



P-ISSN: 2349-8528

E-ISSN: 2321-4902

IJCS 2019; 7(2): 1150-1157

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Received: 13-01-2019

Accepted: 20-02-2019

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International Journal of Chemical Studies

Development of herbal milk using tulsi juice, ginger juice and turmeric powder

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Abstract

Food industries have rather high demand for the products that meet the consumer's demand for a healthy life style for which functional food fortified with the plant ingredients plays an important role. Tulsi, turmeric and ginger are such important medicinal plants whose medicinal usage has been reported in the Indian and British Pharmacopoeias and in the Ayurvedic system of medicine and day to day life of human beings, also having various health benefits including immunomodulatory, anti-inflammatory, anti-arthritis, and anti-bacterial, antioxidant, anti-diabetic and anti-tumor. The present study was anticipated to develop herbal milk with sensorily acceptable levels herbs and assessment for its compositional, physico-chemical properties, sensory attributes and microbiological quality. Preliminary trials were conducted to optimize the levels of tulsi, turmeric and ginger in milk. By preliminary trials range of ingredients selected as well as stabilizer selected. It was observed that addition of tulsi juice (25%), ginger juice (3%) and turmeric powder (0.1%) of sterilized herbal milk exhibited best sensory scores and milk without addition of herbs treated as control. Antioxidant, Viscosity, fat and total polyphenols were significantly ($P < 0.05$) higher than the T_0 (Control). The chemical composition of the optimized product contained fat 3.59%, total solids 14.96%, protein 3.01%, ash 0.70%, carbohydrates 9.30% and energy 81.55 Kcal/100 ml. The antioxidant and total phenolic content were 50.14% DPPH activity and 96.25 mg GAE/100gm respectively. The herbal milk conforms to the FSSAI requirements for 'flavored sterilized' milk. The cost of the newly developed herbal milk was Rs 14.17/200 ml.

Keywords: Tulsi, turmeric, ginger, herbal milk, functional food

1. Introduction

Milk is a complete food that provides a high level of essential nutrients. Today Indian consumer is more conscious towards health and balanced nutrition and has desire for better quality and convenient food products. Milk promotes good health and protect us against various diseases.

Medicinal plants are very rich sources of essential oils which possess therapeutic importance. In India use of plants as a medicine appeared in Rigveda which has been written 3500 - 1600 B.C. Properties of plants as a source of medicine were studied in detailed in Ayurveda which is considered as the foundation of all the medical sciences. The antioxidant compounds or phytochemicals from natural sources like plants, crops and spices are important in the food industry because of their usefulness in various food preparations and health promoting effects (Nile and Park, 2015) [24]. Thus, the demand for natural antioxidants has increased in the food industries which have less side effects and effective against various diseases (Yeh *et al.*, 2014) [37]. Recently, there has been an increasing trend to fortify the milk with herb or spices. Spices are considered as a rich source of minerals and medicinal properties.

Tulsi (*Ocimum sanctum*) is an aromatic plant which has many medicinal properties (Singh *et al.*, 2012) [31]. It contains several phyto-constituents such as cardinene, cubenol, borneol, linoleic acid, orientin, linolenic acid, steric acid, oleic acid, palmitric acid, eugenol, vallinin, vicenin, vitexin, vllinin acid, circineol, gallic Acid, vitamin A, vitamin C, phosphorus and iron (Kadian and Parle, 2012) [14] due to which it possess multifarious medicinal properties such as antiviral, antifungal, antibacterial, antimalarial, anthelmintic, anti-oxidant, anti-cataract, anti-inflammatory, chemopreventive, radio protective, hepato-protective, neuro-protective, cardio-protective, anti-diabetic, anti-hypercholesterolemia, anti-hypertensive, anti-carcinogenic, analgesic, anti-pyretic, anti-allergic, immunomodulatory, anti-asthmatic, diaphoretic, anti-thyroid, anti-fertility, anti-ulcer, anti-emetic, Anti-spasmodic, anti-arthritic, adaptogenic,

