

Influence Of Piper Betel Leaf Extract To Prevent The Premature Spoilage Of Milk

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Abstract: Milk is a nutritious and complete food with minerals and vitamins. Milk provides almost all the nutrients for growth and maintenance of the human body. The milk is a highly perishable commodity and it must be protected from spoilage before reaching the consumers. Currently an interest is going on to use natural products based preservative to prevent spoilage of milk before cooking. In the present study a natural product, betel extract is tested as milk preservative agent. The shelf life of the raw and pasteurized milk added with Piper betel Linn. extract was found to be 5 hours and 11 hours respectively without refrigeration. This is due to the action of the plant extracts on the microbes which causes spoilage.

Index Terms: Raw milk, Pasteurized milk, Lactobacillus acidophilus, Piper betel, Phenolic compounds.

1. INTRODUCTION

Milk is a nutritious and complete food with minerals and vitamins. Milk provides almost all the nutrients for growth and maintenance of the human body. According to United Nations Food and Agriculture Organization, world milk production increased from 800.2 MMT in 2016 to 811.9 MMT in 2017, a growth rate of 1.46 per cent. The per capita availability of milk in India during 2017-18 was 375 gm/day and by 2023-24, it is estimated to increase to 592 gm/day. Livestock economy is important for India, sustaining 73 million rural households in it. According to latest report of the Central Statistics Office, livestock value output is Rs. 917,910 crore at current prices in 2016-17. Milk constitutes 67 per cent of the total value or Rs. 614,387 crore. The contribution of cattle milk is less than 50 per cent of the total milk produced in India. Consuming a litre of milk per day meets the nutrient requirements of human beings. ([https:// www. downtoearth. org.in/news/agriculture/centre-asks-nddb-to-estimate-india-s-milk-demand-62778](https://www.downtoearth.org.in/news/agriculture/centre-asks-nddb-to-estimate-india-s-milk-demand-62778)). The milk is a highly perishable commodity and it must be protected from spoilage before reaching the consumers. From cattle to consumers the milk is exposed to microbial contamination and gets spoiled. Even at the consumer end it has to be preserved from spoilage till cooking or refrigeration. To prevent spoilage during the transportation of raw and pasteurized milk chemical preservatives are used. The chemical preservatives are hazardous to the health of consumers particularly infants and aged. So an alternative method of milk preservation is needed till it is boiled or used or reaching the customers. FAO (1957) recommended the use of hydrogen peroxide after specifying safety measures of using hydrogen peroxide for preservation of milk. Hydrogen peroxide was found to prevent the souring of raw milk and was used for preservation of milk in cheese making (Roushdy, 1959). Saha et al. (2003) reported that the addition of 0.03 to 0.04 per cent hydrogen peroxide in raw milk, extended the shelf life upto 22-24 h. However, the deleterious effect of hydrogen peroxide on sulphur containing amino acids and vitamin C, the use of H₂O₂ for milk preservation is

prohibited. So the use of natural spoilage preventing compounds from herbs and spices are recommended (Smid and Gorris, 1999). Khusniati and Yantiyati, (2008) used ginger, garlic, turmeric, betel vine and Aloe vera extracts to prevent premature spoilage. Gad and Salam (2010) used plant derived phenols as a natural preservatives. Krushna et al. (2007) used honey as natural preservatives. So an interest is going on to use natural products based preservative to prevent spoilage of milk before cooking. In the present study a natural product, betel extract is tested as milk preservative agent.

2. MATERIALS AND METHODOLOGY

2.1. Preparation of the piper betel leaf extracts

The Piper betel leaves of 500 g was washed using the sterile distilled water and shade dried for 3 weeks for the complete removal of the moisture content till the weight was reduced to 30 gm. The dried Piper betel leaves weighing 30g was crushed to powder and added to the warm sterile distilled water of 250 ml. After 24 hours, it was filtered using Whatman filter paper no. 4 for separating the extracts. The obtained extracts were stored in the glass brown bottle at the refrigeration temperature for further use.

