Prevention of microbial spoilage and shelf life extension of dairy productsusing the extracts of MoringaoleiferaLam

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Abstract- Milk gets spoiled easily by microbes if it is not stored properly. To extend the shelf life of milk ,the suppliers use different chemicals that are hazardous to infants in prolonged use. As an alternative method of preservation to extend shelf life ,the extracts of the plant Moringaoleifera was added to raw and pasteurized milk.Normally the raw and pasteurised milk get spoiled within 1 hour if it is kept at room temperature without refrigeration.The use of plant extract was to found to be effective to keep the fresh milk and unspoiled for a period of 7 hours for raw milk and 12 hours for pasteurised milk without refrigeration. The pH acidity and total microbial load were also remain in a favourable range during the protective regime of moringa extract. The bioactive compound derivatives reported , (R)-3-pyrrolidinol, 5- (p- aminophenyl) - 4- (p- tolyl) - 2- thiazolamine, 2'6'- Dihydroxyacetophenone (phenolic compounds)present in the extract were reported to be effective in arresting spoilage causing microbes and extending the shelf life of the milk . The use of bio-preservatives and their mechanism of action on the spoilage microbespromote a healthy life.

Keywords: Raw milk, Pasteurized milk, MoringaoleiferaLam., Phenolic compounds, anti-oxidant property.

I. INTRODUCTION

Milk and their products formally called as Dairy products are rich in Calcium, Vitamin A, Vitamin B (Riboflavin, Thiamine, Niacin), Vitamin C (Ascorbic Acid), Vitamin D, Protein, Carbohydrates, Fat, Phosphorous, Magnesium, enzymes, pigments, sterols etc. and used by all people. In Ayurveda, milk and milk products are considered to be the cure of diseases specially those associated with mental disorder (Kiranet al.2012). The consumption of the milk is used as a preventive measure and an effective treatment for some disorders in both adults and infants (Rozenberg 2014). The highly nutritional and therapeutic milk and its products are contaminated with microbes and subjected to spoilage and affect all milk dependent population. The most commonly found spoilage microbes in the dairy industry are LAB, psychrotrophs, coliforms, fungi, spore forming bacteria and other microorganism (Ledenbach and Marshall 2009). Microbial spoilage involves deterioration of food texture, colour, odour or flavour and degradation of proteins, carbohydrates and fats. Even after pasteurisation some psychotropic organisms like Bacillus spp., Clostridium spp., Arthrobacter spp., Lactobacillus spp., Microbacterium spp., Pseudomonas spp. and their spore and enzymes survive and spoil the milk products (Tortorello2003). Orlaet al., (2013) used plant as effective preservatives, of milk Anderson et al. (2013) described that natural products fortify antioxidant property of the milk and extend shelf life Michael et al. (2013) reported the inhibitory action of plant extracts on the microbes that affect the yogurt starter and other lactobacillus . So there is a need to inhibit the microbial metabolism and their growth in the dairy products to extend the shelf life using non –chemical ingredients which are safe from infants to adults who use milk. Hence in the present study a medicinal plant drumstick extracts are to be tested for its efficacy to extend the shelf life of milk by inhibiting the growth of microbes that induces spoiling.

II. MATERIALS AND METHODS

2.1 Plant extracts

2.1.1 Moringaoleifera

Moringaoleifera generally called Moringa or Drumstick belong to the Monogeneric genus Moringa of the Moringacaefamily.

The Moringaoleifera Lam. leaves (250 g) were collected from 1 year old tree, washed using the distilled water and shade dried for a week for the removal of moisture content.After that the weight was reduced to 45 gm.Then 25gm. of dried leaves were weighed and pulverised into powder for extraction of bioactive compounds.Using sterile distilled water as solvent in a Soxhlet apparatus the aqueous extract was obtained and stored in a brown glass bottle at the refrigeration temperature for further study. The extract was subjected to GC-MS analysis.

2.2 Determination Of The Compounds Present In The Extract By Gc-Ms Analysis

The phenolic compounds present in the plantextract were studied using the GC MS analysis(Shimdzu GC-2010). The compoundspresents are listed in Table 1.

