Effect of optimized formulation (combination of bronopol™ and kathon™) on compositional and physico-chemical parameters of milk samples

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Abstract: The experiment was conducted to study the effect of optimized preservative formulation (combination of bronopol™ and kathon™) on compositional and physico-chemical parameters of milk. The optimized formulation was added at the rate of 0.6% in milk samples and effect was observed on estimation of fat, protein, lactose and ash content during storage at 37°C. For comparison, the legally permitted preservative (by Food Safety and Standard Authority of India, 2011) formalin was also added at the rate of 0.4% in milk samples. There was no significant effect of optimized formulation on estimation of fat and lactose content for 45 days and total solid (TS), ash and protein content for 90 days. However, on addition of formalin, the fat and lactose values of milk decreased after 15 days of storage and no significant effect was observed on TS, ash, protein content for 90 days and lactose content for 60 days.

Keywords: Bronopol™, Kathon™, Formalin, Milk samples

Introduction

Authenticity of dairy products is a major concern for legal authorities as some unscrupulous traders are indulges into malpractice of adulterating milk to combat between great demand and scarce availability of milk. To control such malpractices in India, Food Safety and Standard Act, 2006 has been enacted and

the relevant rules have also been framed in 2011, named as Food Safety and Standard Rules (FSSR, 2011). FSSR, 2011 provides minimum standards for all food and dairy products. To maintain the standards prescribed under the law, the food safety officers have been assigned the job of collecting samples from Food Business Operators and to send them to Food Analyst for analysis. The Food Analyst is usually deluged with a vast number of samples; therefore, delay in the analysis of the sample is bound to occur. As milk and milk products are perishable in nature, they get easily spoiled and since spoiled samples are considered unfit for analysis, the samples collected are, therefore, required to be preserved. Generally, the preservation methods involve physical and chemical techniques. The physical method includes preserving the product under varying the conditions of storage, and the chemical method includes preserving the product by an addition of some external reagents.

Physical method (e.g. refrigeration, chilling and heating) requires considerable economic investment and maintenance facilities and moreover, it is applicable only to preserve the food and dairy products for a short duration. Thus, the physical methods are not applicable for the preservation of food and dairy products for longer period of time. In India, FSSR (2011) only approved 0.4% formalin (37-41% formaldehyde) to be added in milk and milk products samples stored for chemical analysis. Formalin is antimicrobial in nature and used for preservation of organ specimens. It acts through alkylation of amino, carboxyl, or hydroxyl group and probably damages nucleic acids. It inactivates all microorganisms, including spores. According to Haselkorn and Doty (1961) formalin interacts with the amino groups of adenine, cytosine and guanine in the nucleic acid component, denaturing them and resulting in the inhibition of the growth of microorganisms. Preservation action of formalin is very effective but, in the literature, conflicting and contradictory reports are available regarding effect of formalin on the estimation of major milk constituents, particularly milk fat which mainly acts as a base for the commerce of milk and milk products. Singh and Shrivastava (2016) reported that the fat content of milk sample reduced from 6.0% to 5.45% after storage of 1 year with 0.4% formalin at room temperature. It was also concluded that when formalin concentration increased from 0.1% to 0.4%, fat content continuously decreased from 5.45% to 3.05% after 4-6 hr of

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storage at room temperature. However, few researchers (Sandhu et al. 1984; Jandal and Rai, 1988, 1989; Karmakar and Ghatak, 1995, 1997) reported no significant change in Gerber fat values of milk samples preserved with 0.4% formalin. Whereas others (Mulder, 1943; Pien et al. 1976; Krishnamurthy, 1974; Sivakova et al. 1976; Hussain et al. 1984; Des Raj and Singhal, 1988; Bajaj and Rai, 1992; Sharma and Sarwar, 2000) found low fat values in the formalin treated milk samples. The cross-linking between the formaldehyde and milk protein leads to the formation of a cross-linked matrix which in turn hinders the detection of milk fat values by Gerber and Rose-Gottlieb method, which are known to be used for chemical analysis of the milk. Hence, there is a need to develop suitable alternative or substitute of formalin for preserving the dairy products.