anti-stress, anti-cataract, anti-leukodermal and anti-coagulant (Cohen, 2014) [7]. Turmeric (*Curcuma longa*) is a very important spice in India, which is obtained from rhizomes of plant *Curcuma longa*, a member of the *Zingiberaceae* (ginger) family. More than 100 components have been isolated from turmeric. The main component of the root is a volatile oil, containing turmerone, and there are other coloring agents called curcuminoids in turmeric (Lal, 2012) [21]. The volatile oil (5.8%) is obtained by steam distillation and curcumin (3–4%) is responsible for the yellow colour, and comprises curcumin I (94%), curcumin II (6%) and curcumin III (0.3%) (Labban, 2014) [20]. Bioactive components of turmeric have a wide spectrum of biological actions such as anti-inflammatory, anti-oxidant, anti-carcinogenic, anti-mutagenic, anti-coagulant, anti-fertility, anti-diabetic, anti-bacterial, anti-fungal, anti-protozoal, anti-viral, anti-fibrotic, anti-venom, anti-ulcer, hypotensive and hypocholesteremic activities. Thus, both turmeric and curcumin have the potential for the development of modern medicine for the treatment of various diseases (Chattopadhyay *et al.*, 2004) [6]. Ginger, being a major spice, has many uses in food as a flavoring and medicinal product. The aroma of ginger is pleasant with flavor, slightly biting due to antiseptic or pungent compounds present in it, which make it indispensable in the manufacture of number of food products. This rhizome can be processed into a powder, syrup, volatile oil, and oleoresin. Among all spices, it exhibits one of the greatest diversity of uses, such as in dietary supplements, beverages (such as ginger ales), and food products (such as in curry powder, confectionaries, soups, jams, and baked goods). The rhizome contains fats, carbohydrates, protein, fiber, water, and volatile oil. The pungency of fresh ginger results from gingerol and it also may contain paradol. Ginger contains about 1% to 3% volatile oil that imparts a distinctive odor to ginger and which is composed mainly of monoterpenoids and sesquiterpenoids, including camphene, borneol, zingiberene, sesquiphellandrene, and bisabolene (Singletary, 2010) [33]. Ginger was reported to have medicinal properties like anti-microbial, anti-fungal, anti-viral, anti-oxidant, anti-inflammatory, and anti-cancer activities. As ginger is known to be having antioxidant and anti-inflammatory agent; it also exhibits cancer prevention properties, and is used as a postoperative antiemetic. It was found that the ginger contains a number of bioactive phenolic and non-phenolic constituents, which in pure form or its derivatives might be potentially useful in the treatment of various diseases like oxidative stress, diabetes, cancer, arthritis, gout, gastric ulcer, hypercholesterolemia, and pain, microbial or viral infection (Zadeh and Kor, 2014) [38].

Herbal milk is flavored milk made with milk, Tulsi juice, turmeric powder, ginger juice, sugar and stabilizer. It is sterilized which gives it a longer shelf-life. The health benefits of herbal milk, such as improved bone health, reduced risk of cardiovascular disease, cancer, ulcer, types 2 diabetes, allergy and asthma, improves immunity, and many more. Today Indian consumer is more open to new ideas, more conscious towards health and balanced nutrition and has desire for better quality and convenient food products. The demand for milk and milk products is increasing rapidly due to growth in population, changing in its demographic pattern, socio-economic status, income, distribution, taste and preference of the people. So looking to the importance of natural ingredients, efforts have been made to prepare herbal milk using tulsi juice, turmeric powder and ginger juice.

In present study efforts to develop herbal milk using Tulsi, turmeric and ginger was delineated.

2. Materials and Methods

2.1 Materials

Milk has been procured from students' training dairy of the institute while Tulsi, turmeric powder, ginger and sugar has been brought from the local market.

2.2 Methods

2.2.1 Preparation of ginger juice

Ginger rhizomes were washed in running tap water, peeled and then shredded to make juice. The juice was filtered through two fold muslin cloth.

2.2.2 Preparation of Tulsi juice

Tulsi leaves were sorted out and washed with potable water thoroughly and juice was prepared by heating Tulsi at 65°C for 5 min in 1:4 amount of water. The heat treated Tulsi was crushed in the juice maker along with the water to obtain a fine paste. It was then filtered through a clean, sanitized fine double layered muslin cloth to obtain Tulsi juice and kept at refrigeration temperature (7±1°C) until used (Trivedi *et al.*, 2014) [36].

2.2.3 Preparation of herbal milk

The herbal milk was prepared using Tulsi juice (25%), ginger juice (3%), turmeric powder (0.1%), sugar (7%) and stabilizer (carrageenan, 0.025%). The flow chart for preparation of herbal milk using Tulsi juice, ginger juice and turmeric powder is given in Figure 1. Control was prepared by same procedure with addition of sugar in it. Figure 1 illustrate the flow diagram for preparation of herbal milk using Tulsi juice, ginger juice and turmeric powder.

