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Research article

ANTIOXIDANT AND ORGANOLEPTIC PROPERTIES OF TULASI FLAVORED HERBAL MILK

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ABSTRACT: The preparation of milk products by partial substitution of Ocimum sanctum with milk and investigating the proximate quality, textural characteristic, keeping quality and sensory attributes of the developed product. The physico- chemical and organoleptic studies were performed for the Herbal Milk analysis. Laboratory analysis was carried out to study the variation in moisture, protein, fat and ash content. PH, Acidity and Specific Gravity were slightly changed when compared to normal milk. The organoleptic studies appearance, colour, flavor, taste and overall acceptability were studied and overall acceptability was good for Herbalmilk. Microbial studies like total plate count (TPC), yeast and mould count, coliform and E.coli count were carried out to evaluate the safety and keeping quality of the products. Antioxidant and iron chelating activity of the Herbal Milk was determined. The Herbal Milk product was the most preferred and recommended for market exploration.
Key words: Herbal Milk, Organoleptic studies, Antioxidant Properties, Ocimum sanctum

INTRODUCTION

Over the years, the importation of extremely large quantities of milk to satisfy the consumer demands for milk and other dairy products has been the source of genuine concern for the governments, processors and consumers alike because the imported milk is expensive and it drains large sums of foreign exchange reserves [1,2]. It is therefore regarded as urgent and timely to develop dairy-type products from less expensive alternative sources of Herbal plants like Ocimum, Cinnamon, Hemidesmus to compliment the locally produced milk and to develop new milk products with minimum use of the imported dairy ingredients [3, 4]. The new products developed from milk could potentially be of desirable nutritional composition especially in relation to cholesterol inducing fat levels, being as it is that the saturated fat content in milk has been shown to be a good saturated fat, easily metabolized to give the body quick energy [5-9]. New value-added milk products are entering the market. Some examples are milks fortified with calcium, cultured milk fortified with multivitamins and minerals, flavored milks with banana, chocolate and strawberry flavors, new ready-to-drink blends of evaporated milk and black, green and chamomile teas with spices of cinnamon, ginger and clove [10, 11]. Milk powder has been used mainly as a food ingredient, but there is still a gap in the food market, which leads to the opportunity to arrive with a new food product of which milk powder could constitute the major proportion [12]. Natural Herbal milk, composed mainly of milk powder, and Herbal Plants and other nutrients [13]. The Herbal milk represents a great in-between meal and medicine. The flavored Herbal milk contains calcium, phosphorus, iron and other essential nutrients, which makes it a potential food supplement for adults and children. It can be flavored with different Herbal plants to change the medicinal properties [14]. Sensory tests, such as descriptive analysis and consumer affective tests, are regularly used to study food ingredient effects, processing variables and storage changes on the perceived sensory properties of food products [15]. Sensory analysis provides marketers with an understanding of food product quality, directions for product quality, profiles of competing products, and evaluations of product reformulations from a consumer perspective [16, 17]. This research was designed to characterize physic-chemical, organoleptic and sensory properties of Herbal milk and to determine the consumer sensory profile driving product acceptance and purchase intent.

MATERIALS AND METHODS

Sterilization of Milk:

Strerilized Milk was procured in packets from the standard companies. 1000ml Milk was taken and the sterilized again under hygienic conditions. The procedure was repeated for two to three times with 100^{o}C temperature for 15 min. The Milk was packed in individual packs and stored at 5^{o}C for a period of one month and is used for preparation of Herbal Milk.

Preparation of Herbal milk

After storage, the stored milk was mixed with Ocimum sanctum powder. Then the Herbal milk was analyzed both physico-chemically and microbiologically before storage.

Preparation of Ocimum Sanctum Powder

10g of Ocimum leaves were heated on hot pan and dried in room temperature. Then the dried leaves were made into powder. The powder was again filtered through small pored mesh and used to prepare Herbal Milk.

Shelf life Study of Herbal milk

The prepared Herbal milk was stored both at room temperature and cold storage (5-10^{o}C) to study the shelf life. The Herbal milk was initially analyzed chemically, microbiologically and organoleptically before storage.

