

# Studies on Development of Functional Milk

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## **Abstract:**

Background: At present the dairy industry is both actively & articulately involved in product development. This includes new formulations & imitation product designed to compete with or replace existing products based on their superiority inconvenience, cost, quality & health benefits, so the progress in product development. The fast going lifestyle of humans is one of the reasons for health related diseases. So attempt was made to develop functional milk by using the combination of lemongrass & clove both having medicinal properties.

## **Method:**

1. The method of optimization of pasteurized homogenized toned milk with sugar (used as a natural preservative), lemongrass & clove was separately successfully prepared and subjected for sensory evaluation on 9 point hedonic scale by semi trained judges. Different variables of concentrations of sugar, lemongrass & clove were prepared that is 6%, 7%, 8%, 9% & 10% , 0.1%, 0.5%, 1%, 2% & 3%, & 0.05%, 0.1%, 0.2%, 0.3% & 0.4% respectively.

2. Selected concentrations of sugar, lemongrass & clove were combined together for the formulation of functional milk & were finally subjected for sensory evaluation on 9 point hedonic scale by semi trained judges.

Results: 1. Among the different variables of concentrations, 8%, 1% & 0.1% of sugar, lemongrass & clove were selected respectively.

2. 1%: 0.1% concentration of lemongrass : clove were selected with 8% sugar concentration for the preparation of functional milk.

Conclusions: 1. Separate optimization of sugar, lemongrass & clove provides a basic idea to finalize the concentrations each raw material for the formulation of functional milk.

2. 1%: 0.1% concentration of lemongrass: clove with 8% sugar concentration sample showed better results for all sensory parameters. The functional milk has positive effect in improvement of sensorial parameters in taste, mouth-feel, stability & its overall acceptability. Also addition of lemongrass and clove may play vital functional role & exhibit functional properties in the developed product.

**IndexTerms - Milk, Functional Milk, Sugar, Lemongrass, Clove, Optimization.**

## **1. INTRODUCTION**

Nutritionists recognize milk and its products as important constituents of a well-balanced, nourishing human diet. Milk has been described as nature's nearly perfect food in that it provides vital nutrients like proteins essential fatty acids, vitamins, minerals and lactose in balanced proportions. (Anthony et.al., 2013)

Milk is regarded as rich source of nutrients as it contains high quality proteins, lactose, and flavour enriching fat. The perfect composition of milk not only recommends itself for growing children but also suited to satisfy energy needs of adult. In flavoured milks sugar, flavoring agents, coloring matters are added. Milk also provides protection against ill health and promotes good health. (Berry, 2003)

Recently, herbal products either in the form of cosmetics or food has become more popular in the world market. Epidemiological data as well as in-vitro studies strongly suggest that food containing phyto-chemical with anti-oxidation potential has strong protective effect against major disease risks including cancer and cardiovascular disease, (Kaur and Kapoor, 2002).

Lemongrass (*Cymbopogon citratus*) is a widely used herb in tropical countries; especially in it is used in aromatherapy. Some of the reported phytochemicals are essential oils that contain citral  $\alpha$ , citral  $\beta$ , nerolgeraniol, citronellal, terpinolene, geranyl acetate, and myrcene and terpinolmethylheptenone. Two triterpenoids, cymbopogone and cymbopogonol and flavones identified as luteolin and its 6-C-glucoside have also been isolated from leaves of *C. Citratus*. (Hasimet al., 2015).The plant also contains reported phytochemicals such as flavonoids and phenolic compounds, which consist of luteolin, isoorientin 2'-O-rhamnoside, quercetin, kaempferol and apiginin. The compounds identified in *C. citrates* are mainly terpenes, alcohols, ketones, aldehyde and esters. Studies indicate that it possesses various pharmacological activities such as anti-amoebic, anti-bacterial, anti-diarrheal, anti-filarial, anti-fungal and anti-inflammatory properties. Various other effects like anti-malarial, anti-mutagenicity, anti-mycobacterial, antioxidants, hypoglycemic and neurobehaviorial have also been studied (Hasimet al., 2015).

Clove (*Syzygium aromaticum*) is one of the most valuable spices that has been used for centuries as food preservative and for many medicinal purposes. This plant represents one of the richest source of phenolic compounds such as eugenol, eugenol acetate and Gallic acid and possess great potential for pharmaceutical, cosmetic, food and agricultural applications. The biological activities of clove and eugenol. A new application of clove as larvicidal agent is an interesting strategy to combat dengue which is a serious health problem.

