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Formulation of Probiotic Drink for Prevention of Health Complications and Health Promotion

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Abstract: The aim of this research was to provide a non-dairy probiotic drink to attend people that cannot eat dairy products due to lactose intolerance, dietary preferences such as vegetarians, or certain other health issues. While looking for an alternative carrier for probiotics, the suitability of carrot juice and beetroot juice for the production of probiotic food with *L. acidophillus*, *L. casei* and *Bifidum longum* was investigated. Proximate composition of probiotic juice revealed 26% increase in protein content and reduction of 17% in level of carbohydrates as compared to that in the fresh carrot and beetroot juice. The CCRD, RSM and statistical analysis revealed the optimum pH and optimum fermentation temperature for probiotic carrot and beet root juice production, 6.5 and 37°C, respectively. During the study of growth kinetics and physicochemical properties, gradual change in pH (-28%), acidity (+67%), and sugar concentration (-17%) was observed indicating the significant growth of probiotics and production of lactic acid by them. Besides, sensory analysis of fresh and probiotic carrot beetroot juice revealed 20% elevation in the overall acceptability of probiotic carrot beetroot juice over fresh juice. The data obtained from this study provide new insights into projection of fermented carrot beetroot juice as an appropriate media for the growth of probiotics.

Keywords: Carrot juice; Beetroot juice; Probiotic; Acid tolerance; Bile tolerance; Optimization

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1. Introduction

In modern era, with the demand of health improving foods, there is an enhancement in the state of well-being, and thus lowering the risk of diseases (Nasari *et al.* 2014; Te Morenga *et al.* 2014; Hooper *et al.* 2015). This is principally because of the rapid increase in the cost of health care, the desire of elderly people to live improved quality of life and the steady increase in the life expectancy. In this context, the contribution of functional foods like probiotics, prebiotics and symbiotics has received much attention and can be developed as key pillars of the health care system (Pandey *et al.* 2015; Pathan *et al.* 2017; Federik, 2019). These functional foods, along with having the traditional nutrients, have some additional beneficial effects such as prevention and/or reduction of nutrition-related diseases, improving health status and promoting the state of mental and physical well-being. The health professionals are increasingly promoting the food products with added live microbes (probiotics) due to their beneficial effects on human health (Kechagia *et al.* 2013; Peivasteh-Roudsari *et al.* 2019). In the developed countries, with an increase in the consumer vegetarianism, has led in increased demand for the vegetarian probiotic products. Alternative raw materials for probiotics need to be searched, due to the health considerations, from the perspective of cholesterol in dairy products for developed countries and economic reasons for the developing countries. The potential substances like legumes, cereals, fruits and vegetables can be used for the production of re- healthy probiotic drink. This can turn out to be an effective non-dairy probiotic beverage likely to serve as a healthy alternative to the dairy probiotics and also favorable for the lactose intolerant consumers. Probiotics represent the group of functional foods, defined as live microbial feed that provides an intestinal health benefit to the host (Kechagia *et al.* 2013; Peivasteh-Roudsari *et al.* 2019). A number of *in vitro* and *in vivo* studies proves the benefits of probiotic foods for humans by maintaining or improving their intestinal microflora. These micro-organisms have numerous health promoting effects such as improvement of lactose metabolism, prevention of intestinal tract infections and enhancing immunity, reduction of serum cholesterol level, stimulation of calcium absorption, synthesis of vitamins (vitamin B, nicotinic acid, and folic acid), improvement of protein digestibility and counteracting the effects of food-borne pathogens.

