

Development of Probiotic Carrot Juice

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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 other health issues. While looking for alternative carrier for probiotics, the suitability of carrot juice for the production of probiotic food with *L. acidophilus*, *L. plantarium*, *L. casei* and *Bifidum longum* was investigated. Proximate composition of probiotic juice showed increase in level of protein content and reduction in level of carbohydrates than the fresh carrot juice. Response surface methodology (RSM) was employed to analyze the effect of independent variables (temperature and pH) on response variables (biomass and cell viability). The statistical analysis revealed that the optimum pH for probiotic carrot juice production was 6 and optimum fermentation temperature 30°C. During the study of growth kinetics, gradual change in pH, acidity and sugar concentration was observed which indicates the growth of probiotics and production of lactic acid by them. The results suggest that fermented carrot juice can serve as a suitable media for the growth of probiotics.

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

Introduction

Nowadays the concept of nutrition has changed from food providing energy for our body to diets that deliver physiological benefits in management and prevention of diseases. Guarantee of well-being for the consumers demands balanced nutritional intake for the metabolism of the human body to prevent deficiency or excess of certain components, and the idea of functional food was preceded from the above-mentioned conception. Historically, high intake of sugars, salt, saturated and trans-fatty acids, and low intake of fibers, vitamins, and essential minerals affect the nutritional state of populations. These habits are the main causing problems of non-transmissible chronic-degenerative diseases. Hence, to reduce the risk of such illness, the development of new food products that contain biologically active substances has been proposed [1] and probiotification of foods is one of the methods used to produce such new functional foods. Probiotics represent the group of functional foods, which are defined as live microbial feed that provides an intestinal health benefit to the host. A number of *in vitro* and *in vivo* studies are available which proves the benefits of probiotic foods for humans by maintaining or improving their intestinal microflora [2]. These micro-organisms have numerous health promoting effects such as improves lactose metabolism [3], prevents intestinal tract infections and enhance immunity [4], reduces serum cholesterol level [5] stimulates calcium absorption, synthesis of vitamins (vitamin B, nicotinic acid, and folic acid) [6], improves protein digestibility and counteracts the effects of food-borne pathogens [7]. Probiotic products have to be made available for consumers who suffer from food related

disorders like lactose intolerance, so they do not have to abandon benefits of probiotics. This need has led to development of probiotic products from various food matrices including fruits and vegetables. 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 [8].

Carrot (*Daucus carota L.*) one of the more commonly used vegetables of human nutrition was chosen as a vehicle as it is rich in beta 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 [9]. 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). The presence of these carotenoids and other antioxidants may protect humans against certain types of cancer and cardiovascular diseases and may enhance the immune system, protect against stroke, high blood pressure, Osteoporosis, cataracts, arthritis, heart disease, bronchial asthma and urinary tract infections [9]. 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 may be consumed by human who can't take dairy products [10]. Thus, the purpose of the present study was to determine the suitability of a carrot juice as a raw material for production of probiotic vegetable juice by probiotic lactic acid bacteria.

Materials and Method

Raw materials (carrot) were procured from Azadpur vegetable market, New Delhi, India, for juice extraction by using mixer grinder. The extracted juice was filtered and pasteurized at 80°C for 10 minutes. The four strains of lactic acid bacteria (*Lactobacillus acidophilus* NRRL-B-4495, *Lactobacillus plantarum*-C3, *Lactobacillus casei*-B-442 and *Bifidum longum*-35624) were obtained from Space Lab Pvt. Ltd., New Delhi (India). 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 [11]. Fresh carrot juice and probiotic carrot juice were analyzed for moisture, ash, TSS, titrable acidity, carbohydrate, energy value and protein content by AOAC [12].

Inoculums preparation

Inoculums were prepared by transferring a glycerol stock culture tube of *Lactobacillus acidophilus* NRRL-B-4495, *Lactobacillus plantarum*-C3, *Lactobacillus casei*-B-442 and *Bifidum longum*-35624 to a 250 ml flask containing 100 ml MRS broth. The incubator was used for cultivation of cells at 37°C until the cell density, which was recorded spectrophotometrically (590 nm), reached 0.600 that correspond to 9.00 Log CFU/mL, using the scale developed by MacFarland [13].