2.3 Milk Sample Collection

The pasteurized milk (500 ml) was collected from Aavin brand, of Tamilnadu . The raw milk was collected directly from the cow sheds found in the nearby village regions of Periyapalaiyam, Thiruvallur district, of

2.4 Determination Of Inhibiton Zone

The LB agar ,Potato Dextrose Agar and MRS agar were prepared and sterilized at 121 $^{\circ}C$ 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 loaded with the plants extracts of Moringa in 10,20, 40 and 60 % concentrations .The extract doses were loaded in the sterile discs using micro pipettes following standard protocol and were placed over the agar plates containing the sample. These plates were incubated at 35 $^{\circ}C$ for 24 hours for the observation of the result.

2.5 Addition Of Desired Ratio Of Extracts To The Milk

The dose that was foundeffective to inhibit the bacterial growth intheagar plates (40%)was chosen and added to 100 ml of pasteurized and raw milk samples. The required amount of extracts to be added was also optimised by adding different amounts ranging from 500 µl to 10 ml of the extracts to 100 ml of the milk sample. The pH and theacidity% of the milk were analysed for their quality (Amin et al. 2012).

III. RESULTS AND DISCUSSION

The phenolic compounds analysed were found to be (R)-3-Pyrrolidinol (5.493 min) with the highest peak and an area coverage of 38.27%, 2', 6'-Dihydroxyacetophenone (45.574 min) with a peak of 30.02%, 5- (p-Aminophenyl) and - 4-(p- tolyl) -2- thiazolamine (45.745 min) with a peak of 17.50% and other compounds in minor area coverage with least peak height.

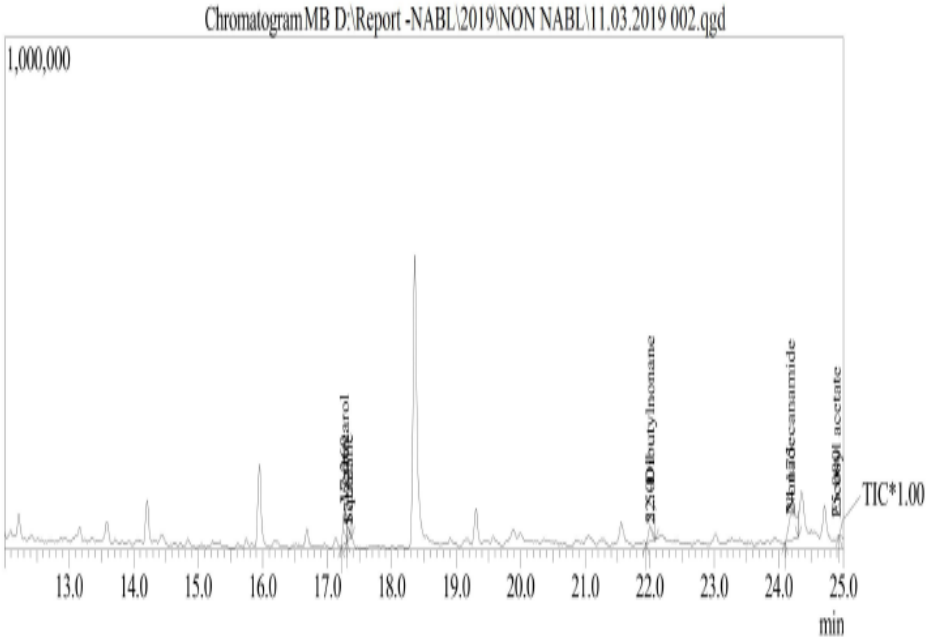


Fig.1. Sample showing peak value for compounds

(R)-3-Pyrrolidinol (5.493 min) with highest peak and area coverage of 38.27%, 2',6'-Dihydroxyacetophenone (45.574 min) with 30.02%, 5- (p- Aminophenyl) - 4-(p- tolyl) -2- thiazolamine (45.745 min) with 17.50%

