A Novel Technology To Prevent Premature Spoilage Of Milk And Shelf Life Extension Of Milk Using The Extracts Of Moringa Oleifera Lam.

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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 Moringa oleifera Lam was added to raw and pasteurized milk and found effective to keep it unspoiled for a period of 5 hours and 11 hours respectively without refrigeration. The bioactive compound derivatives (R)-3-pyrrolidinol, 5- (p- aminophenyl) - 4- (p- tolyl) - 2- thiazolamine, 2'6'- Dihydroxyacetophenone and other 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 microbes promote a healthy life.

Index Terms: Raw milk, Pasteurized milk, Moringa oleifera Lam., Phenolic compounds, anti-oxidant property

1. INTRODUCTION

Milk and milk products forms an important constituent in human food. The milk gets spoiled easily and quickly if it is not properly stored. The journey of milk from cattle to consumer mouth needs adequate protection from exotic, acquired microbes from cattle and milk man zone and through easily deteriorating biochemical parameters (Shan-na Liu et al., 2011). So there is a need to extend the shelf life of milk before being used. To extend the shelf life and preventing microbial spoilage and biochemical changes like hydrogen per oxide formation , pH changes, acidity formation, microbial over loading, and organoleptic changes different strategies are applied for over several years (Datta and Deeth, 1999; Te Giffel and VanDerHorst, 2004; Krushna et., 2007 ; Gad and Salam, 2010 ; Chawla etal.,2011 ; Walkling-Ribeiro etal., 2011; Fernandez et al., 2013; Michael et al., 2013 and Modi etal.,2017). Of all the premature milk spoilage prevention strategies the use of safe natural products derivatives are found the most suitable one (Sivakumar and Dhanalakshmi, 2016 and Samah et al., 2019). Hence in the present study the extracts of the medicinal plant Moringa oleifera is tested to find its efficacy to inhibit microbial invasion and extending the shelf life of both raw and pasteurized milk for a considerable time before use.

2 MATERIALS AND METHODS

2.1 Plant extracts

The Moringa oleifera Lam. leaves (250 g) were 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 g. Then 25 g 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, filtered, concentrated and stored in a brown glass bottle stored in a refrigerator at 4°C for further use.

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2.2 Preliminary phytochemical screening

The aqueous extract was tested for alkaloids, flavonoids, phenols, steroids, tannins, terpenoids, saponins, reducing sugars, volatile oils, carbohydrates and protein/amino acids using standard qualitative analysis.

2.3 Identification of compounds by GC-MS analysis

The phytochemical compound present in the extract was recorded using the GC MS analysis (Shimdzu GC-2010) and the results were compared by using Wiley Spectral library search programme.

2.4 Milk sample

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 Tamilnadu

2.5 Determination of inhibition zone

The LB agar, Potato Dextrose Agar and MRS agar were prepared and sterilized at 121 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. These plates were incubated at 35 for 24 hours for the observation of the microbial colony formation. The developed bacterial and fungal colonies were isolated and identified. They were found to be Lactobacillus sp., Bacillus sp. and Aspergillus colonies. The isolates were stored for further study. To find out the optimum dose of Moringa extract that inhibits the bacterial and fungal colonies effectively empty discs prepared using sterile Whatman filter paper were loaded with different doses of Moringa leaf extracts (25%, 50% and 75%) and were placed over the agar plates containing the bacterial and fungal swabs .The effective dose that inhibit the bacterial and fungal colonies was found to be 75 % dose of Moringa extract .The 75% dose of the extract was mixed with 100 ml of raw and pasteurised milk at room temperature and stored outside for 10hrs. The sample taken from raw and pasteurised milk for every 1h and measured pH using pH meter The microbial growth

was analysed by placing a drop of milk in a Petri plate with agar medium every 1h till 5 h for raw milk and 11 h for pasteurised milk.(Table 4&5).

3 RESULTS AND DISCUSSION

The preliminary phytochemical screening showed the presence of alkaloids, flavonoids, phenols, steroids, tannins, terpenoids , saponins , reducing sugars , volatile oils , carbohydrates and protein/amino acids . GC-MS analysis of the extract of Moringa olefera showed the presence of number of compounds. These compounds were identified through mass spectrometry attached with GC. The various compounds present in the extract includes Triaetin, Isooctylmercapito acetate, (R) - Pyrrolidinol, Resorcinol, Calarene epoxide, Ethyleneglycol-di-n-butyrate,Verrucarol,Squalane,5,5Dibutylnonane, onadecanamide, Eicosyl acetate, Phosphoric acid isodecyldiphenyl ester, Oleylalcohol, Geranylgeraniol, 2,6,Dihydroxyacetophenone, 5 –(P- Aminophenyl -4-(p-totyl) - 2- thiazolamine (Table 1 and Figure1). The major compounds present in the extract were (R)-3-Pyrrolidinol (5.493 min) with highest peak and area coverage of 38.27%, 2',6'Dihydroxyacetophenone (45.574 min) with 30.02%, and 5- (p- Aminophenyl) - 4-(p- tolyl) -2-thiazolamine (45.745 min) with 17.50% and other compounds in minor area coverage with least peak heights. The GC-MS analysis of the of Moringa extract is given in the Table 1 and Figure 1.