Fruits and vegetables have been suggested as suitable media for cultivation of probiotics because they inherently contain essential nutrients; high amount of vitamins, mineral and polyphenolic compounds, free from allergens and easily available with attractive appearance and taste (Mishra *et al.* 2010; Rafiq *et al.* 2016; Bao *et al.* 2019). Carrot (*Daucus carota L.*), one of the more commonly used vegetables of human nutrition, was chosen as a vehicle for being rich in β-carotene, ascorbic acid, tocopherol and classified as vitaminized food. Carrots are good source of carbohydrate, calcium, phosphorous, iron, potassium, magnesium, copper, manganese and sulphur, but lack in protein and fat (Bao *et al.* 2019; Bjarnadottir, 2019). An increased intake of carrot may favour the massive synthesis of vitamin A as it has been reported that 100 g of carrot contains carotenoids 6-15 mg, mainly β-carotene (2-10 mg) (Singh *et al.* 2013). The presence of these carotenoids and other antioxidants may protect humans against certain types of cancer and cardiovascular diseases and may enhance the activity of immune system, protect against stroke, high blood pressure, osteoporosis, cataracts, arthritis, heart disease, bronchial asthma and urinary tract infections (Singh *et al.* 2013). Additionally, the allergenic effect of carrot is very low or lacking and fermentation makes it more suitable by removal of anti-nutritional factors present if any. Thus, carrot and/or beetroot juice(s), may be consumed by human who can't take dairy products (Rafiq *et al.* 2016), although lacking individual or overall acceptability. Besides, Beetroot juice has been considered to contain a huge range of essential vitamins and minerals, antioxidants. Antioxidants reduce oxidative stress, linked to the development of cancer, inflammatory conditions, and heart disease (Mishra *et al.* 2017; Mishra, 2018). Beetroots are a rich source of essential vitamins and minerals, including: folate, which is important for DNA and cell health (Mishra *et al.* 2009; Sharma *et al.* 2009); vitamin B-6, which supports metabolism and red blood cell production; calcium, an essential mineral for bone growth and strength; iron, which allows red blood cells to carry oxygen; magnesium, a mineral that supports immune, heart, muscle, and nerve health; manganese, which contributes to the regulation of metabolism and blood sugar levels; phosphorous, an essential nutrient for teeth, bones, and cell repair; copper, which plays a role in making collagen, maintaining bones and blood vessels, and supporting immune function; zinc, which promotes wound healing, supports the immune system, and encourages normal growth. Therefore, the present study was undertaken to determine the suitability of a carrot and beetroot juice as a raw material for production of probiotic vegetable juice by probiotic lactic acid bacteria.

2. Materials and Methods

Raw materials (Carrot, Beetroot, lemon, ginger, rock salt, black pepper, black salt, ajowan caraway, cumin seed) were procured from Nishatganj vegetable market, Lucknow, UP, India, for juice extraction by using mixer grinder (Brand Philips; Wattage 750 W Type; Model # HL 7707/00). The extracted juice was filtered and pasteurized at 80°C for 10 minutes. The three strains of lactic acid bacteria (*Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidium longum*) were obtained from Microbiology Lab, R-FRAC, Lucknow. The pasteurized juice was inoculated at 40°C by these cultures at 1% (v/v). Culture strains were first analyzed for bile and acid tolerance according to protocol described by Hassanzadazar *et al.* (2012). Fresh carrot and juice and probiotic carrot and beetroot juice were analyzed for moisture, ash, pH, titrable acidity, carbohydrate, energy value and protein content by AOAC (Rafiq *et al.* 2016). Different treatments were made appropriately and ingredients' percentage has been shown in Table 1.

2.1. Preparation of Inoculums

Inoculums were prepared by transferring a glycerol stock culture tube of *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidium longumto* a 250 ml flask containing 100 ml MRS broth. The incubator was used for cultivation of cells at 37°C until the cell density corresponding to 9.00 Log CFU/mL with respect to OD 0.600 recorded spectrophotometrically at 590 nm using the scale developed by MacFarland according to protocol described by Rafiq *et al.* (2016).

