

Development of probiotic carrot-based beverage from *Lactobacillus acidophilus* and *Saccharomyces boulardii*

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Abstract

The carrot juice with 18⁰Bx inoculated using 10% mixed culture of *L. acidophilus* and *S. boulardii* (1:1) and fermented for 20 hours was standardized for development of probiotic carrot beverage. Various physico-chemical attributes of the beverage like TSS, titratable acidity and pH. The color was found to orange red which was most acceptable for carrot based beverage. Further, the microbiological analysis showed that prepared beverage contained optimum level of cultures i.e. 2.4×10^9 CFU/mL and yeast and mold 5.2×10^9 CFU/mL was free from any traces coli-form bacteria. Hence, it can be concluded that the carrot juice has the potential as a carrier of probiotic culture for the preparation of carrot probiotic beverage.

Keywords: probiotic, carrot juice, *L. acidophilus*, *S. boulardii* and beverage

Introduction

Carrot (*Daucus carota* L.) is the most important crop of Apiaceae family. It is a root vegetable that has worldwide distribution. Carrots were first used for medical purposes and gradually used as food. Written records in Europe indicated that carrots were cultivated prior to the tenth century. The colors of the carrot root flesh may be white, yellow, orange, red, purple, or very dark purple. Orange carrots, today more popular, were developed in the 15th and 16th centuries in Central Europe. A rapid rise in the popularity of orange carrots was observed with the recognition of its high pro vitamin A content. Carotenoids and anthocyanins are the major antioxidant pigments found in carrots. Carotenoids are the yellow, orange, or red colored phytochemicals found in most yellow and orange fleshed cultivars. The widely used orange carrot is high in α - and β -carotene and is a rich source of pro vitamin A. Yellow carrot color is due to lutein which plays an important role in prevention of macular degeneration. The red water-soluble anthocyanin pigment and the red water insoluble lycopene pigment present in the roots of some cultivars do not contribute to the pro vitamin A content.

Carrots have also a unique combination of three flavonoids: kaempferol, quercetin and luteolin. They are also rich in other phenols, including chlorogenic, caffeic and p-hydroxybenzoic acids along with numerous cinnamic acid derivatives. Among hydroxycinnamic acid and its derivatives, chlorogenic acid represents 42.2% to 61.8% of total phenolic compounds detected in different carrot tissues. Bioactive polyacetylenes, such as falcarinol (synonymous with panaxynol), and falcarindiol are found in carrots. The concentration of falcarinol in fresh carrots depends on carrot tissue cultivar and water stress. Falcarinol is the most bioactive phytochemical of the carrot polyacetylenes. It is

though that this compound may stimulate cancer-fighting mechanisms in the human body. The mode of action behind the favorable effect of falcarinol may be due to its hydrophobicity and its ability to form an extremely stable carbocation with the loss of water thereby acting as a very reactive alkylating agent toward proteins and other biomolecules. A bitter coumarin compound is formed when carrots are stored.

Among 39 fruits and vegetables carrots have been ranked 10th in nutritional value. Carrot is a good source of dietary fiber and of the trace mineral molybdenum, rarely found in many vegetables. Molybdenum aids in metabolism of fats and carbohydrates and is important for absorption of iron. It is also a good source of magnesium and manganese. Magnesium is needed for bone, protein, making new cells, activating B vitamins, relaxing nerves and muscles, clotting blood, and in energy production. Insulin secretion and function also require magnesium. Potassium and magnesium in carrots help in functioning of muscle (Joao *et al.*, 2014) [4].

Carrot juice contains carbohydrates, dietary fiber, protein, fat, Vitamins A, C, B1, B2, B3, B6 and E. It also contains traditional antioxidants such as ascorbic acid, phytonutrient and beta-carotene (Gopalan *et al.*, 1996) [3].

Probiotic is a relatively new word meaning “for life” and it is generally used to name the bacteria associated with beneficial effects for humans. Probiotics are defined as live microbial feed such as *Lactobacillus plantarum*, *L. casei*, *L. acidophilus*, and *Streptococcus lactis* which are supplemented by food that beneficially affect the host by improving its intestinal balance. Several studies have shown that supplementation of probiotics to food provides several health benefits such as reduction of serum cholesterol, improved gastrointestinal function, enhanced immune

system, and lower risk of colon cancer. This review provides an overview on the current research prospects of LA fermentation of fruits and vegetables with regard to human nutrition and health.

Non-dairy probiotic products have a big worldwide importance due to the ongoing trend of vegetarianism and to a high prevalence of lactose intolerance in many populations around the world (Cargill 2009) [1]. Regular consumption of high levels of probiotic bacteria is required to confer health benefits. It is important that the organisms remain viable in the food product until the time of consumption and be present in significant numbers (at least 10⁷ CFU/mL) in order to confer benefits to the consumer.

Vegetables and fruits have been showed as appropriate for probiotic products as they do not contain any dairy allergens that might prevent usage by part of the population. Also they have several functional food components such as minerals, vitamins, dietary fibers, and antioxidants. In recent years, studies on non-dairy probiotic beverages such as tomato, cabbage, blackcurrant, orange, beet root and carrot juices have been performed in conjunction with different probiotic strains and obtained appealing results (Naga *et al.*, 2014) [6].