2.2. GC-MS study of extracts

The compounds present in the betel leaf extract was analysed using the GC MS analysis (Shimadzu GC-2010) and phenolic compounds (R)-3-Pyrrolidinol and, 2',6'-Dihydroxyacetophenone were in high proportion. The pasteurized milk of Aavin brand of Tamilnadu and raw milk local milk man in Thiruvallur of Tamilnadu were used. The pH and acidity of the pasteurized milk and the raw milk was determined. The samples were tested for the presence of Lactic acid bacteria (LAB) count using the agar plating method. After 48h of incubation at 35-42 °C the colonies developed were isolated and counted. To 15ml of pasteurized and raw milk sample the isolated colonies were inoculated. Luria Bertani (LB) agar was prepared and sterilized at 121°C for 20 minutes using autoclave for plating of microbes associated with the pasteurized and raw milk. The solidified agar plates were spread with the milk sample of 100µl. The discs were prepared with the betel leaf extracts (50%) and placed over the agar plates containing the sample. These plates were incubated at 35°C for 24 h. The diameter of the zone of inhibition was noted. The dose (50%) of extract that was good to inhibit the bacterial growth was added to 100 ml of pasteurized and raw

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milk samples separately. The required amount of extracts to be added was also been optimised by adding different amounts ranging from 500 µl to 10 ml of the extracts to 100 ml of the milk sample.

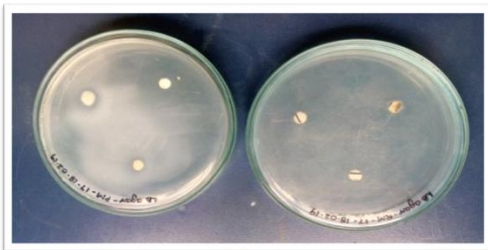


Fig.1 LB agar plated with raw and pasteurized milk with leaf extract containing disks

3. RESULTS AND DISCUSSION

The study showed that the zone of inhibition for LAB on treatment with 500ul Of 50% dose of betel leaf extract was 12 mm for the isolates from raw milk and 10 mm for LAB from pasteurised milk. For the standard antibiotic, Niacin (20%), it was 11mm. When 100ml of raw milk was added with the 500µl of the plant extracts the pH, acidity and LAB load was within the acceptable limit for 5 h in raw milk and 11h for pasteurised milk. (Fig1)

Table 1. pH, Acidity and Microbial Count in the raw milk after the addition of leaf extract.

TIME (in hours)	pH	Acidity %	Microbial Count in MRS (* 10 ⁸ CFU/ml)
0	6.84	0.44	6
1	6.84	0.46	5
2	6.81	0.48	4
3	6.75	0.49	6
4	6.70	0.49	6
5	6.70	0.50	7

Table 2. pH, Acidity and Microbial Count in the pasteurised milk after the addition of leaf extract.

TIME (in hours)	pH	Acidity %	Microbial Count in MRS (* 10 ⁸ CFU/ml)
0	6.98	0.48	0
1	6.97	0.51	3
2	6.96	0.52	4
3	6.88	0.53	4
4	6.84	0.54	5
5	6.76	0.56	6
6	6.72	0.57	7
7	6.72	0.58	8
8	6.71	0.60	9
9	6.70	0.61	9.3
10	6.66	0.58	10
11	6.63	0.57	11

The shelf life of the raw and pasteurized milk added with Piperbetle Linn. was found to be 5 hours and 11 hours respectively without refrigeration(Table 1&2). This is due to the action of the plant extracts on the microbes which causes spoilage (Michael et al.2010). The shelf life of raw and pasteurized milk for standard antibiotic Nisin was 11mm. According to Modi et al. (2017) the plant extracts in trace amount does not cause any change in the nutritional

composition of the milk .In the betel extract phenolic compounds are present in large amount. So phenol enriched betel leaf extract exhibited a good spoilage prevention action.

The compounds do the action of inhibiting the fermentation process in the milk so that the lactose to lactic acid fermentation occurs in the reduced rate of the consumption (Michael et al.2013). Thus the work on the microbial spoilage of dairy products and its prevention of spoilage was found to be possible by the use of leaf extract of Piper betle.

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