Different other preservatives such as mercuric chloride, potassium dichromate, hydrogen peroxide, bronopol™ and azidol for preservation of dairy products. However, until now no such preservative is disclosed which can provide preservation along with maintaining the quality of the sample when used alone at ambient temperature. Among above mentioned preservatives 2-bromo-2-nitropropane-1,3-diol is most widely used (0.02-0.1%) as a milk sample preservative and does not interfere with the normal testing procedures. Bronopol™ is extensively used in Europe and the U.S.A. Kathon™ is a preservative used in cosmetics and hygienic products (Hofmann et al. 2018). Bumbadiya et al. (2017) reported that Bronopol™ and kathon™ have broad spectrum antimicrobial activity and have a potential to be used as preservative in milk and milk products. Since efficiency of analytical methods of fat estimation in formalin preserved milk is questionable, there is need to find out suitable alternative to formalin. As no single preservative can satisfy the entire necessary requirement, thus combination of preservative is a novel approach for efficient preservative action. So in the present investigation, combination of Bronopol™ and Kathon™ preservative was prepared and their effects on compositional & physico-chemical parameters of raw cow milk were analyzed.

Materials and Methods

Chemicals and reagents

Ammonia Solution (30%) and Iso Amyl Alcohol were purchased from S D Fine-Chem. Ltd., Mumbai, India. Boric Acid, Folin's Reagent, Hydrogen Peroxide, Petroleum ether, Methanol, Ethyl alcohol (95%), Diethyl ether, Isopropanol, Sulphuric Acid and Hydro chloric acid were purchased from Merck Darmstadt, Germany. Copper Sulphate Penta Hydrate, Mercuric Chloride, Phenolphthalein Indicator, Potassium Dichromate, Potassium Hydroxide, Sodium Carbonate and Sodium Hydroxide were purchased from SRL Chemicals Mumbai, India. Methyl Red and Methylene Blue were purchased from Qualigens Fine Chemicals Mumbai, India. Bronopol and Kathon preservatives were purchased from Sigma-Aldrich Inc., St. Louis, USA.

A formulation (combination of bronopol and kathon) is optimized and a patent (Patent application no. 201911032383 dated 09.08.2019) has been filed for the same.

Milk sample collection and addition of preservatives in milk

Milk samples (250 ml) were added with optimized formulation (combination of bronopol and kathon) at the rate of 0.6% and stored at 37°C for 3 months. For comparison, the milk samples added with 0.4% formalin (permitted by FSSAI) were also stored at 37°C for 3 months. The samples were analyzed after every 15 days intervals for compositional and physico-chemical parameters. Pooled raw cow milk samples were collected from livestock research centre of the institute (National Dairy Research Institute, Haryana, India) in cleaned autoclaved glass bottles.

Compositional analysis of milk

Estimation of fat in milk was done by Gerber method as given in BIS (IS: 1224-1, 1977) and Rose Gottlieb method as described in BIS (IS 1479-2, 1961). Protein content of milk sample was evaluated by (AOAC 991.23). For Lactose content, the procedure given in BIS (IS: 1479-2, 1961) was used. Estimation of TS was done as per the procedure given in Bureau of Indian Standards (IS: 12333, 1997). For the determination of ash of milk samples, the procedure given in BIS (IS: 1479, 1961, Reaffirmed 2003) was used.

Titrateable acidity of milk

Titrateable acidity of milk samples was analyzed as per the procedure described in BIS (IS: 1479-1 1960).

Estimation of free fatty acid (FFA) content in milk

Estimation of FFA content in milk samples was determined as per the method described by Deeth and Fitzgerald (Lipolysis in Dairy Products, 1976). Accurately 3 ml sample of milk was taken in test tube. Then 10 ml extraction mixture (iso propanol: petroleum ether: 4N H₂SO₄, 40:10:1), 6 ml petroleum ether and 4 ml water were added and shaken vigorously for 15 second. Two layers were allowed to settle (5-10 min) and upper layer (usually 7.5 ml) was withdrawn and transferred to a small flask. 2 drops of 1% methanolic phenolphthalein indicator was added and titrated with 0.02N methanolic KOH solution. Blank reading was obtained using water instead of milk. The FFA content was calculated by following equation.

$$FFA(\text{miliequivalent/ml}) = \frac{V \times N \times 10000}{3P}$$

Where,

V=Volume of 0.02N KOH used for sample

N=Normality of KOH,

P=Proportion of upper layer titrated

Estimation of Tyrosine value in milk

Estimation of Tyrosine value in milk samples was determined as per the method explained by Lowry (Protein Measurement with the Folin Phenol Reagent, 1951). In 5 ml of milk sample, 10 ml of 0.72 N trichloroacetic acid (TCA) and 1 ml of distilled water were added. The contents were shaken and kept undisturbed for 30 min. Then the mixture was filtered through whatman no. 42 filter paper. 0.5 ml of filtrate was transferred to a test tube and then 5 ml of alkaline reagent was mixed and kept at room temperature for 10 minutes. Then to it 0.5 ml of Folin's reagent was added and kept for 30 minutes at room temperature to develop colour. Run the blank test simultaneously using water instead of milk. Colour of sample was measured at 750 nm in a spectrophotometer using the blank solution as reference.