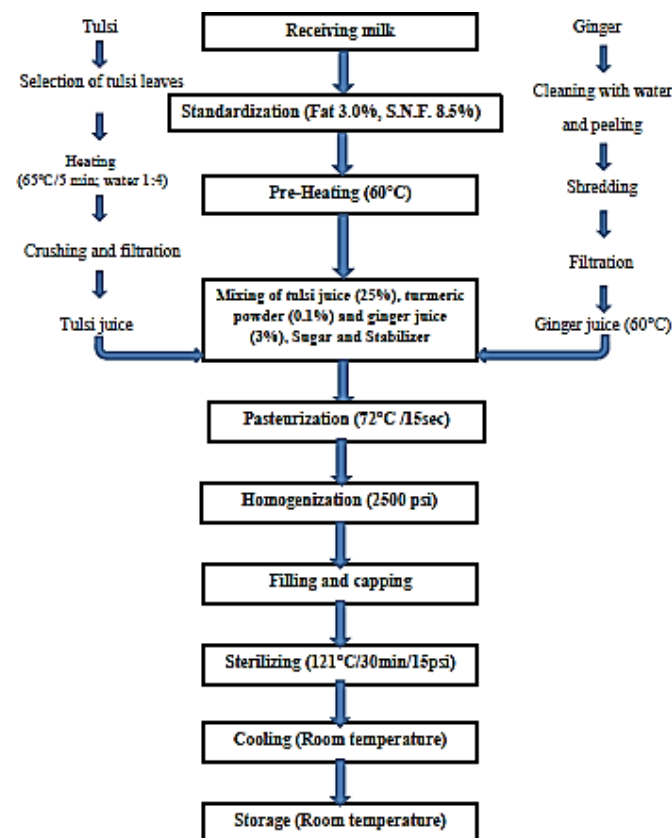


Fig 1: Flow chart for preparation of herbal milk using Tulsi, turmeric and ginger

2.3 Physico-Chemical analysis

Titration acidity and ash was determined by the method described in AOAC (2003). Specific gravity was determined by using relative density bottle method. Viscosity was determined using Ostwald viscosimeter. Fat, protein and total solid was determined by the method described in FSSAI manual (2015) [23]. Carbohydrate was determined by using difference method. Antioxidant content was analyzed by DPPH method suggested by Tabart *et al.* (2009) [35]. Total phenolic content of drinks was analyzed by the Folin Ciocalteu method suggested by Kähkönen *et al.* (1999) [15]. Energy value was calculated using the following formula:
 Energy value (Kcal) = 9 x Fat% + 4 x Protein% + 4 x Carbohydrates%.

2.4 Sensory Evaluation

Herbal milk with Tulsi juice, turmeric powder and ginger juice was evaluated for sensory characteristics by a panel of seven judges selected from the department who had adequate knowledge about the product characteristics and sensory attributes and are regularly involved in the sensory evaluation of dairy products. The samples were evaluated for color and appearance, flavor, consistency, sweetness and overall acceptability using 9-point hedonic scale (Stone and Sidel, 2004) [34].

2.5 Microbiological analysis

Standard plate count, yeast and mould count, coliform count and spore count were analyzed using methods employed in manual of microbiological testing of FSSAI (2012) [22].

2.6 Statistical analysis

All statistical analyses were performed using excel. Results are presented in mean \pm standard error of mean (SEm), and statistical significance was set at $p < 0.05$. Completely Randomized Design (CRD) was used to determine the main effects of treatments (Gacula and Singh, 1984) [10].

3.0 Results and Discussion

Preliminary trials were conducted for level selection of levels of each ingredient are given in Table 1.

3.1 Preliminary Studies

Preliminary trials were conducted to select the levels of Tulsi juice, turmeric powder and ginger juice, sugar and stabilizer level and sterilization temperature. Data pertaining to preliminary trials were summarized in the following Table 1. Preliminary trials were also taken by using Tulsi and ginger powder but particle were not stabilized in the product. Carboxy methyl cellulose (CMC) was also tried but ingredients were not mixed properly by using this.

