Comparative Study on Nutritional Profile Analysis of Herbal Yogurt

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***Abstarct*: - Fermentation of lactose by probiotic microorganisms produces lactic acid, which acts on milk protein to give yogurt its texture and characteristic flavour. Cow's milk is commonly available worldwide, and, as such, is the milk most commonly used to make yogurt. Yogurt is produced using microbial cultures of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. In addition, other *lactobacilli* and *bifidobacteria* are also added during or after culturing yogurt.**

In this present study herbs were used for the preparation of herbal yogurt. Tulsi (*Ocimum basilicum)* and Beet-root (*Beta vulgaris*) extract was used 5 % v/v. Before conducting the study, we have tried to prepare natural yogurt and herbal yogurt in 3 % and 7 % v/v respectively. In spite of the slight antimicrobial property of tulsi and beet root we preferred both the yogurt, but due to undesirable consistency and poor organoleptic value, we rejected 3 % and 7 % extract made yogurt and further study was conducted with 5 % extract. The aim of the study was to find out and differentiate the nutritional aspects, overall acceptability and beneficial effects of the herbal yogurt. A survey based sensory evaluation was carried out for overall acceptability. From this study we have found that beet-root milk powder was much more accepted on the basis of Hedonic scale overall acceptability rather than other yogurt sample. The folic acid and riboflavin content was much higher than normal milk yogurt samples, whereas titrable acidity and fat content showed negligible difference. DPPH scavenging activity was significantly higher in tulsi extract yogurt samples rather than beet-root extract yogurt. Overall we could conclude that both tulsi-extract and beet-root extract samples were superior in nutritional aspects than natural yogurt.

***Keywords*: *Herbal yogurt, Tulsi, Beet root, DPPH, Antioxidant and Nutrients*.**

1. INTRODUCTION

Dahi is a yogurt of the Indian subcontinent, known for its characteristic taste and consistency. Sweet yogurt (misti doi) is common in eastern parts of India, made by fermenting sweetened milk. While cow's milk is considered sacred and is currently the primary ingredient for yogurt, goat and buffalo milk were widely used in the past, and valued for the fat content. Butter and cream were made by churning the milk [1]. Yogurt production begins with the breakdown of lactose into glucose and galactose, a process catalyzed by β-galactosidase. The glucose produced from this catabolic step then enters glycolysis, producing pyruvate. It has been proposed that yogurt bacteria utilize the Embden-Meyerhof-Parnas pathway of glycolysis. Pyruvate then enters lactate fermentation, also known as homolactic fermentation, as it produces only lactic acid molecules.

The production of lactic acid forms the basic structure and texture of yogurt. However, other molecules contribute to the taste of yogurt. These include acetaldehyde, an important flavor substance in yogurt, and tyrosine, a product of proteolytic activity, but can cause bitterness when the concentration is above 0.5 mg/ml [2]. Lactic cultures specially, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in the ratio of 1:1 play an important role for the production of yogurt. Yogurt has so many beneficial effects that play important role for its consumption. Yogurt not only helps to maintain a healthy gut, but also effectively helps in gastrointestinal conditions, like lactose intolerance, constipation, bowel disease and diarrhea. In diabetic women, it has also been suggested that yogurt consumption reduces the risk of vaginal yeast infection, caused by *Candida*, by regulating pH and suppressing *Candida* over growth [3].

Different types of herbal yogurts were developed by mixing standardized milk with pretreated herbs, namely tulsi leaf (*Ocimum sanctum*), pudina leaf (*Mentha arvensis*) and coriander leaf (*Coriandrum sativum*), with leaves separately and a 1:1 (v/v) mixture of the strains of lactic starter cultures *Lactobacillus acidophilus* and *Lactobacillus plantarum* followed by incubation at 40℃ for 6 h. The beta-galactosidase enzymatic activity of the abovementioned herbal yogurts was determined and interestingly noted to exhibit higher enzymatic activity compared with the control yogurt (without any herbs). Among all herbal yogurts, tulsi yogurt had the maximum beta-galactosidase activity [4].

2. MATERIAL & METHODS

2.1. Yogurt preparation

Amul cow milk was collected from local market Jadavpur, Kolkata. To 30 ml of pasteurized cow milk, 5 % (v/v) extract of both tulsi and beet root along with starter culture was added and was allowed to ferment for 12 h for fermentation.

2.2. Yogurt preparation with 5 % w/v milk powder

To 30 ml of pasteurized cow milk, 5 % (v/v) extract of both tulsi and beet root along with 5 % w/v Amul milk powder. The mixture was stirred well and starter culture was added to it and was allowed to ferment for 12 h for fermentation.