Statistical Analysis

Data reported were expressed as mean values with standard errors. In experiments, wherever required, two-way analysis of variance (ANOVA) with a subsequent least significant difference (LSD) test was applied for multiple sample comparison to test for any significant differences ($P < 0.05$) in the mean values of all the groups as described by Snedecor and Cochran (1994) using the statistical program of Microsoft® Excel Version 5.0 (Microsoft Corporation, Redmond, WA, U.S.A.). Graphs were prepared in software Graph Pad Prism version 5.0 (Graph Pad Software, Suite 230 La Jolla, CA 92037, U.S.A.).

Results and Discussion

Effect of preservatives on fat content of milk

The initial fat content of control milk sample was $4.08 \pm 0.05\%$ and $4.10 \pm 0.11\%$ by Gerber and Rose Gottlieb method, respectively. The initial fat content of milk samples preserved with 0.6% of optimized formulation was $4.07 \pm 0.04\%$ and $4.10 \pm 0.04\%$ by Gerber and Rose Gottlieb method, respectively. Addition of optimized formulation (combination of kathon and bronopol) did not have any influence on fat values of milk samples by Gerber and Rose Gottlieb method; however, it was noticed that fat values by Gerber as well as Rose Gottlieb method decreased on addition of 0.4% percent formalin. It is evident from Table 1 that an erratic trend was noticed in the fat content of milk samples added with formalin.

The initial fat content of milk samples preserved with 0.4% of formalin was $4.05 \pm 0.13\%$ and $4.06 \pm 0.04\%$ by Gerber and Rose Gottlieb method, respectively. The fat content in formalin preserved samples decreased significantly after 15 days of storage by both Gerber and Rose-Gottlieb method. However, in case of milk samples added with optimized formulation, the fat content was found to decrease significantly after 60 days of storage at 37°C by both Gerber and Rose-Gottlieb method.

Lower fat values were observed in formalin preserved samples mainly due to hardening of proteins in presence of formaldehyde. Formaldehyde reacts with milk proteins and form practically insoluble high molecular weight compounds (Fraenkel-Conrat and Olcott, 1946, 1948 a, b) and samples correspond to lower fat values due to incomplete dissolution of fat entrapped in milk proteins. In Gerber method, a difficulty was experienced in dissolving the butyrometer contents during shaking. The proteins did not dissolve completely but dispersed in fine particles which deposited below the fat layer after centrifugation. Similarly, in Rose Gottlieb method, turbidity was observed in the fat extraction tubes in case of formalin treated milk samples which may be again be due to incomplete dissolution of proteins with ammonia.

The estimation of fat content in formalin added and stored milk samples indicated that repeatability is affected and no specific trend was observed. The results of the present study are also in consonance with the findings of Mulder, 1943; Pien et al. 1976; Krishnamurthy, 1974; Hussain et al. 1984; Des Raj and Singhal, 1987; Des Raj and Singhal, 1988; Bajaj and Rai, 1992; Chaudhary, 2013 who also reported lower estimations of fat in formalin preserved milk samples during storage of milk samples for variable time durations. However, the literature available on the effect of formalin on the fat estimation by Gerber method is contradictory and some workers (Sandhu et al. 1984; Jandal and Rai, 1988, 1989; Karmakar and Ghatak, 1995, 1997) observed no significant change in the fat estimation by Gerber method in formalin preserved milk samples. Sanchez et al. (2005) observed no significant differences in fat content in case of bronopol-preserved goat milk samples stored at refrigeration temperature. Chaudhary (2013) also reported no change in the fat content (by Gerber method) of milk samples preserved with 0.4% kathon and 0.045% bronopol till 5th week of storage. In contrast, Bertrand (1996) reported higher milk fat of 4.43% vs. 4.37% in cow milk preserved with bronopol compared with that of unpreserved samples.

