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RESEARCH ARTICLE

Studies on suitability to incorporate *Piper betel* leave extract in flavored milk

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Abstract: The present study was planned and executed to evaluate suitability to incorporate *Piper betel* leaves (PBL) in flavor milk and to investigate the antioxidant and phenolic potential of -fortified flavored milk (FM) during its storage. The FM was prepared by fortifying it with aqueous extract of PBL, sugar and carboxy methyl cellulose (CMC) stabilizer @ 5.15, 10.30, and 0.1%, respectively followed by its pasteurization (FM_1). The bottles were stored at 5±1ºC and compared with control (FM_2) i.e. milk without PBL extract. Sensory evaluation was conducted at an interval of 4 days along with proximate composition, physicochemical properties, antioxidant and phenolic potential. It was observed that on 20th day of storage the flavor score for sample FM_2 was <6.00 and that for the sample FM_1 >7.00. The storage period, treatment, and their interaction effect was significantly different (P<0.05) on various quality attributes under study. The viscosity of the FM during storage was altered from 9.62±0.17 to 12.04±0.04 and from 10.85±0.70 to 12.42 ±0.90 cP in FM_1 and FM_2 sample(s), respectively. The change in antioxidant and total phenolic content were also decreased during storage, the values on 0 day of storage for FM_1 was 5.56±0.53 µM/mL and 5.14±0.32 GAE/mL. A significant increase was observed in microbial c ounts from 2.61 ±0.06 to 5.00 ±0.04 $\log_{10}$ cfu/ml during storage for 24 days in FM_1 as compared to the control sample from 2.61±0.03 to 4.75±0.03 $\log_{10}$ cfu/ml. No any coliforms observed in any of the samples during the storage period of 24 days.

Keywords: Antioxidant and phenolic potential; Flavored milk; *Piper betel* leaves; sensory qualities; Storage stability,

Introduction

A trend has been predominantly observed that the children prefer the flavored milk (FM) as compared to the milk, thereby fulfilling the recommended intakes of dairy products. A recent market survey (Theresa et al. 2022) also substantiated these observations. The FM is a sweetened dairy product made from milk, sugar, colorings, and synthetic or natural flavorings. The FM has a remarkable growth @ 27% per annum in the Indian market (Baisya 2005). In India, the flavoured milk market is dominated by several brands (Ravindra et al. 2014). Bisig (2011) defined FM as the ready-to-drink products made from milk of varying fat contents fortified with sweetener, cocoa powder, fruit juice, coffee, aromatics, additives and flavors. Dairy industries developing innovative milk-based beverages and products to meet consumer demand by addressing health concerns through natural ingredients (Keshtkaran et al. 2013).

Natural colors and flavors are the food additives intend to make products more appealing with improved taste. The FM is routinely flavored and colored with synthetic ingredients, may lead to hyperactivity and behavioral problems in children (Weiss, 2012). Therefore the global thrust gaining momentum in the food and beverage industry towards the natural additives owing to their growing demand. Natural flavors and colors are preferable through clean label declarations and these could be easily mixed in milk (Kamble et al. 2019). Various plant-based materials like bark, leaves, seeds, flowers, etc. could be utilized in FM.

The deep green heart-shaped leaves of *Piper betel* leaves (PBL) are popularly known as *Paan* in India. It is also known as *Nagaballi, Nagurvel, Saptaseera, Sompatra, Tamalapaku, Tambul, Tambuli, Vaksha patra, Vettilai, Voojangalata*, etc. in different parts of the country. It's chlorophyll is beneficial in maintaining healthy teeth, refreshes the mouth and throat. It also helps in digestion by enhanced salivation and neutralizing excess

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acid. It is also used as a mouth freshener after the meals (Kumar, 1999). It is rich in essential oils (0.7-2.6 %), carbohydrate (0.5-6.1%), fat (0.4 -1.0%) protein (3.0 -3.5), fiber (2.3%), minerals (2.3-3.3 %), with good source of water and oil soluble vitamins (Guha, 2006 and Ramamurthi and Usha Rani, 2012). Further, the PBL is a rich source of vitamins and minerals (Kruawan and Kangsadalampai, 2006). It is reported that PBL contained a significant amount of antioxidants like hydroxyl chavicol, eugenol, ascorbic acid, and beta carotene (Chakraborty and Shah, 2011). It also contains phenolic compounds, which are bioactive substances that act mostly as radical scavengers (Hodzic et al. 2009) and some act as metal chelators. The presence of an antioxidant is one of the fastest ways to reduce lipid peroxidation to maintain overall product quality (Abdullahi, 2011).

