsistent variation.

4.1.6 Total solids

The mean total solids percentages of fresh cow and buffalo milk samples were 12.45 ± 0.12 and 15.62 ± 0.48 respectively. On addition of formalin the values became 12.46 ± 0.13 and 15.58 ± 0.49 on zero day in cow and buffalo milk samples respectively. At 90 days of storage the total solids contents were 12.51 ± 0.12 and 15.59 ± 0.49 in cow and buffalo milk samples respectively. Total solids content between control and formalin preserved milk samples did not vary significantly.

4.1.7 Solids not fat

Solids not fat content of control samples were 9.05 ± 0.10 and 9.42 ± 0.27 in cow and buffalo milk samples respectively. On addition of formalin the values became 9.14 ± 0.12 and 9.45 ± 0.29 on zero day in cow and buffalo milk samples respectively. The estimated values at 90 days of storage were 9.12 ± 0.10 and 9.41 ± 0.28 in cow and buffalo milk samples respectively. Solids not fat content of control and preserved milk samples did not show any significant difference.

4.1.8 Lactometer reading

The average lactometer reading of control cow and buffalo milk samples were 29.67 ± 0.49 and 28.0 ± 1.39

respectively. On addition of formalin the values became 29.0 ± 0.52 and 27.5 ± 1.09 on zero day. At 90 days of storage the values were 28.67 ± 0.42 and 27.50 ± 1.09. Lactometer reading of control and preserved samples did not vary significantly.

4.1.9 Preservative effect of formalin on pasteurised milk

The pasteurised cow and buffalo milk samples could be preserved by formalin for the entire period of 90 days without any spoilage.

4.2 Potassium dichromate

Potassium dichromate preserved raw milk samples could be stored for a period of 30 days and pasteurised milk samples could be stored for 38 days at room temperature. The raw milk samples became green in colour and curdled after 30 days of storage. The average values with standard errors of different parameter in cow and buffalo milk samples are depicted in Table 4.3 and 4.4 respectively.

4.2.1 pH

The observed average pH values of control cow and buffalo milk samples were 6.58 ± 0.02 and 6.76 ± 0.01 respectively.

On addition of potassium dichromate the values decreased to 6.19 ± 0.02 and 6.27 ± 0.01 on zero day in cow and buffalo milk samples respectively. Afterwards the values increased to 6.42 ± 0.02 and 6.44 ± 0.02 at 30 days of storage in cow and buffalo milk respectively. Cow and buffalo milk samples preserved with potassium dichromate showed a highly significant difference ($p<0.01$) in pH when compared to control.

4.2.2 Titratable acidity

The estimated mean values of titratable acidity in control cow and buffalo milk samples were 0.16 ± 0.01 and 0.013 ± 0.0. On addition of potassium dichromate the values increased to 0.38 ± 0.01 and 0.36 ± 0.01 on zero day. At 30 days of storage the values became 0.28 ± 0.01 and 0.28± 0.01 in cow and buffalo milk samples respectively. Potassium dichromate preserved cow and buffalo milk samples had significantly ($P<0.01$) higher titratble acidity when compared to control.

4.2.3 Clot on boiling test

Potassium dichromate treated cow and buffalo milk samples remained COB negative up to 15 days of storage. Thereafter, it became COB positive.

4.2.4 Fat percentage (Gerber method)

The mean fat percentage of control cow and milk samples were 3.40 ± 0.09 and 6.20 ± 0.22 respectively. Fat percentage of potassium dichromate added samples on zero day were 3.40 ± 0.09 and 6.20 ± 0.22 in cow and buffalo. At the end of 30 days the values were 3.34 ± 0.09 and 6.13 ± 0.21. Fat percentage of control and preserved samples did not show any significant variation.

4.2.5 Fat percentage (Milko-Tester)

The estimated mean fat percentage of control samples were 3.40 ± 0.09 and 6.20 ± 0.22 in cow and buffalo milk respectively. On addition of potassium dichromate the fat contents became 3.38 ± 0.10 and 6.20 ± 0.22 on zero day in cow and buffalo milk respectively. At 30 days of storage the values were 3.25 ± 0.10 and 5.77 ± 0.21 in cow and buffalo respectively. Fat estimation by Milko-Tester showed no significant variation between control and potassium dichromate preserved samples.

4.2.6 Total solids

The estimated mean total solids percentages of control cow and buffalo milk samples were 12.45 ± 0.12 and 15.62 ± 0.48

respectively. On addition of potassium dichromate, total solids content increased to 12.50 ± 0.13 and 15.66 ± 0.48 on zero day in cow and buffalo milk respectively. After 30 days of storage the values were 12.54 ± 0.14 and 15.70 ± 0.48 in cow and buffalo milk respectively. Total solids content between control and potassium dichromate preserved milk samples did not vary significantly.

4.2.7 Solids not fat

The mean solids not fat content of control milk samples were 9.05 ± 0.10 and 9.42 ± 0.27 in cow and buffalo respectively. As soon as the addition of potassium dichromate the solids not fat content became 9.10 ± 0.11 and 9.46 ± 0.27 on zero day in cow and buffalo milk respectively. At 30 days of storage the mean values were 9.19 ± 0.13 and 9.58 ± 0.27 on zero day in cow and buffalo milk respectively. Solids not fat content did not show any significant difference between control and preserved samples.

4.2.8 Lactometer reading

The observed mean lactometer reading of control samples were 29.67 ± 0.49 and 28.0 ± 1.39 in cow and buffalo milk respectively. It increased to 32.33 ± 0.67 and 30.83 ± 1.08 on zero day and 32.83 ± 0.54 and 31.67 ± 1.02 on 30^{th} day of addition of potassium dichromate. Lactometer reading showed significant

($P<0.05$) difference between potassium dichromate preserved milk samples and control.

4.2.9 Preservative effect of potassium dichromate on pasteurised milk

The pasteurised cow and buffalo milk samples could be preserved by potassium dichromate for a period of 38 days without any spoilage.

4.3 Bronopol

Bronopol treated raw cow milk samples could be preserved for 24 days and raw buffalo milk samples could be stored for 16 days at room temperature. Bronopol treated pasteurised cow milk samples could be stored for 30 days and pasteurised buffalo milk samples could be stored for 20 days at room temperature. Both cow and buffalo milk samples became mild pink in colour during storage. The average values with standard errors of different parameters for cow and buffalo milk samples are depicted in Table 4.5 and 4.6 respectively.

4.3.1 pH

The average pH values of control cow and buffalo milk samples were 6.62 ± 0.04 and 6.59 ± 0.04 respectively. On addition

of bronopol the values decreased to 6.64 ± 0.03 and 6.56 ± 0.04 on zero day in cow and buffalo milk respectively. The values continued to decrease and became 6.04 ± 0.02 at 24 days in cow milk and 6.08 ± 0.03 at 16 days in buffalo milk samples. Analysis of variance between control and bronopol preserved samples showed significant ($P<0.01$) difference in pH values.

4.3.2 Titratable acidity

The estimated mean titratable acidity of control samples were 0.17 ± 0.01 in cow and 0.017 ± 0.01 in buffalo milk samples. On addition of bronopol the values became 0.16 ± 0.0 and 0.18 ± 0.01 at zero day. The values became 0.29 ± 0.01 and 0.28 ± 0.01 at 24, 16 days in cow and buffalo milk samples. Titratable acidity increased gradually with the advancement of storage period. Potassium dichromate preserved samples showed significantly ($P<0.01$) different titratable acidity values when compared to control milk samples.

4.3.3 Clot on boiling test

Bronopol treated cow and buffalo milk samples remained COB negative up to eight days. The samples became COB positive after eight days of storage at room temperature.

4.3.4 Fat percentage (Gerber method)

The mean fat percentage of control cow and milk samples were 3.77 ± 0.13 and 6.43 ± 0.12 respectively. On addition of bronopol the values became 3.81 ± 0.14 and 6.42 ± 0.12 at zero day. The values at 24 days of storage in cow milk and 16 days of storage in buffalo milk were 3.78 ± 0.12 and 6.50 ± 0.13 respectively. Milk fat content between control and bronopol preserved samples did not vary significantly.

4.3.5 Fat percentage (Milko-Tester)

The mean fat percentage of control samples were 3.75 ± 0.14 and 6.43 ± 0.12 in cow and buffalo milk samples respectively. On addition of bronopol the values became 3.80 ± 0.10 and 6.47 ± 0.13 at zero day. The values became 3.80 ± 0.12, 6.50 ± 0.12 after 24, 16 days of storage in cow and buffalo milk respectively. Fat percentage in bronopol preserved milk samples did not vary significantly with control.

4.3.6 Total solids

The average total solids percentages of control cow and buffalo milk samples were 13.19 ± 0.16 and 16.72 ± 0.32 respectively. On addition of bronopol the values

became 13.17 ± 0.15 and 16.77 ± 0.31 at zero day. Further the values became 13.23 ± 0.13 at 24 days in cow milk and 16.78 ± 0.33 at 16 days of storage in buffalo milk. Total solids content between control and preserved samples did not vary significantly.

4.3.7 Solids not fat

The calculated average solids not fat content of control milk samples were 9.42 ± 0.11 in cow and 10.29 ± 0.29 in buffalo. On addition of bronopol the solids not fat content became 9.36 ± 0.07 and 10.35 ± 0.28 at zero day. The values were 9.46 ± 0.11 and 10.28 ± 0.29 at 24 and 16 days of storage in cow and buffalo milk samples respectively. Solids not fat content did not show any significant difference between control and bronopol preserved milk samples.

4.3.8 Lactometer reading

The average lactometer reading of control cow and buffalo milk samples were 28.17 ± 0.31 and 29.33 ± 0.56. On addition of bronopol the values became 28.83 ± 0.31 and 29.17 ± 0.48 at zero day. The value at 24 days in cow milk was 28.33 ± 0.33 and at 16 days in buffalo milk was 28.83 ± 0.48. Bronopol preserved cow and buffalo milk samples did not have any

significant change in lactometer reading when compared to control.

4.3.9 Preservative effect of bronopol on pasteurised milk

The pasteurised cow milk could be preserved by bronopol for a period of 30 days without any spoilage while buffalo milk sample could be preserved for 20 days.

Table 4.1. Mean values* of different parameters in formalin preserved cow milk

Parameter	Control	Storage period in days						
		0	15	30	45	60	75	90
P^H	6.58 ±0.02[a]	6.46±0.01[b]	6.21±0.01[b]	6.11 ±0.02[b]	6.09 ±0.02[b]	6.08 ±0.02[b]	6.06 ±0.03[b]	6.06 ±0.02[b]
Titratable acidity (% lactic acid)	0.16 ±0.01[a]	0.21 ±0.01[b]	0.22 ±0.0[b]	0.24 ±0.0[b]	0.25 ±0.01[b]	0.26 ±0.0[b]	0.27 ±0.0[b]	0.27 ±0.0[b]
Clot on boiling test (COB)	– ve	– ve	– ve	– ve	– ve	– ve	– ve	– ve
Fat % (Gerber)	3.40 ±0.09	3.32 ±0.1	3.33±0.09	3.36±0.09	3.38±0.09	3.39±0.08	3.38±0.09	3.38±0.09
Fat % (Milko-Tester)	3.4 ±0.09[a]	3.33±0.10[a]	3.35±0.11[a]	3.38±0.01[a]	3.28±0.24[a]	3.88±0.17[b]	3.35 ±0.24[a]	3.43±0.27[a]
Total solids %	12.45±0.12	12.46±0.13	12.45±0.11	12.46±0.11	12.45±0.12	12.48±0.12	12.50±0.12	12.51±0.12
SNF %	9.05 ±0.10	9.14 ±0.12	9.12 ±0.10	9.10 ± 0.10	9.07 ±0.11	9.09 ±0.10	9.11 ±0.10	9.12 ±0.10
Lactometer Reading	29.7±0.49	29.0±0.52	28.7±0.42	29.2±0.60	28.7 ±0.42	28.7 ±0.42	28.7 ±0.42	28.7 ±0.42

* Values are averages of six observations

[ab] Means bearing same superscript within the same row do not differ significantly ($P<0.05$)

Table 4.2. Mean values *of different parameters in formalin preserved buffalo milk

Parameter	Control	Storage period in days						
		0	15	30	45	60	75	90
P^H	6.76±0.01[a]	6.58±0.03[b]	6.28±0.01[b]	6.26 ±0.01[b]	6.24 ±0.01[b]	6.23 ±0.01[b]	6.21 ±0.01[b]	6.21 ±0.01[b]
Titratable acidity (% lactic acid)	0.13 ±0.0[a]	0.18±0.01[b]	0.20 ±0.0[b]	0.21 ±0.0[b]	0.22 ±0.0[b]	0.24 ±0.01[b]	0.25 ±0.01[b]	0.25 ±0.01[b]
Clot on boiling test (COB)	– ve	– ve	– ve	– ve	– ve	– ve	– ve	– ve
Fat % (Gerber)	6.20 ± 0.22	6.13 ± 0.22	6.12 ± 0.22	6.13 ± 0.23	6.18 ± 0.22	6.18 ± 0.23	6.18 ± 0.22	6.18 ± 0.22
Fat % (Milko-Tester)	6.20 ±0.22	6.13 ±0.21	6.10 ±0.22	6.10 ±0.22	6.45 ±0.32	6.18 ±0.28	6.15 ±0.42	6.32 ±0.34
Total solids %	15.62±0.48	15.58±0.49	15.60±0.50	15.59±0.48	15.58±0.48	15.60±0.50	15.63±0.50	15.59±0.49
SNF %	9.42 ±0.27	9.45 ±0.29	9.48 ±0.29	9.46 ± 0.26	9.40 ±0.27	9.40 ±0.28	9.45 ±0.29	9.41 ±0.28
Lactometer Reading	28.0 ±1.39	27.5 ±1.09	27.3±1.09	27.7±1.15	27.5 ±1.09	27.5 ±1.09	27.5 ±1.09	27.5 ±1.09