Effect of preservative on protein content of milk

The total protein content in control milk sample was found to be $3.88 \pm 0.01\%$. Immediately after addition of formalin and optimized formulation, the protein content of milk was found 3.79 ± 0.05 and $3.83 \pm 0.03\%$, respectively. It is apparent from Table 1 that addition of optimized formulation and formalin did not have any effect on protein content of milk and also the protein content remained fairly constant during the storage period at 37°C in milk samples with preservatives (combined formulation and formalin). Similar results were reported by Bector and Narayanan (1973). They observed no significant difference in protein content of milk either on addition of formalin or during storage up to 6 months analyzed by Kjeldahl method. Furthermore, when formalin was added in cow (Karmakar and Ghatak, 1997) and buffalo milk (Karmakar and Ghatak, 1995) showed no change in milk proteins content by kjeldahl method during storage for one month at $7-8^\circ\text{C}$.

Effect of preservative on Total Solids of milk

In control milk sample the total solid content was found to be $13.67 \pm 0.01\%$. After addition of preservatives (optimized formulation and formalin), no significant change was observed in total solid content of milk in comparison to control milk. After addition of formalin and optimized formulation, the total solid content of milk was 13.67 ± 0.01 and $13.72 \pm 0.02\%$, respectively. It is evident from Table 1 that the total solid content remained fairly constant all over the storage period at 37°C in milk samples with preservatives (combined formulation and formalin). This finding is in agreement with those found by Sandhu et al. 1984; Bector and Narayanan, 1973; Bajaj and Rai 1992 who confirmed that the total solid content analyzed using gravimetric method was not affected in formalin preserved milk. Gupta and Gupta (2010) also reported no significant change was observed in total solid content of milk preserved with 0.3% and 0.5% formalin during storage up to 48 h.

Effect of preservative on ash content of milk

The total ash content in control milk sample was found to be $0.70 \pm 0.01\%$. Immediately after addition of preservatives (optimized formulation and formalin), no significant variation was

observed in total ash content of milk in comparison to control milk. After addition of formalin and optimized formulation, the ash content of milk was 0.71 ± 0.02 and $0.70 \pm 0.01\%$, respectively. It is apparent from Table 1 that the ash content remained fairly constant all over the storage period at 37°C in milk samples with preservatives (optimized formulation and formalin).

Effect of preservative on lactose content of milk

In control milk sample the lactose content was found to be $4.62 \pm 0.02\%$. Immediately after addition of preservatives (optimized formulation and formalin), no significant variation was observed in lactose content of milk in comparison to control milk. After addition of formalin and optimized formulation, the lactose content of milk was 4.60 ± 0.02 and $4.59 \pm 0.05\%$ respectively.

It is evident from Table 1 that decrease in lactose content was observed in milk samples added with preservative (optimized formulation and formalin) during storage. A significant difference was observed after 60 days of storage and there after it remained constant till the end of storage period. At the end of 90 days of storage, the lactose content was found 4.44 ± 0.05 and $4.48 \pm 0.06\%$ in milk samples added with formalin and optimized formulation, respectively. The findings are similar with several workers (Bector