Pasteurized FM gets spoiled at refrigerated temperatures due to the activities of psychotropic bacteria, particularly *Pseudomonas* spp. (Datta and Wallace 2002). The average shelf life of stored FM is approximately 7 days at refrigerated temperature (Khusniati et al. 2008). It is attributed to the extracellular enzymes of the putrefactive organisms (Datta and Wallace 2002). By incorporating the PBL food matrix, it is expected that it can increase the biochemical properties as well storage stability of flavored milk by suppressing the psychotropics. In view of this the present investigation was planned and executed.

Materials and methods

Preparation of ethanolic extract

The ethanolic extract of PBL of Culcatta cultivar was prepared as per the method suggested by Chakaraborty and Shah (2011) with some modifications. About 1 g of PBL was mixed in 10 ml ethyl acetate followed by crushing for a minute and transferred in a centrifuge tube (10 ml capacity). The tube was placed in a dark place for 2 h followed by its centrifugation at 22000 rpm for 5 min. The supernatant was taken (as a sample) for estimation of antioxidant properties.

Preparation of methanolic extract

The methanolic extract of PBL was prepared as above by replacing ethyl acetate with methanol as solvent. The extraction was carried out for 3 h and used for antioxidants estimation.

Preparation of aqueous extract

The aqueous extract of PBL was prepared by mixing 1g of PBL in 10 ml distilled water followed by crushing in mortar and pastel for a min. The solution was filtered through a muslin cloth. The filtrate was transferred in a centrifuge tube and it was placed in a dark place for 2 hr and centrifuged at 22,000 rpm for 10 min. The supernatant was collected (as a sample) for the estimation of antioxidant properties.

Preparation of PBL extract for milk enrichment

Fresh PBL were washed under running tap water and a 10 g leaf sample was crushed by mixing 100 ml of distilled water for 2-3 min. Then leaves extract was allowed to filter through four folded muslin cloths. The filtered extract (10 %) was termed as PBL Extract and was used for flavoring and coloring the milk.

Preparation of flavored milk using (PBLE)

The BLE added FM was prepared as per protocol developed by Kamble et al. (2019) as shown in **Fig. 1**. The product was fill in sterilized glass bottles (200 ml) and sealed by crown cork. These sealed milk bottles were pasteurized at 68°C for 30 min and stored at 5±1ºC (FM_1) and compared with control (FM_2) i.e. milk without added PBLE. In both the samples sugar and stabilizer were mixed @ 10 and 0.1%, respectively. Each sample of FM was evaluated at an interval of 4 days for sensory, physico-chemical, and microbial qualities along with change in antioxidant and phenolic properties.

Estimation of antioxidant and phenolic properties PBL and of FM

Total antioxidant activity was measured by FRAP assay as per Benzie and Strain (1999). Total phenolic content expressed as Oxygen Radical Absorbance Capacity (ORAC) of flavored milk was analyzed by the Folin-Ciocalteu method (Kahkonen et al. 1999).

Physico-chemical analysis of FM

Fig. 1 Flow diagram for preparation of flavored milk added with PBLE (Piper betel leaves extract)

The proximate analysis, as well as pH, acidity, specific gravity, and viscosity of both the milk, were analyzed as per the method of AOAC (2005). The method described by Keeney and Bassette (1959) was used for the measurement of HMF (Hydroxy methyl furfural) value of milk during storage. The TBA value of milk measured as per the method developed by King (1962) with suitable modifications.

Sensory evaluation

Sensory evaluation of PBLE FM was carried out by a semi-trained panel of judges from the Institute using a 9-point Hedonic scale as described by Amerine et al. (1965). Samples were served in the coded number flasks.

Microbial quality analysis

The samples were evaluated for commercial sterility. They were evaluated for total viable counts, Coliform, Yeast and Mould counts by the method described by FSSAI (2016).

Statistical analysis

The data obtained were analyzed statistically in SPSS software (Version 20.0) as per the standard

Results and Discussion

Antioxidants and phenolic properties of *Piper betel* leaves

The methanolic extract of PBL was found to possess a higher concentration of antioxidants (41.22±0.87µg/ml) than ethanolic (34.08±15.9µg/ml) and aqueous extract (14.27±2.77 µg/ml). These findings are in close conformity with those reported earlier (Dasgupta and De, 2004 and Chakaraborty and Shah, 2011). Along with PBL, the antioxidant properties of milk were also determined and it had low antioxidant activity compared to PBL (Fig. 2a and 2b).