* Values are averages of six observations

[ab] Means bearing same superscript within the same row do not differ significantly ($P<0.05$)

Table 4.3. Mean values *of different parameters in potassium dichromate preserved cow milk

Parameter	Control	Storage period in days		
		Zero	15	30
P^H	6.58 ±0.02[a]	6.19 ±0.02[b]	6.33±0.01[b]	6.42 ±0.02[b]
Titratable acidity (% lactic acid)	0.16 ±0.01[a]	0.38 ±0.01[b]	0.31 ±0.01[b]	0.28 ±0.01[b]
Clot on boiling test (COB)	– ve	– ve	– ve	+ ve
Fat % (Gerber)	3.40 ± 0.09	3.40 ± 0.09	3.38 ± 0.10	3.34 ± 0.09
Fat % (Milko-Tester)	3.40 ±0.09	3.38 ±0.10	3.33 ±0.10	3.25 ±0.10
Total solids %	12.45±0.12	12.50±0.13	12.53±0.14	12.54±0.14
SNF %	9.05 ±0.10	9.10 ±0.11	9.15 ±0.13	9.19 ± 0.13
Lactometer Reading	29.7±0.49[a]	32.3 ±0.67[b]	32.7±.49[b]	32.8±0.54[b]

* Values are averages of six observations

[ab] Means bearing same superscript within the same row do not differ significantly ($P<0.05$)

Table 4.4. Mean values* of different parameters in potassium dichromate preserved buffalo milk

Parameter	Control	Storage period in days		
		Zero	15	30
P^H	6.76 ±0.01 [a]	6.27 ±0.01 [b]	6.37±0.01 [b]	6.44 ±0.02 [b]
Titratable acidity (% lactic acid)	0.13 ±0.0 [a]	0.36 ±0.01 [b]	0.31 ±0.0 [b]	0.28 ±0.01 [b]
Clot on boiling test (COB)	– ve	– ve	– ve	+ ve
Fat % (Gerber)	6.20 ± 0.22	6.20 ± 0.22	6.18 ± 0.23	6.13 ± 0.21
Fat % (Milko-Tester)	6.20 ±0.22	6.20 ±0.22	5.90 ±0.21	5.77 ±0.21
Total solids %	15.62±0.48	15.66±0.48	15.67±0.48	15.70±0.48
SNF %	9.42 ±0.27	9.46 ±0.27	9.49 ±0.27	9.58 ± 0.27
Lactometer Reading	28.0±1.39 [a]	30.8 ±1.08 [b]	31.3±0.99 [b]	31.7±1.02 [b]

* Values are averages of six observations

[ab] Means bearing same superscript within the same row do not differ significantly ($P<0.05$)

Table 4.5. Mean values* of different parameters in bronopol preserved cow milk

Parameter	Control	Storage period in days						
		0	4	8	12	16	20	24
P^H	6.62±0.04 [a]	6.64±0.03 [b]	643±0.04 [b]	6.28±0.03 [b]	6.23 ±0.03 [b]	6.17±0.04 [b]	6.09 ±0.01 [b]	6.04 ±0.02 [b]
Titratable acidity(% lactic acid)	0.17 ±0.01 [a]	0.16 ±0.0 [b]	0.21 ±0.01 [b]	0.23 ±0.01 [b]	0.24 ±0.01 [b]	0.26 ±0.01 [b]	0.29 ±0.01 [b]	0.29 ±0.01 [b]
Clot on boiling test (COB)	– ve	– ve	– ve	– ve	– ve	– ve	– ve	– ve
Fat % (Gerber)	3.77 ± 0.13	3.81 ± 0.14	3.82 ± 0.13	3.82 ± 0.14	3.79 ± 0.12	3.78 ± 0.13	3.78 ± 0.12	3.78 ± 0.12
Fat % (Milko-Tester)	3.75 ±0.14	3.80 ±0.10	3.77 ±0.14	3.80±0.12	3.80 ±0.13	3.80 ±0.12	3.80 ±0.12	3.80 ±0.12
Total solids %	13.19±0.16	13.17±0.15	13.18±0.16	13.22±0.15	13.23±0.14	13.22±0.14	13.25±0.14	13.23±0.13
SNF %	9.42 ±0.11	9.36 ±0.07	9.36 ±0.09	9.40 ± 0.09	9.44 ±0.08	9.44 ±0.12	9.48 ±0.11	9.46 ±0.11
Lactometer Reading	28.2 ±0.31	28.8 ±0.31	28.5±0.22	28.5±0.22	28.3 ±0.33	28.3 ±0.33	28.2 ±0.31	28.3 ±0.33

* Values are average of six observations

[ab] Means bearing same superscript within the same row do not differ significantly ($P<0.05$)

Table 4.6. Mean values* of different parameters in bronopol preserved buffalo milk

Parameter	Control	Storage period in days				
		0	4	8	12	16
P^H	6.59±0.04[a]	6.56±0.04[b]	6.33±0.03[b]	6.22±0.02[b]	6.16 ±0.02[b]	6.08±0.03[b]
Titratable acidity (% lactic acid)	0.17 ±0.01[a]	0.18 ±0.01[b]	0.21 ±0.01[b]	0.25 ±0.02[b]	0.27 ±0.02[b]	0.28 ±0.01[b]
Clot on boiling test (COB)	– ve	– ve	– ve	– ve	+ ve	+ ve
Total solids %	16.72±0.32	16.77±0.31	16.76±0.32	16.77±0.31	16.76±0.34	16.78±0.33
Fat % (Gerber)	6.43 ± 0.12	6.42± 0.12	6.43 ± 0.12	6.48 ± 0.14	6.50 ± 0.13	6.50± 0.13
Fat % (Milko-Tester)	6.43 ±0.12	6.47 ±0.13	6.43±0.11	6.50±0.11	6.48±0.11	6.50 ±0.12
SNF %	10.29±0.29	10.35 ±0.28	10.33 ±0.27	10.30 ± 0.28	10.26 ±0.30	10.28 ±0.29
Lactometer Reading	29.3 ±0.56	29.2 ±0.48	29.5±0.56	28.7±0.49	28.8 ±0.48	28.8 ±0.48

* Values are averages of six observations

[ab] Means bearing same superscript within the same row do not differ significantly ($P<0.05$)

Table 4.7. Comparison between three preservatives

Preservative	Species	Duration of preservation (Days)	pH		Titratable acidity (% lactic acid)		Fat % (Gerber)		Fat % (Milko-Tester)		Total solids		SNF		Lactometer Reading	
			Zero day	Last day	Zero day	Last day	Zero day	Last day	Zero day	Last day	Zero day	Last day	Zero day	Last day	Zero day	Last day
Formalin	Cow	90	6.46	6.06	0.21	0.27	3.32	3.38	3.33	3.43	12.46	12.51	9.14	9.12	27.5	27.5
	Buffalo	90	6.58	6.21	0.18	0.25	6.13	6.18	6.13	6.32	15.58	15.59	9.45	9.41	27.5	27.5
Potassium dichromate	Cow	30	6.19	6.42	0.38	0.28	3.40	3.34	3.38	3.25	12.50	12.54	9.10	9.19	32.3	32.8
	Buffalo	30	6.27	6.44	0.36	0.28	6.20	6.13	6.20	5.77	15.66	15.70	9.46	9.58	30.8	31.7
Bronopol	Cow	24	6.64	6.04	0.16	0.29	3.81	3.78	3.80	3.80	13.17	13.23	9.36	9.46	28.8	28.3
	Buffalo	16	6.56	6.08	0.18	0.28	6.42	6.50	6.47	6.50	16.77	16.78	10.35	10.28	29.2	28.8

Fig. 4.1 Effect of formalin on pH in cow and buffalo milk

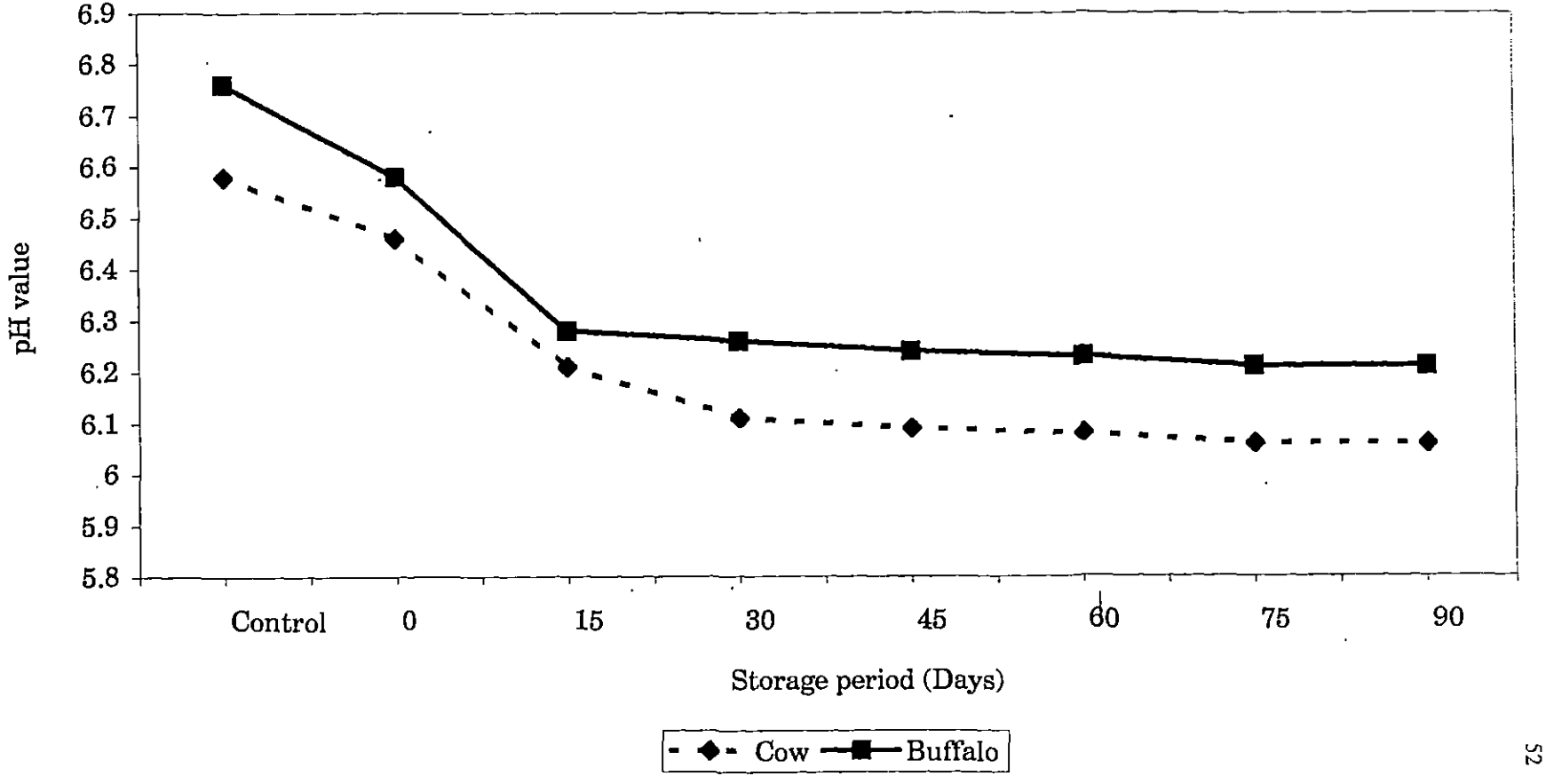


Fig. 4.2 Effect of formalin on titratable acidity in cow and buffalo milk

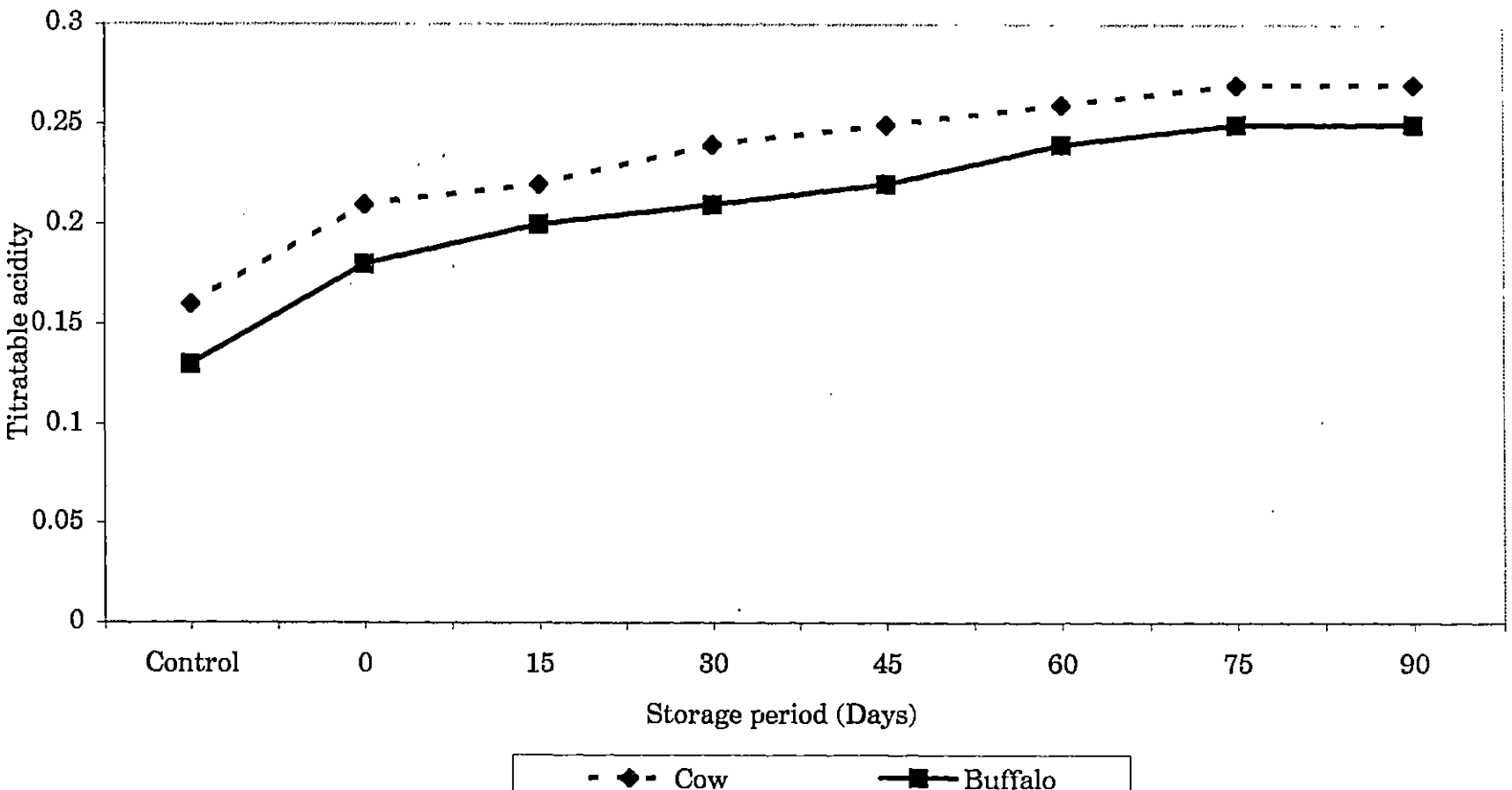


Fig. 4.3. Effect of formalin on fat percentage (Milko-Tester) in cow and buffalo milk