The addition of antibacterial and aromatic supplements, to reduce the spoilage, can be added to the milk and also medicinal plants like lemongrass containing phytochemicals and spices like clove having antibacterial, antimicrobial properties were used for the development of the functional milk having functional properties and nutritional value like functional food.

## 2. MATERIALS AND METHODS

All the raw material Pasteurized homogenized toned milk, lemongrass leaves, clove powder and sugar were procured from local market of Kolhapur & were analysed for various parameters which are tabulated below.

### 1. Pasteurized homogenized toned milk

Table: 1 Physical parameters of Pasteurized homogenized toned milk

Sr. No	Parameters	pasteurized homogenized toned milk
1	Colour	White
2	Flavour	Pleasant and clean
3	Taste	Clean and slightly sweet
4	Appearance	Liquid

Table: 2 Chemical Analysis of Pasteurized homogenized toned milk

Sr. No	Parameter	Pasteurized homogenized toned milk
1	Acidity(as Lactic acid)	0.126 %
2	Fat (by Gerber)	3.0 %
3	Solid not fat (SNF) by gravimetric method	8.53 %
4	Protein (Pyne's methods)	3.06 %
5	pH (at 25°C)	6.65
6	Phosphatase test	Negative
7	M.B.R.T test	6.00 hrs
8	Calcium	150 mcg
9	Phosphorus	100 mcg
10	Sodium	50 mcg
11	Thiamine (Vitamin B1)	42.5 mcg
12	Riboflavin (Vitamin B2)	120 mcg
13	Niacin	100 mcg
14	Vitamin A	7.5 mcg

Table: 3 Microbial parameters of Pasteurized Homogenized toned milk

Sr. No	Parameters	pasteurized toned milk	homogenized
1	SPC and TPC	7500 cfu/ml	
2	Coliform	Nil /0.1ml	
3	E.Coli	Absent /ml	

## 2. Lemongrass leaves

Table : 4 Chemical Analysis of Lemongrass leaves

Sr.N o.	Parameter	Lemongrass leaves
1	Moisture	11.35 ± 0.01%
2	Ash	7.15 ± 0.21%
3	Carbohydrates	19.64 ± 0.51gm
4	Protein	22.59 ± 0.01%
5	Fat	2.43 ± 0.04%
6	Crude Fibre	37.53 ± 0.67%
7	Vitamin A	1.25 ± 0.02 mcg
8	Vitamin C	2.43 ± 0.06 mcg
9	Vitamin B1	2.33 ± 0.04 mcg
10	Vitamin B2	0.23±0.06 mcg
11	Manganese	2.57±0.04 mcg

Table: 5 Qualitative tests for Phytochemical of Lemongrass leaves

Sr. No	Phytochemical components	Test	Observation	Inference
1	Alkaloids	Wagnersdragendi offs test	Reddish brown test	+ ve
2	Saponin	Frothing test	Stable froth emulsion	+ ve
3	Tannin	Acid test	Reddish brown	+ ve
4	Glycoside	Sodium Picrate	Yellow dish to reddish	+ ve
5	Sterol	Salkowsk is test	Red color not interface	-ve
6	Phenol	Ferric chloride	Greenish brown precipitate	+ ve
7	Flavonoids	Sodium hydroxide	Yellow color	+ ve

## 3. Clove powder

Table: 6 Chemical Analysis of clove powder

Sr. No.	Parameter	Clove Powder
1	Moisture	14.8±0.20 %
2	Ash	12.6±0.10 %
3	Carbohydrates	41.3±0.30 gm.
4	Protein	12.4±0.20%
5	Fat	16.2±0.30 %
6	Crude Fibre	17.5±0.20 %
7	Vitamin K	3 ±0.04 mcg
8	Vitamin C	1.692±0.005 mcg
9	Manganese	196.8±0.020 mcg
10	Calcium	117.5 mcg
11	Iron	8.3±0.003 mcg

Table: 7 Qualitative tests for Phytochemical of clove powder.

Sr. No	Phytochemical component	Test	Observation	Inference
1	Alkaloids	Wagnersdragendi offs test	Reddish brown test	+ ve
2	Saponin	Frothing test	Stable froth emulsion	+ ve
3	Tannin	Acid test	Reddish brown	+ ve
4	Glycoside	Sodium Picrate	Yellow to reddish brown	+ ve
5	Sterol	Salkowsk is test	Red color interface	+ve
6	Phenol	Ferric chloride	Greenish brown precipitate	+ ve
7	Flavonoids	Sodium hydroxide	Yellow color	+ ve

## 4. Sugar

Table: 8 Physical Parameter of Sugar

Sr. No	Parameters	Sugar
1	Colour	White
2	Appearance	Crystalline solids
3	Taste	Pleasant and sweet
4	Extraneous Matter	Free from extraneous matter and smell

Preparation of Functional Milk.

