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Development of Probiotic Pomegranate Beverage and Its Physico-Chemical and Microbial Characterization

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ABSTRACT

The pomegranate juice with 13°Bx inoculated using 10% mixed culture of L. bulgaricus and L. plantarum (1:1) and fermented for 7 hours was standardized for development of probiotic pomegranate beverage. Various physico-chemical attributes of the beverage like TSS, titratable acidity, pH, glucose and fructose content, total sugars, reducing sugars and non reducing sugars, ascorbic acid, polyphenol and tannin content were found to be 13 °Bx, 0.540 per cent, 3.512, 60.35 g/L, 62.82 g/L, 13.14 per cent, 12.32 per cent, 0.82 per cent, 9.53 mg/100mL, 206.64 mg/100mL and 0.114 per cent, respectively. The color was found to red which was most acceptable for pomegranate based beverage. Further, viscosity of beverage was found to be decreased with increase in temperature. The microbiological analysis showed that prepared beverage contained optimum level of cultures i.e. 6.5 x10^9 CFU/mL and was free from any traces of yeast, mold and coli-form bacteria. Hence, it can be concluded that the pomegranate juice has the potential as a carrier of probiotic culture for the preparation of pomegranate probiotic beverage.

Key words: *Pomegranate juice, Probiotics, Physico-chemical properties, Color, Viscosity*

INTRODUCTION

Currently food industry is targeting for the development of more healthy foods due to consumers' awareness towards the relationship between food and health and their demands of decreasing the use of chemical preservatives. Nowadays, healthy foods mean "functional foods" which exert the beneficial effects on specific body functions, in addition to the traditional nutritional effects. Well-known examples of functional foods are those which contain bioactive compounds, like phytochemicals, oligosaccharides, dietary fiber and "friendly" bacteria i.e. probiotics[7].

Probiotic foods are defined as those that contain microorganisms which influence beneficially the consumer's health by improving their intestinal microbial balance[5]. A commercial probiotic product is considered as functional only if it contains 10^7 CFU/ml at the time of consumption[2].

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Dairy is a very nutritious substrate for LAB but expanding the trend of vegan lifestyles; the issues of lactose intolerance and demand for low-fat and low-cholesterol foods have created a need for non-dairy probiotic products[14].

Fresh fruits and vegetables are considered good matrices and can provide ideal substrates for probiotics and do not contain any dairy allergens[9]. Pomegranate (*Punica granatum* L.) is known to be a superfruit having health-promoting properties with antimicrobial, antiviral, anticancer, antioxidant and antimutagenic effects[12]. Pomegranate juice is a good source of sugars, organic acids, amino acids, polyphenols as well as enzymes, proteins, pectins and insoluble complexes. One hundred twenty two phytochemicals have been identified in pomegranate and has been reported to be a rich source of antioxidants. These antioxidants are more potent than many other antioxidants including vitamin C, vitamin E, coenzyme Q-10 and alpha-lipoic acid[1]. The antioxidant level in pomegranate juice was found to be higher than in green tea and red wine[6].

A wide range of studies have been carried out to find the potential of fruit juices such as tomato, beet and cabbage juices as raw materials for the production of probiotic drinks. Results have shown that all the strains (*L. plantarum, L. acidophilus* and *L. delbrueckii except L. casei*) are capable of growth in the fruit juices mentioned[22,23,24].

Since, in addition to being delicious and nutritious, the pomegranate juice may be an excellent medium for the supplementation of existing nutraceutical components with probiotic culture. Thus, pertaining to the above discussion, in response to the demand from increasingly health conscious consumers for nutritive value and medicinal properties of pomegranate fruit, it is, therefore, felt logical to seek its potentiality for developing probiotic pomegranate beverage.

MATERIALS AND METHODS

Preparation of pomegranate juice

Freshly harvested pomegranate fruits (cv. *Bhagwa*) were procured from local market of Parbhani (Maharashtra). Pomegranate juice was prepared by blending the juicy arils in the domestic mixer. Its total soluble solids was maintained to 13^{o}Bx and stored at 4^{o}C before use.

Probiotic strains

Lactobacillus isolates, *Lactobacillus bulgaricus and L. plantarum* were isolated and identified using phenotypic and genotypic methods in Department of Food and Industrial Microbiology, College of Food Technology, VNMKV, Parbhani in collaboration with Department of Microbiology, Shivaji College, Parbhani. Stock solution was prepared by adding sterile glycerol (50% v/v) to the activated culture. The glycerol stock culture was stored at -20 ^{o}C in sterile screw cap tubes.

Preparation of starter culture

The starter culture was prepared with the help of method described by Mousavi *et al.*[11], with slight modifications. *L. plantarum* and *L. bulgaricus* was cultivated separately in the MRS broth for 24-h at 37^{o}C. To obtain the biomass, 10 mL of the separately cultivated MRS broths were mixed in equal proportion (1:1) and centrifuged at 4000 rpm for 10 min. The obtained biomass was washed with sterile saline solution twice to remove the residual MRS media. Thus, inoculum was prepared.