Physico – chemical analysis of the Herbal milk

The physical characteristics of Herbal milk sample were determined after they were brought to the laboratory. All determinations were carried out according to AOAC (2000)'s methods. Briefly, moisture content was determined by the difference between the known weight of milk sample and the determined weight of the total solid after evaporating the liquid component of the milk sample on a hot plate. The pH measurement was made using a digital pH-meter calibrated with pH 4 and 7 buffers. Titratable acidity was measured by titrimetric method, and expressed as percent of lactic acid. Specific gravity, conductivity and viscosity were determined by the standard methods (AOAC, 2000).

Lactose content was determined by using Fehling's solution method (Triebold, 2000). The ash content was obtained by incineration of the sample placed in the muffle furnace at 550 °C for 6 h (AOAC, 2000). For minerals analysis, the milk solid contents were taken and digested using two volumes of concentrated nitric acid. After adding one volume of perchloric acid, the contents were heated gently on a hot plate followed by a vigorous heating till dryness. This digestion technique makes no attempt to dissolve any silicate-based material that may be present in the samples. After cooling, the digested samples were quantitatively transferred to a flask and diluted to 100 ml with deionized double distilled water and then filtered. Atomic absorption spectrophotometer equipped with standard burner, air-acetylene flame and hollow cathode lamps, as radiation source, was used for the analysis of minerals (Fernandez et al., 2002).

Proximate Analysis

Proximate analyses were carried out on 100% milk. All analyses were carried out in triplicates.

Moisture Content

Moisture content of milk samples was determined in triplicate using the Official Methods of Analysis (AOAC, 2000) for food. Three ml of milk was pre-dried into moisture dish with tight-fit cover. Samples were partially dried and weighed on a steam bath prior to oven combustion at 105^{o}C for 8 hrs. Moisture content was determined by difference and expressed as a percentage of the initial weight of Herbal Milk.

Crude Protein Content

Percent nitrogen content of the Herbal Milk was determined using the micro Kjedahl method (AOAC, 1990) and crude protein content.

Fat Content

De-moisturised samples were transferred into 22×80 mm paper and were placed in thimbles. A small ball of cotton wool was placed into the thimble hole to prevent lose of sample. Anti bumping granules were added to a previously dried 250 ml round bottom flask and weighed accurately. A quantity of 150 ml of petroleum ether was added to the flask and the apparatus assembled. A condenser was connected to a Soxhlet extractor and refluxed for 16 hrs at 40oC on the heating mantle. The flask was removed and evaporated on a steam bath. The flask and oil then heated for 1 hr in an oven at 80oC. The flask and its contents were cooled at room temperature of 26^{o}C in a dessicator and accurately weighed.

Total solids (^{0}brix)

The percentage of total soluble solids were determined by using "Erma" hand refractometer and expressed as percent total soluble solids (^{o} brix).

Minerals

For mineral analysis 5 ml of respective samples were digested in concentrated HNO_3. The digest was quantitatively transferred to a 50 ml volumetric flask and made up to volume with distilled water. A blank digest was carried out in the same way. All minerals were determined using atomic absorption spectrometry against aqueous standards. The mineral concentration was expressed as mg mineral/100gm dry weight.

Sugars (%):

Reducing sugars and total sugars were determined by the method of "Lane and Eynon" (1923). To 25g of the sample in a volumetric flask 100ml of water was added and neutralized with 1N NaoH. 2ml of 66% lead acetate solution was added and kept for 10 minutes. Excess lead acetate was precipitated by necessary amount of 20% potassium oxalate, made upto the volume with water, filtered and taken in burette.

10ml of mixed Fehling's solution was taken in 250ml conical flask. Little quantity of the sample was run into flask and heated to boil moderately for 2 minutes. 3 drops of methylene blue solution was added and completed the titration until the indicator was completely decolourized. Brick red colour of the solution indicates the end point.

Total sugars

For total sugars 50 ml of filtered sample was taken in a 250 ml conical flask to which 50 ml water and 5g of citric acid was added, boiled gently for 10 minutes to complete the inversion of sucrose, transfered to 250 ml volumetric flask and neutralized with 1N NaOH. The volume was made upto the mark and determined the total sugars as invert sugars.

Organoleptic evaluation of the Herbal Milk:

The Herbal Milk developed from Milk was assessed every month. The qualities considered during the study were appearance, colour, flavor, taste and overall acceptability.