The phenolic contents in terms of gallic acid equivalent (GAE)/ml and the ethanolic extract of PBL showed the highest 41.22±0.87 phenolic activity whereas in methanolic and aqueous extract it was 12.82±0.06 and 13.49±0.41 GAE/ml, respectively. As expected the milk sample had lower phenolic content as was expected. Chakaraborty and Shah (2011) monitored the phenols of PBL and reported that the methanolic extract possesses a high concentration of phenolic and flavonoids in moderate concentration and tannins in limited concentrations.

Changes in sensory qualities of FM During Storage

Changes in color and appearance

The color and appearance are most important for the consumers. As it create the first impression towards dairy products. It is clear from Fig. 3a that the color and appearance score was decreased significantly ($p<0.05$) from 8.34±0.10 to 7.06±0.48 in milk added with PBLE (FM_1) whereas, the score of control samples (FM_0) had changed from 8.01±0.16 to 7.38±011. It was observed that the storage period, the addition of PBLE, and their interaction exerted a significant effect ($P<0.05$) on the color and appearance score during storage. The judges reported that as the storage period progressed the color of the FM_1 sample was significantly decreased from the 16th day onwards. It might be because of progress in the Maillard reaction and subsequent increase in HMF (Hydroxy methyl furfural) level as earlier reported by Singh and Patil (1989).

Change in consistency score

Consistency is an important parameter in evaluating fluid products like the FM. The length of the storage period, type of sample, and its interaction with treatment showed a significant effect on consistency score (Fig. 3a). The lower consistency score of FM_1 sample may be because of the addition of PBLE, as was supported by Kumar et al. *(*2017) and Rejesh et al. *(*2017).

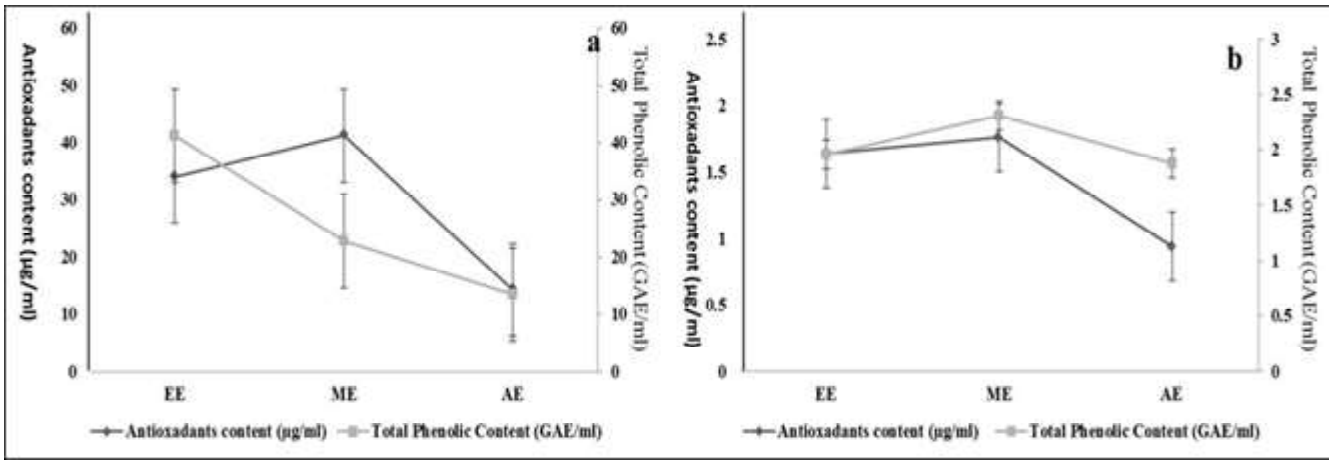

Fig.2: Antioxidants (µg/ml) and Total phenolic content (GAE/ml sample) of (a) *Piper betel* leaves and (b) milk (EE-Ehtanolic extract, ME-Methanolic extract, and AE- Aqueous extract)

The initial consistency score was comparatively lower than the middle period of storage (score 8.06 on 12th days of storage). It may be because of age gelation in the product or aggregation of protein particles. However at the end of the storage period in both the samples the score was significantly lowered.

Effect on sweetness score

The sweetness score for the FM_1 sample was decreased from 8.22±0.10 to 7.04±0.46 during the entire storage period whereas, for the control sample it was decreased from 8.30±0.04 to 7.60±0.53 (Fig. 3a). The storage period had a significant ($P<0.05$) effect on sweetness score ($P>0.05$). Tamilarasi (2001) reported that inverse relationship between sweetness score and storage period might be because of bacterial decomposition of the products.