Table 1: Preliminary studies for selection of ingredients

Ingredients	Levels (%)	Levels/range selected	Remarks
Sugar	6.0, 6.5, 7.0, 7.5, 8.0	6.5-7.0	At lower levels- less sweet At higher levels- too sweet
Stabilizer (Carrageenan)	0.015, 0.020, 0.025, 0.01, 0.03, 0.05, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18, 0.20,	0.015, 0.020, 0.025	At lower levels- more sediments At higher levels- gelation observed
Tulsi juice	3, 4, 5, 7, 10, 15, 20, 25, 30	25, 30	At lower levels – no significant flavor At higher levels- dark green color and high flavor.
Ginger juice	1.0, 1.5, 2.0, 2.5, 3.0	2.5, 3.0	At lower levels – no significant flavor At higher levels- high pungency.
Turmeric powder	0.3, 0.4, 0.5, 1.0	0.3, 0.4, 0.5	At lower levels – no significant flavor At higher levels- more sediments and bitter taste.
Sterilization time (min)	15,20,25,30	30	Short time duration- not proper sterilization

From the preliminary trials levels of tulsi juice, ginger juice and turmeric powder were selection for better formulation. Sugar level (7%), Carrageenan Stabilizer (0.025%) level and Sterilization time (30 min) was also selected by preliminary trials and these were fixed for all trials.

3.2 Effect of Tulsi juice on Sensory Scores of Herbal Milk

There was a significant ($p < 0.05$) difference in the color and appearance, flavor and overall acceptability scores of the herbal milk containing different levels of Tulsi juice (Table 2). Tulsi juice addition improved the color and appearance scores significantly ($p < 0.05$). The increasing Tulsi juice level imparted green color. The flavor scores were also improved significantly ($p < 0.05$) by the addition of Tulsi juice being highest for 25% level of Tulsi juice. Thereafter, the scores declined for 30% Tulsi juice because of high Tulsi flavor over the innate flavor of milk. Similar trend was observed in overall acceptability. The scores decreased beyond 25% level of Tulsi juice, due to high flavor of Tulsi that was not liked by the panelists. Therefore, this level was selected for the further experimentation on herbal milks. Kumari *et al.*

(2011) [16, 19] reported that the addition of Tulsi paste improved color and appearance, body and texture and also overall acceptability of herbal yoghurt. Johri and Chauhan (2014) [13] reported that color acceptability of herbal Tulsi doi was highest. Kumar *et al.* (2013) [17] observed the highest score of color and appearance at 3.0% level of Tulsi extract; the level of Tulsi extract increases, decrease the color and appearance score of the ice cream sample. He also found that in Tulsi ice cream, flavor increased up to 3% level. They reported that 4% level led to decrease in score due to intense flavor. Ahalawat *et al.* (2009) reported the desirability of use of 8% of sugar level in a non-acidic mango milk beverage. Trivedi *et al.*, (2014) [36] reported that incorporation of basil juice in the ice cream compared to control increased flavor preferences, when added up to a level of 6.0% (w/w) only. Thereafter there is a decrease in flavor score. The reason for less score of flavor was due to stronger flavor as there was higher rate of addition of basil juice in that. He also reported that progressive increase in rate of addition of basil juice in ice cream decreased the body and texture scores of ice cream.

Table 2: Effect of addition of different levels of tulsi juice, ginger juice and turmeric powder on sensory quality of herbal milk

	Treatment	Level (%)	CA	Flavor	Consistency	Sweetness	OA
Tulsi juice	T ₀ (control)	0	7.25 ^b ±0.07	7.01 ^a ±0.12	7.48 ^a ±0.10	7.62 ^a ±0.10	7.22 ^a ±0.09
	T ₁	20	8.14 ^c ±0.09	7.63 ^b ±0.09	7.80 ^a ±0.08	7.37 ^a ±0.07	8.10 ^b ±0.10
	T ₂	25	8.07 ^c ±0.04	8.76 ^b ±0.05	8.11 ^a ±0.05	7.99 ^a ±0.05	8.69 ^b ±0.05
	T ₃	30	6.44 ^a ±0.09	6.92 ^a ±0.11	7.63 ^a ±0.10	7.48 ^a ±0.06	7.3 ^a ±0.10
	C.D.			0.80	0.83	0.78	0.63
Ginger juice	T ₀ (control)	0	7.52 ^b ±0.12	7.59 ^a ±0.10	7.50 ^a ±0.12	7.8 ^a ±0.08	7.69 ^b ±0.12
	T ₄	2.5	7.17 ^a ±0.10	7.44 ^a ±0.12	7.52 ^a ±0.13	7.69 ^a ±0.16	7.28 ^a ±0.12
	T ₅	3.0	8.23 ^c ±0.05	8.11 ^b ±0.15	7.63 ^a ±0.07	7.88 ^a ±0.06	7.90 ^b ±0.06
	T ₆	3.5	7.11 ^a ±0.13	7.32 ^a ±0.13	7.47 ^a ±0.16	7.64 ^a ±0.14	7.22 ^a ±0.14
	C.D.			0.31	0.34	0.34	0.33
Turmeric powder	T ₀ (control)	0	7.59 ^a ±0.09	7.63 ^a ±0.09	7.51 ^a ±0.11	7.74 ^a ±0.08	7.58 ^a ±0.09
	T ₇	0.1	8.59 ^b ±0.05	8.28 ^b ±0.05	8.11 ^a ±0.05	8.26 ^a ±0.05	8.38 ^b ±0.07
	T ₈	0.2	8.60 ^b ±0.11	7.57 ^a ±0.09	7.5 ^a ±0.09	7.61 ^a ±0.06	7.63 ^a ±0.06
	T ₉	0.3	8.46 ^b ±0.09	6.92 ^a ±0.12	7.45 ^a ±0.10	7.57 ^a ±0.10	6.90 ^a ±0.12
	C.D.			0.85	0.87	0.85	0.79