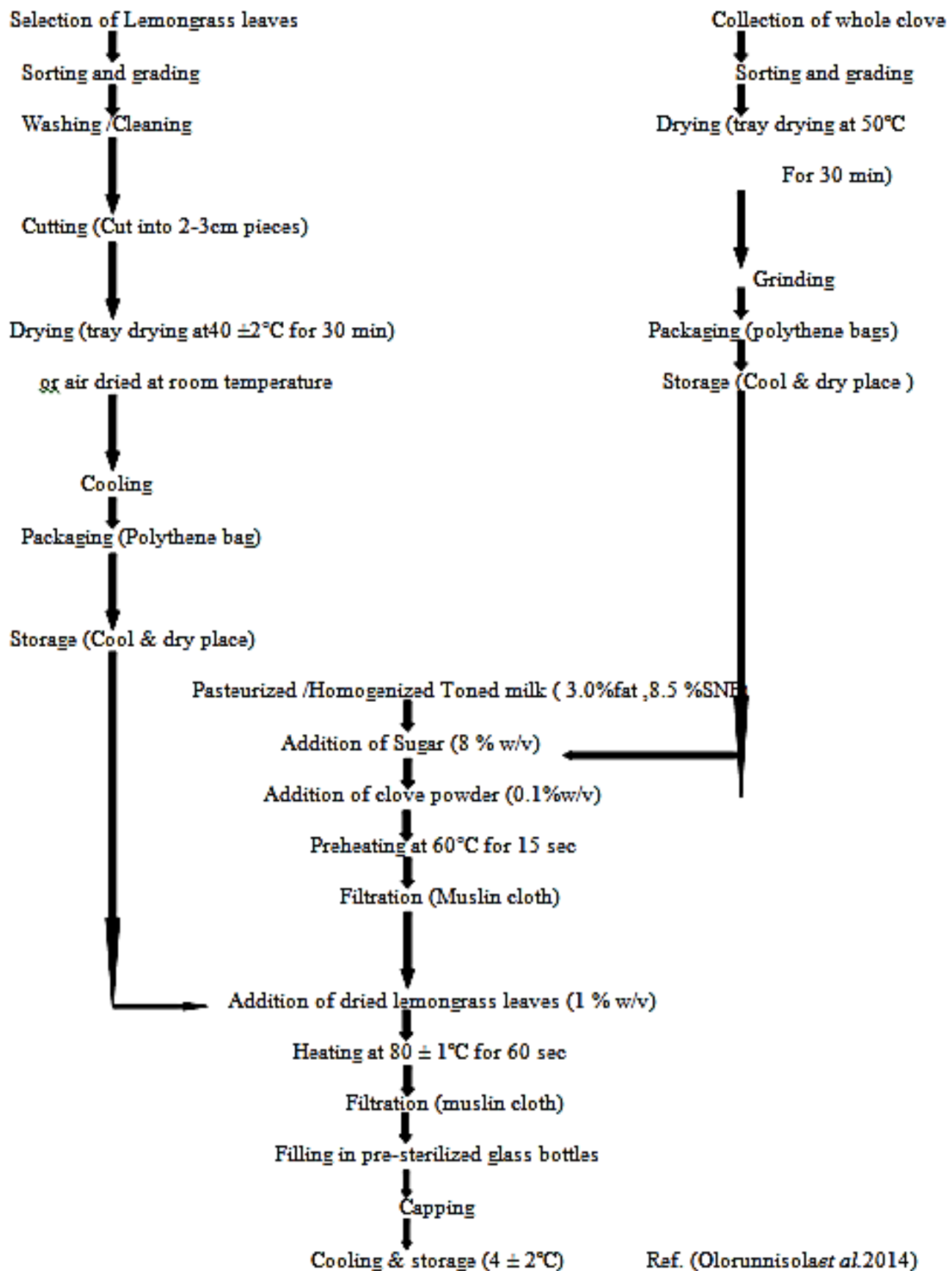


Figure.1 Process flow chart for preparation of Functional Milk

## 1. Preparation of Control Sample

Table : 9 Optimization with sugar

Trial Percentage(w/v)	Appearance	Color	Flavour	Taste	Consistency	Overall Acceptability
6 %	6.5	7.5	7.0	7.0	7.5	7.0
7 %	7.5	7.5	8.0	8.0	8.0	8.0
8 %	8.0	8.0	8.5	8.5	9.0	8.5
9 %	7.5	7.5	8.0	8.0	7.5	8.0
10 %	7.0	7.0	6.5	6.0	7.0	6.5

Observation-8% (W/V) concentration were selected due to it's better results.