Changes in flavour score

The mean flavour score of the treated (FM_1) sample, as depicted in Fig. 3b, indicates that the storage period and treatment have significant effect ($P<0.05$). The sample without PBLE fetched scores less than 6, while those with PBLE recorded a score of 7 in the Hedonic scale. It was observed that there was development of a slight staleness at the end of storage period. The flavor score of FM_1 was not changed significantly up to 16th day of storage, thereafter it was significantly ($p<0.05$) decreased. Our findings are in agreement with a study by Ravindra et al. (2014) who reported that the flavored milk showed decrease in flavor score with the increase in storage period.

Changes in overall acceptability

It could be seen from the data presented in Fig. 3b that the maximum overall acceptance score was 8.36±0.01 and 8.08±0.07 for FM_1 and FM_0 sample on 0 day and 6.17 ±0.23 and 6.00 ±0.12 on 24 and 20th day of storage for respective sample. Hassan et al. (2015) recorded the decreasing trend in overall acceptability score during refrigerated storage. Perez and Sanz (2001) described the slight changes in flavor, taste and overall acceptability of FM. It was stated that it may be due to the degradation of ascorbic acid and furfural during storage.

Kumar et al. (2017) found that storage period did not affect significantly ($P<0.05$) the quality characteristics and sensory scores till 15 days of storage during which the control sample recorded overall acceptability score 5.45 ±0.12 on 20th days, which was less than '6' on '9' point i.e. neither like nor disliked hence it was discontinued from the study at this point. Similar action was taken on FM_1 sample on 24th days of storage.

Changes in physico-chemical properties of flavored milk during storage

Changes in specific gravity

It could be seen from **Table 1** that the specific gravity of sample F1 was decreased. The rate of decrease was higher in control sample than that of the treated one. It was found that the effect of storage period and treatment was significant during the storage. Palthur et al. (2014a) also reported the change in specific gravity of the ginger extract added FM as compared to its normal variant. Anandh et al. (2014) observed that the specific gravity of the rose flavored milk (1.035-1.037) showed a slight rising tendency over the control during storage.

Changes in viscosity

It could be seen from the data on viscosity presented in Table 1 that it was increased from 9.62±0.17 to 12.04 ±0.04 P and from 10.85 ±0.70 to 12.42 ±0.90 P in FM_1 and FM_0 samples, respectively. The rate of increase in viscosity was higher in control samples than that of PBL flavored sample. Effect of storage period, treatment and their interactions were significantly ($P<0.05$) affected the viscosity of the product. It is attributed to proteolysis, aggregation enzymatic action and interaction of milk fat and protein. Our findings are supported by Dey and Karin (2013).

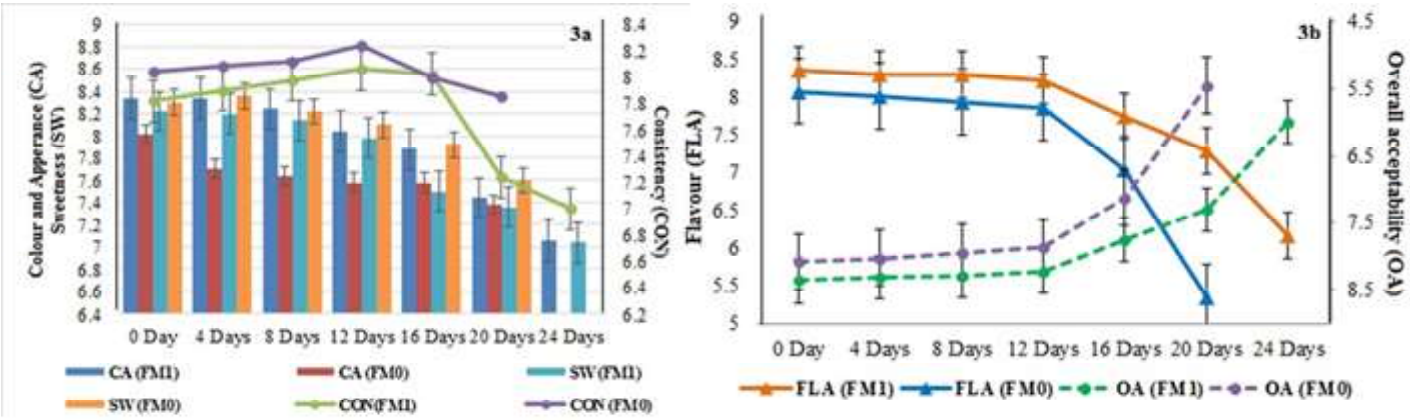

Fig. 3: Changes in sensory attributes (score) of flavored milk during storage at 5±1ºC

