DEVELOPMENT AND ANALYSIS OF PROBIOTIC AND SYNBIOTIC FRUIT JUICE

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Abstract

Probiotics are reported to have beneficial effect in the gut health, lipid profile and breast cancer. Prebiotics have the ability to beneficially alter the gut microbiota and also have the potential to reduce the risk of colorectal cancer, stimulate immune response, alleviate symptoms of inflammatory bowel disease, modify serum triglycerides and cholesterol, enhance mineral absorption in the intestine and thereby reduce the risk of intestinal infectious diseases, cardiovascular disease, non-insulin dependent diabetes, obesity, osteoporosis and cancer. Fruit juices are very healthy and nourishing beverages. Orange juice is one of the most popular fruit juice and accepted by people of every age group. It has several health and nutritional benefits. The present study was planned to study the physicochemical and sensorial quality of control, probiotic and synbiotic orange juice. The probiotic juice was prepared using probiotic strain *L. Acidophilus* and synbiotic juice was prepared using *L. Acidophilus* and Inulin as a prebiotic source. The juices were studied for pH, titratable acidity, total soluble solids (Brix), Ascorbic Acid, Total Phenol, Flavonoid, Ferric Reducing Antioxidant Power Assay (FRAP), microbial analysis, Sensory Evaluation and Color Analysis on 0th, 3rd and 6th day of storage. For storage study, juices were stored under refrigeration temperature (4°C). Result showed that, pH of control, probiotic and synbiotic juices ranged from 4.00-4.01 whereas titratable acidity was 0.5-0.6% which decreased during storage. Total phenolic content of the juices ranged from 24.80 mg GAE/100 ml to 40.34 mg GAE/100 ml. The maximum flavonoid content was 20.96 mg RE/100 ml while, minimum was 11.85 mg RE/100 ml amongst fresh and stored samples. There was a significant (P<0.01) decrease in the flavonoid content during storage. The antioxidant capacity using FRAP assay ranged from 37.69 mg TE/100 ml to 50.65 mg TE/100 ml. The ascorbic acid content ranged from 8.13 mg/100 ml to 6.88 mg/100 ml. Brix of the juice ranged from 16.50-17.75. During storage, the *Lactobacillus* count in the juices decreased but the viable count of the synbiotic juices did not fall below 10⁶ cfu/ml and retained its probiotic property while in probiotic juices the viable count was not maintained for its probiotic property. The overall acceptability of the juices was significantly (P<0.05) higher at 0 day but reduced after 6 days of storage. Color analysis of all the juices revealed that there was retention of color in all the juices during storage. The present study concludes that the addition of probiotic strains and prebiotic to the orange juice was found to be acceptable as control orange juice. It can be served as a functional food.

Keywords

Orange juice, Probiotic, Synbiotic, *L. Acidophilus*, Inulin
Introduction

Fruits provide a significant part of human nutrition, as they are important sources of nutrients, dietary fiber, and phytochemicals. Risk of certain chronic diseases like hypertension, chronic heart disease, stroke, overweight, type 2 diabetes mellitus, eye diseases, dementia, risk of osteoporosis, asthma, chronic obstructive pulmonary disease, *rheumatoid arthritis*, inflammatory bowel disease, glaucoma and diabetic retinopathy can be reduced by increased consumption of fruits (Boeing et al., 2012). Fruit juice is the unfermented but fermentable liquid obtained from the edible part of sound, appropriately mature and fresh fruit or of fruit maintained in sound condition by suitable means including post-harvest surface treatments applied in Codex Alimentarius Commission (2005).

The National Academy of Sciences’, Food and Nutrition Board defined functional foods as “any modified food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains” (Hasler, 2014). Some of the nutraceuticals for functional foods are as follows (International Food Information Council Foundation, 2011) – carotenoids, dietary (functional and total) fiber, fatty acids, flavonoids, isothiocyanates, minerals, phenolic acids, plant stanols/sterols, polyols, prebiotics, probiotics, phytoestrogens and soy protein.