Mean ± S.E, n=3, CA- Color and Appearance, OA- Overall Acceptability.

* Different letters indicated the significant difference ($p < 0.05$) among the column.

Table 3: Effect of addition of different levels of tulsi juice, ginger juice and turmeric powder on acidity, viscosity and specific gravity of herbal milk

	Treatment	Level (%)	Acidity	Viscosity	Specific Gravity
Tulsi juice	T ₀ (control)	0	0.12 ^a ± 0.0039	3.56 ^a ± 0.014	1.18 ^a ± 0.001
	T ₁	20	0.12 ^a ± 0.0039	3.74 ^a ± 0.099	1.12 ^a ± 0.005
	T ₂	25	0.12 ^a ± 0.0039	3.21 ^a ± 0.038	1.11 ^a ± 0.017
	T ₃	30	0.12 ^a ± 0.0039	3.02 ^a ± 0.074	1.11 ^a ± 0.021
	C. D.			0.80	0.18
Ginger juice	T ₀ (control)	0	0.12 ^a ± 0.0035	3.55 ^a ± 0.013	1.22 ^a ± 0.009
	T ₄	2.5	0.12 ^a ± 0.0032	3.31 ^a ± 0.027	1.09 ^a ± 0.017
	T ₅	3.0	0.13 ^a ± 0.0027	3.26 ^a ± 0.057	1.12 ^a ± 0.011
	T ₆	3.5	0.12 ^a ± 0.0026	3.35 ^a ± 0.105	1.12 ^a ± 0.015
	C. D.			0.31	0.15
Turmeric powder	T ₀ (control)	0	0.12 ^a ± 0.0036	3.52 ^a ± 0.009	1.08 ^a ± 0.013
	T ₇	0.1	0.12 ^a ± 0.0028	4.05 ^a ± 0.485	1.12 ^a ± 0.011
	T ₈	0.2	0.12 ^a ± 0.0035	4.12 ^a ± 0.384	1.12 ^a ± 0.025
	T ₉	0.3	0.12 ^a ± 0.0049	4.09 ^a ± 0.403	1.11 ^a ± 0.014
	C. D.			0.85	0.17

Mean ± S.E, n=3

* Different letters indicated the significant difference ($p < 0.05$) among the column.

3.3 Effect of ginger juice on sensory scores of Herbal milk

There was a significant ($p < 0.05$) difference in the color and appearance, flavor and overall acceptability scores of the herbal milk containing different levels of ginger juice (Table 2). Tulsi juice addition improved the color and appearance scores significantly ($p < 0.05$). The flavor scores were also improved significantly ($p < 0.05$) by the addition of ginger juice being highest for 3% level of ginger juice. Thereafter, the scores declined for 3.5% ginger juice because of high pungency of ginger. Similar trend was observed in overall acceptability. The scores decreased beyond 3% level of ginger juice, due to high flavor of ginger that was not liked by the panelists. Therefore, this level was selected for the further experimentation on herbal milks. Jadhav *et al.* (2017) [12] found that the score for general appearance, flavor and overall acceptability increased up to 5% addition of ginger juice and, thereafter, score was declined simultaneously. The differences in score for color and appearance and flavor of milk shake due to different levels of ginger juice were significant. He also reported that milk shake without addition of ginger juice showed less acceptability product which may be due to very thin consistency of milk shake. Similar result was found in (Gavhane *et al.*, 2014) [11] ginger flavored peda in which the color score decrease in the order of T₀ (0%) > T₁ (2%) > T₂ (4%) > T₄ (6%) and flavor score decrease in the order of T₀