(CA-Color and Appearance, SW- Sweetness, CON- Consistency, FLA- Flavour, and OA- Overall acceptability)

Table 1: Changes in proximate composition of flavored milk during storage at 5±1ºC

Parameter (Score)	Sample	Storage period (Day)						
		0	4	8	12	16	20	24
Protein *(%)	FM_1	3.52 ±0.19	3.50 ±0.27	3.44 ±0.27	3.42 ±0.20	3.40 ±0.24	3.36 ±0.16	3.30 ±0.09
	FM_0	3.54 ±0.29	3.52±0.18	3.50 ±0.15	3.46 ±0.21	3.40 ±0.19	3.30 ±0.12	--
	CD (P<0.05)	Storage period NS		Treatment NS		Storage period x Treatment NS		
Fat *(%)	FM_1	1.68 ±0.02	1.68 ±0.04	1.66 ±0.02	1.65 ±0.02	1.63 ±0.03	1.60 ±0.03	1.50 ±0.02
	FM_0	1.72 ±0.05	1.69 ±0.02	1.60 ±0.02	1.58 ±0.01	1.56 ±0.03	1.53 ±0.02	--
	CD (P<0.05)	Storage period 0.32		Treatment 0.17		Storage period x Treatment 0.46		
Lactose *(%)	FM_1	4.60 ±0.09	4.60 ±0.16	4.40 ±0.22	4.22 ±0.15	4.00 ±0.05	3.92 ±0.07	3.88 ±0.19
	FM_0	4.64 ±0.17	4.62 ±0.24	4.42 ±0.21	4.24 ±0.19	4.14 ±0.10	3.98 ±0.18	--
	CD (P<0.05)	Storage period NS		Treatment NS		Storage period x Treatment NS		
Sucrose *(%)	FM_1	10.74 ±0.05	10.74 ±0.17	10.72 ±0.15	10.69 ±0.14	10.69 ±0.25	10.68 ±0.16	10.65 ±0.16
	FM_0	10.80 ±0.06	10.80 ±0.10	10.79 ±0.24	10.80 ±0.46	10.78 ±0.25	10.72 ±0.16	--
	CD (P<0.05)	Storage period NS		Treatment NS		Storage period x Treatment NS		
Ash *(%)	FM_1	0.86 ±0.01	0.89 ±0.01	0.93 ±0.02	0.94 ±0.03	0.94 ±0.01	0.95 ±0.04	0.95 ±0.03
	FM_0	0.85 ±0.09	0.84 ±0.06	0.86 ±0.05	0.87 ±0.03	0.89 ±0.01	0.89 ±0.02	--
	CD (P<0.05)	Storage period 0.18		Treatment 0.09		Storage period x Treatment 0.25		
Total solids *(%)	FM_1	21.67 ±0.05	21.61 ±0.07	21.34 ±0.03	21.06 ±0.25	20.77 ±0.54	20.57 ±0.52	20.20 ±0.69
	FM_0	21.64 ±0.29	21.58 ±0.18	21.25 ±0.18	20.93 ±0.06	20.74 ±0.39	20.31 ±0.50	--
	CD P<0.05)	Storage period 0.69		Treatment 0.37		Storage period x Treatment 0.97		
Specific gravity	Sample (FM_1)	1.064 ±0.012	1.059 ±0.042	1.054 ±0.039	1.052 ±0.028	1.050 ±0.019	1.050 ±0.059	1.050 ±0.059
	Control (FM_0)	1.071 ±0.031	1.068 ±0.044	1.068 ±0.035	1.057 ±0.022	1.054 ±0.016	1.052 ±0.033	--
	CD (P<0.05)	Storage period NS		Treatment NS		Storage period x Treatment NS		
Viscosity *(cP)	Sample (FM_1)	9.62 ±0.17	10.80 ±0.49	11.00 ±0.32	11.50 ±0.55	11.88 ±0.56	11.92 ±0.29	12.04 ±0.04
	Control (FM_0)	10.85 ±0.70	11.00 ±0.55	11.40 ±0.40	11.80 ±0.58	12.06 ±0.92	12.42 ±0.90	--
	CD (P<0.05)	Storage period 1.15		Treatment 0.61		Storage period x Treatment 1.63		