Materials and Methods

Preparation of Carrot Juice

Freshly harvested carrot fruits were procured from local market of Parbhani (Maharashtra). Carrot juice was prepared by blanching of carrot at 60°C for 20 min. after filtration through muslin and to adjust TSS to 18°Bx and stored at 4°C before use.

Probiotic strains

Probiotic isolates, *Lactobacillus acidophilus* and *Saccharomyces boulardii* were identified using phenotypic and genotypic methods in Department of Food and Industrial Microbiology, College of Food Technology, VNMKV, Parbhani. Stock solution was prepared by adding sterile glycerol (50% v/v) to the activated culture. The glycerol stock culture was stored at -20 °C in sterile screw cap tubes.

Preparation of starter culture

The starter culture was prepared with the help of method described by Thakur M and Sharma (2017), with slight modifications. *L. acidophilus* and *S. boulardii* was cultivated separately in the MRS broth and Potato Dextrose Broth for 24-h at 37°C. To obtain the biomass, 10 mL of the separately cultivated MRS broths (5ml) and Potato dextrose broth (5ml) 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 and Potato dextrose media. Thus, inoculum was prepared.

It was then introduced into pasteurized carrot juice (100 mL) for making it 10% concentration of probiotics. The inoculated juice was then incubated at 37°C for 24 h and

was treated as starter culture for preparation of final beverage.

Preparation of probiotic beverage

Above prepared starter culture (10mL) was then added to the pasteurized carrot juice (100 mL) to obtain 10% inoculation. It was allowed to ferment in incubator at 37°C for 20 h. After incubation, the beverage was kept at refrigeration temperature for future use.

Sensory analysis of Probiotic Beverage

The sensory evaluation of carrot and tomato based probiotic beverage was carried out by 10 semi-trained panel members comprised of postgraduate students and academic staff members of the faculty who had some previous experience in sensory evaluation. The panel members were requested in measuring the terms identifying sensory characteristics and in use of the score. Judgment were made through rating products on a 9 points Hedonic Scale with corresponding descriptive terms ranging from 9 “like extremely” to 1 “dislike extremely” with respect to different quality attributes such as colour, flavour, taste, aroma, mouthfeel and overall acceptability, as per the method recommended by Ranganna (1986) [7].

Physico-chemical analysis of probiotic beverage

Following physico-chemical properties of fresh carrot and tomato 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 9.015 (Ranganna S.1991) [8].

Microbial analysis of probiotic 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 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°C for 48-72 hours (Chris *et al.*, 2006) [2].

Results and Discussion

Sensory evaluation of Probiotic Beverage

The probiotic beverage were used to sensory analyzed because the overall acceptability of the developed probiotic beverage is to be checked by different sensory evaluation panel and find out which is more delicious and tasty out of two prepared samples.

Table 1: Organoleptic Characteristics of Probiotic Beverage

Sample	Color	Taste	Appearance	Flavor	Consistency	Overall acceptability
A	9	9	9	8	8	8
B	8	7	8	7	7	7

From table 1, it is observed that the sample A having 18⁰ Bx TSS before fermentation got more scores in color, Taste, appearance, flavor, consistency as well as overall acceptability that are 9 where as sample B having 19⁰ Bx TSS before fermentation got less score. So, from this sensory evaluation data of probiotic beverage it is showed that the sample A carrot probiotic juice was more acceptable.

Physico-chemical characteristics of probiotic beverage

The probiotic 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 2.

Table 2: Physico-chemical characteristics of probiotic beverage

Parameter(s)	Observations
Color	Orange-Red
T.S.S	16.10 ⁰ Bx
PH	3.685
Acidity	0.45 %

From the Table 2, it is revealed that the TSS of probiotic beverage was found to 16.10⁰ Bx as it was changed during the fermentation 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 (Swientek B. 1998) [10] and storage (Langthasa S. 1999) [5].

The titratable acidity was 0.45 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 3.685.

Microbial analysis of probiotic carrot and tomato 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 5.

Table 3: Microbial analysis of probiotic beverage

S. No.	Parameters	Observations
1	Total Plate Count (TPC)	2.4 x 10 ⁹
2	Yeast and Molds	5.2 x 10 ⁹
3	Coli form Count	ND

In the present work, the count of beneficial bacteria was detected as 2.4 x 10⁹ CFU/mL and 5.2 x 10⁹ CFU/mL of yeast and mold beverage. This count was in range for a product to be called as probiotic (shah 2001).

On the other hand, 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 present investigation focuses on the development of probiotic beverage which imposes potential health benefits. It was concluded that prepared probiotic carrot based

beverage having good organoleptic quality and high nutritional value. The shelf life study of probiotic beverage in glass bottle was carried out by keeping the product at refrigerated temperature for four weeks storage period. It is found that the viability of cultures decreased during storage, but the count was within the limits (10⁷-10⁹).

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