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EFFECT OF BETEL LEAF EXTRACT ON PHYICO-CHEMICAL AND MICROBIAL QUALITY OF PASTEURIZED MILK STORED AT REFRIGERATED TEMPERATURE

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Abstract: A study was conducted to assess the efficacy of betel leaf extract in extending the shelf life of pasteurized milk. Raw milk was collected from organized dairy farm was laboratory pasteurized was added with 0.5 percent of betel leaf extract and stored at refrigerated temperature. The physico-chemical and microbiological parameters were studied at regular intervals of 0,2,4,6 days respectively. It was found that addition of 0.5 percent betel leaf extract extended the shelf life of pasteurized milk by 5 days by clot on boiling test. There was a gradual decrease in pH and increase in titratable acidity was observed in the milk added with the extract during storage. Standard plate count and psychrotrophic count showed restricted increase during the storage period. Statistical analysis showed highly significant difference ($P \leq 0.01$) in treatments during 2^{nd} and 4^{th} days of storage.
Keywords: Pasteurized milk, Betel leaf, Standard plate count, Psychrotrophic count.

Introduction

Milk is an easily perishable raw material. Contaminating bacteria may multiply rapidly and render it unsuitable for processing or unfit for human consumption. The psychrotrophic bacteria were killed during pasteurization however, after the pasteurisation process, particularly if hygiene inthe processing plant is of a poor standard, these psychrotrophic bacteria can re-contaminate milk (Craven and Macauley, 1992). The growth of these bacteria, especially *Pseudomonas* spp. Occurred during the time of milk storage, higher the growth and enzyme activities of the psychrotrophic bacteria in the milk, which may result in spoilage of the milk in storage (Deeth *et al*., 2002). The spoilage of pasteurized milk in refrigerated storage may result in the microbial and chemical changes, and the volatile compounds of the milk (Reinheimer *et al*., 1993). The biochemical changes of milk during spoilage may be a result of the activities of extracellular enzymes especially protease

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which degrades protein (Janzen *et al*.,1982) and lipase which degrades lipid (Bucky *et al*., 1986).

Natural antibacterial substances like extracts of herbs can be used in preservation of milk. The mode of action of these substances is inhibition of microbial growth, oxidation and certain enzymatic reactions occurring in milk. Phenols and polyphenols are water soluble compounds which can be easily mixed with milk. The use of plant extracts as a source of phenols is preferred as a natural method (Gad and Salam, 2010). Herbal extracts when added to milk in storage can suppress the growth and enzymatic activities of psychrotrophic bacteria, thus increasing the quality and shelf life of pasteurised milk. The present study was carried out to study use of betel leaf extract to extend the shelf life of pasteurized milk stored at refrigerated temperature.

Materials and Methods

Fresh betel leaves (*Piper betel* Linn) procured from the local market in Chennai was shade dried and powdered as per the method of Preethi *et al.* (2010). Betel leaf powder (10 g) was immersed in 100 ml of sterile distilled water, mixed and allowed to soak for 24 hours, then filtered through whatman No.4 filter paper to obtain a clear extract. The extract was diluted with sterile distilled water to a concentration of 100 mg/ml and then stored in air tight containers at refrigerated temperature

The milk samples collected from the farm was laboratory pasteurized at a temperature of 63ºC for 30 minutes and cooled to 4°C. 0.5 per cent aqueous betel leaf extract was added and a control sample without extract were stored at refrigerated temperature. The samples were analysed for physico-chemical and microbiological parameters like Clot on boiling test (COB), pH, Titratable acidity, Standard plate count and psychrotrophic count done on 0,2,4 and 6 days as per the (BIS, SP: 18 (Part XI)-1981.

Results and discussion

Table 1 shows that the pasteurized milk (control) remained acceptable up to 2nd day of storage and samples (T1) added with 0.5 per cent aqueous extract of betel leaves showed an increase in shelf life up to the 5th day of storage at refrigerated condition. The average shelf life of pasteurized milk at refrigerated temperature was 2 to 3 days (Foreman, n.d). Khusniati and Yantyati (2008) reported that additions of aromatic materials viz. Cinnamon, ginger, turmeric, pepper etc, increased the shelf life of commercial whole milk up to 5 days at 4°C. These studies support the findings of the present research.

The pH decreased from 6.56 ± 0.014 to 5.95 ± 0.005 during 4 days of storage in pasteurized milk (control), whereas the milk (T1) treated with 0.5 per cent aqueous extract of betel leaves showed gradual decrease in pH from 6.56 ± 0.012 to 6.10 ± 0.003 during 6 days of storage (Table -2). This observation is in accordance with the finding of Shari *et al.* (1996) who reported that pasteurized, unhomogenised whole milk stored at 7 °C showed a decrease in pH during 6 days of storage.