pH	Sample (FM_1)	6.30 ±0.04	6.30 ±0.05	6.16 ±0.05	6.11 ±0.11	6.01 ±0.12	6.00 ±0.17	5.98 ±0.17
	Control (FM_0)	6.32 ±0.07	6.30 ±0.07	6.10 ±0.12	6.00 ±0.12	5.95 ±0.17	5.70 ±0.21	--
	CD (P<0.05)	Storage period 0.27		Treatment 0.14		Storage period x Treatment 0.38		
Acidity *(% LA)	Sample (FM_1)	0.157 ±0.004	0.159 ±0.004	0.165 ±0.007	0.168 ±0.006	0.179 ±0.005	0.186 ±0.003	0.193 ±0.003
	Control (FM_0)	0.154 ±0.005	0.159 ±0.005	0.166 ±0.003	0.172 ±0.005	0.180 ±0.003	0.189 ±0.003	--
	CD (P<0.05)	Storage period 0.009		Treatment 0.004		Storage period x Treatment 0.012		

CD: Critical difference NS: Non-significant *: Significant difference

Table2. Changes in Antioxidant*(µM/mL) and Phenolic content *(GAE/ml) of flavoured milk during storage at 5±1ºC

Parameter	Treatment	Storage period (Days)						
		0	4	8	12	16	20	24
Antioxidant* (µM/mL)	Sample (FM_1)	5.56 ±0.53	5.49 ±0.59	5.42 ±0.44	5.39 ±0.29	5.33 ±0.25	5.22 ±0.30	5.18 ±0.89
	Control (FM_2)	4.15 ±0.22	4.06 ±0.28	4.07 ±0.04	4.00 ±0.48	4.00 ±0.55	3.91 ±0.44	--
CD P<0.05)		Storage period 0.49		Treatment 0.46		Storage periodxreatment 0.63		
Phenolics* (GAE/ml)	Sample (FM_1)	5.14± 0.32	5.08± 0.35	5.02± 0.22	5.00± 0.56	4.98± 0.14	4.90± 0.23	4.88± 0.24
	Control (FM_2)	4.34± 0.39	4.29± 0.19	0.98± 1.79	4.00± 0.34	3.96± 0.04	3.89± 0.58	--
CD P<0.05)		Storage period 0.65		Treatment 0.66		Storage period x Treatment 0.77		

The possible increase in viscosity of PBLE added milk may be related to interaction of herb component with milk constituents and its destabilizing effect.

Changes in pH

The average pH value was found higher in control sample than that fortified with PBLE. A highly significant ($p<0.05$) decrease in pH value was noticed from 16th and 8th days of storage period in flavored and control sample, respectively (Table 1). Average pH value was found lower and statistically different in PBLE added flavored milk i.e., 6.17 than control. Gupta et al. (2017) and Sawale et al.(2017) observed decreasing pH of functional dairy drinks during storage. Wegrzyn et al. (2008) found that the pH of pasteurized apple flavor milk decreased with storage period of 12th week. These workers also reported a drop in pH of herb-fortified flavor milk.

Changes in proximate composition of milk during storage

The protein content of treated sample (FM_1) was significantly decreased as compared to the control sample (FM_0). The changes in protein content were not significant during storage period (Table 1). These finding are in close conformity with the report of Hassan et al. (2015). These workers observed that during refrigerated storage the mean protein content of beverages showed slight decrease in protein content, but the difference was nonsignificant ($p > 0.05$). The fat content of treated sample 0, 4, 8, 12,16, 20 and 24 days were 1.68±0.02, 1.68±0.04, 1.66±0.02, 1.65±0.02, 1.63 ±0.03, 1.60 ±0.03 and 1.50±0.02%, respectively. The lactose content of treated (FM_1) milk sample was 4.60±0.09% on the 0 day storage and in control milk (FM_0) sample lactose content was 4.64 ±0.17. Slight decline in lactose content during storage might be due to utilization of lactose by bacteria and its conversion into lactic acid, such type of effect of slightly observed in milk treated with PBLE. Similar results were reported by Shukla et al. (2018). The sucrose content of the treated and control sample milk were ranges from 10.74 to 10.80%, respectively.