2.2. Optimization of Probiotic Carrot and Beetroot Juice Production

The optimum fermentation conditions were determined using central composite rotated experimental design (CCRD), where the initial pH and temperature were changed from 4 to 7 and 10-45°C, respectively. The experimental domain was selected since the *Lactobacillus* can grow in such pH and temperature conditions. Acetic acid (0.1 N) was added to Erlenmeyer's flasks containing 100 ml of clarified carrot and beetroot juice to reach the Initial pH values of experimental design. The acetic acid treated clarified carrot juice was inoculated with pre-determined concentration recommended for probiotic foods (Nguyen *et al.* 2016). Fermentations were carried out statically in an incubator set for 72 hrs at different temperatures of experimental design. Response surface methodology (RSM) was applied to the response variables (biomass and cell viability) followed by process optimization through an experimental design changing initial pH and fermentation temperature.

2.3. Growth and Productivity of Probiotic Strains

Using the optimized factor values (i.e., temp 37°C and initial pH 6.5), culture were grown in pasteurized carrot and beetroot juice (400:300 mL in 1 L Erlenmeyer flasks) by inoculating with the probiotic strains (10%, v/v) at 37°C for 72 hours. Samples for estimating the growth and viable cells count (Panghal *et al.* 2017) were taken at the interval of every two hours. Growth was estimated by utilization of sugar content, change in pH and titratable acidity. Change in pH was monitored every 2 hrs. Titratable acidity was evaluated by doing titration against 0.1 N NaOH. Change in sugar content was determined by DNS (dinitrosalicylic acid) method by reading absorbance at 540 nm (Jain *et al.* 2020).

2.4. *Sensory Quality Evaluation*

The different treatments of carrot and beetroot juices were inoculated with *Lactobacillus* and fermented at 37°C for 72 hours in an incubator. The sensory evaluation was conducted by the panel of trained judges and the score were given according to the Hedonic Rating Test Scale. Sensory evaluation of the probiotic drink was made by a 9-number sensory panel. The panel members were requested to evaluate the probiotic drink on the bases of its appearance, aroma, consistency, taste, mouthfeel, number of viable cells and overall acceptance.

3. Statistical Analysis

The results for physico-chemical, microbiological and sensory attributes of carrot and beetroot probiotic drinks were analysed with the help of Graph Pad Prism (La Jolla, CA, USA) (version 5.01) software. 2-way ANOVA was conducted for statistical significance for mean differences. The significance level was set at 5 % ($P<0.05$) for all calculations.

4. Results and Discussion

Based on preliminary experiments resulted in Table 1, further study was undertaken with combination T2. Three species of lactic acid bacteria (*Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidium longum*) were found to be capable of growing well on pasteurized carrot and beetroot juice without any specific nutrient requirement. Viability of three strains on different pH level was observed (Table 2) and it was concluded that at pH 6.5, microbial count was more than 300 for all three strains. The CCRD and RSM analysis revealed the optimum pH and optimum fermentation temperature for probiotic carrot and beet root juice production, 6.5 and 37°C, respectively. So, pH 6.5 was selected for further product development at 37°C.

4.1. *Proximate Analysis of Fresh and Probiotic Juice*

As shown in Table 3, additions of probiotic in carrot and beetroot juice result in small reduction in proximate composition of carrot and beetroot juice. Moisture content (70.20) in fresh juice of carrot and beetroot was slightly higher than the carrot and beetroot probiotic drink (70.10). Fresh blended carrot and beetroot juice and probiotic drink were evaluated for proximate composition (Table 3) and antioxidant activity. There was an augmentation in protein per cent value in probiotic drink 4.74±0.008 as compared to that in fresh juice 3.77±0.009, probably might be due to presence of probiotic microorganisms and their metabolites (Fenster *et al.* 2019). Acidity of sample was increased from 0.45±0.02 to 0.75±0.01 (Table 3). There was increase in antioxidant activity, total phenols and flavonoid content was observed. The

Table 1: Different treatments and ingredients composition

Code	Control	T1	T2	T3	T4
Carrot	45 mL	50 mL	60 mL	70 mL	80 mL
Beetroot	45 mL	40 mL	30 mL	20 mL	10 mL
Lemon	5 mL	5 mL	5 mL	5 mL	5 mL
Ginger	5 mL	5 mL	5 mL	5 mL	5 mL
Salt mix	4 g	4 g	4 g	4 g	4 g

Table 2: Viability of *Lactobacillus* strain at variable pH level after 24 hrs.