Table 1 Effect of addition of preservatives on compositional parameters of milk

Fat (%)	Gerber method							
	Days	0	15	30	45	60	75	90
Protein (%)	FPM	4.05±0.13 ^{aA}	3.88±0.03 ^{bA}	3.74±0.04 ^{cA}	3.76±0.05 ^{cA}	3.69±0.03 ^{cA}	3.79±0.01 ^{cA}	3.71±0.02 ^{cA}
	OPM	4.07±0.04 ^{aA}	4.09±0.05 ^{aB}	4.08±0.02 ^{aB}	4.06±0.12 ^{aB}	3.99±0.05 ^{bB}	3.93±0.02 ^{bB}	3.89±0.02 ^{cB}
	Rose Gottlieb method							
	Days	0	15	30	45	60	75	90
	FPM	4.06±0.04 ^{aA}	3.82±0.15 ^{bA}	3.78±0.07 ^{cA}	3.75±0.05 ^{cA}	3.65±0.15 ^{cA}	3.73±0.12 ^{cA}	3.68±0.07 ^{cA}
	OPM	4.10±0.04 ^{aA}	4.10±0.05 ^{aB}	4.09±0.02 ^{aB}	4.07±0.12 ^{bB}	4.04±0.05 ^{bB}	4.01±0.02 ^{bB}	4.01±0.05 ^{bB}
	Days	0	15	30	45	60	75	90
	FPM	3.79±0.05 ^{aA}	3.82±0.08 ^{aA}	3.85±0.05 ^{aA}	3.89±0.01 ^{aA}	3.89±0.04 ^{aA}	3.83±0.02 ^{aA}	3.82±0.06 ^{aA}
	OPM	3.83±0.03 ^{aA}	3.82±0.03 ^{aA}	3.89±0.02 ^{aA}	3.91±0.03 ^{aA}	3.98±0.03 ^{aA}	3.87±0.01 ^{aA}	3.85±0.04 ^{aA}
	Total solid (%)	Days	0	15	30	45	60	75
	FPM	13.67±0.01 ^{aA}	13.67±0.02 ^{aA}	13.65±0.01 ^{aA}	13.66±0.02 ^{aA}	13.67±0.06 ^{aA}	13.68±0.09 ^{aA}	13.68±0.01 ^{aA}
	OPM	13.72±0.02 ^{aA}	13.73±0.04 ^{aA}	13.71±0.04 ^{aA}	13.74±0.01 ^{aA}	13.74±0.01 ^{aA}	13.72±0.08 ^{aA}	13.74±0.04 ^{aA}
Ash (%)	Days	0	15	30	45	60	75	90
	FPM	0.71±0.018 ^{aA}	0.71±0.004 ^{aA}	0.70±0.002 ^{aA}	0.70±0.008 ^{aA}	0.69±0.007 ^{aA}	0.70±0.012 ^{aA}	0.70±0.004 ^{aA}
	OPM	0.70±0.007 ^{aA}	0.70±0.004 ^{aA}	0.69±0.004 ^{aA}	0.70±0.008 ^{aA}	0.70±0.013 ^{aA}	0.70±0.005 ^{aA}	0.70±0.009 ^{aA}
Lactose (%)	Days	0	15	30	45	60	75	90
	FPM	4.59±0.02 ^{aA}	4.59±0.01 ^{aA}	4.56±0.03 ^{aA}	4.56±0.07 ^{aA}	4.51±0.06 ^{bA}	4.49±0.03 ^{bA}	4.44±0.06 ^{bA}
	OPM	4.59±0.02 ^{aA}	4.59±0.05 ^{aA}	4.53±0.04 ^{aA}	4.51±0.01 ^{aA}	4.49±0.04 ^{bB}	4.45±0.07 ^{bB}	4.48±0.05 ^{bB}

Data are presented as means $\pm$ S.E (n=3). a-c Means with different superscript are significantly different ($p < 0.05$) from each other in row

A-B Means with different superscript are significantly different ($p < 0.05$) from each other in column for each parameter

FPM: Formalin Preserved Milk, OPM: Optimized Formulation Preserved Milk

Fig. 1 Effect of preservatives on Acidity (% Lactic acid) of milk during storage at $37\pm1^{\circ}\text{C}$, Data are presented as means $\pm$ S.E. (n=3), FPM: formalin preserved milk, OPM: optimized formulation preserved milk

and Narayanan, 1973; Sandhu et al. 1984; Bansal and Singhal, 1991; Bajaj and Rai 1993) who reported that storage of milk samples preserved with 0.4% formalin either at room or refrigeration temperatures for six months to 1 year had no effect on lactose content of the milk samples estimated by Lane-Eynon method.

Effect of preservative on Titratable Acidity of milk

The % titratable acidity of control milk sample was found to be $0.13\pm0.01\%$ lactic acid. After the addition of optimized formulation, no major variation was observed, however on addition of formalin, the % titratable acidity was found to be slightly higher than % titratable acidity of control milk samples. This increase in initial acidity on addition of formalin was due to the liberation of hydrogen ions when formaldehyde reacts with primary amino groups, amide groups and guanidyl groups of protein (Jenness and Patton, 1969; Bansal and Singhal, 1991; Upadhyay et al. 2014). Although the acidity of milk increased after addition of formalin (0.4%) but statistically there was no significant difference in the initial acidity of control milk with that of the milk samples added with formalin (0.4%).

It is clear from Figure no 1 that an increasing trend was found in the % titratable acidity of milk samples added with optimized formulation and formalin. In case of 0.4% formalin preserved milk samples, the initial % titratable acidity was $0.14\pm0.02\%$ lactic acid, which significantly increased after 15 days and continued to increase till 90 days of storage at 37°C . After 90 days the % titratable acidity was $0.31\pm0.05\%$ lactic acid. Whereas, milk samples preserved with optimized formulation (0.6%), the initial % titratable acidity was found $0.13\pm0.06\%$ lactic acid, which

Fig. 2 Effect of preservatives on FFA (milliequivalents/ml) of milk during Storage at $37\pm1^{\circ}\text{C}$, Data are presented as means $\pm$ S.E. (n=3), FPM: Formalin Preserved Milk, OPM: Optimized Formulation Preserved Milk

significantly increased after 15 days and continued to increase till 90 days of storage at 37°C . After 90 days the % titratable acidity was $0.33\pm0.09\%$ lactic acid.