Before conducting the study, we have tried to prepare natural yogurt and herbal yogurt with 3 % and 7 % v/v

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extraxt of both tulsi and beet root respectively. However, due to undesirable consistency and poor organo-leptic values of the prepared yogurt, we rejected 3 % v/v/ and 7 % v/v extract yogurt and further study was conducted with 5 % v/v extract.

3. SENSORY EVALUATION

A sensory analysis was used to evaluate differences among the three types of yogurt samples by forty untrained panellists using a 9-point hedonic scale. Panellists were asked to rate samples for sourness, bitterness, flavor, viscosity, firmness, and overall preference with the words “low” (1-3), “medium” (5), and “high” (7-9) for each yogurt sample. Sensory evaluation was conducted in individual booths to prevent rate score bias. Sensotool (Sensometrics. Co., Ltd, Korea) was used for statistical analysis of sensory analysis [5].

4. BIOCHEMICAL ANALYSIS

4.1. Determination of Titrable acidity

Reagents

(1) Standard Sodium Hydroxide Solution – 0.1 N.

(2) Phenophthalein Indicator – 0.5 g phenolphthalein was dissolved in100 ml of 50 % (v/w) ethyl alcohol.

Procedure

To 10 g of curd sample 30 ml of warm water was added. 1 ml of phenolphthalein indicator was added to the mixture. The mixture was shaken well and titrated against standard NaOH solution.

Calculation

Titrable acidity as Lactic acid = 9 AN/ W

Where A = Volume of standard NaOH required for titration

N = Normality of Standard NaOH solution

W = weight of the sample taken for test

(Ref: I.S. 1166 – 1973) [6].

Protein in the yoghurt samples was measured by Lowry method [7].

4.2. Estimation of folic acid

Folic acid content was measured as per procedures of USP XVIII [8]. 1ml of homogenized sample was mixed with 25 ml of distilled water and shaken well. Then KOH solution was added drop wise to the diluted sample, a sticky layer appeared then dissolve completely, a clear solution obtained. After this volume made to 100 ml with distilled water and 1 gm Zn dust was added to it. Allow it for waited 1 hour. Then 1 ml 5N HCL, 1ml $NaNO_2$ (0.2 %) were mixed well and added to the previous solution. 1ml of ammonium sulfonate was added along with 1 ml of 5 % EDTA & 0.1% N NED. Mixture was shaken for 2 minutes after each addition, a purple color was developed and then OD was taken at 550 nm.

4.3. Measurement of riboflavin

Concentration of Riboflavin in sample was measured by spectroflourimeter method described by Kodicek et.al (1979) [9]. 25 g sample was dissolved in 50 ml of distilled water in a 500 ml of volumetric flask. Then 1 ml glacial acetic acid was added to it. The solution was then mixed with further 500 ml distilled water and stirred until completely dissolved and the volume was made upto 1 l with distilled water. Now OD was taken in spectroflourimeter at 524 nm.

4.4. Determination of antioxidant activity scavenging of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical

The DPPH radical scavenging activity was evaluated using the method of Jhong et al. (2008) [10]. DPPH radical solution (0.004 %, w/v) in 95 % ethanol was prepared. A volume of 2 ml of DPPH in ethanol was added to 2 ml of sample, well vortexed and incubated for 30 min in dark room at room temperature. Absorbance of each sample at 517 nm was measured using UV-visible spectrophotometer (Varian Carry 50 Conc.). Ethanol was used as a blank, while DPPH solution in ethanol served as control. The antioxidant activity was expressed as percentage of DPPH activity calculated as [(Absorbance of blank - absorbance of sample) × 100] / (Absorbance of blank).

5. INFLUENCE OF THE LACTIC ACID AND OTHER FERMENTING ORGANISMS UPON THE COLOR OF THE BEETROOT JUICE

Beta vulgaris contain calcium, sulfur, iron, potassium, beta-carotene, and vitamin C, therefore is famous for their ability to clean the blood. It is an excellent source of vitamin A, of soluble and insoluble dietary fiber and it is used against constipation. Beets are also high in minerals that strengthen the liver and gall bladder, and are the building blocks for blood corpuscles and cells. It is also an important source of boron which is useful for the secretion of sexual hormones. The sugar content of the beetroots (6.76 g/100 g) is superior. The pigments found in the beet root are called betalains and they are classified into two categories: the beta-cyanins that have a red-violet color and the betaxanthins which have a yellow-orange color [11]. All the lactic cultures were found capable of rapidly utilizing beet juice for cell synthesis and lactic acid production. However, *L. acidophilus* and *L. plantarum* produced a greater amount of lactic acid than other cultures and reduced the pH of fermented beet juice from an initial value of 6.3 to below 4.5 after 48 h of fermentation at 30 °C. Although the lactic cultures in fermented beet juice gradually had lost their viability during cold storage [12].