Development of score card:

In order to evaluate the sensory qualities of developed Herbal Milk, descriptive test, which analytically describes the sensory qualities of Herbal Milk, was used. In order to rank the sensory qualities, ordinal scoring method (ranking) was used (Peryam and Pilgrim, 1957). Five point scale was used for ranking and details of ranks/scores were given as 5- Excellent; 4- Good; 3- Fair; 2- Poor; 1-Very poor. A score card was developed to evaluate the acceptability of the Herbal Milk. The analysis was carried out in a room, which was free from all disturbances in mid afternoon (3pm).

Microbiological evaluation of the Herbal Milk:

Microbiological studies were conducted at 1st and 3rd month of storage. Total plate count (TPC), yeast and mould count, coliform and E.Coli were undertaken. The procedure of Cruick Shank et.al, (1975) was used for total plate count and yeast and mould count.

Total plate count and yeast and mould count by pour-plate method

Procedure

Ten fold serial dilution of the bacterial suspension was prepared. Normal saline was used as diluents for the organism. 9 ml of the diluents was pipette into several sterile test tubes. The bacterial suspensions were uniformly mixed. Using a sterile 1 ml pipette, 1 ml of the suspension was transferred into the first tube of diluents and mixed thoroughly. From this mixed dilution, 1 ml was transferred to the next diluents. Similar dilutions were made in the same way using fresh pipettes.

1 ml of each dilution was pipetted into sterile petri plates and 15 ml of molten agar medium which was cooled around 45^{o}C was poured into plates containing diluted samples. The agar medium was immediately distributed by gently mixing the petridish in circular movements both clock wise and anti clock wise on a flat bench and then allowed to set evenly and the inverted plates were incubated for 1-2 days and 3-5 days at 37^{o}C for bacterial and yeast and moulds respectively.

For total plate count, total plate count agar and potato dextrose agar for yeast and mould count was used.

Coliform and E.coli count:

Procedure

10 ml of the sample was inoculated in double strength lactose broth in 5 test tubes, 1 ml in 5 test tubes single strength lactose broth and 0.1 ml in other set of lactose broth test tubes and incubated at 35^{o}C for 24+/- 2hours. After incubation they were observed for gas production. Gas production in Durham's tubes indicates positive test. Positive test tubes were separated and inoculated in Brilliant green lactose bile broth and incubated for 24-48 hours. Presence of gas production indicates positive test. From the positive tubes, inoculated in EMB agar and streaking was done. The plates were incubated for 24 – 48 hours. After the development of colonies, they were differentiated by observing colony morphology and Gram's staining.

Sedimentation

To make this measurement you take a fixed volume of milk and filter through a screen made of lintine paper which has been mounted in the base of a large funnel. The filter1 is held in place by a bushing and by the funnel being tightened into place with a ¼ turn. The bushing used to test liquid milk is a set of two plastic rings: one serves as a bottom washer and the other ring comes with a small hole (aperature) in the center. The filter / filter card is held in place between these 2 rings. The size of the aperature is determined by the volume of milk that will be tested: 1, 2, 4 or 16 oz. The filter can be a simple disc of lintine paper or it can be a disc mounted in a card (Sediment Tester Card) that allows you to record all the necessary information for that milk sample and provides a convenient storage for your records.

Scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical

The scavenging of DPPH free radical of the samples was measured using the method of McCue and Shetty (14) with some modifications. A 0.1 mM DPPH radical solution in ethanol was prepared. 8ml of ethanolic DPPH solution was mixed with 2ml of sample or ethanol (as control), vortexed well, and then incubated for 30 min at room temperature. The samples were then centrifuged for 10 min at 9500 rpm at room temperature. After filtration through a Whatman No 40 filter, absorbance of each sample at 517 nm was measured. Trolox at a concentration of 0.25 mg/ml was used for comparison. Radical-scavenging activity was calculated as follows:
DPPH radical-scavenging activity (%)

= (absorbance of control-absorbance of sample) / (absorbance of control) X 100

Chelation of metal ions (Fe2+)

Sample extracts were prepared according to Hernández-Ledesma et al. (6). The chelating activity of samples on Fe2+ was measured according to El and Karakaya (3) with some modifications. Briefly, one millilitre of sample (1 g/ml) was mixed 3.7 ml deionized water. Each sample was incubated with 0.1 ml FeCl24H2O (2 mM) for 40 min. After incubation, the reaction was initiated by addition of 0.2 ml ferrozine (5 mM). The mixture was shaken vigorously and left at room temperature for 10 min. The absorbance of the mixture (formation of the ferrous iron-ferrozine complex) was measured at 562 nm. The control was performed in the same way using FeCl24H2O and water. The lower the absorbance of the reaction mixture means the higher the Fe2+ chelating ability. EDTA (0.1 mg/ml) was also run in the same way for comparison. The chelating activity was calculated using the following equation (15):
Fe2+ chelating activity (%)