Optimization of probiotic carrot 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. HCL (0.1 N) was added to Erlenmeyer's flasks containing 100 ml of clarified carrot juice to reach the Initial pH values of experimental design. The HCL treated clarified carrot juice was inoculated with pre-determined concentration recommended for probiotic foods [14] i.e., 7.00 log CFU/ml (1 ml of MRS broth containing 9.00 CFU/ml of *L. acidophilus*, *L. plantarium*, *L. casei*, *Bifidum longum*). Fermentations were carried out statically in an incubator set for 24 h at different temperatures of experimental design. Response surface methodology (RSM) was applied to the response variables (biomass and cell viability) and process optimization was done through an experimental design changing initial pH and fermentation temperature.

Growth and productivity of probiotic strains

Using the optimized factor values (i.e., temp 30°C and initial pH 6), culture were grown in pasteurized carrot juice (700 mL in 1 L Erlenmeyer flasks) by inoculating with the probiotic strains at 10% (v/v) and incubating at optimum temp i.e., 30°C for 24 hours. Samples for estimating the growth and viable cells count [15] were taken every

two hour. Growth was estimated by utilization of sugar content, change in pH and titrable acidity. Change in pH was checked every 2 hrs by digital pH meter. Titrable acidity was evaluated by doing titration against 0.1 N NaOH. Change in sugar content was determined by DNS (dinitrosalicylic acid) method. DNS reagent was prepared by mixing the potassium sodium tartarate (30 gm in 50 ml water) and DNS (1 gm in 20 ml of 2 M NaOH) and volume up to 100 ml was made. After the preparation of DNS reagent, 1 ml of DNS reagent was taken and mixed with 3 ml of sample and the solution was kept in water bath at 100°C for 5 mins. Absorbance was taken at 540 nm. A standard solution (dextrose) of different dilutions (25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml) was also prepared to develop a standard curve.

Sensory quality evaluation

Sensory analysis was carried out by using semi-trained panelists drawn from faculty members and post graduate students of the Department based as described by Larmond [16] to measure sensory characteristics viz. color, texture, flavor and overall acceptability of juices. The panelists were asked to record their observations on the sensory sheet based on a 5 point hedonic scale (5 and 1 points showing like extremely and dislike extremely).

Results and Discussion

Proximate analysis of fresh and probiotic juice

As the Table 1 displayed additions of probiotic in carrot juice cause small reduction in proximate composition of carrot juice. Moisture content (90.10) in fresh juice of carrot was slightly higher than the fresh carrot juice one (90.05). The protein content in probiotic juice was highest (1.12) than the fresh one, which showed, that increased level of protein content might be due to activity of probiotic organisms and their metabolites. Fat and carbohydrates content in probiotic carrot juice were slightly lower than fresh one carrot juice. The ash content in probiotic carrot juice was highest (0.34) than fresh one (0.23). The energy content determined by difference method was highest in fresh carrot juice than the probiotic juice which was due to the higher content of fat in fresh carrot juice.

Sensory analysis of fresh and probiotic carrot juice

The Table 2 shows that probiotic had least acceptance than the fresh one, but the benefit of probiotic cultures in carrot juice adds value in terms of health aspects. The highest mean color (3.4) and texture (3.3) was observed in fresh carrot juice than the probiotic one (2.7). The mean flavor was highest in probiotic carrot juice (3.2) than fresh one carrot juice. These results indicated the suitable potential of microorganisms in development of flavors for future uses. The overall acceptability of both juices of carrot were least different.

Sample	Moisture (%)	Protein (%)	Ash (%)	Fat (%)	Carbohydrate (%)	Energy (kcal)
Fresh carrot juice	90.10 ± 6.45 ^a	0.76 ± 0.68 ^a	0.23 ± 0.12 ^a	0.95 ± 0.11 ^a	8.10 ± 0.78 ^a	46.28
Probiotic carrot juice	90.05 ± 6.20 ^a	1.12 ± 0.67 ^a	0.34 ± 0.13 ^a	0.79 ± 0.12 ^a	7.94 ± 0.68 ^b	40.83

^{a,b}Means in column with same superscript are not significantly different (p<0.05)

Table 1: Proximate analysis of fresh and probiotic carrot juice.

Sample	Color	Texture	Flavor	Overall acceptability
Fresh carrot juice	3.4 ± 0.73 ^a	3.3 ± 0.72 ^a	2.8.0 ± 0.70 ^a	3.0 ± 0.71 ^a
Probiotic carrot juice	2.7 ± 0.68 ^b	2.8 ± 0.69 ^a	3.2 ± 0.67 ^a	2.9 ± 0.67 ^a

^{a,b}Means in column with same superscript are not significantly different (p<0.05)

Table 2: Sensory analysis of fresh and probiotic carrot juices.