The titratable acidity (per cent lactic acid) of the pasteurized milk (control) increased from initial value of 0.16 ± 0.002 to 0.24 ± 0.004 on 4^{th} day of storage period, whereas 0.5 per cent betel extract treated milk sample (T1) showed titratable acidity of 0.21 ± 0.003 on 6^{th} day of storage. The statistical analysis showed that there was a highly significant difference ($P \leq 0.01$) between treatments during 2^{nd} and 4^{th} days of storage. The values for titratable acidity was almost similar compared with reports of Khusniati and Yantyati (2008) who observed that acidity of pasteurized milk supplemented with aromatic materials increased slowly when compared to control.

Standard plate count of pasteurized milk (control) was 4.22 ± 0.027 $\log_{10}$cfu/ml which increased during the storage period up to 4^{th} day (4.77 ± 0.047 $\log_{10}$cfu/ml) whereas, milk samples (T1) treated with 0.5 per cent aqueous extracts of betel leaves showed a restricted increase in standard plate count during the storage period of 6 days from 4.22 ± 0.027 to 4.72 ± 0.0036 $\log_{10}$cfu/ml. The results were in agreement with the study of Ultee *et al.* (1999) who interpreted that phenolic compounds exhibited higher antibacterial activity at 4°C.

Psychrotrophic count in control increased from 3.09 ± 0.019 $\log_{10}$cfu/ml to 3.89 ± 0.009 $\log_{10}$cfu/ml on 4^{th} day of storage, whereas, the pasteurized milk sample(T1) treated with 0.5 per cent aqueous extract of betel leaves showed an increase from 3.09 ± 0.019 $\log_{10}$cfu/ml to 3.87 ± 0.065 $\log_{10}$cfu/ml on 6^{th} day of storage. The increase in psychrotrophs during refrigerated storage influence the shelf life of efficiently refrigerated pasteurized milk as per the report of Foreman (n.d). Statistical analysis showed that there was a highly significant difference ($P \leq 0.01$) between treatments during 2^{nd} and 4^{th} days of storage. This was similar with findings of Khusniati and Yantyati (2008) who reported that the aromatic compounds added to whole milk suppressed the bacterial count, protease and lipase activities of psychrotrophic bacteria during storage period of 5 days even after the expiry date.

Conclusion

In the present study it was concluded that the addition of betel leaf extract at 0.5 percent level in pasteurized milk extended the shelf life of milk to 5 days at refrigerated

storage. The addition of extract results in the suppression of bacterial growth and inhibition of protease and lipase activity of psychrotrophic bacteria in milk.

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Table -1
Clot on boiling test for pasteurized milk with 0.5 per cent (v/v) aqueous extract of betel leaves at refrigerated storage

Storage time (Days)	Control pasteurized milk	T1 Pasteurized milk with 0.5% aqueous. extract of betel
0	-ve	-ve
1	-ve	-ve
2	-ve	-ve
3	+ve	-ve
4	+ve	-ve
5	+ve	-ve
6	+ve	+ve

@Average of six trials

Table- 2

pH, titratable acidity, standard plate count and psychotrophic count of pasteurized milk with 0.5 per cent(v/v) aqueous extract of betel leaves at refrigerated storage (Mean±SE)[@]

Storage time (Days)	pH			Titratable acidity[#]			Standard PlateCount (log_{10}cfu/ml)			Psychrotrophic Count (log_{10}cfu/ml)		
	Control pasteurized milk	T1 Pasteurized milk with 0.5% aqueous. extract of betel	t value	Control pasteurized milk	T1 Pasteurized milk with 0.5% aqueous. extract of betel	t value	Control pasteurized milk	T1 Pasteurized milk with 0.5% aqueous. extract of betel	t value	Control pasteurized milk	T1 Pasteurized milk with 0.5% aqueous. extract of betel	t value
0	6.56±0.014	6.56±0.012	0.00^{NS}	0.16±0.002	0.16±0.003	0.00^{NS}	4.22±0.027	4.22±0.027	0.00^{NS}	3.09±0.019	3.09±0.019	0.00^{NS}
2	6.10±0.003	6.46±0.007	9.90^{**}	0.20±0.005	0.17±0.003	4.29^{**}	4.48±0.006	4.31±0.047	3.47^{**}	3.54±0.009	3.33±0.064	3.51^{**}
4	5.95±0.005	6.35±0.005	10.65^{**}	0.24±0.004	0.19±0.002	6.26^{**}	4.77±0.047	4.48±0.034	4.91^{**}	3.89±0.009	3.65±0.031	6.99^{**}
6	-	6.10±0.003		-	0.21±0.003		-	4.72±0.036		-	3.87±0.065	

NS – Non significant (P>0.05)

** Highly significant (P≤0.01)

Acidity expressed as percentage of lactic acid