Table 1 : Phenolic compounds present in the extracts

Peak Report TIC

Peak#	R.Time	Name	Area	Area%	Height	Height%
1	5.493	Triacetin	409286	1.07	44792	0.58
2	5.742	Isooctyl mercaptoacetate	46272	0.12	15504	0.20
3	5.836	(R)-3-Pyrrolidinol	14613886	38.27	2335354	29.99
4	6.242	Resorcinol	526747	1.38	54659	0.70
5	8.092	Calarene epoxide	126100	0.33	38447	0.49
6	10.087	Ethylene glycol di-n-butyrate	737126	1.93	155727	2.00
7	17.269	Verrucarol	237216	0.62	75170	0.97
8	17.325	Squalane	52186	0.14	25693	0.33
9	22.001	5,5-Dibutylnonane	120927	0.32	31954	0.41
10	24.175	Nonadecanamide	441692	1.16	47733	0.61
11	25.000	Eicosyl acetate	147175	0.39	36392	0.47
12	30.785	Phosphoric acid, isodecyl diphenyl ester $$ Isc	1058346	2.77	238827	3.07
13	32.548	Oleyl alcohol	339161	0.89	61674	0.79
14	43.622	Geranylgeraniol	1181032	3.09	279491	3.59
15	45.574	2',6'-Dihydroxyacetophenone	11463968	30.02	2103263	27.01
16	45.742	5-(p-Aminophenyl)-4-(p-tolyl)-2-thiazolamine	6681936	17.50	2242409	28.80
			38183056	100.00	7787089	100.00

IV. NUTRITIONALCOMPOSITION

The milk samples collected for the study were analysed for the nutritional content. (Table .2)

Table 2. Nutritional Profile of pasteurized milk (500ml)

1	DESCRIPTION	Standardised Full Cream milk
2	PACK COLOUR	Red
3	QUANTITY	500ml
4	FAT (%)	6
5	SNF (%)	9
6	pH	6.94
7	ACIDITY (%)	0.80
8	LAB MICROBIAL COUNT	10 (*10^8 CFU/ml)
9	AEROBIC PLATE COUNT	1 (*10^8 CFU/ml)
10	FAT (per 100ml)	6g
11	PROTEIN (per 100ml)	3.4g
12	CARBOHYDRATE (per 100ml)	4.9g
13	MINERALS (per 100ml)	740mg
14	ENERGY VALUE (per 100ml)	90KCal

Table 3.Nutritional Characteristics of raw milk

1	DESCRIPTION	Raw milk
2	pH	6.87
3	ACIDITY (%)	0.90
4	FAT (%)	14
5	FAT (per 100ml)	3.6g
6	CARBOHYDRATE (per 100ml)	4.8g
7	PROTEIN (per 100ml)	3.6g
8	LAB MICROBIAL COUNT	26 (*10^8CFU/ml)
9	AEROBIC PLATE COUNT	4 (*10^8CFU/ml)

Table 4.The zone of inhibition in (mm) for the plant extract treated pasteurized and raw milk samples in different agar medium..

S. NO	TEST doses (%)	PASTEURIZED MILK			RAW MILK		
Microbes		Bacillus spp.,	Lactobacillus spp.	Fungal spp.	Bacillus spp.	Lactobacillus spp.	Fungalspp.
1	10	7	6.0	3.5	5.0	5.0	4.8
2	20	9	7.0	6.0	7.0	7.0	7.2
3	40	13	15.0	9.0	12.0	12.0	10.0
4	60	11	11.0	8.0	9.5	9.3	8.6
4	Nisin (10%)	6.3	10.0	5.5	0.8	8.0	7.0
5	Natamycin 10%)	7.5	7.9	8.6	5.4	8,6	9.0

V. NUTRIENT CONTENTS IN RAW AND PASTEURISED MILK

5.1 Pasteurised Milk

The pasteurised milk was found to contain 6% fat, 9% SNF, pH of 6.94, 0.80% of acidity, LAB microbial count of 10 (*10^8CFU/ml), aerobic plate count of 1 (*10^8CFU/ml), 6 g of fat, 3.4 g of protein, 4.9 g of carbohydrate, 470 mg of minerals and energy value of 90 KCal for 100 ml of milk.

Raw milk

The raw milk was found to contain 14% fat, pH of 6.87, 0.90% of acidity, LAB microbial count of 26 (*10^8CFU/ml), aerobic plate count of 4 (*10^8CFU/ml), 3.6 g of fat, 3.6 g of protein and 4.8 g of carbohydrate for 100 ml of milk.