When the formalin was added, an instant increase in acidity of milk sample occurs, which continued during subsequent storage. Similar results were reported by Venkateswara rao et al. 1950; Armandola, 1969; Minzner and Kroger, 1974; Fahmi et al. 1982; Bansal and Singhal, 1991 who observed a gradual increase in acidity while preserving composite samples of cow and buffalo milk with 0.2, 0.3, 0.4, 0.5 % (v/v) formalin in glass bottles at room temperature ($16-30^{\circ}\text{C}$) up to one year.

Effect of addition of preservative on FFA content of milk

The FFA content in control milk sample was found to be 0.05 ± 0.001 milliequivalents/ml milk. No any significant difference was observed in FFA content of milk in comparison to control milk immediately after addition of preservative. It is evident from Figure no 2 that an increasing trend was found in the FFA content of milk samples added with optimized formulation and formalin.

In case of 0.4% formalin preserved milk samples, the initial FFA content was 0.05 ± 0.006 milliequivalents/ml milk, which significantly increased after 30 days and continued to increase till 90 days of storage at 37°C . After 90 days the FFA content was 0.25 ± 0.01 milliequivalents/ml milk. Whereas, In case of milk samples preserved with optimized formulation (0.6%), the initial FFA content was 0.05 ± 0.002 milliequivalents/ml milk, which significantly increased after 30 days and continued to increase till 90 days of storage at 37°C . After 90 days the FFA content was 0.26 ± 0.08 milliequivalents/ml milk.

Effect of addition of preservative on tyrosine value of milk

The Tyrosine value in control milk sample was found to be 42.39 ± 0.74 mg/100 ml milk. After addition of formalin (0.4%), no significant variation was observed in tyrosine value of milk, however on addition of optimized formulation (0.6%), the tyrosine value was found to be 28.80% higher than tyrosine value of control milk samples. The higher values of tyrosine may be due to reaction of isothiazoline group of Kathon™ with folin's reagent.

It is evident from Figure no 3 that the tyrosine value of milk samples added with optimized formulation and formalin was increased significantly. In case of 0.4% formalin preserved milk samples, the initial tyrosine value was 42.83 ± 1.39 mg/100 ml milk, which significantly increased after 15 days and continued to increase till 90 days of storage at 37°C. After 90 days the tyrosine value was 167.05 ± 1.97 mg/100 ml milk. In case of milk samples preserved with optimized formulation (0.6%), the initial tyrosine value was 54.60 ± 0.49 mg/100 ml milk, which significantly increased after 15 days and continued to increase till 90 days of storage at 37°C. After 90 days the tyrosine value was 345.33 ± 4.96 mg/100 ml milk.

Conclusions

From this study, it was ascertained that during storage at 37°C, there was no significant effect of optimized formulation (0.6%) on estimation of fat (Gerber and Rose Gottlieb) and lactose content for 45 days, on estimation of total solids, ash and protein content for 90 days. On addition of formalin (0.4%), the fat values of milk decreased significantly after 15 days of storage and also during storage at 37°C, no specific trend was observed. The lactose content was also found to decrease significantly after 60 days of storage. However, there was no any significant effect of formalin (0.4%), on estimation of total solids, ash and protein content of milk for 90 days. The addition of optimized formulation (0.6%) and formalin (0.4%) did not have any significant effect on titratable acidity and FFA content of milk. However, on addition of optimized formulation (0.6%), the tyrosine value was found to be 28.80% higher than tyrosine value of control milk samples. Therefore, it may be concluded that the optimized formulation is able to prevent deterioration of milk upto 45 days at ambient temperature without interfering with the chemical composition of the same and it can serve as a suitable alternative of formalin as preservative.

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Fig. 3 Effect of preservatives on Tyrosine Value (mg/100 ml) of milk during Storage at $37 \pm 1^\circ\text{C}$, Data are presented as means $\pm$ S.E. (n=3) FPM: Formalin Preserved Milk, OPM: Optimized Formulation Preserved Milk

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