Probiotics are live microbes that can be formulated into many different types of products, including foods, drugs, and dietary supplements. Species of *Lactobacillus* and *Bifidobacterium* are most commonly used as probiotics, also the yeast *Saccharomyces cerevisiae* and some *E. coli* and *Bacillus species* are also used as probiotics. Prebiotics are non-starch polysaccharides and oligosaccharides poorly digested by human enzymes) that nurture a selected group of microorganisms especially probiotics living in the gut. They favor the growth of beneficial bacteria over that of harmful ones. Synbiotics are appropriate combinations of prebiotics and probiotics. A synbiotic product exerts both a prebiotic and probiotic effect (Gaurner et al., 2012).

Worldwide nondairy probiotic products are gaining importance due to the ongoing trend of vegetarianism and to a high prevalence of lactose intolerance in most of the populations around the world. Fruit juices, desserts, and cereal-based products may be other suitable media for delivering probiotics (Cargill, 2009).

Orange (*citrus sinensis*) is well known for its nutritional and medicinal properties throughout the world. Orange is also rich in vitamin C and other antioxidants (Milind et al., 2012).

**Material and Methodology**

The present study was planned to formulate probiotic and synbiotic orange juice using the selected probiotic strain *L. Acidophilus* and prebiotic source Inulin followed by the storage studies.
Maintenance and Preservation of Probiotic culture - This procedure was carried out for the preservation of the probiotic strain *L. Acidophilus*. The culture was activated in MRS broth. MRS broth was prepared and it was autoclaved at 15 psi for 20 minutes. It was incubated at 37°C for 24 to 48 hours. The vials were wrapped properly and carefully with parafilm and stored at -20°C.

Activation of Culture - Culture of *L. Acidophilus* were obtained. A stock culture was activated for the study by sub culturing them weekly in sterile 10% juice tubes.

Method of preparation of Control Fruit Juice
Orange was procured from the local market of Anand. Juice was extracted from the fruits and to it required amount of powdered sugar was added. The juice was sterilized (121°C, 15 psi for 20 minutes). The prepared juices were filled in sterile amber colored glass bottles. The juices were stored under refrigeration temperature (4°C).

The probiotic juice was prepared using probiotic strain *L. Acidophilus* and synbiotic juice was prepared using *L. Acidophilus* and Inulin as a prebiotic source. Juices were analyzed for the following parameters at 0th, 3rd and 6th Day.

Physicochemical parameters like pH, Titratable Acidity (BIS 1981), Total Phenols (Singleton and Rossi, 1965), Flavonoids (Singleton et al, 1999), Total Antioxidants-FRAP (Ferric Reducing Antioxidant Power) (Benzie et al, 1996), Ascorbic Acid (S. Ranghanna, 2000), Total Soluble Solids (Refractometer) were used for analysis.

Microbial analysis was done using Total Plate Count (Nutrient Agar), Lactobacillus Count (de Man Rogosa Sharpe; MRS Agar), Yeast and Mold count (Potato Dextrose Agar; PDA Agar).

Sensory evaluation parameters included sensory attributes like Color, Consistency, Taste/After Taste, Flavor, Mouthfeel, Overall Acceptability and Color Analysis.

Statistical analysis was expressed as Mean ± SD. The data was analyzed by student’s paired T test. A value of P ≤0.05 was considered as statistically significant.

RESULT

The data of pH, Titratable Acidity and Brix of juices are expressed in Table 1-

<table>
<thead>
<tr>
<th>Juice</th>
<th>Day</th>
<th>pH</th>
<th>Titratable Acidity</th>
<th>Brix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>4.00</td>
<td>0.6</td>
<td>16.75</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>4.28</td>
<td>0.5</td>
<td>17.75</td>
</tr>
<tr>
<td>Probiotic</td>
<td>0</td>
<td>4.00</td>
<td>0.5</td>
<td>16.50</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>4.29</td>
<td>0.5</td>
<td>16.25</td>
</tr>
<tr>
<td>Synbiotic</td>
<td>0</td>
<td>4.01</td>
<td>0.6</td>
<td>17.75</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>4.27</td>
<td>0.5</td>
<td>17.75</td>
</tr>
</tbody>
</table>

Table 1- pH, Titratable Acidity and Brix of Control, Probiotic and Synbiotic Juice

pH of juices ranged from 4.00 to 4.01. The highest pH was observed for synbiotic juice (4.01) compared to control and probiotic juice. There was no significant difference observed in pH of juices on zero day. No significant difference was observed in all the three juices on zero day as compared to the difference observed during the storage study. This could be due to equilibration time given during the storage of freshly prepared juices. Rekha et al (2012) noticed that the pH of citrus fruit juices ranged from 3.50 to
4.33 and was found to be lesser in case of unripe fruits when compared to ripe fruits (pH of bitter orange < lemon < mandarin orange < orange).

