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Research Article

Development of Probiotic RTS Beverage from Ber Fruit (Ziziphus Mauritiana)

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2. Key words

Probiotic; Ziziphus mauritiana; Lactobacillus acidophilus

1. Abstract

Investigation was performed to develop probiotic ready to drink beverage from ber fruit (*Ziziphus mauritiana*). Fruit syrup (concentrate) was prepared by adding sugar and citric acid to the extracted juice. Hot process was used to extract pulp and juice of ber fruit, followed by addition of sugar and citric acid to prepare syrup (concentrate). Prepared syrup samples were fermented by inoculating *L. acidophilus* culture at 1% (T2), 1.5% (T3) and 2% (T4) which were incubated at 37^{0}C for 48 hours. Finally, the concentrate samples pre sterilized PET bottles at the rate of 80 ml and chilled carbonated water was added and sealed. The prepared drink kept under refrigeration (4 ^{0}C) for a period of 45 days no artificial preservative was added. The total viable counts of *L. acidophilus* for freshly prepared drink were calculated as 15.8 X10^7, 20X 10^7 and 22.9 X 10^7 cfu / ml for T2, T3 and T4, respectively.

3. Introduction

For people living in villages, the underutilized fruits are the only source of protective food to meet their vitamins and minerals requirements in their poor diet. Because of their curative properties, these fruits have been used in Indian system of medicine such as Ayurvedic and Unani since time immemorial. Apart from their nutritive and medicinal values quite a few of these underutilized fruits have excellent flavour and very attractive colour. In spite of these quality attributes most have not undergone any conscious phase of domestication and human selection. Being tolerant to biotic and abiotic stresses, these fruit species are suitable for growing in the disaster- and drought-prone areas. These arid vegetables and fruits just grow wild on barren land and are available free of cost to provide food and nutrition security but they are underutilized and down to drain. Although some fruits have already been recommended for commercial planting, it is apparent that there are a lot more fruit types that await future exploitation.

Ziziphus mauritiana, also known as Ber, Chinese date, Chinee/ Chinkee apple, jujube, Indian plum, and Indian jujube, is one of such underutilizd fruit grow in the arid region of India. *Ziziphus mauritiana* belongs to the family Rhamnaceae. It is a spiny, evergreen shrub or small tree up to 15 m high, with trunk 40 cm or more in diameter; spreading crown; stipular spines and many drooping branches. Fruit is drupe, globose to ovoid, up to 6 x 4 cm in cultivation, usually much smaller when wild; skin smooth or rough, glossy, thin but tough, yellowish to reddish or blackish; flesh white, crisp, juicy, sub-acid to sweet, becoming mealy in fully ripe fruits [1].

The genus *Ziziphus* comprises about 40 species distributed throughout the tropical and subtropical regions of the world. In India itself there are 90 or more cultivars are produced. India ranks second among ber growing countries in the world after China, occupying approximately one lakh hectare area [2]. The top eleven cultivars are 'Banarasi (or Banarsi) Pewandi', 'Dandan', 'Kaithli' ('Patham'), 'Muria Mahrara', 'Narikelee', 'Nazuk', 'Sanauri 1', 'Sanauri 5', 'Thornless' and 'Umran' ('Umri') [3]. The research on the utilization of ber fruit to prepare value added products is very scant which provide sample promise of using the ripe fruit for processing into value added nutritive products.

Probiotics can be defined as microbial cells that have a beneficial effect on the health and wellbeing of the host. A wide number of products like curd, cheese, pickle, vinegar, wine, cider, yoghurt, etc. are produced through microbial fermentation. Fermented food products, besides having good nutritional profile, provide good resistance to human body against certain hardcore

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diseases. The probiotics come under the category of functional food, which benefit the human health beyond the level of basic nutrition. Lactic acid bacteria are among the most common types of microbes used as probiotics. These help in maintaining health-promoting microbial population in the digestive tract and protect the body from various forms of pathogenic infections and intestinal diseases. Fruits and vegetables offer an alternative for the production of probiotic foods due to their large distribution and nutritive value. Lactic acid fermentation can help to improve the safety, shelf life, and nutritional and sensory properties of vegetable.