It was then introduced into pasteurized pomegranate juice (100 mL) for making it 10% concentration of probiotics. The inoculated juice was then incubated at 37^{o}C for 24 h and was treated as starter culture for preparation of final beverage.

Preparation of probiotic pomegranate beverage

Above prepared starter culture (10mL) was then added to the pasteurized pomegranate juice (100 mL) to obtain 10% inoculation. It was allowed to ferment in incubator at 37^{o}C for 7 h. After incubation, the beverage was kept at refrigeration temperature for future use.

Physico-chemical analysis of probiotic pomegranate beverage

Following physico-chemical properties of fresh pomegranate juice were determined:

Total soluble solids (T.S.S.), Titratable acidity and pH

Total soluble solids were measured immediately after extraction using hand refractometer (ERMA make). Titratable acidity, expressed as per cent lactic acid, was determined by titration against 0.1N NaOH using phenolphthalein as an end point indicator. The pH value was obtained by using a digital pH meter (ELICO LI612) after standardizing it with buffers of pH 4.0 and 9.0[15].

Glucose and fructose

The glucose and fructose content were determined in juice by phenol sulfuric acid method[13].

Total Sugars, Reducing Sugar and Non-reducing sugars

Total carbohydrate/sugars was estimated by standard procedure using phenol sulphuric acid[13]. The amount of reducing sugar of fresh juice was calculated by Nelson – Somogyi method[20] and non-reducing sugar was obtained by subtracting reducing sugars from total sugars.

Ascorbic acid (vitamin C)

Ascorbic acid contents of samples were determined according to the titration method using 2, 6-dichlorophenol indophenols[15].

Total phenolic content

The concentration of phenolic compounds was determined by the Folin-Ciocalteu colorimetric method[18] where 5g of sample was homogenized in 25 mL of 50% (v/v) ethanol/water solution. The sample (100 μL) was mixed with 5 mL of the 0.2N Folin-Ciocalteu reagent and 4 mL of 7.5% sodium carbonate. The mixture was kept for 2 h at room temperature in the dark before the absorbance was measured at 765 nm spectrophotometrically. The total phenolic content was expressed as mg gallic acid equivalents (mg GAE/100 mL).

Tannin content

Total tannin content of sample was measured by Folin Denis method[16] which is based on the measurement of blue color formed by the reduction of phosphotungstomolybdic acid by tannin like compounds in alkaline solution.

Color

The colour of juice was determined by a Color HunterLab L*a*b* (Hunterlab ColorFlex EZ)[21]. The equipment was calibrated using a white and black standard ceramic tile. The color is expressed as L*, a*, b*, c*, and h* color values. L* defines lightness, a* and b* define red-greenness and blue-yellowness, respectively, and c* defines saturation whereas hue angle (h*) is the attribute according to which colours have been traditionally defined as reddish, greenish, etc.

Consistency (Viscosity)

Viscosity was determined using the Brookfield viscometer DV-E at constant speed of 100 rpm and varying temperature with a spindle no S-62 [21]. Viscosity was expressed in terms of centipoises (cP). Parameters used for viscosity measurement of pomegranate juice were as follows:

- Shear rate: 10
- Speed: 100 rpm
- Temperature: 20, 25, 30, 35 and 40^{o}C

Microbial analysis of probiotic pomegranate beverage

The viable count of mixed culture was determined by the standard plate count method using Man-Rogosa-Sharpe agar (MRS agar) and the results were expressed as CFU ml^{-1} juice. The yeast and mold count of beverage was determined using potato dextrose agar medium. The coli-form and basically *E. coli* are the indicator microbes of water contamination by feces. The coli-form gives red pink color colonies on the MacConkey agar. Plates were incubated at 37^{o}C for 48-72 hours[3].

RESULTS AND DISCUSSION

Physico-chemical characteristics of probiotic pomegranate beverage

The probiotic pomegranate beverage was used to analyze its chemical characteristics because the nutrients present in beverage would be a source of food for probiotic organisms to maintain their viability in product besides quality of end product. The chemical properties of the prepared product are presented in Table 1.

Table 1 Chemical characteristics of probiotic pomegranate beverage

S. No.	Paramter(s)	Observations
1	Total Soluble Solids (°Brix)	13.0
2	Acidity (% of lactic acid)	0.540
3	pH	3.512
4	Glucose (g/L)	60.35
5	Fructose (g/L)	62.82
6	Total sugars (%)	13.14
7	Reducing sugars (%)	12.32
8	Non reducing sugars (%)	0.82
9	Ascorbic Acid (mg/100mL)	9.53
10	Total phenolic content (mg/100mL)	206.64
11	Tannin (%)	0.114

-Each value is an average of three determinations

From the Table 1, it is revealed that the TSS of probiotic pomegranate beverage was found to 13 °Bx as it was kept fixed during the preparation of beverage. The titratable acidity is a measure of shelf life of the product and guard against the attack of micro-organisms. It also helps to ensure some chemical changes during preparation[19] and storage[8]. The titratable acidity was 0.540 percent expressed in terms of lactic acid, produced during the metabolic activity of probiotic organisms. The pH is inversely proportional to the acidity of any medium. The pH value observed was 3.512.