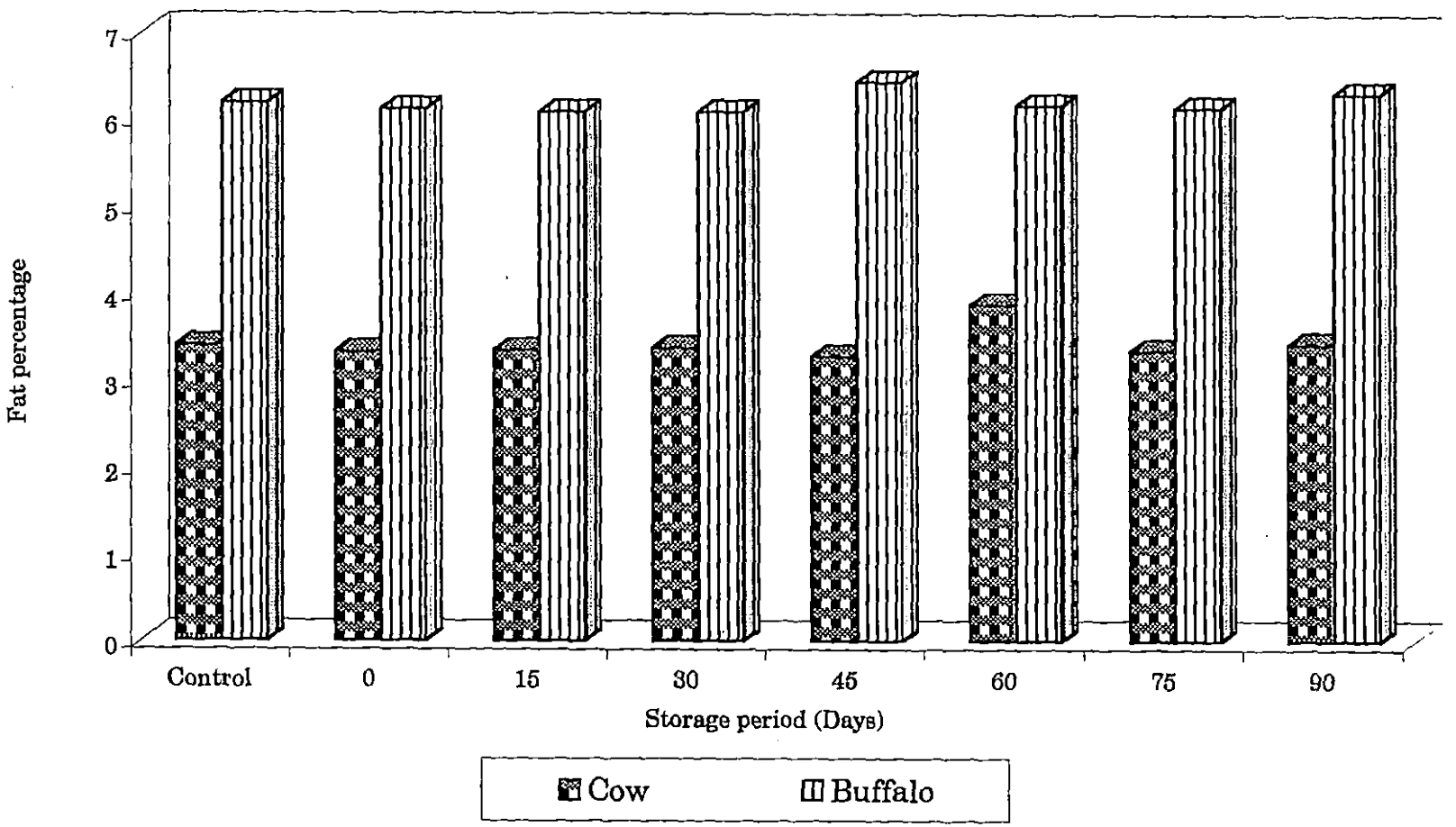


Fig. 4.4 Effect of potassium dichromate on pH in cow and buffalo milk

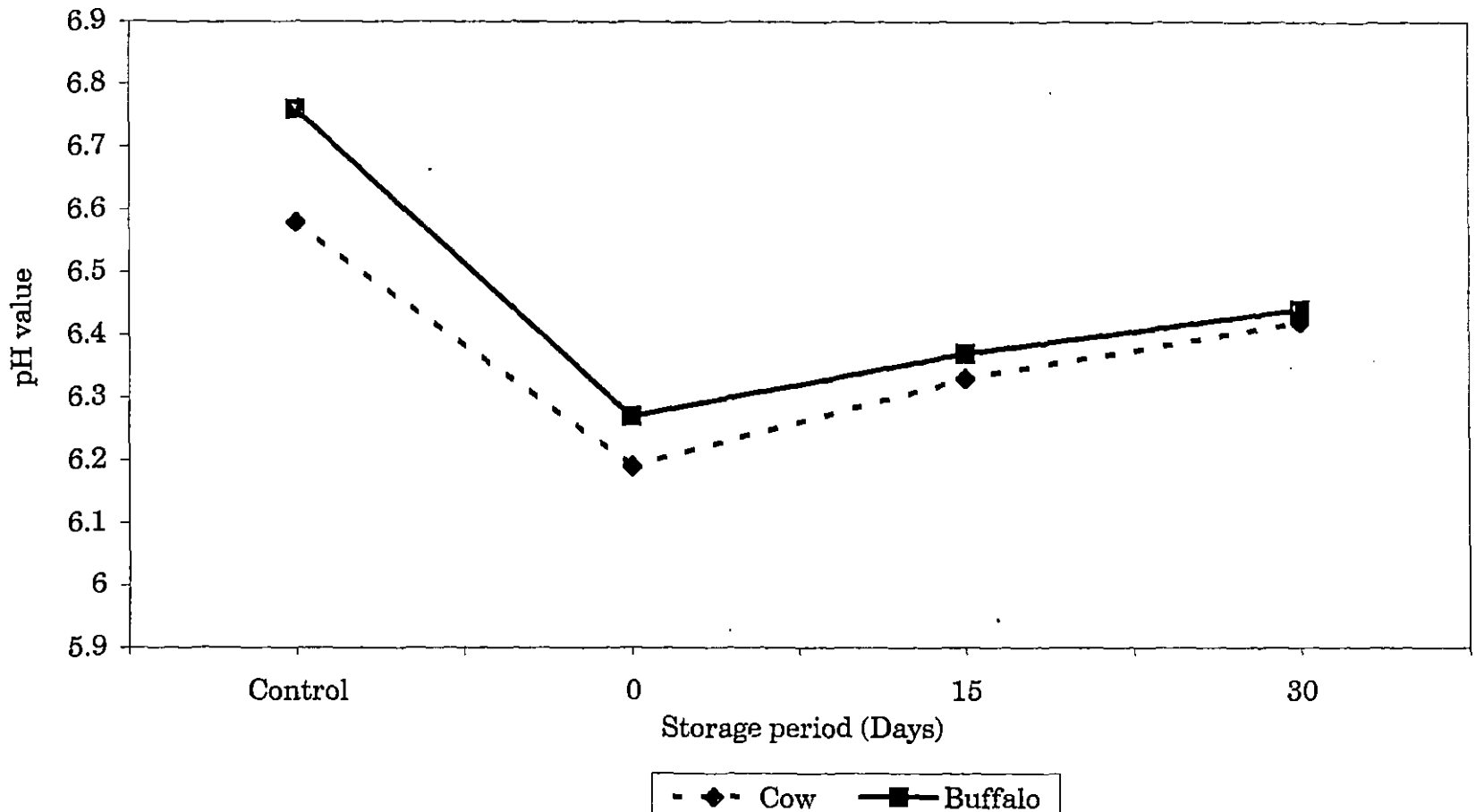


Fig.4.5 Effect of potassium dichromate on titratable acidity in cow and buffalo milk

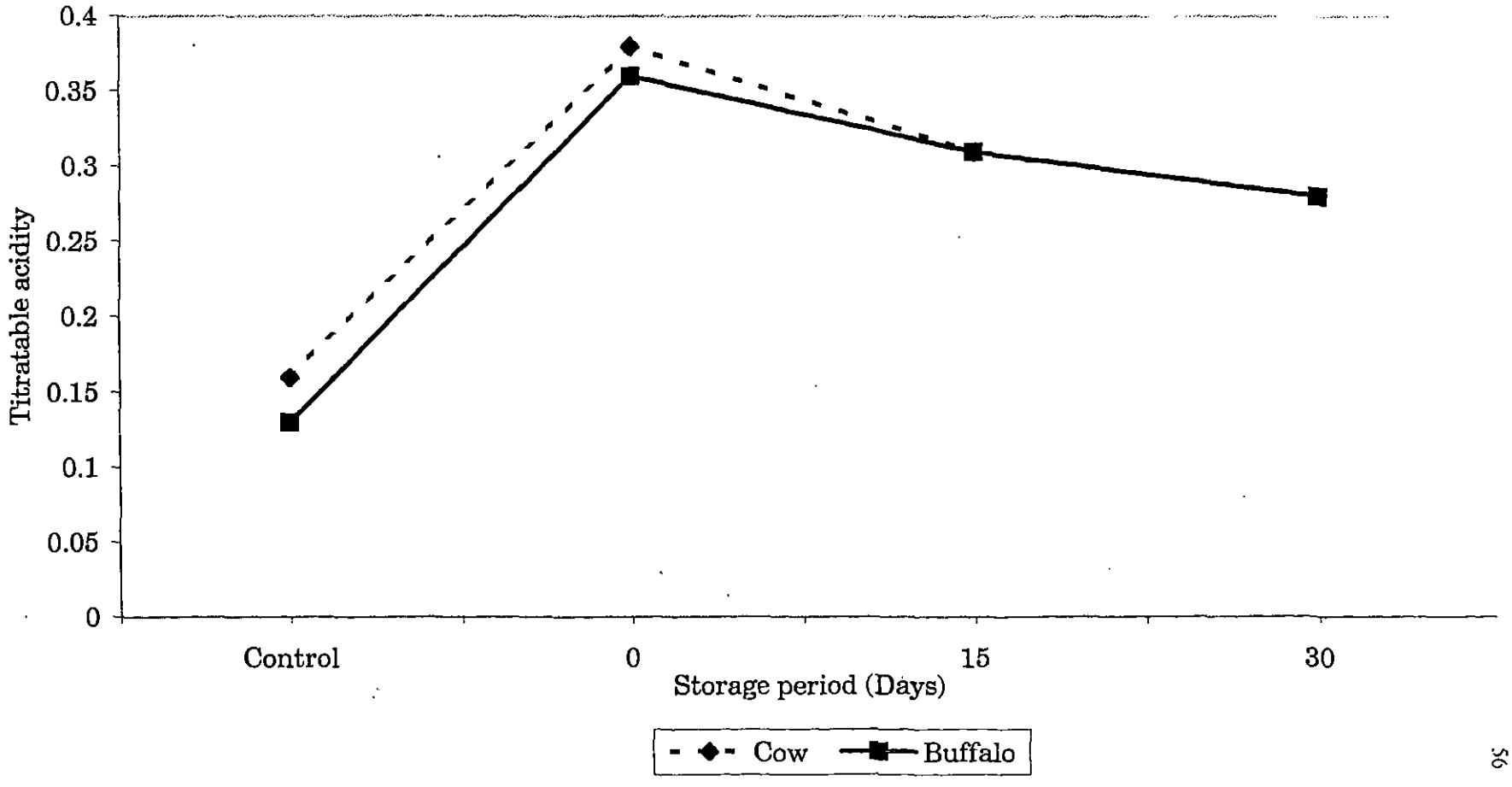


Fig 4.6 Effect of potassium dichromate on lactometer reading in cow and buffalo milk