## 2. Preparation and Optimization of clove powder for functional milk

Table: 10 Optimization of clove powder

Trial Percentage (w/v)	Appearance	Color	Flavor	Taste	Consistency	Overall Acceptability
0.05 %	7.5	7.0	7.0	7.0	7.5	7.5
0.1	8.0	8.0	8.5	8.5	8.0	8.5
0.2	7.5	7.5	8.0	8.0	7.5	7.5
0.3	6.5	7.0	6.5	6.5	7.0	6.5
0.4	6.5	6.0	6.5	6.0	6.0	6.0

Observation- 0.1% (w/v) concentration was selected due to its better results of sensory and overall acceptability.

## 3. Preparation and optimization of lemongrass leaves for functional milk

Table : 11 Optimization of dried lemongrass leaves

Trial Percentage (w/v)	Appearance	Color	Flavor	Taste	Consistency	Overall Acceptability
0.1	7.5	8.0	6.5	6.5	7.5	7.0
0.5	7.0	7.5	7.0	7.0	7.5	7.0
1	8.0	8.0	8.0	8.5	9.0	8.5
2	8.0	8.0	8.0	8.0	8.0	8.0
3	8.0	8.0	7.0	7.0	7.5	7.5

Observation- 1% (w/v) concentration were selected due due to its better results for sensory characteristics.

## 4. Development of Functional Milk

Functional milk was prepared using the pre-optimized concentrations of raw materials by following the steps mentioned in the flowchart.

## 5. Packaging of Functional Milk

200 ml glass bottles were procured from the market .Filling the prepared functional milk into that glass bottle after that capping .

## 6. Storage

Filled bottles were stored at  $4 \pm 2^\circ\text{C}$  for the best use and to avoid microbial growth due to high storage temperature.



Figure.2 Prepared Functional Milk

## 3 . RESULTS

Prepared functional milk was analyzed for phychemical, microbiological parameters and qualitative phytochemical screenings were also tested.

1. Effect of addition of lemongrass leaves and Clove powder on sensory parameters.

Table : 12 Optimization of functional milk

Sample	Functional milk prepared
Parameters	
Appearance	8.5
Colour	9.0
Taste/Flavour	8.5
Mouth feel	9.0
Consistency	9.0
Overall Acceptability	9.0

Table : 13 Quantitative analysis of total phenolic content in lemongrass leaves and clove powder

Sr. No	Parameter	Dried lemongrass leaves	Clove powder
1.	Total phenolic content (mg/g extract )	5.75	17.20

Table : 14 Activity of antioxidant for Lemongrass leaves and clove powder

Sr. No	Parameter	Dried lemongrass leaves	Clove powder
1.	Total antioxidant capacity (%)	0.88	2.12

Table : 15 Antimicrobial activity for lemongrass leaves and clove powder

Sr. No	Sample Name	Unit	Zone of Inhibition	
			<u>Escherichia coli</u>	<u>Staphylococcus Aureus</u>
1.	Lemongrass leaves	mm	0	0
2.	Clove powder	mm	14	0

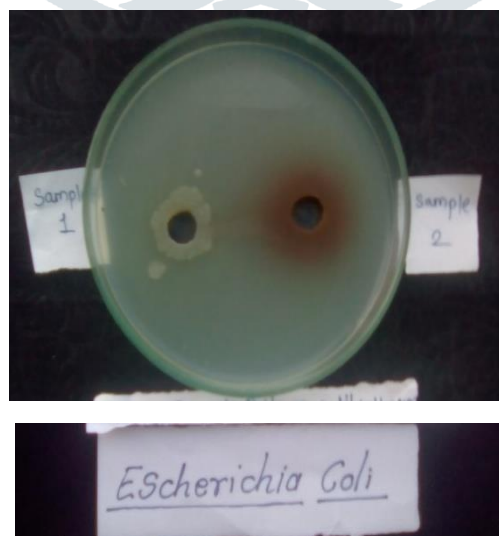


Fig no. 3 Zone of inhibition of Gram negative bacteria and Gram positive bacteria