The glucose was used as a carbon source by bacteria so, it had influencing role on growth and viability of bacteria. The high concentration of glucose and fructose were found appropriate for probiotic viability[4]. The value of main sugars glucose and fructose were 60.35 and 62.82 g/L, respectively. These values are almost in line with those followed by Mousavi *et al.*[11] for the preparation of probiotic pomegranate drink.

The probiotic pomegranate beverage contained 13.14, 12.32 and 0.82 percent of the total sugars, reducing sugars and non reducing sugars, respectively. Ascorbic acid, total phenols and tannin content which contribute to antioxidant property of pomegranate juice were found to 9.53 mg/100mg, 206.64 mg/100mL and 0.114 percent, respectively.

Color characteristics of probiotic pomegranate beverage

In the present work, fresh pomegranate juice was used as substrate. This juice was very clear and didn't contain any suspend solid. Its color was red. After fermentation, the prepared pomegranate beverage turned to pinkish/bright red color. The values of color parameters (L*, a*, b*, c* and h*) for the product are depicted in Table 2.

Table 2 Color characteristics of probiotic pomegranate beverage

Parameters	L* value	a* value	b* value	c* value	h* value
Observations	24.39	15.14	2.57	19.50	31.13

-Each value is an average of three determinations

l*=luminosity;+a=red; −a=green; +b=yellow; −b=blue, c* =Chroma; h* =Hue

From the Table 2, it is found that the luminosity of final beverage was observed to 24.39, whereas a* value and b* value were 15.14 and 2.57, respectively. On the other hand, c* value and h* value of the product were observed as 19.50 and 31.13, respectively. Thus based on the color values, the probiotic pomegranate beverage was found be red in color (Fig. 1).

Fig. 1: Probiotic pomegranate beverage

Viscosity characteristics of probiotic pomegranate beverage

The viscosity is an important characteristic to determine in food industry because it is related to the appearance and density of product. In the present investigation, the effect of temperature on the viscosity of probiotic pomegranate beverage was estimated. The data related to viscosity change is tabulated in Table 3.

Table 3 Effect of temperature on viscosity characteristics of probiotic pomegranate beverage

S. No.	Temperature (^{o}C)	Viscosity (cP)
1	20	7.9
2	25	7.1
3	30	6.2
4	35	5.0
5	40	3.9
	F-value	481.31
	SE±	0.073
	CD	0.2297

- Each value is an average of three determinations

As shown in the above table, the viscosity changed significantly with the increase in temperature. At temperature 20^{o}C, the viscosity of beverage was significantly higher (7.9 cP) than that observed at 40^{o}C temperature (3.9 cP). The viscosity observed at 25, 30 and 35^{o}C were found be 7.1, 6.2 and 5 cP showing a declining trend as the temperature increased. The reason for viscosity reduction was that the heat causes the molecules to speed up as they bump and move around each other. Hence, more temperature means more movement of molecules and thus reducing their resistance to flow. According to Magerramov *et al.*[10], the viscosity of tangerine and lemon juices monotonically decreases with the temperature.

Microbial analysis of probiotic pomegranate beverage

The growth of undesirable organisms will spoil the product and may lead to food borne diseases affecting the healthy lives. Therefore, performing microbial analysis is mandatory in probiotic based products to assess their safety. The data related to microbiological analysis of probiotic beverage is tabulated in Table 4.

Table 4 Microbial analysis of probiotic pomegranate beverage

S. No.	Parameters	Observations
1	**Total plate count** (CFU/mL)	6.5 x10^9
2	**Yeast and mold count** (CFU/mL)	ND
3	**Coli-form count** (MPN/mL)	ND

-Each value is an average of three determinations
ND: not detected

In the present work, the count of beneficial bacteria was detected as 6.5 x10^9 CFU/mL of beverage. This count was in range for a product to be called as probiotic[17].

On the other hand, the yeast and mold count and coli-form count was also determined. And they were not detected in the sample, which showed that the product was free of any pathogenic microbes and safe for consumption.

CONCLUSION

The pasteurized pomegranate juice was inoculated with probiotic cultures (10%) of *L. bulgaricus* and *L. plantarum* (1:1) and fermented for 7 h. Results showed that the chemical parameters were in sufficient amount for providing nutrition and bioactive components to consumers. Red color was dominating in the prepared beverage. And viscosity was also increased negligibly on the addition of probiotics in the pomegranate juice. Microbiological analysis found that the beverage contained the desired level of probiotic cultures (10^9CFU/mL) which is helpful for maintaining the health of gastro intestinal tract. Further, the prepared beverage didn't contain any traces of yeasts and molds and also coli-form bacteria, thus indicating that beverage is containing only health benefitting bacteria.

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