(0%) > T₁ (2%) > T₂ (4%) > T₄ (6%). Agrawal *et al.* (2016) [11] that use of ginger juice in ice cream increased its flavor preference, when added up to 4% only. Addition of the juice at 5% level led to decrease in flavor score. Pinto *et al.* (2004) reported flavor and overall acceptability preference for ginger juice when added at the rate of 4%, 3%, 2% and 1% in ice cream when judged against vanilla as control. He reported overall acceptability score in ice cream prepared with 4% ginger juice was higher than rest of treatments. In another study, Pinto *et al.*, (2010) reported increase in flavor when treated ginger shreds were added up to 6%. David (2014) [8] reported that the scores for body and texture of ice cream did not differ significantly when ginger juice is incorporated at the rate of 2%, 4% and 6%. Palthur *et al.* (2014b) [26] reported that overall acceptability of the ginger flavored herbal milk was found to be good and recommended for market exploration.

3.4 Effect of turmeric powder on sensory scores of herbal milk

There was a significant ($p < 0.05$) difference in the color and appearance, flavor and overall acceptability scores of the herbal milk containing different levels of turmeric powder (Table 2). Turmeric powder addition improved the color and appearance scores significantly ($p < 0.05$). The

flavor scores were also improved significantly ($p < 0.05$) by the addition of turmeric powder being highest for 0.1% turmeric powder. Thereafter, the scores declined for 0.2% turmeric powder because of high bitterness due to turmeric powder. Similar trend was observed in overall acceptability. The scores decreased beyond 0.1% level of turmeric powder, due to high sediments and bitter flavor of turmeric powder that was not liked by the panelists. Therefore, this level was selected for the further experimentation on herbal milks. Foda *et al.*, (2007) [9] reported that the adding turmeric powder increases firmness in the turmeric incorporated milk. He observed that turmeric in different concentrations had significant effect on acceptability of fresh and stored yoghurt. Singh *et al.* (2014) reported that sharp decline in sensory score of paneer as concentration of turmeric increases but it is still acceptable and safe for usage. He observed addition of turmeric powder in paneer has significant effect on body.

3.5 Optimized Herbal Milk

From the above results the optimum level selected for Tulsi juice is 25%, 3% ginger juice and 0.1% turmeric powder. Selected levels of Tulsi juice, ginger juice and turmeric powder are given in Table 4.

Table 4: Selected levels of ingredients of optimized product

Variables	Level of variation			Level selected
Tulsi juice	20	25	30	25
Ginger juice	2.5	3	3.5	3
Turmeric powder	0.1	0.2	0.3	0.1

Formulations of optimized flavored milk (herbal milk) on the basis of sensory parameters are summarized in the Table 5.

Table 5: Formulation of optimized flavored milk (herbal milk)

Treatments	Milk (%)	Sugar%	Stabilizer%	Tulsi juice%	Ginger juice%	Turmeric powder%
T ₀ (Control)	100	7.0	-	-	-	-
T ₁ (Optimized sample)	100	7.0	0.025	25	3.0	0.5

Table 6: Proximate analysis of herbal milk in comparison with the control

Parameter	Control	Optimized product
Physico-chemical parameters		
Total solids (%)	21.74 ^a ± 2.17	14.96 ^b ± 0.72
Fat (%)	3.02 ^a ± 0.14	3.59 ^b ± 0.02
Protein (%)	3.21 ^a ± 0.07	3.01 ^b ± 0.17
Ash (%)	0.71 ^a ± 0.0052	0.70 ^a ± 0.005
Carbohydrates (%)	14.99 ^a ± 2.08	9.30 ^b ± 0.50
Acidity (% L.A.)	0.107 ^a ± 0.0018	0.096 ^a ± 0.0015
Specific gravity	1.2373 ^a ± 0.0011	1.2207 ^a ± 0.0052
Viscosity (cp)	2.22 ^a ± 0.04	6.07 ^b ± 0.20
Energy (Kcal)	99.98	81.55
Nutritional parameters		
Antioxidant (% DPPH Activity)	-	50.14
Total phenolic content (mg GAE/100g)	-	96.25
Microbiological parameters		
SPC (CFU/ml)	Nil	Nil
Coliform (CFU/ml)	Nil	Nil
Yeast and mold count (CFU/ml)	Nil	Nil
Spore count (CFU/ml)	Nil	Nil

*Data expressed as Mean ± SE, n=3. Values with different superscripts (a, b) in a row are statistically significant at $p < 0.05$.



