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BETEL LEAF EXTRACT AS SHELF LIFE EXTENDER OF RAW MILK

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Abstract: A study was conducted to assess the efficacy of betel leaf extract in extending the shelf life of raw milk. Raw milk collected hygienically from an organized dairy farm was assessed by addition of aqueous extracts of betel leaves at different concentrations viz., 0.25, 0.5, 0.75 and 1 per cent and studied for physico-chemical parameters at regular intervals at 37 °C. It was found that addition of aqueous extract of betel leaves extracts at 0.5 per cent (v/v) extended the shelf life of raw milk by 5 hours by clot on boiling test. There was a controlled increase in titratable acidity in the milk samples added with 0.5%, 0.75% and 1% level of betel leaves extract. The Statistical analysis of data revealed that acidity, standard plate count of the aqueous extract of betel leaves treated raw milk showed highly significant difference ($P \leq 0.01$) between treatments from 2nd hour of storage period and within the treatments at different hours of storage.
Keywords: Betel leaves, Clot on boiling test, titratable acidity, standard plate count.

Introduction

Milk is a highly nutritious and complete food which provides almost all the nutrients for growth and maintenance of the human body. It is a suitable medium for the growth of almost all spoilage as well as pathogenic organisms. Since milk is perishable, special measures and considerations are necessary to ensure that it reaches the market in an acceptable condition. The preservation of milk in the tropical countries like India is a major bottleneck especially where refrigeration facilities are limited and increased environmental temperature. Currently there is a growing interest to use natural antibacterial compounds like extracts of herbs and spices for the preservation of food. The mode of action of natural preservatives 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). The present study was carried out to study use of betel leaf extract to extend the shelf life of raw milk.

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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.

Raw milk was collected hygienically from the organized dairy farm in sterilized sample bottles (100 ml) with aqueous betel extract at 0.25, 0.5, 0.75 and 1 per cent (v/v) concentrations. One sample was maintained as a control without extract, and all the samples were stored at a temperature of 37 °C. The samples were analysed for physico-chemical parameters like Clot on boiling test (COB), titratable acidity and Standard plate count done as per the (BIS, SP: 18 (Part XI)-1981).

Results and Discussion

The control raw milk samples were acceptable up to 6 hours at 37 °C with a negative COB test. At 0.5 per cent level addition of aqueous extract of betel leaves to the raw milk, it remained acceptable up to 11 hours of storage. Addition of higher concentrations up to 0.75 and 1 per cent level of the aqueous extracts of betel leaves did not increase the shelf life of raw milk beyond 11 hours. These findings were in accordance with Ray (2008) who stated that the control milk sample remained acceptable up to 4 hours of storage at 30 ± 2 °C, whereas banana pseudo stem treated milk samples extended the shelf life only for 2 hours than that of control. He also stated that higher concentrations of the banana pseudo stem juice did not increase the shelf life of raw milk.

Control raw milk samples were acceptable up to 6 hours at 37 °C with 0.19 ± 0.003 titratable acidity (Table -1) and this was similar to the findings of Ray (2008).Milk samples treated with 0.5, 0.75 and 1per cent remained acceptable up to 11 hours of storage period. Statistical analysis of data revealed that there was no significant difference up to 1 hour of storage, which was in agreement with the observations of Urbiene and Leskauskaite (2006). Highly significant difference ($P \leq 0.01$) was found between treatments from 2nd hour of storage and within the treatments during the storage period between 2 to 12 hours.

The standard plate count of the untreated samples showed a gradual increase, whereas the treated samples showed a restricted increase of standard plate count up to 12 hours of storage (Table -2) This correlates with the findings of Ray (2008) who found the restricted increase

in standard plate count during the storage period in the milk treated with banana pseudostem juice. Statistical analysis showed a highly significant difference ($P \leq 0.01$) between treatments during the storage period. The restricted increase in standard plate count in treated samples may be due to the phenolic compounds possessing broad spectrum of antimicrobial activity present in the betel extracts (Chandra *et al*., 2012). The action involves interaction of the cytoplasmic membrane with phenolic compounds and activity is selectively higher against gram positive bacteria (Sharma *et al.,* 2010).

Conclusion

In this present study it was concluded that addition of 0.5 percent betel leaf extract (v/v) to raw milk remained acceptable upto 11 hoursof storage at 37°C. Addition of higher concentrations up to 0.75 and 1 per cent level of the aqueous extracts of betel leaves did not increase the shelf life of raw milk beyond 11 hours.