Growth kinetics

The four species of lactic acid bacteria were found capable of growing well on sterilized carrot juice without any nutrient supplementation. The change in pH, acidity and sugar content were monitored to determine the growth kinetics of mixed culture. For monitoring the fermentation process, the pasteurized carrot juice was inoculated with culture of mixed strains (*L. acidophilus*, *L. plantarium*, *L. casei*, *Bifidum longum*). The suitability of probiotic cultures was examined by assessing their acid and bile tolerance and the results indicated that they are resistant to such conditions as shown in Figure 1 and Table 3, respectively. To optimize the maximum specific growth rates of these probiotic strains during carrot juice fermentation, response surface methodology was used. The statistical analysis revealed that the optimum pH for probiotic carrot juice production was 6 and optimum fermentation temperature 30°C. The process variables investigated during fermentations were temperature and pH as indicated in Table 4. The kinetics of cell growth was evaluated in terms of sugar consumption, acid production and change of pH.

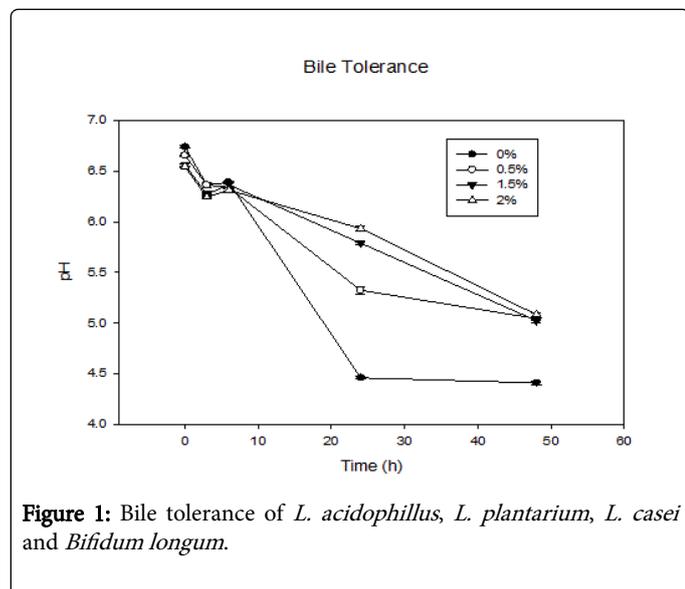


Figure 1: Bile tolerance of *L. acidophilus*, *L. plantarium*, *L. casei* and *Bifidum longum*.

As shown in Figure 2, it was observed that the culture pH was dropped based on the production of acid in culture accordingly. The initial pH of culture was adjusted to 6.08 and decreased gradually to 4.39 in 24 hrs. Similar results for pH decline were reported by Devi et al. [17], for whey based fruit beverages stored at refrigeration temp and Shukla et al. [18] in probiotic beverage from whey and pineapple juice. Similarly, a gradual decline in pH of soy milk and whey blended papaya RTS was reported by Kumar et al. [19].

The initial acidity of the carrot juice was 0.192 and within 24 hrs it reached to 0.536 as shown in Figure 3 which indicated the production of lactic acid. The sugar concentration was decreased from 41.6 to 38.6 as shown in Figure 4.

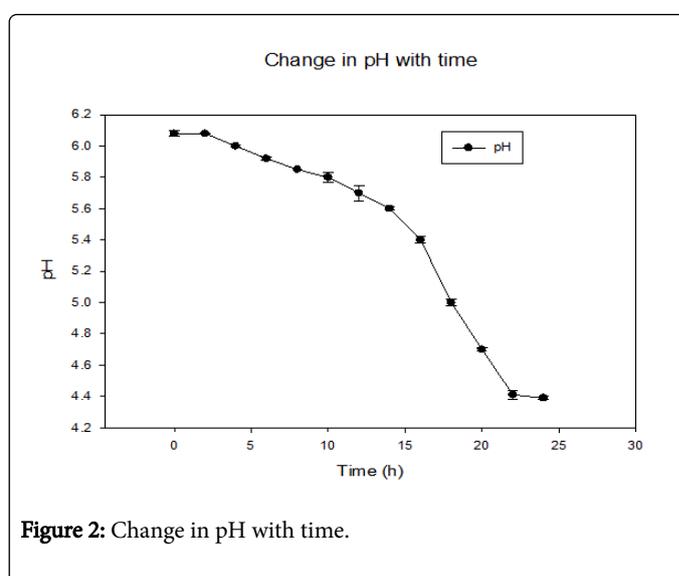


Figure 2: Change in pH with time.