Sucrose content of milk sample was decreased with increased storage period. It was observed that the ash content of the sample was slightly increased in treated sample (FM_1) as compared to control (FM_0). The initial average values of TS of treated samples and control samples are shown in Table 1 which shows that the TS of the control sample is slightly higher than the treated sample due low moisture content. Ammra et al. (2009) reported a non-

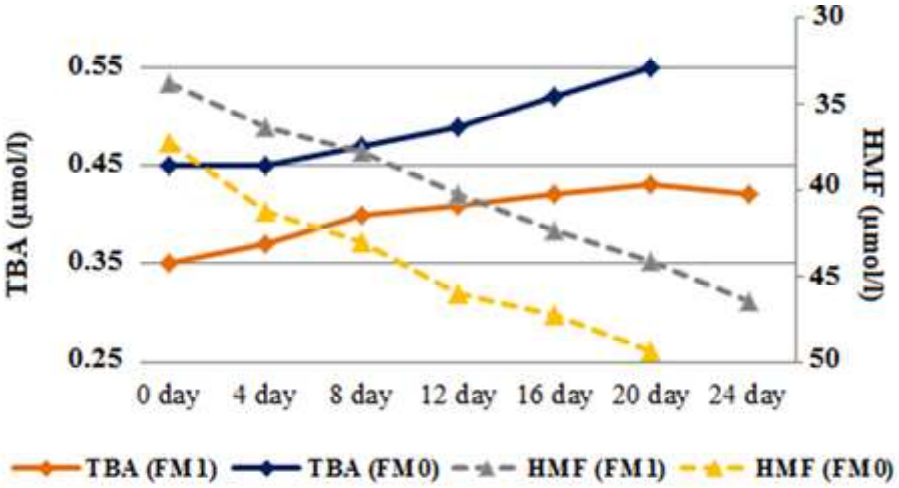

Fig.4 Changes in TBA*(µmol/l), HMF*(µmol/l), Antioxidant*(µM/mL), Phenolics* (GAE/ml) milk during storage at 5±1ºC

significant decline in TS content of UHT milk during the storage period. Singh et al. (2014) also observed same trends.

Effect on chemical parameters

Lipid oxidation

The extent of lipid oxidation during storage was measured in terms of thio-barbituric acid (TBA) value. The changes in TBA value (in terms of absorbance) of samples stored at refrigeration are presented in Fig. 4. In all samples, a steady and significant increase in TBA value was observed during storage. The effect of storage period, treatment and interaction showed significant changes in TBA values. Results are in well agreement with earlier report (Bandyopadhyay et al. 2007) who incorporated herbs such as turmeric (*Curcumalonga L.*), coriander (*Coriandrumsativum L.*), curry leaf (*Murrayakoenigii L.*), spinach (*Spinaciaoleracea*) and Aonla (*Emblica officinalis*) at 10% levels in Sandesh. In the present study, a decreased rate of oxidation in case of herb supplemented samples might be related to antioxidant compounds of herbs which inhibited the lipid oxidation. Dasgupta and De (2004) reported similar results.

Effect on HMF

It was observed that the HMF content of the products increased significantly ($p<0.05$) during storage (Fig.4) in treated and control samples. The interaction between treatment and days of storage was found to be significant ($P<0.05$) in case of HMF. Similar findings are reported by Richards et al. (2016) and Shukla et al. (2018) in case of storage of UHT milk and flavored dairy drinks.

Changes in antioxidant and phenolic properties during storage

Changes in antioxidant activity

The antioxidant activity was 5.56±0.53 and 4.15±0.22 µM/mL in FM_1 and $FM_{0,}$ respectively on 0 day of storage (Table 2). During storage period their rate of ferric reducing antioxidant power of both the milk was decreased. The storage period, treatment and their interaction had exerted a significant ($P<0.05$) effect on antioxidant content of the product. The herb PBLE contains bioactive compounds such as Chavicol, Chavibetol, Chavibetol acetate, and Eugenol, etc. (Swapna et al. 2012) which could be responsible for the higher radical scavenging activity of PBLE added sample as compared to their controls. In the present study, higher antioxidant activity could be due to the interaction of herb or milk constituents with oxygen or any other component that might have quenched radicals during heating leading to increased antioxidant activity. The antioxidant properties of the PBLE leaves were also reported by Kruawan and Kangsadalampai, (2006); Niranjan et al. (2002), Dasgupta and De, (2004) and Chakraborty and Shah (2011).

Changes in phenolic content

Phenolic compounds in herbs and spices act mostly as radical scavengers and metal cheaters are considered as potential protectors against lipid oxidation (Abdullahi, 2011). In the present study, on the day first higher phenol content (5.14±0.32 GAE/ mL) was found in PBLE added sample (FM_1), than in the control ($FM_{0)}$. In both, the sample the total phenols were decreased and the rate of degradation of total phenol content was significantly (<0.05) lower in a treated sample over control (Table 2). Changchub and Maisuthisakul (2011) reported that encapsulation of mango seed kernel using maltodextrin, gum arabic and tween-80 by spray drying technique had obtained significantly ($p>0.05$) low phenol content compared to non-encapsulated form.