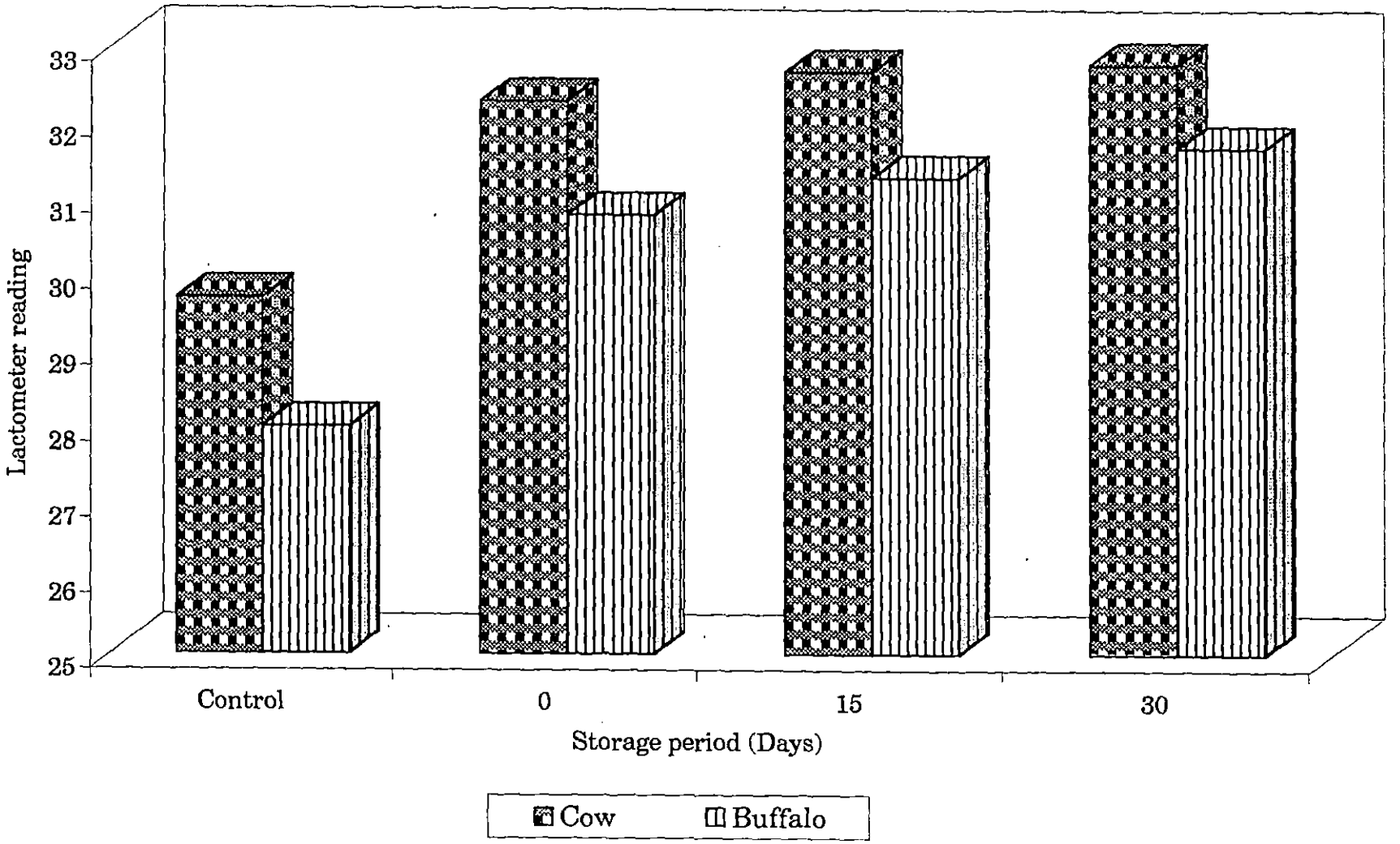


Fig. 4.7 Effect of bronopol on pH in cow and buffalo milk

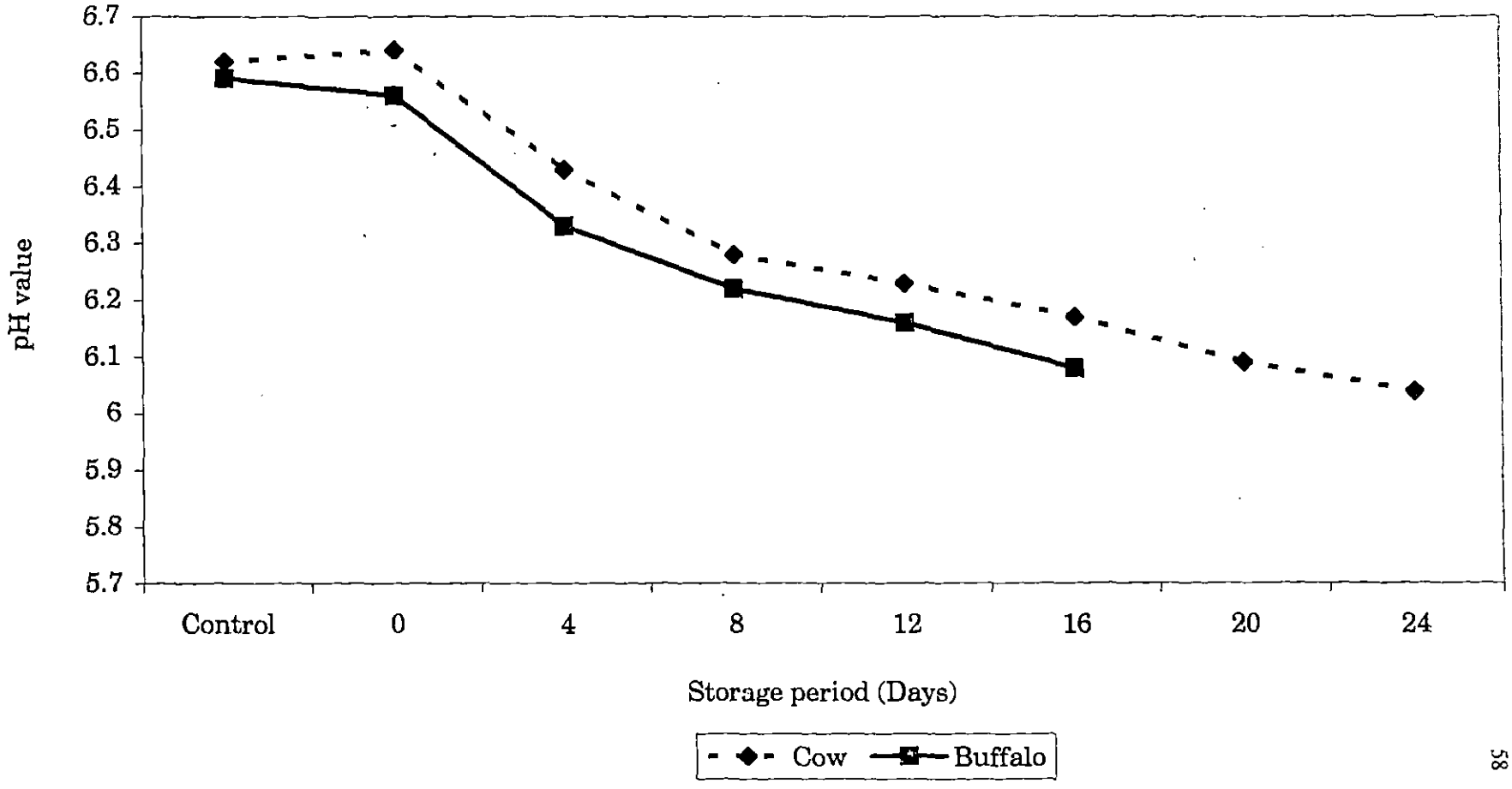
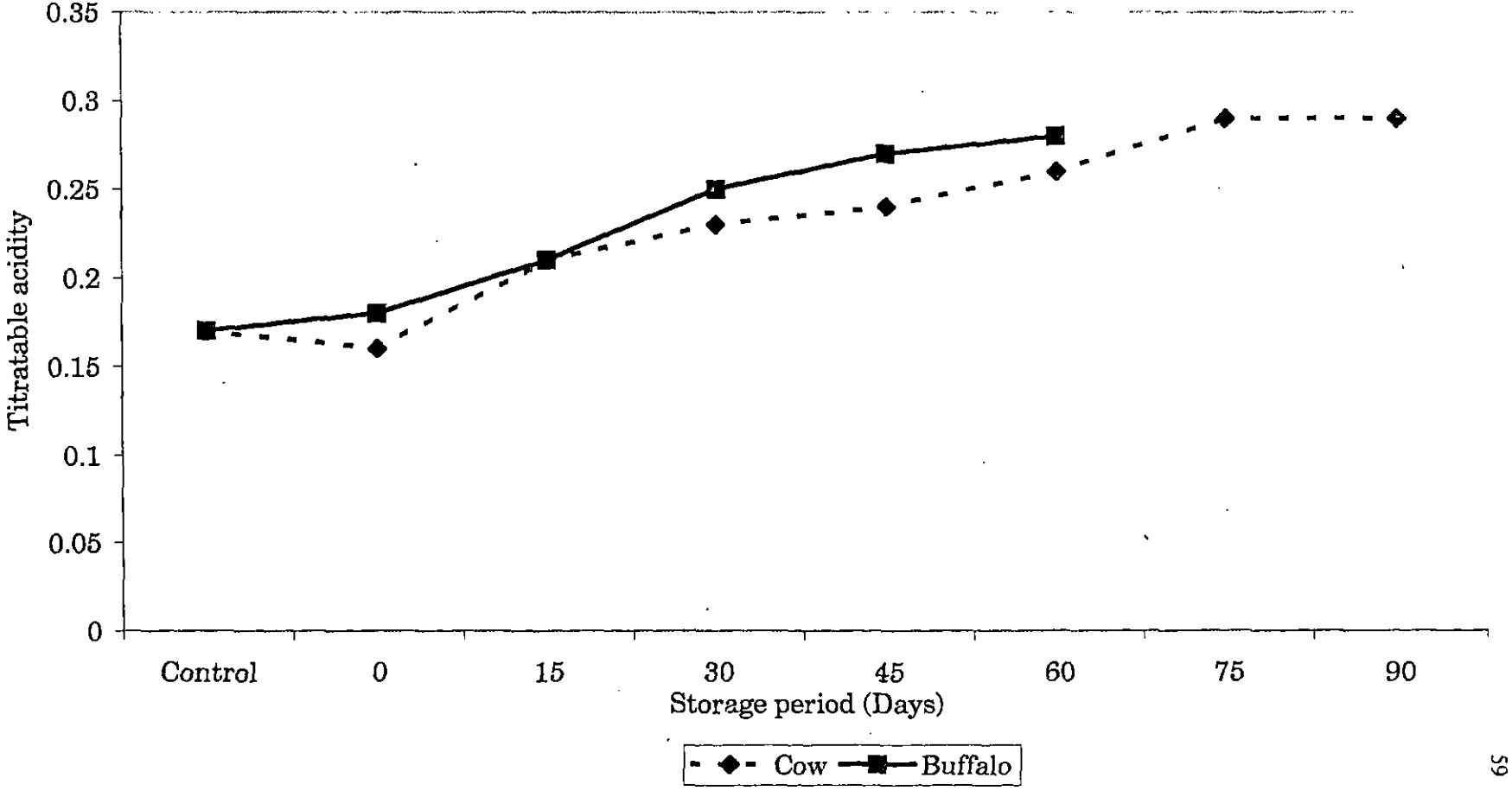


Fig. 4.8 Effect of bronopol on titratable acidity in cow and buffalo milk



Discussion

5. DISCUSSION

5.1 Formalin

No colour change was noticed in cow and buffalo milk samples preserved with formalin even after the storage period of 90 days at room temperature. Cream plug formed in all the samples after 24 hours. During storage the milk samples started separating into three layers after about a month. The upper creamy layer was quite prominent but, the middle layer was not so distinct. The bottom layer was a white sedimentation probably due to casein, and whey proteins, which appeared after one month of storage.

Sandhu *et al.* (1984) and Bansal (1989) have reported no colour change in formalin preserved samples even after the storage period of one year at room temperature. They have also reported three distinct layers in the milk samples after one year of storage. The layers were top layer of cream plug, middle layer of milk serum and bottom layer of casein and whey proteins. Other workers also reported similar observations (Jandal and Rai, 1989; Bajaj and Rai, 1993).

5.1.1 Effect of formalin on pH

The pH value or hydrogen ion concentration gives a measure of the true acidity of milk. Normal pH values of cow and buffalo milk are 6.6 and 6.8 respectively (BIS, 1981).

Average pH values of control milk samples were 6.58 ± 0.02 and 6.76 ± 0.01 for cow and buffalo. The values decreased to 6.46 ± 0.01 and 6.58 ± 0.03 on zero day and then to 6.06 ± 0.02 and 6.21 ± 0.01 at 90 days of storage (Table 4.1 and 4.2). This decrease in pH was statistically significant ($P<0.01$). Rate of decrease was faster up to first 15 days (0.25, 0.30 units) and thereafter it slowed down. Decrease in pH immediately on addition of formalin is due to the acidity of formalin and later decrease may be due to the reaction of formalin with milk proteins.

Decrease in pH in the formalin preserved milk samples was also reported by Jandal and Rai (1988), but they have not suggested any reason for this phenomenon. These authors noticed a significant drop in pH even under refrigeration pointing out that bacterial multiplication is not the reason for decrease in pH.

5.1.2 Effect formalin on titratable acidity

The estimation of titratable acidity is a criterion to assess the suitability of milk for processing. Higher titratable acidity of milk indicates its lower keeping quality and higher chances for curdling on heating.