Fermented fruit and vegetable products are equally good as probiotic materials. Dairy products are generally associated with problems of cholesterol and lactose intolerance, while fruit and vegetable products are rich in vitamins and minerals. Most probiotic foods available today are milk based, but consumer's preference today lie more with botanical dietary supplements, which are either free from or have minimal cholesterol content.

It is necessary that the probiotic culture contain sufficient number of viable microbial cells. The probiotic cell viability dramatically decreases because of exposure to detrimental environmental factors such as organic acids, hydrogen ions, molecular oxygen and antibacterial components. The beneficial effects of probiotic microorganisms appear when they arrive in the intestinal medium, viable and in high enough number, after surviving the above mentioned harsh conditions. According to the International Dairy Federation (IDF) recommendation, this index should be $\geq 10^7$ cfu /g up to the date of minimum durability [4].

Foods claiming to be organic must be free of artificial food additives, and are often processed with fewer artificial methods, materials and conditions, such as chemical ripening, food irradiation, and genetically modified ingredients. Pesticides are allowed as long as they are not synthetic. The developed RTS beverage fulfils the above requirement of being an organic drink.

3.1. Objectives

- Development of probiotic RTS beverage from ber fruit (*Ziziphus mauritiana*).
- Quality evaluation of the freshly developed RTS.

4. Methodology

1. Procurement of raw materials

i) Selection and Procurement of fruit: Ber fruit of Gola variety was selected because of its high juice content.

ii) Procurement of probiotic culture: The strain of *Lactobacillus acidophilus* was selected as a suitable probiotic culture as studies have suggested that it doesn't alter the taste of beverage [4]. It was purchased from the Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh. These strains were anaerobic and were maintained in *Lactobacillus* MRS.

iii) MRS broth: For revival of probiotic culture the required MRS broth was procured from the local chemical distributor.

1) Preparation of the ber fruit syrup: Freshly ripened ber fruits were used for the preparation of the juice, as suggested by [5]. In order to produce clear juice the softened pulp was passed through a stainless steel sieve. The juice was preserved by adding sugar and citric acid to it so as to reach the ratio of 50^0 Brix TSS. The syrup was kept under sun for 2-3 days and then filled in sterilized bottles.

2) Reviving of microbial culture: *Lactobacillus acidophilus* was reactivated by sub culturing in MRS broth overnight at 37 °C. For high density harvest, cells were grown in MRS broth with 10% seed inoculum and incubated at 37 °C for 48 h. The cells were harvested by centrifugation at 6500 rpm for 15 min [4].

3) Inoculation of probiotic culture: Probiotication of ber fruit syrup samples was done by inoculating 500 mL of the sample each with 1% , 1.5 % and 2 % (v/v) equivalent to 5 mL, 7.5 ml and 10ml of the probiotic *L. acidophilus* starter culture (0.5 McFarland standard containing 1.5^{*} 10^8 cfu/mL), respectively. The inoculated samples with the probiotic were labeled as T2 (with 1% inoculation), T3 (1.5% inoculation) and T4 (with 2% inoculation) and T1 (the controlled group with no inoculation).

4) Incubation of inoculated syrup: The inoculated samples were incubated at 37 °C for 48 h and population was determined by standard plate count method on MRS medium for lactic acid bacteria.

5) Bottle filling and corking: 80 ml of the each sample syrup was filled in the pre sterilized 250 ml PET bottles. Carbonation was performed and simultaneously crown corking was done with head space of 2-3 cm. the developed RTS drink was subjected to following quality characteristics:

6) **Sensory evaluation:** The sensory qualities were evaluated by panel of judges for assessing the acceptability of the product [7].