= [1- (absorbance of sample / absorbance of control)] X 100

Statistical Analysis

The data were analyzed using univariate and multivariate statistical analysis. Analysis of Variance (ANOVA, proc mixed, SAS version 8.2, 2001) was performed to determine significant effects of the attribute intensities in each of the products. A significant F-ratio ($\alpha < 0.05$) from the ANOVA indicated that an attribute was used to find differences among the products. Multivariate Analysis of Variance (MANOVA) was used to determine differences among the products, expressed in terms of mean vectors of the sensory attributes. Descriptive Discriminant Analysis (DDA, proc candisc SAS version 8.2, 2001) was applied to identify sensory attributes that essentially emphasized differences among the products. When applying this technique, canonical coefficients are calculated.

RESULTS AND DISCUSSION

Herbal milk

Herbal Milk was developed at laboratory level and stability of the product at two different storage conditions was evaluated. The results were subjected to appropriate statistical analyses.

Physico–chemical characteristics of Herbal Milk

The physical characteristics such as moisture, total solids, specific gravity, pH, conductivity, viscosity and titratable acidity are important parameters in studying the physicochemical compositions and nutritional aspects of milk. Table 1 shows the various physical parameters of the Herbal Milk. Initially for all the processed products of Milk, analysis was carried out to determine the values for different parameters. The initial values of the product before storage are presented in the table1.

Table 1: Physico-chemical properties of different dietetic herbal milk

pH	6.69±0.012
Acidity	0.17±0.004
Specific gravity	1.085±0.02
Fat (%)	2.16±0.007
Protein (%)	3.45±0.017
Lactose (%)	4.33±0.022
Total solids (%)	17.59±0.032
Total ash (%)	0.69±0.004
Calcium (%)	0.26±0.007
Phosphorus (%)	0.15±0.006
Sodium (mg/100g)	58.28±0.015
Potassium (mg/100g)	142.63±0.212
Iron (µg/lt)	278.43±0.047
Sedimentation	0.4mg/0.1filtering area

Organoleptic evaluation of processed Herbal Milk

The qualities considered during the study were appearance, colour, flavor, taste and overall acceptability. A maximum score of 5 was taken as standard for considering the quality of the Herbal Milk. Processed Herbal milk ranked excellent in all the qualities. Data pertaining to the initial organoleptic evaluation of the Milk are presented in table 2.

Table–2: Organoleptic characteristics of Herbal Milk before storage.

Attributes	Herbal Milk
Appearance	5
Colour	5
Flavour	4
Taste	5
Overall acceptability	5

Microbial evaluation of processed Herbal Milk

The Herbal Milk developed was analyzed initially for microbial quality. Microbial studies like total plate count (TPC), yeast and mould count, coliform and E.coli count were carried out to evaluate the safety and keeping quality of the Herbal Milk. Data pertaining to microbial evaluation of processed herbal milk presented in Table 3.

Table 3: Microbiological characteristics of Herbal Milk before storage.

Products	Total plate count (CFU/ml)	SPC (CFU/ml)	Yeast and mould count (CFU/ml)	Coliform (CFU/Ml)
Herbal Milk	15	8500CFU/ml	10	60

AntiOxidant Proeprty

DPPH method was used to study the Antioxidant and Iron chelating activity of the Herbal Milk and the results were tabulated in Table 4.

Table 4: Antioxidant activity of Herbal Milk

Products	Activity	% of DPPH free radical Scavenging activity and iron chelating activity
Control	Anti oxidant activity Iron chelating	40% 55%
Herbal milk	Anti oxidant activity Iron chelating	40% 55%

CONCLUSION

The results of the study lead to the conclusion that the partial substitution of Tulasi powder with milk sufficiently developed a significant product. Initially for all the processed products of Milk, analysis was carried out to determine the values for different parameters. The qualities considered during the study were appearance, colour, flavor, taste and overall acceptability. Microbial studies like total plate count (TPC), yeast and mould count, coliform and E.coli count were carried out to evaluate the safety and keeping quality of the Herbal Milk. Antioxidant and Iron chealating activity of Herbal Milk was studied.

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