Table 5: Determination of pH, Acidity, & Microbial count of the raw milktreated with extract

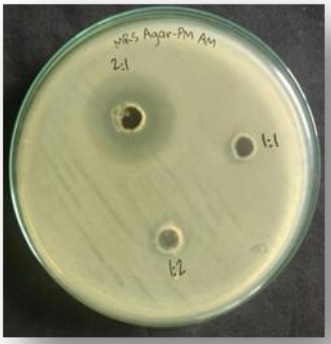
TIME (in hours)	pH	Acidity -%	Microbial Count in MRS $(*10^8 CFU/ml)$
0	6.85	0.45	5
1	6.83	0.46	7
2	6.80	0.47	4
3	6.76	0.49	9
4	6.72	0.49	10
5	6.72	0.50	12

Table 6.pH, Acidity and Microbial Count in the pasteurized milk treated with extract

TIME (in hours)	pH	Acidity %	Microbial Count in MRS $(*10^8 CFU/ml)$
0	6.97	0.49	1
1	6.94	0.52	4
2	6.90	0.53	7
3	6.89	0.54	3
4	6.82	0.56	8
5	6.75	0.58	11
6	6.73	0.57	15
7	6.72	0.59	13
8	6.70	0.60	9
9	6.68	0.60	10
10	6.66	0.58	11
11	6.63	0.57	12

Plate 1 Bacterial zone of inhibition in (mm) in the plant extract treatedmilksamples pasteurized(P) and raw milk (R)

P



R

The results shows that the different doses of moringa were found to inhibit the bacterial isolates in a dose dependent manner..Effective inhibition at 40% dose for Bacillus spp was13mm, Lactobacillus spp was 15.0mm and for fungal cultures was 9mm. The raw milk added with 40 % of the extract was found to have an inhibition with 12 mm of the zone of inhibition against the Bacillus spp. and Lactobacillussppand 10 mm for the fungal isolates . The standardantibiotics such as nisin and natamycin were also found to show a good inhibition zone closer to the plant extracts.

VI. ADDITION OF THE PLANT EXTRACTS IN DESIRED RATIO TO PASTEURIZED AND RAW MILK

6.1 Identification of the desired quantity of the plant extracts

The different doses of plant extracts were added to pasteurized and raw milk and were found to alterthe colour , odour and pH of milkThis shows that there is a relationship between the pH and the acidity% of the milk.The observation shows that the use of 40% of the plant extracts is agood biopreservative.The raw andpasteurized milksample of500 ml added with the 100µl of the plant extract(40%) was found to delay the spoilage time. effectively.Normally the raw and pasteurised milk get spoiled within 1 hour if it is kept at room temperature without refrigeration .The rapid spoilage of the milk may lead to financial constraint and health problems. The shelf life enhancement of milk thus leadsto a reduction in the spoilage of other related dairy products.

The shelf life of the raw and pasteurized milk added with Moringaoleifera extract (40%) 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 (Micheal,etal. 2013). The shelf life of raw and pasteurized milk can be alsoenhanced by the addition of nisin and natamycin as chemical preservatives and storage at low temperature. But the chemical preservatives cause side effects and so the uses of chemicalpreservatives can be reduced by using moringa extract. The use of plant extracts in trace amount does not cause any change in the nutritional composition of the milk (Modiet al., 2017).

The plant extracts analysed for the phenolic compound study using the GC-MS analysis were found to have (R) - 3 – pyrrolidinol (Compound 3), 2’ 6’ – Dihydroxyacetophenone (Compound 15), Squalene (Compound 8), andIsodecyldiphenyl phosphoric acid (Compound 12) inMoringaolerifera as reported by Junairiahet al., (2017). The compoundswere identified in the maximum concentrations have peak range of 29.99 (38.27), 27.01 (30.02) and 28.80 (17.50) respectively.

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 (Michael et al., 2013). The desired elements in the plant extracts were also been found to inhibit the fungal growth (Ersozet al., 2011). Thus the work on the microbial spoilage of milk and its prevention of spoilage was found to be possible by the use of extracts of the Moringaoleifera.

In future, this mode of preservation can be used as bio – preservative in the form of probiotic and the use of such natural products still remains unknown at present. .

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