Fig 2: Flavoured milk (Vanilla flavour)-(Control)



Fig 3: Herbal milk containing Tulsi and Ginger juice and Turmeric powder

3.6 Physico-chemical analysis of optimized product

The control (T₀) as well as experimental samples (T₁) was analyzed for their total solids, fat, protein, ash, total carbohydrates, energy value, specific gravity, viscosity and acidity content. The average values of compositional analysis of herbal milk as well as control milk are detailed in Table 6. The total solids content in control milk was found to be 21.74%. The total solids content of herbal milk (14.96%) showed decreasing trend due to low total solids content in Tulsi juice and ginger juice. The total solids content in herbal milk differed significantly ($P < 0.05$) that is the treatment T₀ was significantly different from T₁ treatment. Palthur *et al.* (2014a) [25] observed total solid content of 17.59% which was a bit higher than our findings when milk was prepared by partial substitution of *Ocimum sanctum* powder.

Johri and Chauhan (2014) ^[13] determined total solid content of 14.96% in misthi doi prepared with Tulsi extract which was in accordance with our findings. Palthur *et al.* (2014b) ^[26] observed total solid content of 17.57% which was a bit higher than our findings when milk was prepared by using ginger juice.

The average fat content of herbal milk was 3.59%. It is seen that fat content was the higher than control milk (3.02%) which is due to the addition of Tulsi, turmeric and ginger although the juice had very low fat content. It can be seen that by addition of Tulsi, turmeric and ginger, the fat content increased significantly ($P < 0.05$). Palthur *et al.* (2014a) ^[25] observed fat content of 2.16% which was lower than our findings when milk was prepared by partial substitution of *Ocimum sanctum* powder. Trivedi *et al.* (2014) ^[36] observed that there was a marginal decline in fat content in the experimental samples (0.12 to 0.62%) on addition of basil powder compared to control. Palthur *et al.* (2014b) ^[26] observed fat content of 2.05% which was a lower than our findings when milk was prepared by using ginger juice. Kumar *et al.* (2017) ^[18] observed fat content of 1.42% in milk prepared by addition of wheat grass juice which was lower than our findings.

The addition of herbal preparations had insignificant effect ($P > 0.05$) on the protein content of herbal milk. The protein content of herbal milk showed decreasing trend with the addition of Tulsi, turmeric and ginger. The protein content 3.21% was observed for control milk (T_0) and 3.01% was observed at treatment T_1 and differed insignificantly from each other. Palthur *et al.* (2014a) ^[25] observed protein content of 3.45% which was higher than our findings when milk was prepared by partial substitution of *Ocimum sanctum* powder. Trivedi *et al.* (2014) ^[36] observed that there was a decline in protein content in the experimental samples on addition of basil powder compared to control which is in accordance with our findings. Palthur *et al.* (2014b) ^[26] observed protein content of 3.48% which was higher than our findings when milk was prepared by using ginger juice.

The addition of herbal preparations had insignificant effect on the ash content of herbal milk. The ash content of herbal milk showed decreasing trend with the addition of Tulsi, turmeric and ginger. The ash content 0.7116% was observed for control milk (T_0) and 0.6972% was observed at treatment T_1 and does not differed significantly from each other. Palthur *et al.* (2014b) ^[26] got ash content of 0.67% which was lower than our findings when milk was prepared by using ginger juice. Johri and Chauhan (2014) ^[13] determined ash content of 0.78% which was higher than our findings when misthi doi prepared with Tulsi extract. Palthur *et al.* (2014a) ^[25] got ash content of 0.69% which was in accordance with our findings when milk was prepared by partial substitution of *Ocimum sanctum* powder.

The addition of herbal preparations had significant effect ($P < 0.05$) on the Carbohydrates content of herbal milk. The carbohydrates content of herbal milk showed decreasing trend with the addition of Tulsi, turmeric and ginger. It may be due to the dilution by addition of tulsi and ginger juice. The Carbohydrate content 15.003% was observed for control milk (T_0) and 9.32% was observed at treatment T_1 and differed significantly from each other. Kumar *et al.* (2017) ^[18] observed carbohydrate content of 10.69% in milk prepared by addition of wheat grass juice which was higher than our findings.

The energy content of herbal milk showed decreasing trend with the addition of Tulsi, turmeric and ginger. The energy

content 99.98% was observed for control milk (T_0) and 81.55% was observed at treatment T_1 . Kumar *et al.* (2017) ^[18] observed calorific value of 64.43 Kcal in milk prepared using wheat grass juice which was lower than our findings.