The acidity of control and preserved milk samples has been expressed as percent lactic acid and presented in Table 4.1 and 4.2 for cow and buffalo. The average acidity of fresh cow and buffalo milk samples were 0.16 ± 0.01 and 0.13 ± 0.0 respectively. On addition of formalin the titratable acidity of both cow and buffalo milk increased to 0.21 ± 0.01 and 0.18 ± 0.01 on zero day. On subsequent storage the acidity continued to increase slowly and at 90 days the values became 0.27 ± 0.00 and 0.25 ± 0.01. After 75 days of storage no further increase in acidity was noticed. This trend was common for both cow and buffalo milk samples. Increase in titratable acidity was statistically significant ($P<0.01$).

Increase in acidity in the milk samples on addition of formalin can be explained with the same reasons pointed out for decrease in pH. Formalin contains formic acid as free acid. This may be the reason for increase in acidity immediately after the addition of formalin. Because of this, formalin is neutralized in formol titration method for estimation of protein in order to avoid

errors in results. Jandal and Rai (1988) compared the changes in titratable acidity of formalin preserved milk samples at 30°C and 5°C for 90 days. They found that titratable acidity was 0.28 and 0.22 respectively while the control value was 0.16. So, refrigeration could not prevent the development of acidity even though bacterial multiplication was very slow at this temperature. Jandal and Rai (1989) could notice only 0.1 per cent increase in titratable acidity of cow's milk immediately on addition of formalin and they attributed this increase to reaction of formalin with different functional groups of proteins. In the present study increase in acidity was much more (0.5 per cent) both in cow and buffalo milk immediately on addition of formalin. Since same workers got an increase of 0.12 per cent increase in acidity at the end of 90 days as we have observed in the present study, it is assumed that the acidity of formalin used by them may be different.

Bansal and Singhal (1991) reported very low bacterial counts (650/ml) in formalin preserved samples even though the acidity of the samples were high. The reported bacterial counts were too less to be considered responsible for the degradation/ hydrolysis of lactose producing lactic acid. They have also reported higher proteolytic activity in formalin preserved samples. These authors noticed a higher acidity on zero day when the level of formalin was increased from 0.2 per cent to 0.5 per cent. But after three months the acidity was almost same with all levels of

formalin. Further, other workers reported no change in lactose content of formalin preserved samples (Sandhu *et al.*, 1984; Bajaj and Rai, 1993). This excludes the possibility of lactic fermentation as the cause of increase in acidity. This indicates that acidity of formalin is more contributing to a higher acidity in milk than the proteolysis caused by formalin which is a gradual process.

Bajaj and Rai (1993) reported that the sudden increase in acidity immediately after the addition of formalin is due to the fact that formalin reacts with primary amino, amide and guanidyl groups, whereas it does not react with secondary amide groups (peptide linkages). Karmakar and Ghatak (1995, 1997) have reported increase in tyrosine value in formalin preserved samples which indicates proteolysis and this may be the reason for slow increase in acidity on further storage.

5.1.3 Effect of formalin on clot on boiling test

The clot on boiling test was performed to assess whether the milk samples preserved with formalin were in the condition suitable for analysis, as the COB positive samples are considered unfit for analysis. Both cow and buffalo milk samples remained COB negative for the entire storage period of three months at room temperature.

Normally milk samples will become COB positive at an acidity of above 0.17 per cent lactic acid. However in the present study milk samples preserved with formalin did not became COB positive even at an acidity of 0.27 per cent.

As discussed above, the initial increase in acidity in the formalin preserved milk samples was not due to lactic fermentation, but because of the formic acid in formalin as revealed in the study of Jandal and Rai (1988) where, formalin preserved samples were COB negative up to 90 days under refrigerated storage. Jandal and Rai (1989) observed that formalin increases the heat stability of milk and this may be the reason for COB negative test.

The results are in agreement with Jandal and Rai (1989); Karmakar and Ghatak (1995 and 1997).

Bansal and Singhal (1991) reported COB negative test in formalin preserved samples even at an acidity of 0.34 per cent lactic acid. However they have reported a positive COB test in formalin preserved buffalo milk samples after five months of storage at room temperature while they could not observe positive test in samples of cow milk even after storage of one year. They have also reported proteolytic activity in COB positive samples. Bajaj and Rai (1993) have also reported a positive COB test in

buffalo milk samples after one month of storage. They have reported proteolysis as the reason for COB positive test.

5.1.4 Effect of formalin on milk fat percentage by Gerber method

The milk samples detained by PFA authorities are preserved with formalin and judged for its quality on the basis of fat and solids not fat content. The effect of formalin on Gerber fat test has been a controversial subject in the recent past. It is there fore essential to study the effect of formalin on the fat values determined by Gerber method.

Fat percentage in the control and preserved milk samples was determined by Gerber method after increasing the concentration of Gerber's Sulphuric acid to 94 per cent as suggested by Jandal and Rai (1988) and Bansal (1989).

The estimated mean fat percentage of control samples were 3.4 ± 0.09 and 6.2 ± 0.22 in cow and buffalo milk samples. Immediately on addition of formalin the fat percentage decreased to 3.32 ± 0.1 and 6.13 ± 0.22. At 90 days of storage the values became 3.38 ± 0.09, 6.18 ± 0.22 (Table 4.1 and 4.2). The extent of decrease of fat content at the end of storage was 0.02 units in both cow and buffalo milk samples respectively. These changes were

not statistically significant. The results of this study are in agreement with Hussain *et al.* (1984).

Sandhu *et al.* (1984) observed no significant change in fat percentage of formalin preserved samples even up to a storage period of twelve months at room temperature which fully supports the present findings.

No significant change in fat percentage by Gerber method for the entire storage period of three months was also reported by Jandal and Rai (1989).

However several other authors reported a significant decrease in the fat percentage of formalin preserved milk samples. Desraj and Singhal (1987) reported consistently low fat values in both cow and buffalo milk samples preserved with formalin. Bansal, (1989) reported a significant (17%) decrease in fat percentage in formalin preserved samples after one year of storage at room temperature. They used standard Gerber method for the estimation of fat percentage. Bajaj and Rai (1992) reported 4.16 and 1.9 per cent decrease in fat values of cow and buffalo milk samples after three months of storage at room temperature when analysed by modified Gerber method. So it can be concluded that by using 94 per cent sulphuric acid, formalin preserved milk

samples can be readily tested by Gerber method with out any significant change in fat results upto 90 days of storage.

5.1.5 Effect of formalin on Milko-Tester method

Milko-Tester method of fat estimation is fast and avoids the use of hazardous chemicals. It is a popular instrumental method used even at milk co-operatives. It is therefore essential to evaluate the influence of preservation of milk samples with formalin on the fat results of Milko-Tester.

Working principle of Milko-Tester is based on the photometric measurement of light scattered by the fat globules in milk. The milk sample is diluted by a chelating agent like EDTA dissolves the protein to avoid the influence of protein content. The measuring system requires a constant globule size to provide a fixed relation between the amount of light scattered to the fat content. This is achieved by the homogenization of diluted milk sample.

The fat percentage of fresh milk samples were 3.4 ± 0.09 and 6.2 ± 0.22 in cow and buffalo respectively. Immediately on addition of formalin there was a slight decrease in fat percentage and it was 3.33 ± 0.10 and 6.13 ± 0.21. Up to 30 days of storage no significant change in fat percentage was noticed.

Afterwards the change in fat percentage was inconsistent. The values at 45, 60, 75 and 90 days were 3.28 ± 0.24, 3.88 ± 0.17, 3.35 ± 0.24 and 3.43 ± 0.27 for cow milk and 6.45 ± 0.32, 6.18 ± 0.28, 6.15 ± 0.42 and 6.32 ± 0.34 for buffalo milk (Table 4.1 and 4.2).

Higher fat values by Milko-Tester may be due to the reduced solubility of milk protein in the diluent solution. Where as lower fat values may be due to the changes in nature of fat globule membrane. The denaturation or damage to the fat globules caused by the reaction of formalin with milk proteins may reduce both the number and surface area of fat globules. This may be the reason for decrease in fat percentage by Milko-Tester as reported by Bansal (1989).

Milk proteins might have underwent polymerisation which made the molecules difficult to dissolve with chelating agent. So protein molecules also scattered some quantity of light leading to higher fat percentage. Afterwards proteolysis may lead to dissolution of these protein molecules and we could get values which are not significantly different from control values.

Use of Milko-Tester for the estimation of fat in the formalin preserved milk samples was reported unsatisfactory by most of the workers (Kvapilik and Sachanek, 1975; Bansal, 1989 ;

Bajaj and Rai, 1992). Incomplete dissolution of hardened protein in the diluent solution is the suggested reason by Bajaj and Rai (1992) for erratic values.

5.1.6 Effect of formalin on the total solids

The total solids content of milk is an important factor in assessing the quality of the milk. The total solids percentage of fresh cow and buffalo milk samples were 12.45 ± 0.12 and 15.62 ± 0.48 respectively. On addition of formalin the values became 12.46 ± 0.13 and 15.58 ± 0.49. At 90 days of storage the values were 12.51 ± 0.12 and 15.59 ± 0.49. Total solids content did not show any significant change neither on addition of formalin nor during storage as shown in Table 4.1 and 4.2. As the level of formalin added was very low, this might not have increased the weight of the samples appreciably. Therefore no substantial change in total solids percentage was noticed. The results are in agreement with Bector and Narayanan (1973), Bansal (1989), Bajaj and Rai (1992). Kvapilik and Suchanek (1975) recommended the use of formalin as a preservative for milk samples intended for total solids estimation.

5.1.7 Effect on solids- not- fat percentage

The milk samples detained under PFA Act are preserved with formalin and are judged on the basis of their fat

and solids not fat percentages. Minimum standards for solids not fat content of cow and buffalo milk are 8.5 and nine per cent respectively throughout the country. Hence it is utmost important to study the influence of formalin on solids not fat content of milk. Solids not fat percentage of fresh cow and buffalo milk samples were 9.05 ± 0.10 and 9.42 ± 0.27. On addition of formalin the values increased to 9.14 ± 0.12 and 9.45 ± 0.29 and at 90 days of storage, the values were 9.12 ± 0.10 and 9.41 ± 0.28 (Table 4.1 and 4.2).

Change in solids not fat percent was not statistically significant as shown in Table 4.1 and 4.2. The slight increase in solids not fat may be due to changes in milk proteins. Similar results were also reported by earlier workers (Sandhu *et al.*, 1984 and Jandal and Rai, 1989). Jandal and Rai (1988) found out SNF percentage of formalin preserved milk samples by lactometer reading and they could find only a non-significant difference of 0.09% in SNF percent. However Hussain *et al.* (1984) and Bansal (1989) reported a significant increase in solids not fat content of formalin preserved samples due to the low fat values obtained by Gerber method.

5.1.8 Effect of formalin on Lactometer reading

The estimated mean lactometer reading of fresh cow and buffalo milk was 29.67 ± 0.49 and 28 ± 1.39 respectively. On

addition of formalin lactometer reading decreased to 29.0 ± 0.52 and 27.5 ± 1.09. At 90 days of storage the values became 28.67 ± 0.42 and 27.50 ± 1.09 (Table 4.1 and 4.2). This result fully agrees with the report of Jandal and Rai (1988). The changes in fat and solids not fat contents of formalin added milk along with the dilution of milk may be the reason for decrease in lactometer reading.

5.1.9 Preservative effect of formalin on pasteurised milk

The pasteurised cow and buffalo milk samples could be preserved by formalin for the entire period of 90 days without any spoilage. It is very clear that formalin prevents any microbial growth in milk samples and no additional effect is noticed on pasteurisation of samples.

5.2 Potassium dichromate

Cow and buffalo milk samples preserved with potassium dichromate could be stored for 30 days at room temperature. Milk samples became orange yellow in colour immediately after the addition of potassium dichromate. Cream plug formed with in 24 hours of storage. There was a gradual change in colour and milk samples became green in colour from its original orange yellow colour towards the end of storage period. At

about 30 days signs of curdling appeared and milk samples became too viscous.

Kroger (1971) also reported similar colour change in potassium dichromate treated cow milk samples stored at room temperature. The colour change from yellow to green was an indication of reduction of the orange hexavalent chromium anion (Cr_2O_7) in the dichromate to the trivalent chromium ion (Cr^{+++} or Cr $(OH)^-_4$), which may appear as green in solution. This reduction is most likely caused by reducing bacteria as reported by Kroger (1971).

In the present study milk samples treated with potassium dichromate showed signs of curdling after 30 days of storage at room temperature. Similar changes were also reported by earlier workers (Desraj and Singhal, 1990) at room temperature and (Karmakar and Ghatak 1995) at refrigerated condition in cow and buffalo milk samples.

5.2.1 Effect of potassium dichromate on pH

The average pH values of fresh cow and buffalo milk samples were 6.58 ± 0.02 and 6.76 ± 0.01. The values decreased to 6.19 ± 0.02 and 6.27 ± 0.01 immediately after the addition of potassium dichromate at zero day. Thereafter pH gradually

increased to 6.42 ± 0.02 and 6.44 ± 0.02 at 30 days of storage in cow and buffalo milk samples respectively (Table 4.3 and 4.4). The decrease in pH was statistically significant ($P<0.01$) in cow and buffalo milk samples.

In the present study a significant sudden decrease in pH was noticed immediately after the addition of potassium dichromate. The rate of decrease was higher (0.49 units) in buffalo than in cow milk (0.39 units). Thereafter a slight but successive increase in pH was noticed with the advancement in storage period. The pH at the end of 30 days storage period was 0.16 units and 0.32 units lesser than the control in cow and buffalo milk samples respectively. The reasons for decrease in pH are not clear.

5.2.2 Effect of potassium dichromate on titratable acidity

The estimated mean values of titratable acidity for control cow and buffalo milk samples were 0.16 ± 0.01 and 0.13 ± 0.0. After the addition of potassium dichromate the values increased to 0.38 ± 0.01 and 0.36 ± 0.01 at zero day. At 30^{th} day it became 0.28 ±0.01 in both cow and buffalo milk samples (Table 4.3 and 4.4).