7) **Physico-chemical analysis :**

- **TSS:** The content of total soluble solids (TSS) of probiotic beverage samples was determined using a digital hand refractometer.
- **pH:** It is the measurement of the logarithm of inverse of hydrogen ion concentration in the solution. The electronic pH

meter (Systronic), was used for the purpose.

- **Titratable acidity (TA):** It is an approximation of total acidity of a solution. Five mL of sample was taken and the final volume was made to 20ml and titrated against 0.1 Normal NaOH using phenolphthalein as indicator to a light pink color. It was expressed as per cent acidity (AOAC, 2004).

$$\%\text{Acidity} = \frac{\text{Titer x Normality of NaOH x 0.064 x 100}}{\text{Volume of sample}}$$

- **Nutrient analysis:**

- **Sugars:** reducing sugars, non-reducing sugar and total sugar in all products were estimated by Lane and Eynon's method reported by [7].

- **Ascorbic acid:** The amount of ascorbic acid presented in the samples was estimated by standard method [8].

1) **Total viable count of *Lactobacillus acidophilus* :** After fermentation, viable cell counts (cfu /ml) were determined by standard plate count method or serial dilution agar plate technique with nutrient agar media after 48 hrs of incubation at 37°C. Then colonies of viable cells were observed by plate count or Serial Dilution Agar Plate Technique on nutrient agar (NA) media. Procedure of plate count or serial dilution technique was followed. Number of colonies in the plates was counted by placing on the plate form of Quebec colony counter and expressed the results as cfu /ml.

$$\text{Number of cells (cfu /ml)} = \frac{\text{number of colonies}}{\text{Amount plated X dilution}}$$

10) **Shelf life assessment of probiotic carbonated RTS beverage:** The developed probiotic carbonated RTS beverage samples were stored in refrigerator for a period of 45 days.

11) **Statistical analysis:**

4.1. Results and discussion

1) Physico- chemical evaluation

Titeratable acidity: Acidity values recorded in different treatments are given in the (**Table 1**).

Table 1: Titratable acidity of the probiotic carbonated RTS.

Treatment	Mean ± SD		
	TA	TSS	pH
T1	0.376 ± 0.013	13.675 ± 0.532	3.248 ± 0.061
T2	0.451 ± 0.024	14.025 ± 0.171	3.050 ± 0.008
T3	0.398 ± 0.006	14.175 ± 0.171	3.305 ± 0.122
T4	0.429 ± 0.017	14.325 ± 0.171	3.018 ± 0.017

- Initially, TA for all treatments was maintained around 0.3 percent to 0.4percent. The highest acidity percentage was recorded in T2 and lowest was calculated in T1.

- **Total Soluble Solids**: It was found that the maximum TSS was recorded in T4 with maximum percentage of inoculation as 14.1 °Brix and least was recorded in T1 (controlled) as 12.9 °Brix.

- **pH value:** pH of beverage was measured just after final preparation. Highest pH was found in T3 treatment as 3.42, then T1 3.3, T2 3.06 and the lowest was of T4 treatment as 3.04. Treatment T3 had the highest mean pH value whereas T4 had the lowest as shown in the Table 1. Among treatments there is significant pH difference was observed at confidence level of 0.1%.

- **Nutritional analysis**

- Ascorbic acid content: The mean vitamin C content of the freshly prepared beverage was calculated as 1.3275. Sample T1 and T2 contained the highest amount of vitamin C as 1.34 mg/100 ml then T3 as 1.33 mg/100 ml and T4 1.30 mg/100 ml (Table 2). The results are in agreement with the results reported by Kavitha in 2011.

- Sugar content: Freshly prepared samples were tested for the reducing sugar, non- reducing sugar and total sugar content. The enumerated total sugar (%) was highest in the T1 sample and minimum in the T4 sample as in given table. The reducing sugar (%) were calculated as in T1 2.68, in T2 and T4 2.7 and T3 had 2.71

1) **Sensory evaluation**

The data (Table 3) clearly portrays the fact that the samples T1, T2 and T3 have obtained mean score above 8.0 on the scale for sensory evaluation. This proved that the T1, T2 and T3 were got extreme likeness by the consumers. Even the T4 sample also scored 7.82 mean values on the hedonic scale out of 9.0 rating, That showed moderate likeness. Thus, it is evident from the scores that all samples were accepted well by the consumers.