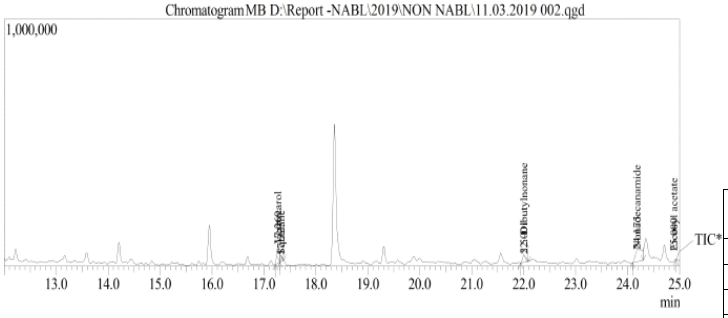

Fig 1: *GC-MS Chromatogram of the sample showing peak value of compounds.*

Table 1: Phenolic compounds present in the extract

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

NUTRITIONAL COMPOSITION:

The milk samples collected for the study were analysed for the nutritional profiling (Table 2&3)

TABLE 2: *Nutritional Profile of pasteurized milk (500ml)*

1	DESCRIPTION	Standardized 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 (*)
9	AEROBIC PLATE COUNT	1 (*)
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 (*)
9	AEROBIC PLATE COUNT	4 (*)

NUTRIENT CONTENTS IN RAW AND PASTEURISED MILK

PASTEURISED MILK

The milk was found to contain 6% fat, 9% SNF, pH of 6.94, 0.80% of acidity, LAB microbial count of 10 (*), aerobic plate count of 1 (*), 6 g of fat, 3.4 g of protein, 4.9 g of carbohydrate, 470 mg of minerals and energy value of 90 K Cal for 100 ml of milk. The raw milk was found to contain 14% fat, pH of 6.87, 0.90% of acidity, microbial count of 26 (*), aerobic plate count of 4 (*), 3.6 g of fat, 3.6 g of protein and 4.8 g of carbohydrate for 100 ml of milk (Table 4).

TABLE 4: *The zone of inhibition in (mm) developed for the bacterial colonies on treatment with Moringa extract in pasteurized and raw milk*

S. NO	Test dose Extract	Diameter of zone of inhibition in the isolates from pasteurised milk			Diameter of zone of inhibition in the isolates from raw milk		
		Lactobacillus sp.	Bacillus sp.	Fungal sp.	Lactobacillus sp.	Bacillus sp.	Fungal sp.
1	25%	5	5	8	6	7	8
2	50%	8	9	11	9	10	9
3	75%	9	9	11	9	10	9
4	Nisin (10%)	7	7	7	7	8	8
5	Natamycin (10%)	7	7	9	8	8	8

TABLE 5: *Determination of the raw milk after treating with Moringa leaf extract*

TIME (in hours)	pH	Acidity %	Microbial Count in MRS
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. *Determination of pH ,Acidity %and Microbial Count in the pasteurized milk after treating with Moringa leaf extract*

TIME (in hours)	pH	Acidity %	Microbial Count in MRS
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

P R

Fig 2: *Zone of inhibition in bacterial strains isolated from plant extract treated milk samples -Pasteurized (P) and Raw milk (R)*

The results shows that the ratio of Moringa leaf extracts have a good inhibitory effects on the Bacillus spp., Lactobacillus spp. and fungal cultures, whereas the 75% dose of the of the extract was found to have a good inhibitory effect against the Bacillus sp . , Lactobacillus sp. and fungal sp (Fig 2). The standards antibiotics such as nisin and natamycin were also found to show inhibition effect closer to the plant extracts (Table 4). The plant extracts dose of 75% added to pasteurized and raw milk was found to alter colour , odour and pH of milk (Table 5 and 6). The shelf life of the raw and pasteurized milk added with Moringa oleifera Lam. 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, 2010). The shelf life of raw and pasteurized milk can be enhanced by the addition of nisin and natamycin as chemical preservatives and storage at low temperature. But the chemical preservatives cause side effects. The use of plant extracts in trace amount does not cause like any side effects. The extract was found to contain phenolic compounds in a major proportion. This includes (R) - 3 – pyrrolidinol , 2' 6' – Dihydroxyacetophenone), Squalene , and Isodecyl diphenyl phosphoric acid as reported (Junairiah et al., 2017). The compounds do the action of inhibiting the fermentation process in the milk so that the lactose to lactic acid fermentation occurs in a reduced rate (Michael et al. 2013). The phenolic compounds in the plant extracts were also been found to inhibit the bacterial and fungal growth. The phenolic compounds the microbial growth and extended the shelf life period as reported (Ersoz et al., 2011).

4 CONCLUSION

In future, the extract of Moringa leaf can be used as bio – preservative in the form of probiotic natural products to extend the shelf life period of milk before boiling or use.

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