There was a sudden increase in titratable acidity of cow and buffalo milk samples immediately on addition of potassium dichromate. The increase was 0.22 units in cow milk and 0.23 units in buffalo milk samples. The level of acidity showed a slow but gradual decreasing trend towards the end of 30 days storage. The increase in titratable acidity was statistically significant ($P<0.01$).

Karmakar and Ghatak (1995, 1997) reported very little change in acidity immediately on addition of potassium dichromate but there after they observed a steady and progressive increase in titratable acidity. The increase in acidity of preserved samples is not due to bacterial growth because, level decreased with the increase of storage period. Further, Dunham and Bechtle (1978) reported a drop in standard plate count from 1500/ml to 300/ml at the end of 10 days of preservation by potassium dichromate. This excludes the bacterial spoilage. The reasons for increase in titratable acidity in potassium dichromate preserved samples has to be investigated further.

5.2.3 Effect of potassium dichromate on Clot on boiling test

Potassium dichromate treated cow and buffalo milk samples remained COB negative up to 15 days of storage and there after they became COB positive as shown in Table 4.3 and 4.4.

The present results are in agreement with the findings of two studies by Karmakar and Ghatak (1995 and 1997). Who reported a COB Positive test after 15 days of storage in cow and buffalo milk samples preserved with potassium dichromate at refrigerated condition.

Their reports further indicate that the samples were becoming COB positive not due to bacterial spoilage because observations were same under refrigerated storage.

5.2.4 Effect of potassium dichromate on milk fat percentage by Gerber method

The estimated mean fat percentage of cow and buffalo milk samples were 3.40 ±0.09 and 6.20 ±0.22 before the addition of potassium dichromate. No change in fat percentage was noticed immediately after the addition. Fat percentage slightly decreased to 3.34±0.09 and 6.13±0.21 after 30 days of storage in cow and buffalo milk samples respectively (Table 4.3 and 4.4).

A gradual but non-significant decrease in fat content was noticed in cow and buffalo milk samples (0.06, 0.07 units). The decrease in fat content can be attributed to the degradation of milk fat by the strong oxidative property of potassium dichromate.

Such findings were also reported by several earlier workers. Desraj and Singhal (1990) reported 0.2 and 0.1 per cent decrease in fat content of buffalo and cow milk samples after 30 days of storage at ambient temperature. They opined that hardening of proteins as well as degradation of milk fat by oxidative property of potassium dichromate may be the reasons for decrease in fat percentage. But the effect was almost same for both cow and buffalo milk in this study. Karmakar and Ghatak (1995 and 1997) reported similar findings with buffalo milk at refrigerated temperature. But they observed a non-significant increase in fat content of preserved cow milk samples stored at refrigerated temperature.

5.2.5 Effect of potassium dichromate on Milko-Tester

The estimated mean fat percentage of control samples were 3.40 ± 0.09 and 6.20 ± 0.22. After the addition of potassium dichromate the fat percentage was 3.38 ± 0.10 and 6.20 ± 0.22 at zero day. It decreased further to 3.25 ± 0.10 and 5.77 ± 0.21 at 30 days in cow and buffalo milk samples respectively (Table 4.3 and 4.4).

The decrease in fat content was 0.15 units in cow and 0.43 units in buffalo milk. The decline was progressive in both cow and buffalo milk samples. In cow milk maximum decrease of 0.08

units was noticed between 15 and 30 days of storage. In buffalo milk samples maximum decrease of 0.30 units was noticed between zero and 15th day. Changes were not statistically significant.

The decrease in fat percentage may be due to changes in physical structure of aged milk samples. Kroger (1971) reported 0.18 per cent depression in milk fat after seven days of storage. Dunham and Bechtle (1978) reported a gradual decline in fat percentage in milk samples preserved with potassium dichromate when testing was done by Milko-Tester. Similar observations were made in refrigerated samples also. Similar trend of steady decline was also reported by many authors (Bakke, 1968; Uzonyi, 1977; Vaitnus and Kazlanskaite, 1982).

5.2.6 Effect of potassium dichromate on total solids percentage

The mean total solids percentage of control samples were 12.45 ± 0.12 and 15.62 ±0.48 in cow and buffalo milk samples respectively. The total solids percentage at zero and 30 days after addition of potassium dichromate were 12.50 ± 0.13, 12.54 ± 0.14 in cow milk and 15.66 ± 0.48, 15.70 ± 0.48 in buffalo milk samples respectively (Table 4.3 and 4.4).

The total solids percentage showed a non-significant increase in potassium dichromate treated cow and buffalo milk

samples. The increase was maximum immediately after the addition of potassium dichromate. Thereafter a slight increase was noticed and the extent of increase was 0.09 units in cow and 0.08 units in buffalo milk samples. The slight increase in total solids content may be due to the weight contributed by the solid preservative.

The present findings are in agreement with the reports made by Kroger (1985) in potassium dichromate treated cow milk samples stored at refrigerated temperature.

5.2.7 Effect of potassium dichromate on solids not fat percentage

Mean solids not fat percentage of control samples were 9.05 ± 0.1 and 9.42 ± 0.27 in cow and buffalo milk samples. After addition of potassium dichromate the values increased to 9.10 ± 0.11 and 9.46 ± 0.27 at zero day and thereafter to 9.19 ± 0.13 and 9.58 ±0.27 at 30 days of storage (Table 4.3 and 4.4). Solids not fat of content of potassium dichromate treated cow and buffalo milk samples showed an increasing trend throughout the entire storage period. But the increase was not statistically significant.

The rate of increase in solids not fat percentage was constant in cow milk but it was not uniform in buffalo milk

samples. The extent of increase was 0.16 units in buffalo and 0.14 units in cow milk samples after 30 days of storage when compared to control samples. These findings are in agreement with Hussain *et al.* (1984). The slight increase in solids not fat content in potassium dichromate treated milk samples may be due to the weight contributed by the solid preservative.

5.2.8 Effect of potassium dichromate on lactometer reading

The mean Lactometer reading of control samples were 29.7 ± 0.49 and 28 ±1.39 in cow and buffalo milk samples respectively. The average Lactometer reading at zero, 30 days were 32.3 ± 0.67 and 32.8 ± 0.54 in cow milk. The corresponding values in buffalo milk were 30.8 ± 1.08 and 31.7 ± 1.02 respectively (Table 4.3 and 4.4).

The rate of increase was maximum immediately after the addition of potassium dichromate. Thereafter a slight but progressive increase was noticed in cow and buffalo milk samples up to 30 days of storage. The increase was more in buffalo milk (3.7 units) than in cow milk (3.1 units). Increase in lactometer reading was statistically significant in cow and buffalo milk samples ($P<0.05$).

Desraj and Singhal (1990) made similar observations and they opined that milk samples preserved with potassium

dichromate are not suitable for lactometer measurements because samples had become too viscous. Since potassium dichromate is a soluble salt it increased the specific gravity of milk and there by increased the lactometer reading. However Armandola (1969) described potassium dichromate as a chemical preservative with least effect on density of milk.

5.2.9 Preservative effect of potassium dichromate on pasteurised milk

The pasteurised cow and buffalo milk samples could be preserved by potassium dichromate for a period of 38 days without any spoilage. Hence pasteurisation is influencing period of storage by reducing initial count of bacteria in those milk samples.

5.3 Bronopol

Bronopol treated cow milk samples could be preserved for 24 days and buffalo milk samples could be preserved for 16 days at room temperature. The samples became mild pink in colour and signs of curdling appeared towards the end of the storage period.

Several workers (Ardo, 1979; Jeunet and Grappin, 1979; Ruttan, 1993 and Gencurova *et al.* 1995) preserved milk samples with 0.02 per cent bronopol up to ten days at room

temperature. In the present study the milk samples were preserved with 0.1 per cent bronopol.

Foltys *et al.* (1995) preserved milk samples with bronopol for 14 days at room temperature and 17 days at refrigerated temperature. From the available literature it was found that many workers reported a shorter duration of preservation by bronopol even at refrigerated temperature.

5.3.1 Effect of bronopol on pH

The average pH values of control samples were 6.62 ± 0.04 and 6.59 ± 0.04 in cow and buffalo milk respectively. The values changed to 6.64 ± 0.03 and 6.56 ± 0.04 on zero day of addition of bronopol. Further the values decreased to 6.04 ± 0.02 at 24^{th} day in cow milk and 6.08 ± 0.03 at 16^{th} day in buffalo milk samples (Table 4.5 and 4.6). There was a decreasing trend in pH of bronopol preserved milk samples.

An overall 0.58 unit declined was noticed in cow milk samples at 24^{th} day. The value was 0.51 units at 16^{th} day in buffalo milk. There was no noticeable change in pH immediately after the addition of bronopol. This indicates that the decrease in pH is not due to the chemical nature of bronopol, but due to multiplication

of bacteria in milk and production of lactic acid. The decrease in pH was statistically significant ($P<0.01$).

5.3.2 Effect of bronopol on titratable acidity

The estimated mean titratable acidity of control samples were 0.17 ± 0.01 and 0.17 ± 0.01 in cow and buffalo milk samples. Immediately after addition of bronopol the values changed to 0.16 and 0.18 ± 0.01 respectively. The acidity increased to 0.29 ± 0.01 after 24 days of storage in cow milk and 0.28 ± 0.01 at 16 days in buffalo milk samples (Table 4.5 and 4.6). Titratable acidity increased gradually as the storage period progressed. The buffalo milk samples attained maximum acidity by 16th day and curdled. This indicates higher rate of increase in acidity in buffalo milk samples. The reason for this may be the higher total solids content in buffalo milk facilitating more rapid multiplication of bacteria. The increase in acidity was statistically significant ($P<0.01$). There was no appreciable change in titratable acidity immediately after the addition of bronopol. The increase in acidity during storage may be due to the multiplication of bacteria and production of lactic acid.

5.3.3 Effect of bronopol on clot on boiling test

Bronopol treated cow and buffalo milk samples remained COB negative immediately after the addition. The

samples remained COB negative up to eight days of storage and there after the samples became COB positive as shown in Table 4.5 and 4.6. Increase in acidity due to the bacterial multiplication may be the reason for COB positive test at an earlier stage.

5.3.4 Effect of bronopol on milk fat percentage by Gerber method

The initial fat percentage of fresh cow and buffalo milk samples were 3.77 ± 0.13 and 6.43 ± 0.12 respectively. On addition of bronopol the values became 3.81 ± 0.14 and 6.42 ± 0.12. The values at 24 days of storage in cow milk and 16 days of storage in buffalo milk were 3.78 ± 0.12 and 6.50 ± 0.13 respectively (Table 4.5 and 4.6).

The changes in fat percentage were non significant. So it can be concluded that bronopol had no influence on fat percentage of the preserved samples. There are different reports on changes in fat percentage in milk samples preserved with bronopol. All these workers used methods other than Gerber method for analysis of fat. Ardo (1979) reported that bronopol at 0.02 per cent concentration affects fat estimation by Rose-Gottlieb method, but not the instrumental methods. He also observed that decrease in fat content increased with the increase in concentration of bronopol. In the present study bronopol had not affected the fat percentage even at a concentration of 0.1 per cent.

This study proves that Gerber method can be used as a reference method in samples preserved with bronopol.

5.3.5 Effect of bronopol on milk fat percentage by Milko-Tester

The mean fat percentage of control cow and buffalo milk samples were 3.75 ± 0.14 and 6.43 ± 0.12 respectively. At zero day the fat content was 3.80 ± 0.1 and 6.47 ± 0.13 and the values became 3.80 ± 0.12 after 24 days of storage in cow milk and 6.50 ± 0.12 after 16 days of storage in buffalo milk samples (Table 4.5 and 4.6).

A slight increase in fat percentage by Milko-Tester was noticed in bronopol preserved cow and buffalo milk samples. However this increase was not statistically significant. Ardo (1979) reported that use of 0.02 per cent bronopol had not at all affected the fat percent by Milko-Tester method on the same day. Several earlier workers have also reported similar findings (Kyla-Siurola, 1982; Lee *et. al.*, 1986; Monardes *et. al.*, 1996). The results of this study recommend the use of bronopol for the preservation of milk samples meant for fat estimation by Milko-Tester.

5.3.6 Effect of bronopol on total solids percentage

The average total solids percentage of fresh cow and buffalo milk samples were 13.19 ± 0.16 and 16.72 ± 0.32

respectively. On addition of bronopol the values became 13.17 ± 0.15 and 16.77 ± 0.31 at zero day. Further the values became 13.23 ± 0.13 at 24 days in cow milk and 16.78 ± 0.33 at 16 days of storage in buffalo milk (Table 4.5 and 4.6). The increase in total solid percentage was not statistically significant.

As the level of preservative added was very low it might not have added any measurable weight to the sample. Hence no significant increase in total solid content was noticed. The available literature did not give any information regarding the effect of bronopol on total solids content.

5.3.7 Effect of bronopol on solids not fat percentage

The calculated average solids not fat content of fresh milk samples were 9.42 ± 0.11 in cow milk and 10.29 ± 0.29 in buffalo milk. At zero day of storage the solids not content was 9.36 ± 0.07 and 10.35 ± 0.28. The values became 9.46 ± 0.11 in cow milk at 24 days of storage and 10.28 ± 0.29 at 16 days of storage in buffalo milk (Table 4.5 and 4.6).

No appreciable change in solids not fat percentage was observed either immediately on addition of bronopol or during storage. The changes were not statistically significant. Ardo (1979) reported no change in protein percentage of samples preserved

with bronopol up to nine days of storage. This supports the findings of this study.

5.3.8 Effect of bronopol on lactometer reading

The average Lactometer reading of control cow and buffalo milk samples were 28.2 ± 0.31 and 29.3 ± 0.56. Immediately after the addition of bronopol the values became 28.8 ± 0.31 and 29.2 ± 0.48 and the value at 24 days in cow milk was 28.3 ± 0.33 and at 16 days in buffalo milk was 28.8 ± 0.48 (Table 4.5 and 4.6). Bronopol preserved cow and buffalo milk samples did not show any significant change in Lactometer reading compared to control.