Table 2: Total sugar, reducing sugar, non reducing sugar and ascorbic acid content of developed probiotic RTS beverage.

S.No.	Variables	Total sugar (%)	Reducing sugar (%)	Non-reducing sugar (%)	Ascorbic acid content (mg/100 ml)
1	T1	10.56	2.68	7.88	1.34
2	T2	10.5	2.7	7.8	1.34
3	T3	10.5	2.71	7.79	1.33
4	T4	10.4	2.7	7.7	1.3

Table 3: Organoleptic scores of different treatments of probiotic carbonated beverage.

Sample	Sensory attributes						Mean
	Colour	Appearance	Taste	Flavour	Mouthfeel	Overall acceptability	
T1	8.9	8.9	8.9	8.8	8.9	8.06	8.74
T2	8.96	8.9	8.9	8.8	8.0	7.06	8.43
T3	8.86	8.9	8.0	7.0	8.0	8.0	8.12
T4	8.96	8.9	7.06	8.0	7.0	7.0	7.82
Mean	8.92	8.9	8.21	8.15	7.97	7.53	

3) Total viable count of *Lactobacillus acidophilus*: Total viable count was calculated by using plate technique. Enumerated colony counts of freshly prepared samples on 6^{th} dilutiion are given in the Table 4. On the 6^{th} dilution mean colony count value for T2 was calculated as 19.900 ± 6.032 (10^6), for T3 21.300 ± 5.817(10^6), and T4 was calculated as 22.400 ± 5.766 (10^6). The difference between T2 and T3 was calculated as non-significant, where as between T2 and T4 was found to be significant at five percent level of confidence. Again the difference between T3 and T4 was non significant.(**Table 4**) and (**Table 5**)

Table 4: Mean Colony count of Lactobacillus acidophilus (cfu /ml).

Treatment	(Mean ± SD) x 10^6	(Mean ± SD) x 10^7
T2	19.900 ± 6.032	14.450 ± 3.078
T3	21.300 ± 5.817	17.050 ± 3.508
T4	22.400 ± 5.766	17.050 ± 4.806

Table 5: Difference in the colony count among the treatments.

Pair	(10^6 cfu /ml)		(10^7 cfu /ml)	
	Abs. diff	Result	Abs. diff	Result
T2- T3	1.40	NS	2.60	NS
T2-T4	2.50	*	2.60	NS
T3-T4	1.10	NS	0.00	NS

Total viable count values for 7^{th} dilution are shown in the **table 4**. It is exihibited that because of 2% concentration, T4 had highest colony count, i.e. 22.9 X10^7 cfu/ ml. On 7^{th} dilution, the non-significant difference amongst the viable count of treatments explained that all three treatments can be used equally for the purpose excluding the organoleptic scores.

The above table showed that among the treatments the difference of change in the viable count appeared to be non significant.

5. Conclusion

The freshly prepared RTS beverage had value of total viable count of *L. acidophilus* as 15.8 X10^7, 20X 10^7 and 22.9 X 10^7 cfu / ml for T2, T3 and T4, respectively. The physico-chemical properties of RTS beverage such as titratable acidity, TSS, pH value, vitamin C, total sugar, reducing sugar and non reducing sugar were found to be 0.42, 13.7 ^{0}Brix, 3.2, 1.3 mg/100ml, 10.49 %, 2.69%, and 7.79 %, respectively. The values are in consensus with the standards mentioned by [9] for the RTS beverage. The overall acceptability of consumers was scaled on the nine point hedonic scale. The results were above 7.0. This showed the positive response of consumers. The developed probiotic beverage was free from any artificial preservative.

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