The conversion of carbohydrate to lactic acid, carbon dioxide and other organic acids occurs under the action of the lactic bacteria. The homofermentative bacteria produce only lactic acid through the Embden–Meyerhof route and the heterofermentative bacteria produce ethanol, CO_2 and other volatile compounds besides the lactic acid the 6-phosphoglucanate/phosphoketolase route.

The stability of the beetroot juice depends on a series of factors: pigment structure and concentration, pH value, water activity, light, temperature, metal cations and decolorizing enzymes and betalain-stabilizing compounds [13].

6. RESULTS & DISCUSSION

Table 1. Comparative analysis of herbal yogurt

Biochemical properties	Amul cow milk	Cow milk curd	Cow milk curd treated with 5 % v/v *Ocimum basilicum* (tulsi) extract	Cow milk curd treated with 5 % v/v *Beta vulgaris* (Beet root) extract
Riboflavin	0.075 mg/ dl	1.2 mg/ dl	1.32 mg/ dl	1.4 mg/ dl
Folic acid	0.042 mg/ dl	0.06 mg/ dl	0.08 mg/ dl	1.42 mg/ dl
Titrable acidity (g/ 100 ml)	0.14	0.17	0.16	0.16
Fat content (%)	0.16	0.19	0.21	0.18
Protein content (%)	0.18	0.22	0.21	0.24
DPPH (%)	16.2	18.03	40.32	44.25

All the experiment was done in triplicate.

Table 2. Hedonic rating scale of Herbal yogurt

Attributes	Cow milk curd	Cow milk curd treated with 5 % v/v *Ocimum basilicum* (tulsi) extract	Cow milk treated curd with 5 % v/v *Beta vulgaris* (Beet root) extract
Appearance	8	8.5	9
Flavor	7.5	8	8.5
Color	7.5	8	9
Odor	7.5	8	8.5
Mouthfeelness	7	7.5	8
Overall acceptability	7	8	9

All the experiment was done in triplicate.

From Table 1, it was observed that, folic acid and riboflavin content was much more higher than normal milk yogurt samples, but titrable acidity and fat content showed negligible difference.
DPPH scavenging activity was higher in cow milk curd with tulsi extract samples rather than Beet-root extract sample.
From Table 2, it was observed that beet-root yogurt was much more accepted on the basis of Hedonic scale overall acceptability rather than other yogurt sample.

Influence of the lactic cultures and other fermenting organisms upon the color of the beetroot juice
Beta vulgaris contains calcium, sulfur, iron, potassium, choline, beta-carotene, and vitamin C, therefore is famous for their ability to cleanse the blood. It is an excellent source of vitamin A, of soluble and insoluble dietary fiber and it is used against constipation. Beets are also high in minerals that strengthen the liver and gall bladder, and are the building blocks for blood corpuscles and cells. It is also an important source of boron which is useful for the secretion of sexual hormones. The sugar content of the beet-roots (6.76 g/100 g) is superior. The pigments found in the beet root are called betalains and they are classified into two categories: the betacyanins that have a red-violet color and the betaxanthins which have a yellow-orange color [11].
All the lactic cultures were found capable of rapidly utilizing beet juice for cell synthesis and lactic acid production. However, *L. acidophilus* and *L. plantarum* produced a greater amount of lactic acid than other cultures and reduced the pH of fermented beet juice from an initial value of 6.3 to below 4.5 after 48 h of fermentation at 30 °C. Although the lactic cultures in fermented beet juice gradually lost their viability during cold storage, the viable cell counts of these lactic acid bacteria [12].
The conversion of carbohydrate to lactic acid plus carbon dioxide and other organic acids occurs under the action of the lactic bacteria. The homofermentative bacteria produce only lactic acid through the Embden–Meyerhof route and the heterofermentative bacteria produce ethanol, CO_2 and other volatile compounds besides the lactic acid the 6-phosphoglucanate/ phosphoketolase route.
The stability of the beetroot juice depends on a series of factors: pigments structure and concentration, pH value, water activity, light, temperature, metal cations and decolorizing enzymes and betalain-stabilizing compounds [13].

7. CONCLUSION

Yogurt is among the most popular fermented milk products consumed all over the world because of its excellent sensory properties, as well as high nutritive and therapeutic values. The aim of the present study was to produce yogurt with the addition of different concentration of tulsi and beet root (1 %, 3 %, 5 %) v/v and to determine the effect of selected herbs on the antioxidant activity, nutritional aspects and organoleptic acceptance of the fermented milk products. Overall we can conclude that both tulsi yogurt and beet-root extract yogurt samples are superior in biochemical aspects of cow milk curd.

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