5.3.9 Preservative effect of bronopol on pasteurised milk

The pasteurised cow milk could be preserved by bronopol for a period of 30 days without any spoilage while buffalo milk sample could be preserved for 20 days. This is a satisfactory period for carrying out routine analyses on milk. Pasteurisation could prolong the keeping quality of bronopol preserved samples by six days in cow milk and four days in buffalo milk. This finding also suggests the slow multiplication of microbes in bronopol preserved pasteurised milk samples.

Conclusion

Based on the results of the study it can be seen that all the three preservatives are having influence over the pH and titratable acidity of milk samples to varying extents. All these preservatives increased the acidity and decreased the pH of the milk samples. Pasteurisation of milk samples before addition of preservatives prolonged the duration of preservation in case of potassium dichromate and bronopol.

Among the three preservatives formalin had the maximum duration of preservative effect. When formalin was used, in spite of the increase in acidity, keeping quality was not affected. Also formalin had no adverse effect on the estimation of important parameters like fat (modified Gerber method) and solids not fat (Gravimetric method). But modified Gerber method is not an officially approved method.

However formalin had interfered with the estimation of fat by Milko-Tester method. There are reports indicating interference of formalin on estimation of protein, lactose and other constituents by instrumental methods. Apart from this formalin has been considered as a potential carcinogen by the International Agency for Research on Cancer (IARC).

Potassium dichromate had shorter duration of preservative effect than formalin. It did not interfere with the estimation of fat by Gerber method and solids not fat by gravimetric method though a non-significant decrease in fat percent and a proportional increase in solids not fat percentage were noticed. But it caused significant changes in lactometer reading.

The major disadvantage with potassium dichromate is it is highly toxic, allergenic and carcinogenic. Further it is difficult to estimate titrable acidity in milk samples preserved with potassium dichromate because, the colour of the sample was too intense to detect the correct endpoint.

Bronopol had very short duration of preservative effect than the other two preservatives. It had not interfered with the estimation of important parameters like fat and solids not fat. It was also suitable for the estimation of fat by Milko-Tester. Among the three preservatives studied bronopol is less toxic. Because of its less toxicity and suitability for instrumental methods it is preferred in several developed countries. But bronopol is expensive.

With the development of technology, the conventional gravimetric and volumetric methods are being replaced by

instrumental methods. So bronopol will be the preservative of choice in future, since it is the only preservative suitable for instrumental methods.

In recent years dairy plants are certified by agencies like ISO, not only for quality of dairy products but also for quality of service. So use of chemical preservatives which are potentially toxic, irritant or causing environmental pollution are also to be discouraged.

Summary

6. SUMMARY

Chemical preservatives such as formalin, potassium dichromate and bronopol were studied to know their efficiency of preservation of milk at room temperature. The influence of these preservatives on milk components like fat, total solids and solids not fat were assessed for different storage periods. Physical properties of milk such as pH, titratable acidity, clot on boiling test and lactometer reading were also studied. Duration of preservation for each preservative was also noted down.

Pooled milk samples were collected from cow and buffaloes maintained at the University Livestock Farm at weekly intervals. Calculated levels of preservatives viz. formalin (0.4 per cent), potassium dichromate (0.4 per cent) and bronopol (0.1 per cent) were added to samples and stored at room temperature. The results of the present investigation are summarized below:

1. Milk samples treated with formalin could be stored up to 90 days without any change in colour. But, a cream plug after 24 hours and a white sedimentation at the bottom after one month were noticed. Potassium dichromate added samples could be stored for 30 days and the colour became green with signs of curdling at the end of storage period. Cow and

buffalo milk samples preserved with bronopol could be stored for 24 and 16 days respectively. Samples became mild pink in colour as the storage period progressed.

2. The average pH values of milk samples were, 6.58 ± 0.02, 6.76 ± 0.01 in control milk samples of cow and buffalo respectively. The values became 6.46 ± 0.01, 6.58 ± 0.03 at zero day and 6.06 ± 0.02, 6.21 ± 0.01 at 90 days of storage in formalin treated cow and buffalo milk samples respectively. The corresponding values for potassium dichromate added milk samples were, 6.19±0.02, 6.27±0.01 at zero day and 6.42 ± 0.02, 6.44 ± 0.02 at 30 days of storage. Control samples had pH value of 6.58 ± 0.02, 6.76±0.01. Bronopol preserved samples had mean pH values of 6.64 ± 0.03, 6.56 ± 0.04 at zero day in cow and buffalo milk samples respectively. The values became 6.04 ± 0.02 at 24 days in cow milk and 6.08±0.03 at 16 days in buffalo milk respectively. Control samples had a pH value of 6.62 ± 0.04 and 6.59 ± 0.04 for cow and buffalo milk samples.

3. Significant decrease in pH was observed in all the preserved milk samples ($P<0.01$). The decrease in pH was steady and progressive in formalin and bronopol treated samples whereas, potassium dichromate preserved samples showed a sudden decrease in pH immediately after the addition and thereafter the values increased slightly.

4. The estimated titratable acidity of control samples were 0.16 ± 0.01 and 0.13 ± 0.0 in cow and buffalo milk samples respectively. On addition of formalin the acidity increased to 0.21 ± 0.01, 0.18 ± 0.01 at zero day and 0.27 ± 0.0, 0.25 ± 0.01 at 90 days of storage in cow and buffalo milk samples respectively. Potassium dichromate preserved cow and buffalo milk samples had 0.38 ± 0.01, 0.36 ± 0.01 per cent acidity at zero day and 0.28 ± 0.01, 0.28 ± 0.01 per cent at 30 days of storage. The mean acidity of control samples for bronopol was 0.17 ± 0.01 in both cow and buffalo milk samples. After the addition of bronopol the acidity became 0.16 ± 0.0, 0.18 ± 0.01 at zero day and 0.29 ± 0.01, 0.28 ± 0.01 at 24, 16 days in cow and buffalo milk respectively.

5. The increase in titratable acidity was highly significant ($P<0.01$). The increase in acidity was gradual and progressive in formalin and bronopol preserved milk samples whereas, potassium dichromate preserved samples showed a sudden increase in acidity immediately after the addition of preservative and thereafter a successive decrease was noticed.

6. Formalin treated cow and buffalo milk samples remained COB negative throughout the entire storage period of 90 days. Potassium dichromate added samples remained COB

negative up to 15 days of storage and thereafter it became COB positive. Bronopol preserved samples remained COB negative up to eight days of storage and became COB positive thereafter.

7. Fat percentage estimated by Gerber method in control milk samples were 3.40 ± 0.09, 6.20 ± 0.22 in cow and buffalo milk respectively. At zero day the values became 3.32 ± 0.10, 6.13 ± 0.22 and at 90 days of storage the values were 3.38 ± 0.09, 6.18 ± 0.22 in cow and buffalo milk respectively. In potassium dichromate added samples the values at zero day were 3.40 ± 0.09, 6.20 ± 0.22 and at 30 days of storage were 3.34 ± 0.09, 6.13 ± 0.21 percentage in cow and buffalo milk respectively. Milk fat percentage before and after addition of bronopol were 3.77 ± 0.13, 6.43 ± 0.12 and 3.81 ± 0.14, 6.42 ± 0.12 in cow and buffalo milk samples respectively. The values became 3.78 ± 0.12 and 6.50 ± 0.13 after 24 and 16 days of storage in cow and buffalo milk samples respectively.

8. No significant variation was observed in milk fat percentage estimated by Gerber method due to the addition of chemical preservatives, though a slight decrease was noticed in potassium dichromate preserved cow and buffalo milk samples.

9. The fat percentage estimated by Milko-Tester in control samples were 3.40 ± 0.09, 6.20 ± 0.22 in cow and buffalo milk respectively. After addition of formalin the fat percentages were 3.33 ± 0.10, 6.13 ± 0.21 at zero day and 3.43 ± 0.27, 6.32 ± 0.34 at 90 days of storage in cow and buffalo milk samples respectively. Potassium dichromate added samples had fat percentages of 3.38 ± 0.10, 6.20 ± 0.22 at zero day and 3.25 ± 0.10, 5.77 ± 0.21 at 30 days of storage in cow and buffalo milk respectively. Cow and buffalo milk samples preserved with bronopol had mean fat percentages of 3.80 ± 0.10, 6.47 ± 0.13 at zero day. The values after 24 and 16 days in cow and buffalo milk samples were 3.80 ± 0.12 and 6.50 ± 0.12 respectively. Control samples had fat percentage of 3.75 ± 0.14 and 6.43 ± 0.12.

10. Significant difference was noticed in the milk fat percentage of formalin preserved samples by Milko-Tester method ($P<0.05$). The values were inconsistent after 30 days of storage in formalin treated samples.

11. Total solids percentage of fresh cow and buffalo milk samples were 12.45 ± 0.12, 15.62 ± 0.48 respectively. The percentages after the addition of formalin were 12.46 ± 0.13, 15.58 ± 0.49 at zero day and 12.51 ± 0.12, 15.59 ± 0.49 at 90 days of storage in cow and buffalo milk samples respectively. Total solids content of potassium dichromate treated cow

and buffalo milk samples were 12.50 ± 0.13, 15.66 ± 0.48 on zero day and 12.54 ± 0.14, 15.70 ± 0.48 at 30 days of storage respectively. Bronopol preserved samples had total solids percentage of 13.17 ± 0.15, 16.77 ± 0.31 at zero day and 13.23 ± 0.13, 16.78 ± 0.33 at 24 and 16 days of storage in cow and buffalo milk samples.

12. A non-significant increase in total solids content was observed in potassium dichromate preserved milk samples.

13. Solids not fat contents of control samples were 9.05 ± 0.10, 9.42 ± 0.27 in cow and buffalo milk samples respectively. The estimated values at zero day on addition of formalin were 9.14 ± 0.12, 9.45 ± 0.29 and at 90 days of storage were 9.12 ± 0.10, 9.41 ± 0.28 in cow and buffalo milk samples respectively. Potassium dichromate treated samples had solids not fat percentage of 9.10 ± 0.11, 9.46 ± 0.27 at zero day and 9.19 ± 0.13, 9.58 ± 0.27 at 30 days of storage in cow and buffalo milk respectively. Bronopol preserved samples had solids not fat percentage of 9.36 ±0.07, 10.35±0.28 at zero day and 9.46 ± 0.11, 10.28 ± 0.29 at 24 and 16 days of storage. The control samples had values of 9.42 ± 0.11, 10.29 ± 0.29 for cow and buffalo milk samples respectively.

14. A non-significant increase in solids not fat percentage was noticed in potassium dichromate treated milk samples.

15. The average lactometer reading of fresh cow and buffalo milk samples were 29.7 ± 0.49, 28.0 ± 1.39 respectively. The lactometer reading of formalin added samples at zero and 90 days were 29.0 ± 0.52, 27.5 ± 1.09 and 28.7 ± 0.42, 27.5 ± 1.09 in cow and buffalo milk samples respectively. Potassium dichromate added milk samples had lactometer reading of 32.3 ± 0.67, 30.8 ± 1.08 at zero day and 32.8 ± 0.54, 31.7 ± 1.02 at 30 days of storage in cow and buffalo milk respectively. Bronopol preserved samples had lactometer reading of 28.8 ± 0.31, 29.2 ± 0.48 at zero day and 28.3 ± 0.33, 28.8 ± 0.48 at 24 and 16 days of storage in cow and buffalo milk samples respectively.

16. A significant variation ($P<0.05$) was noticed in lactometer reading of potassium dichromate preserved milk samples.

17. Pasteurisation of milk samples before addition of preservatives prolonged the duration of preservation in case of potassium dichromate and bronopol.

From the above findings, it can be summarized that formalin is the preservative with longest preservative effect and it can be used without much impact on fat and solids not fat content of milk samples. Potassium dichromate has comparatively shorter duration of preservative effect than formalin. It can also be used without much influence on milk solids. Bronopol is a short term

preservative and it is suitable for conventional as well as instrumental methods of estimating milk solids. This study has revealed that bronopol can be used in milk samples for estimation of fat by Gerber method which is also a reference method, while earlier reports indicated that this preservative is not suitable for fat estimation by Rose-Gottlieb method.

Formalin appears to be ideal for the existing standard methods of estimating milk solids. With the popularization of instrumental methods for fast and accurate analysis of milk constituents, formalin will not be suitable as a milk sample preservative in future. Further, formalin and potassium dichromate have deleterious effects on human health and environment. Even though bronopol is little expensive, it is best suited for instrumental analysis of milk constituents and safe for handlers. So, bronopol is recommended as a preservative for the near future.

References

REFERENCES

Ardo, Y. (1979). Bronopol as a preservative in milk samples. *Milchwissenschaft.* **34**: 14-16.

Ardo, Y. (1982). Bronopol as a preservative in milk samples for the determination of cell count using Fossomatic. *Milchwissenschaft.* **37**: 139-142.

Armandola, P. (1967). Preservation of raw milk samples *Minerva Diet.* **7**: 256-257 (*Cited in Dairy Sci. Abstr.* **31**:727).

Armandola, P. (1969). Preservation of milk samples for analytical purposes. *Industria Latta.* 5: 33-38 (*Cited in Dairy Sci. Abstr.* **31**: 3144).

Ashworth, U.S., Rupert S. and Erb, R.E. (1960). An improved procedure for the determination of milk proteins by Dye-binding. *J. Dairy Sci.* **43**(5): 614.

Bajaj, V.K. and Rai, T. (1992). Comparative efficiency of various analytical methods for fat and total solids determined gravimetrically in formalin preserved milk samples. *Indian J. Anim. Sci.* **62** (11): 1096-1098.