Table : 16 physicochemical analysis of functional milk

Sr. No	Parameters	Functional milk
1.	Total solid	19.24 %
2.	SNF	15.83 %
3.	protein	3.24 %
4.	Fat	3.41%
5.	Saturated fat	2.413 %
6.	Acidity (as lactic acid )	0.14 %
7.	PH	6.74
8.	Total carbohydrates	11.95%
9.	Ash content	0.64 %
10.	Energy	91.45 Kcal
11.	Vitamin B2	646.9 mg/100 ml
12.	Vitamin A	1062.5 mg/100 ml
13.	Calcium	196.81 mg/100 gm
14.	Sodium	41.4 mg/100 gm
15.	Iron	9.43 mg/kg
16.	Potassium	78.22 mg/100 gm
17.	Magnesium	47.18 mg/kg
18.	Citral	Absent
19.	Eugenol	Present
20.	Antioxidant	Absent
21.	Coliform	<1 cfu/ml
22.	Total plate count	67 cfu/ml
23.	Yeast & mould	< 1 cfu/ml
24.	Total phenolic content (mg%)	01.85

Table : 17 Qualitative Phytochemical Analysis

Sr. No	Parameters	Functional milk
1.	Tannins	+
2.	Saponins	+
3.	Alkaloids	-
4.	Glycosides	-
5.	Flavonoids	+

Table : 18 Quantitative analysis of total phenolic content in functional milk

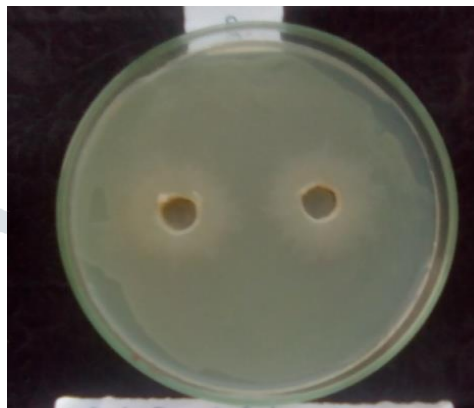
Sr. No	Phytochemicals	Total content
1.	Total phenolic content (mg/g extract )	01.85

Table : 19 Activity of antioxidant for functional milk

Sr. No	Parameters	Total content
1.	Total antioxidant capacity (mg %)	1.68

Table : 20 Antimicrobial activity for functional milk

Sr. No	Sample Name	Unit	Zone of inhibition	
			<u>Escherichia coli</u>	<u>Staphylococcus aureus</u>
1.	Functional milk	mm	18	0



Escherichia coli



Staphylococcus aureus

**Storage study of functional milk**

Table : 21 Effect of refrigeration storage on physicochemical parameter of the milk .

Storage days	Physico-chemical parameters		
	TS	Acidity (%)	pH
0	19.24	0.140	6.74
3	19.22	0.140	6.72
6	19.22	0.142	6.71
9	19.18	0.145	6.67
12	19.15	0.149	6.63
15	19.15	0.153	6.54
18	14.20	0.190	4.70

Table: 22 Effect of refrigeration storage on microbial quality of the product.

Storage days	Microbial parameter	
	Total plate count (cfu /ml)	Yeast and mold count (cfu/ml)
0	67	<1
3	72	<1
6	77	1
9	94	2
12	1500	2
15	11200	2
18	To Many To Count (TMTC)	100

#### 4. FINAL DISCUSSION ON RESULTS

- 1) Addition of lemongrass & clove gradually increased the nutritional value of the milk.(ref .results of analysis of functional milk)
- 2) Phytochemicals, antioxidant values, antimicrobial values present in lemongrass & clove shows that the prepared product have the medicinal potential value to use as functional product in the health treatment. (Ref. Table of Qualitative tests of phytochemicals, antioxidant, and antimicrobial analysis of functional milk).
- 3) Storage study shows that there is increase in the shelflife of product under the refrigeration temperature. ( Results of storage study)
- 4) Gradual increase in nutritional value of prepared Product have the positive effect on sensory parameters , stability, consistency of the prepared milk.( Ref. Sensory evaluation of functional milk).
- 5) Microbial analysis of prepared milk shows that the improvement in the microbial quality of milk over the milk used.(Ref. Analysis of functional milk & Analysis Of PHTM).
- 6) There is increase in values of parameters like fat, SNF, protein, total solids over the milk used. (Ref. Analysis of functional milk).
- 7) Energy value of prepared milk shows that the prepared product is Rich source of energy.( Ref. Analysis of functional milk).

#### 5. CONCLUSION

The conducted study suggests that ,pasteurized homogenized toned milk can effectively combined with different concentrations of medicinal plants like lemongrass and spice like clove to produce a option for functional food having highly acceptable sensorial parameters ,nutritive value ,good shelf life and functional properties of better health.

Commercialization of this study with market survey will add an innovation in dairy industry due to it's high potential value and present lifestyle of human beings.

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