The average acidity of milk irrespective treatments and replications was 0.107%. The acidity of herbal milk showed decreasing trend with the addition of Tulsi, turmeric and ginger. It may be due to the addition of Tulsi and ginger juice. The acidity of herbal milk was 0.096% and does not differed significantly with respect to the control. Palthur *et al.* (2014a) ^[25] observed acidity of 0.17% which was higher than our findings when milk was prepared by partial substitution of *Ocimum sanctum* powder. Pugazhenth and Jothylingam (2013) ^[28] observed that there was insignificant ($P > 0.05$) difference in titrable acidity of different low calorie herbal flavored milk and control. The mean values of acidity of dietetic herbal flavored milk ranged from 0.14 - 0.15% respectively.

The addition of herbal preparations had insignificant effect ($P > 0.05$) on the specific gravity of herbal milk. The specific gravity of herbal milk showed decreasing trend with the addition of Tulsi, turmeric and ginger. This may occur due to low solid content of Tulsi and ginger juice. The specific gravity 1.2373 was observed for control milk (T_0) and 1.2207 was observed at treatment T_1 but does not differed significantly from each other. Palthur *et al.* (2014a) ^[25] observed specific gravity of 1.085 which was lower than our findings when milk was prepared by partial substitution of *Ocimum sanctum* powder. Palthur *et al.* (2014b) ^[26] observed specific gravity of 1.078 which was lower than our findings when milk was prepared by using ginger juice. Ardali *et al.* (2014) ^[5] reported that the specific gravity of milk increases with increase in the concentration of date palm juice which is not in accordance with our findings.

Viscosity of herbal milk and control milk was measured at 25 °C using Ostwald's viscosimeter. There was a significant increase in viscosity of herbal milk as compared to control. Milk shows a viscosity of 2.22 and 6.07 centipoises in control and herbal milk, respectively. Increase in viscosity of herbal milk might be due interaction of milk constituents with phytochemicals (saponins, polyphenols, phyto-sterols, flavonoids and ascorbic acid) of herb extract and also due to addition of carrageenan (@ 0.025%). The large increase in viscosity with increasing hydrocolloid content is observed. Pugazhenth and Jothylingam (2013) ^[28] found viscosity of different dietetic herbal flavored milk were lower than the control. This was in concurrence with our findings.

3.7 Antioxidant and phenolic content in optimized product

The antioxidant activity of herbal milk as a% DPPH activity was found to be 50.14. Palthur *et al.* (2014a) ^[26] studied the antioxidant activity by DPPH method of milk prepared by partial substitution of *Ocimum sanctum* powder and found 40% activity. Samaddar *et al.* (2015) ^[30] observed that the antioxidant activity of Trans- Cinnamaldehyde and Eugenol enriched flavored milk were that is 0.1495 and 1.2860 μM of trolox/ml of milk respectively of the product and ultimately enhances the shelf life of the product. Also total phenolic content of herbal milk was found to be 96.25 mg GAE (Gallic acid equivalent) /100g.

3.8 Microbiological analysis

The herbal milk developed was analyzed initially for microbial quality. Microbial studies like standard plate count (SPC), yeast and mould count, coliform and Spore count were

carried out to evaluate the safety and keeping quality of the herbal milk. Standard plate count (SPC), yeast and mould count, coliform and spore count were absent in the herbal milk after sterilization. It may also be due antimicrobial effect of Tulsi, turmeric and ginger. Kumara *et al.* (2011) [16] observed that there was less number of yeast and mould and coliform were absent because of proper maintenance of sanitary condition. It is also due to the anti-microbial and anti-bacterial properties of herbal paste (Tulsi) added in low fat herbal yoghurt.

4. Conclusion

From the results of the present investigation, it is concluded that Tulsi juice, ginger juice and turmeric powder could be successfully utilized for preparation of herbal milk which is nutritionally rich, wholesome and reasonable. Present investigation also revealed that herb played an important role in altering the functional properties of milk fortified with it. Addition of Tulsi juice, ginger juice and turmeric powder in milk gave typical flavor and improved the sensory quality and acceptability of the product. The most acceptable quality herbal milk can be prepared by using 25% Tulsi juice, 3% ginger juice and 0.1% turmeric powder. Having good amount of antioxidant and total phenolic content it can give various health benefits such as anti-microbial, anti-inflammatory, cardio-protective, anti-carcinogenic, anti-pyretic. The cost of production of herbal milk with natural flavor and color was worked out as Rs 14.17 per 200 ml that means the product is reasonable and nutritious with respect to its health benefits. The herbal milk confirms to the FSSAI requirements and such novel flavored milk has natural flavor as well as very high consumer acceptability. Results will facilitate the development of herb fortified functional dairy products.

5. References

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