Chang et al. (2010) observed a rise in phenolic content of the *Ginseng jungkwa* extract after heat treatment (100ºC for 3, 6 and 12 h). It was attributed to phenolic content, which leads to increase the free and conjugated phenolic acid and maltol content during heat treatment. Increase in phenolic content of sample could be ascribed to the release of the bound phenolic compounds from PBLE during its pasteurization at 68°C/30 min. In case of

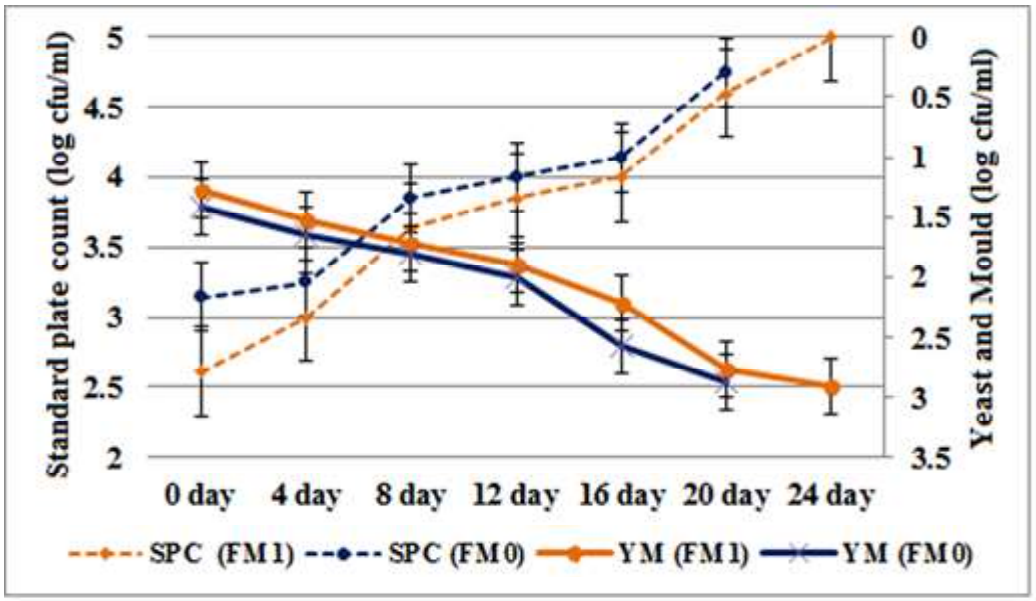

Fig. 5: Changes in microbial quality of flavored milk during Storage at 5±1ºC

encapsulated dairy drink, maltodextrin-gum arabic matrix might have resisted the release of phenolic compounds into outer medium thus resulted in lower amount of phenolic content during the analysis.

Changes in microbial quality of flavored milk during storage at 5±1ºC

It could be seen from the data presented in Fig. 5 that there was a sharp increase in standard plate count from an initial count of 2.61±0.06 cfu/ml to 5.00±0.04 log cfu/ml at the end of shelf life (24 days) in case of FM_1 and in case of FM_0 count were increased (Fig. 5). There was no any coliform in both treated and control sample up to 20th and 24th day of storage period. There was sharp increase in yeast and mould count from initial of 1.28 ±0.07 logcfu/ml to 2.91±0.02 log cfu/ml at the end of storage period which is attributed to an increase in acidity during fermentation process which possibly might have provided suitable conditions for growth (Sengupta, et al. 2013 and Nagarajeppa and Butulla, 2017). Datta et al. (2011) and Sripradha (2014) studied various properties of PBL include antioxidant, antifungal, and antimicrobial properties, it was reported that the *Piper betel* appears to be a promising and valuable source of antimicrobial compounds which acts against undesirable microorganisms in milk.

Conclusions

The antioxidant properties and phenolic activity of aqueous extract of PBL were 4.27±2.77 µg/ml and 3.49±0.41 GAE/ml, respectively. Based on sensory evaluation, the milk added with PBL aqueous extract could be stored up to 20th days as against the plain sweet milk up to 16th days of storage at 5±1ºC. During storage there were increased in the values of acidity, viscosity, TBA and HMF content, whereas, the values of specific gravity, pH, protein, fat, lactose, sucrose and total solid content were decreased. The antioxidant and phenolic properties of milk were decreased as storage period progress. The standard plate count and yeast and mould counts in both the samples were increased during storage.

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