S.No.	*pH*	*Lactobacillus acidophilus*	*Lactobacillus casei*	*Bifidium longum*
1	4.0	120	100	80
2	5.0	150	120	150
3	6.0	220	180	200
4	6.5	>300	>300	>300
5	7.0	<250	<220	<200

All values are Mean ± SD of three sets of experiments with triplicates in each set.

Table 3: Nutritional composition of fresh and probiotic drink.

Treatments	*pH*	*Acidity (%)*	*Carbohydrate/ Sugar (%)*	*Protein (%)*	*Antioxidant activity (%)*	*Flavonoid content (%)*	*Total Phenol (%)*
Fresh	6.5±0.03	0.45±0.02	9.95±0.07	3.77±0.009	78.8±0.05	09.12±0.02	3.85±0.01
Probiotic	4.7±0.03	0.75±0.01	8.25±0.07	4.74±0.008	82.8±0.05	11.30±0.04	4.25±0.01

All values are Mean ± SD of three sets of experiments with triplicates in each set.

Table 4: Sensory analysis of fresh and probiotic juice

Treatments	*Appearance*	*Aroma*	*Consistency*	*Taste*	*Mouth feel*	*Number of viable cells (CFU/mL)*	*Overall acceptability*
Fresh	7.0±0.34	7.0±0.28	7.5±0.36	7.0±0.42	7.0±0.39	1.2×10^{8}	7.0±0.42
Probiotic	8.5±0.31	8.5±0.34	8.0±0.32	8.8±0.42	8.7±0.32	2.8×10^{9}	8.5±0.44

All values are Mean ± SD of three sets of experiments with triplicates in each set.

increase in total antioxidants occurred due to fermentation leading to increase in phenolic and flavonoids compounds due to microbial hydrolysis reaction (Verni *et al.* 2019). Fermentation also results in structural disintegration of cell walls leading to either liberation or synthesis of different antioxidant compounds. As antioxidants possess free radical scavenging capacity and health promoting benefits, so the probiotic drink is highly valuable. Recently, similar results have been reported by Yang *et al.* (2020) providing authenticity of data obtained from the present study. The ash content in probiotic carrot juice was highest (0.40) than fresh one (0.35). The energy content determined by difference method was highest in fresh carrot juice than the probiotic juice which might probably be due to the higher content of fat in fresh carrot and beetroot juice (Panghal *et al.* 2017).

4.2. Sensory Analysis

The probiotic drink was less accepted (7.0±0.42) as compared to the fresh one (8.5±0.44), although the benefit of probiotic cultures in beetroot juice has been reported with value addition in terms of health aspects (Panghal *et al.* 2017). The highest mean appearance, aroma, consistency, taste, mouth feel was observed in fresh beetroot juice than the probiotic one (Table 4). Taken together previous study (Ray *et al.* 2016) and data obtained from the present study it is depicted that microbial fermentation may be used as suitable technology in formulation of health promoting foods.

5. Conclusions

Probiotic carrot and beetroot mixed probiotic drink appears to fulfill all requirement of FSSAI regulations and guidelines (2017) as well as NIH and FDA related dietary supplement label database, and policy regarding quantitative labeling of dietary supplements containing live microbials, respectively (2018) from the point of view of health prevention and promotion, and thus may be recommended to be a good alternative to serve lactose intolerant and individuals unable to consume probiotic dairy products for certain allergic reactions. Data obtained from present study also provide new insights into projection of fermented carrot and beetroot juice as an appropriate media for the growth of probiotics, and more comprehensive research to be conducted regarding sensory improvement, storage stability and commercialization of probiotic drink with specific doses in view of prevention and/or treatment of six major health conditions viz., atopic dermatitis, pediatric acute infectious diarrhea, antibiotic-associated diarrhea, irritable bowel syndrome, hypercholesterolemia and obesity. Further studies along these notions and objectives are in progress.

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