Time (h)	Dilutions	Viable cell count
0	10 ⁻⁶	>300
	10 ⁻⁷	>300
1	10 ⁻⁶	>300
	10 ⁻⁷	>300
2	10 ⁻⁵	>300
	10 ⁻⁶	>300
5	10 ⁻⁵	296
	1	284
6	10 ⁻⁴	264
	10 ⁻⁵	256

Table 3: Evaluation of acid tolerance of *L. acidophilus*, *L. plantarium*, *L. casei* and *Bifidum longum*.

No.	Fermentation temperature (°C)	pH
1	27.5	5.5
2	39.87	6.6
3	15.13	6.6
4	27.5	4
5	39.87	4.4
6	27.5	5.5
7	27.5	5.5
8	45	5.5
9	15.13	4.4
10	10	5.5
11	27.5	7
12	27.5	5.5
13	27.5	5.5

Table 4: Fermentation parameters for optimization.

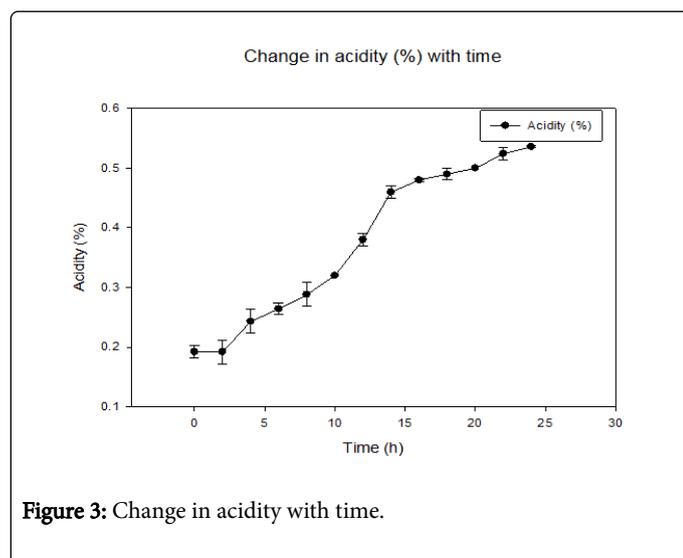


Figure 3: Change in acidity with time.

Conclusion

Mixed culture of *L. acidophilus*, *L. Plantarium*, *L. Casei* and *Bifidum longum* fermented well and survived in carrot juice at optimum conditions. The effect of probiotic strains on proximate composition of fresh carrot juice was found to be non-significant and the strains were capable of being viable in carrot juice without any nutrient supplementation. This result makes carrot juice a promising vehicle for probiotic bacteria and can act as an alternative to serve the persons who are unable to consume probiotic dairy products due to allergic reactions and lactose intolerance. Further research is required on improvement of taste, storage stability and packaging of such probiotic juice.

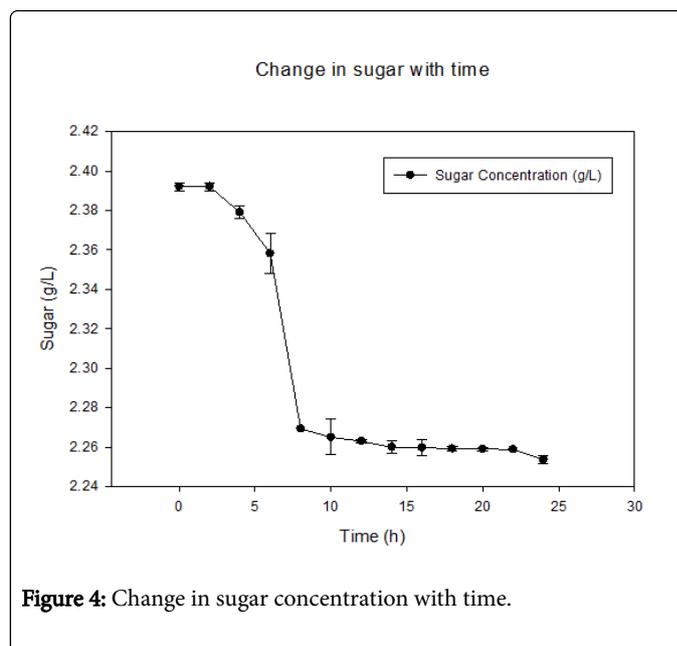


Figure 4: Change in sugar concentration with time.

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