Bajaj, V.K. and Rai, T. (1993). Effect of formalin on comparative efficiency of protein and lactose estimation. *Indian J. Dairy Sci.* **46**(1): 21-25.

Bakke, F. (1968). Testing the Milko-Tester MK II for milk fat determination. *Meieri posten*, 57:565-572, 594-601. (*Cited in Dairy Sci. Abstr.* **31**:3146).

Bansal, A. (1989). Effect of formalin on different constituents of cow and buffalo milk with particular reference to fat globule membrane. Ph.D. Thesis submitted to the National Dairy Research Institute, Karnal, India. pp. 20-35.

Bansal. A and Singhal, O.P. (1991). Preservation of milk samples with formalin – Effect on acidity. *Indian J. Dairy Sci.* **44** (9): 573-576.

Bector, B.S. and Narayanan, K.M. (1973). Studies on the changes in composition of milk containing formalin during storage. *Fd. Industry J.* **5**: 5-6.

Benda, P. (1996). The effect of some preservatives on natural micro flora in milk samples *Veterinarni Medicina* **40**(11): 359-364 (*Cited in Dairy Sci. Abstr.* **58** (7): 553).

BIS (1981) Hand book of food analysis Part XI: *Dairy products* SP: 18 (Part –XI) 1981. pp. 1-66.

Borsi, M. and Varga, G. (1971) Use in the dairy Industry of the Miko-Tester to determine fat by turbidimetry. *Elelmi-Kozlem.* **17**:157-163 (*Cited in Dairy Sci. Abstr.* **35**:1006).

Brunt, K.D. and Higton, F.R. (1984). Milk preserving tablet *UK patent applications*, GB 2 131 947 A. (*Cited in Dairy Sci. Abstr.* **47**: 3706).

Cerna, Eva and Pisckey, J. (1966). Determination of butterfat content of milk by Milko-Tester. *Potravin. 17*:298-300 (*Cited in Dairy Sci. Abstr.* **28**:3312).

Desraj and Singhal, O.P. (1987). Effect of formalin on Gerber fat test. *J.Fd. Sci. Technol.*, **24**:183-184.

Desraj and Singhal, O.P. (1990). Effect of potassium dichromate, mercuric chloride and hydrogen peroxide on the Gerber fat test. *Indian J. Dairy Sci.*, **43**(3): 364-368.

Dunham, J.R., Bechtle, R.M. and Michael oberlander, (1978) Chlorinated quaternized hexamine and potassium as preservatives for milk samples. *J. Dairy Sci.*, **61**:1696-1699.

Foltys, V., Pazmova, J. and Bezakova, E. (1995). Evaluation of the preservative substances potassium dichromate and bronopol in the infrared analysis of milk. *Pol'nohospodarstvo* **40***(2):* 144-152 (*Cited in Dairy Sci. Abstr.* **57**(5): 403).

Gencurova, V., Hanus,O., Matous, E., Gabriel, B. and Kopecky, J. (1995). Should bronopol replace potassium dichromate in the preservation of milk samples in the Czech Republic. *Vyzkum chovu skotu* **36**(4): 7-14 (*Cited in Dairy Sci. Abstr.*, **57**(6):497.

Hamdy, A., Radii, M. and Safwat, I. (1961). Preservatives for milk samples. *Agric. Res. Review,* Cairo. **39**:202.

Hanus,O., Gencurova, V. and Zvacova, I. (1993). Tests of Milkofix- a new preservative substance for milk samples used for the purposes of an infrared analysis of basic milk composition. Part-II, Checks of preservative effects in relation to the infrared analysis. *Vterinarni Medicina* **37**(1): 33-43 (*Cited in Dairy Sci. Abstr.* **55** (3): 208).

Hedrick, T.I. and Koch, U. (1967). Rapid milk fat testing. *Milk Dlr.* 57:23-24. (*Cited in Dairy Sci. Abstr.* 31:1513).

Herzog, J., Dunne, J., Aber, R., Claver, M. and Marks, J.G. (1988). Milk tester's dermatitis. *J. Amer. Acad. Dermatol.* **19**(3): 503-508.

Hussain, M.D.Z., Mallayyasastri, P., Rao, G.N. and Rao, V.R. (1984). Effect of certain preservatives of composite milk samples stored at room temperature. *Indian J. Dairy Sci.,* **37**(1): 30-32.

Jandal, J.M. and Rai, T. (1988). Effect of storage temperatures on compositional changes in milk samples added with preservatives. *Indian J. Anim. Sci.,* **58** (10): 1245-1247.

Jandal, J.M. and Rai, T. (1989). Compositional changes in milk as affected by the addition of some chemical preservatives and subsequent storage. *Indian J. Dairy Sci.* **42** (2): 203-205.

Jeyachandran, T., Krishnamurthi, P.S. and Sadasivam, P. (1984). The effect of addition of formalin on the determination of protein content of milk by Pro-Milk method. *Cheiron.* **13** (1): 40-42.

Jeunet, R. and Grappin, R. (1979). Preservation of milk samples with bronopol. *Revue Laitiere Francaise.* 380:37-38 (*Cited in Dairy Sci. Abstr.*).

Karmakar, B. and Ghatak, P.K. (1995). Effect of chemical preservatives on chemical quality of buffalo milk during storage. *Indian J. Dairy Sci.* **48** (9): 559-561.

Karmakar, B. and Ghatak, P.K. (1997). Effect of chemical preservatives on different constituents of cow milk during storage under refrigerated conditions. *Cherion.* **26** (5&6): 89-93.

Kreuzer, K. (1981). Preservation of milk for cell counting. *Welt. Der. milch* **35**: 5-6. (*Cited in Dairy Sci. Abstr.* **43**(10): 842).

Kroger. M (1971). Instrumental milk fat determination. I. Effects of potassium dichromate concentration and sample storage time on Milko-Tester results. *J. Dairy Sci.*, **54** (5): 615.

Kroger. M (1985). Milk sample preservation. *J. Dairy Sci.*, **68**: 783-787.

Kvapilik, J. and Suchanek, B. (1975). Effect of different methods of sample preservation on results of milk analysis. *Ziveciina Vyroba.* **19**:31-38 (*Cited in Dairy Sci. Abstr.* **37**: 1393).

Kyla-Siurola, A.L. (1982). Bronopol as a preservative in milk samples. *Proceedings of XXI. Int. Dairy Cong.* **1**: 176-177.

Lee, K.L., Dayton, K.P., Kroll, G. and McGilliard, M.L. (1986) Effects of preservatives, storage time and storage temperature on milk fat percent, protein percent and somatic cell count determination. *J. Dairy Sci.,* **69** supple. 1: 211.

Monardes, H.G., Moore, R.K., Corrigan, B. and Rioux, Y. (1996). Preservation and storage mechanisms for raw milk samples for use in milk recording schemes. *J. Food Protec.* **59** (2): 151-154.

Ng-Kwai-Hang, K.F. and Hayes, J.F. (1983). Effects of levels of potassium dichromate and sample storage time in fat, protein and casein determination in Bovine milk. *Proceedings of XXI. Int. Dairy Cong.* **1**: 206.

Ol' Shevskii, P.A. and Men' Shikov, N.G. (1974). Effects of some factors on accuracy of fat estimation in milk. *Moloch. Prom.* **8**: 27-28. (*Cited in Dairy Sci. Abstr.* **37**: 2172).

Rakshy, S.E.S.E. and Hassan, N. (1971) Studies on the use of preservatives in the dairy industry. I-A preliminary report on the effect of formaldehyde on certain factors of importance in the manufacture of Domiatti cheese. *Indian J. Dairy Sci.*, **24** (4): 172-175.

Rao,V.R., Dastur, N.N. and Dharmarajan, C.S. (1950). Composition of milk of Indian animals. II. Effect of preservatives on freezing point of milk. *Indian J. Vet. Sci.* **20**: 263-269.

Roto, P. and Sala,E. (1996). Occupational laryngitis caused by formaldehyde. *Amer. J. Industrial Medicine* **29** (3): 275-277.

Ruttan,G.R.S. (1993). Milk sample preservative. United States Patent US 5 196344 (*Cited in Dairy Sci. Abstr.* **55**(9): 720).

Sandhu, J.S., Nasir, N.M., Narayanaswamy and Kapur, O.P. (1984). Study on the effect of formalin as a preservative on different constituents of raw milk samples during storage. *J. Fd. Sci. Technol.* **21**: 424-425.

Sathian, C.T. (2001): Somatic cell count and its influence on the quality of milk in crossbred cows. Ph.D. Thesis submitted to the Kerala Agricultural University. p. 88.

Smith, J.W., Plowman, R.D. and Spencer, S.B. (1969). Current status of weighing sampling and testing device for the use in National DHI programme. *J. Dairy Sci.*, **52**: 128-137.

Snedecor, G.W. and Cochran, W.G. (1956). Statistical Methods 5th Ed. *Oxford and IBH Publishing Co.*, London. pp. 237-266

Sotlar, M. and Arsov, A. (1971). Standardization of Laboratory control methods in the dairy industry – Possibility of using new routine methods for milk fat and protein determination. *Mljekarstvo* **21**:149-156 (*Cited in Dairy Sci. Abstr.* **33**: 804).

Tarassuk, N.P. and Abe, N. (1963). Factors affecting dye-binding capacity of milk proteins. *J. Dairy Sci.*, **46**:612.

Toxicological Profile for Formaldehyde (1999). Agency for Toxic Substances and Disease Registry, *United States Public Health Service*. pp. 1-10.

Uzonyi, G. (1977). Preservation of farm milk samples for instrumental determination of protein and fat. *Tejitar* **26**: 28-30. (*Cited in Dairy Sci. Abstr.* **39**:7701).

Vaitnus, V. and Kazlauskaite, E. (1982). Use of the Milko-Tester mark II instrument for determination of milk fat. *Promyshlennosti.* **11**: 154. (*Cited in Dairy Sci. Abstr.* **44**(1): 288.

Viveksharma and Desraj (1999). Fat and solids not fat estimation as affected by formalin treatment and storage of lacto-peroxidase system activated milk. *J. Fd. Sci. Technol.* **37** (2): 204-206.

Waite, R. and Taylor, M.M. (1966). The chemical composition and microbial flora of milk stored in the presence of potassium dichromate. Proceedings of *XVII. Int. Dairy Cong.* **B**: 87-93.

Wolfschoon-Pombo, A, F. (1979). Determination of milk protein with the pro milk *MK II. Revista do Instituto Delaticinios candido Tostes* **34**:3-14. (*Cited in Dairy Sci. Abstr.*, **42**:1822).

Yusa, K. and Tsuchiya, T. (1963). Studies on preservatives of cow's milk. I. Comparison of the effect of various preservatives. *Anim. Husb. Tokyo* 17: 1489-1490. (*Cited in Dairy Sci. Abstr.*, 27:1294).

EFFECT OF PRESERVATIVES ON MILK SOLIDS IN COW AND BUFFALO MILK

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ABSTRACT OF THE THESIS

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ABSTRACT

Pooled milk samples were collected from cow and buffaloes maintained at the University Livestock Farm at weekly intervals. Three chemical preservatives viz., formalin (0.4 per cent), potassium dichromate (0.4 per cent) and bronopol (0.1 per cent) were studied for their efficiency of preservation.

Calculated levels of preservatives were added and the samples were stored at room temperature in dark place. Major milk constituents like fat, total solids and solids not fat were estimated in control and preserved samples. Physical properties of milk such as pH, titratable acidity, clot on boiling test, lactometer reading as well as efficiency of preservation were studied in control and preserved milk samples.

Milk samples treated with formalin could be stored up to 90 days without any spoilage changes. A cream plug formed after 24 hours of storage and a white sedimentation at the bottom appeared after one month of storage.

Potassium dichromate treated samples could be stored for 30 days. There after the samples curdled and became green in colour towards the end of the storage period.

Bronopol preserved cow and buffalo milk samples could be stored for 24 and 16 days respectively and samples became mild pink in colour as the storage period advanced.

There was a significant increase in titratable acidity in cow and buffalo milk samples preserved with all the three chemical preservatives. The increase in acidity was steady and progressive in formalin and bronopol preserved samples. But an abrupt increase in acidity was noticed in potassium dichromate preserved samples immediately after the addition of preservative and there after a successive decrease was noticed.

The pH values showed a significant decline during storage in preserved milk samples. Decline in pH was abrupt in potassium dichromate treated samples whereas it was gradual in samples treated with the other two preservatives.

Formalin treated milk samples remained COB negative throughout the storage period of 90 days, whereas potassium dichromate and bronopol treated samples became COB positive after 15 and eight days of storage respectively.

No significant variation was noticed in fat percentage of preserved milk samples estimated by Gerber method. But a slight decrease in fat per cent was observed in formalin and

potassium dichromate treated samples. The concentration of Sulphuric acid used was increased to 94 per cent for estimating fat percentage in formalin preserved milk samples.

Formalin preserved samples showed inconsistent changes in fat percentage estimated by Milko-Tester. So this method cannot be recommended for formalin preserved milk samples. Bronopol treated milk samples showed lesser variation in milk fat percentage estimated by Milko-Tester when compared to potassium dichromate and formalin.

There was a non-significant increase in total solids and solids not fat content in potassium dichromate preserved samples.

Potassium dichromate preserved samples showed significant increase in lactometer reading, where as formalin and bronopol treated samples did not show any significant changes in lactometer reading.

Formalin appears to be ideal for the existing standard methods of estimating milk solids. With the popularization of instrumental methods for fast and accurate analysis of milk constituents, formalin will not be suitable as a milk sample preservative in future. Further, formalin and potassium dichromate have deleterious effects on human health and environment. Even though bronopol is little expensive, it is best suited for instrumental analysis of milk constituents and safe for handlers. So, bronopol is recommended as a preservative for the near future.