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Table 1

Acidity[#] of the raw milk with addition of aqueous extract of betel leaves at different concentrations (v/v) at 37°C (Mean±SE)[@]

Storage time (Hrs)	Control raw milk	T1 0.25% aqueous extract of betel leaves	T2 0.5% aqueous. extract of betel leaves	T3 0.75% aqueous extract of betel, leaves	T4 1% aqueous. extract of betel, leaves	F value
0	0.14 ± 0.002^{aA}	0.14 ± 0.003^{aA}	0.14 ± 0.003^{aA}	0.14 ± 0.002^{aA}	0.14 ± 0.002^{aA}	0.577^{NS}
1	0.15 ± 0.002^{abA}	0.15 ± 0.002^{aA}	0.14 ± 0.002^{aA}	0.14 ± 0.003^{aA}	0.14 ± 0.004^{aA}	1.579^{NS}
2	0.16 ± 0.003^{bC}	0.15 ± 0.002^{aBC}	0.15 ± 0.004^{aAB}	0.14 ± 0.003^{aA}	0.14 ± 0.004^{aA}	6.235^{**}
3	0.18 ± 0.003^{cB}	0.17 ± 0.002^{bB}	0.16 ± 0.003^{bA}	0.15 ± 0.003^{abA}	0.15 ± 0.005^{aA}	17.417^{**}
4	0.18 ± 0.002^{cC}	0.17 ± 0.003^{bB}	0.16 ± 0.002^{bAB}	0.15 ± 0.004^{bcA}	0.15 ± 0.003^{aAB}	9.423^{**}
5	0.19 ± 0.003^{cC}	0.17 ± 0.005^{bcAB}	0.17 ± 0.003^{bAB}	0.16 ± 0.004^{bcA}	0.16 ± 0.003^{aB}	4.866^{**}
6	0.19 ± 0.003^{cdB}	0.17 ± 0.002^{bcdA}	0.17 ± 0.002^{bA}	0.16 ± 0.005^{bcA}	0.17 ± 0.004^{aA}	5.878^{**}
7	0.20 ± 0.004^{dD}	0.18 ± 0.003^{bcdeC}	0.17 ± 0.002^{bAB}	0.16 ± 0.003^{cA}	0.18 ± 0.006^{aBC}	12.056^{**}
8	0.21 ± 0.003^{eB}	0.18 ± 0.003^{cdeA}	0.17 ± 0.005^{bA}	0.17 ± 0.004^{dA}	0.18 ± 0.002^{aA}	23.333^{**}
9	0.22 ± 0.005^{egC}	0.19 ± 0.003^{defB}	0.17 ± 0.002^{bA}	0.17 ± 0.006^{dA}	0.19 ± 0.004^{aB}	17.158^{**}
10	0.23 ± 0.003^{cG}	0.19 ± 0.003^{efAB}	0.19 ± 0.004^{cAB}	0.18 ± 0.003^{deA}	0.19 ± 0.005^{aB}	17.269^{**}
11	0.25 ± 0.004^{hB}	0.20 ± 0.004^{fA}	0.19 ± 0.002^{cA}	0.19 ± 0.005^{efA}	0.19 ± 0.003^{A}	30.070^{**}
12	0.34 ± 0.004^{iC}	0.24 ± 0.003^{gB}	0.20 ± 0.005^{dA}	0.21 ± 0.004^{fA}	0.20 ± 0.002^{A}	137.115^{**}
F value	135.215^{**}	34.375^{**}	24.792^{**}	23.593^{**}	21.534^{**}	

Different superscripts in a row (capital caps) and column (small caps) differ significantly
NS – Non significant (P>0.05)
** Highly significant (P≤0.01)# Titratable acidity expressed as percentage of lactic acid

Table 2

Standard Plate Count (log_{10}cfu/ml) of the raw milk with aqueous extract of betel leaves at different concentrations (v/v) at 37°C (Mean±SE)[@]

Storage time (Hrs)	Treatments					
	Control raw milk	T1 0.25% aqueous extract of betel leaves	T2 0.5% aqueous .extract of betel leaves	T3 0.75% aqueous. extract of betel leaves	T4 1% aqueous. extract of betel leaves	F value
0	5.27^{Aa}±0.017	5.27^{Aa}±0.017	5.27^{Aa}±0.017	5.27^{Aa}±0.017	5.27^{Aa}±0.017	0.00^{NS}
3	5.81^{Cb}±0.011	5.41^{Bb}±0.015	5.37^{ABa}±0.018	5.35^{Aa}±0.012	5.39^{ABa}±0.019	156.567^{**}
6	6.08^{Bc}±0.003	5.67^{Ac} ±0.011	5.56^{Ab}±0.013	5.60^{Ab}±0.014	5.73^{Ab}±0.155	13.531**
9	6.16^{Cd}±0.018	5.87^{Bd}±0.035	5.61^{Ab}±0.030	5.81^{Bb}±0.029	5.82^{ABb}±0.065	12.594^{**}
12	6.78^{Ce}±0.033	6.44^{Be}±0.048	6.12^{Ac}±0.085	6.21^{Ac}±0.097	6.26^{ABc}±0.088	12.128^{**}
F value	874.886^{**}	254.371^{**}	29.529^{**}	17.736^{**}	20.00^{**}	

Different superscripts in a row (capital caps) and column (small caps) differ significantly
NS – Non significant (P>0.05)
* - Significant (P≤0.05)
** Highly significant (P≤0.01)