The titratable acidity decreased slightly as the storage time was extended. At 0 day, it was noticed that the titratable acidity of the control and symbiotic juice was the same (0.6 % citric acid) whereas the titratable acidity of the probiotic juice (L. Acidophilus) was 0.5 % citric acid. No significant difference was noticed. Rekha et al (2012) studied that the total acidity was found to be higher in lemon juice followed by mandarin orange, orange and bitter orange. In all fruit juices, the total acidity was higher in unripe fruits when compared to ripe fruits. The titratable acidity ranged from 0.563 % to 3.404 %.

Brix of all juices ranged from 16.50 of probiotic juice to 17.75 of symbiotic juice. No significant difference was observed. The orange and apple containing concentrate along with Lactobacillus acidophilus bacteria in juice by brix 11 and 15 and juice sample was lasting to 6 days by depending directly upon the optimum temperature of bacterial growth (Marhamatizadeh et al., 2012).

**Ascorbic Acid and Antioxidant profile of juices are showed in figure 1 to 4**

As shown in figure 1, it was observed that the highest ascorbic acid content was observed for control juice (8.13 mg/100 ml) and the lowest ascorbic acid was noticed for symbiotic juice (6.88 mg/100 ml). Rekha et al (2012), studied that the ascorbic acid content in the fruit juices, as estimated by volumetric method, ranged from 6.34 to 26.01 g/100 ml. Unripe fruits contained high ascorbic acid compared to ripe fruits. Among fruits, high ascorbic acid content was observed in bitter orange followed by orange, lemon and mandarin orange.

As shown in figure 2, at 0 day, the phenolic content ranged from 24.80 mg GAE/100 ml of probiotic juice to 40.34 mg GAE/100 ml of symbiotic juice. The highest phenolic content was found in symbiotic juice which was significantly higher than the probiotic juice. Rekha et al (2012) studied the total phenolic content of juices. The total phenolic content ranged from 532 to 960 µg GAE/mL of fruit juice. High phenolic content was observed in unripe fruits. Among Citrus fruits, high phenolic content was observed in orange followed by bitter orange, lemon and mandarin orange.

**Figure 1 – The Ascorbic Acid values (mg/100 ml) of Control, Probiotic and Synbiotic juice**

**Figure 2 – The Total Phenolic values (mg GAE/100 ml) of Control, Probiotic and Synbiotic juice**
As shown in figure 3, the flavonoid content of the three juices, ranged from 15.89 mg RE/100 ml probiotic juice to 20.96 mg RE/100 ml of control juice. The flavonoid content varied significantly (P<0.01) in all the juices. Synbiotic juice contained significantly higher flavonoids compared to probiotic juice. Rekha et al (2012) depicted that, during refrigerated storage of fresh juice (4°C), 50% of the soluble flavanones precipitate and integrate into the cloud fraction. Commercial orange juices contain only 81-200 mg/L soluble flavanones (15-33%) and the content in the cloud (206-644 mg/L) is higher (62-85%), showing that during industrial processing and storage the soluble flavanones precipitate.

As shown in figure 4, FRAP activity ranged from 37.69 mg TE/100 ml of synbiotic juice to 50.65 mg TE/100 ml of probiotic juice. There was significant difference (P<0.05) noticed in the FRAP values of all the juices.

**Microbial Analysis:** Highest LAB count was observed for synbiotic juice (9.95×10⁶ cfu/ml) and the lowest count was observed for probiotic juice (5.61×10⁶ cfu/ml) on zero day. On 6th day, the highest LAB count was observed in synbiotic juice (1.43×10⁶ cfu/ml) and lowest count was observed in probiotic juice (0.55×10⁶ cfu/ml). Looking at the impact of storage on LAB count of juice, the viability of *L. Acidophilus* population decreased, the viable count of the synbiotic juice did not fall below 10⁶ cfu/ml. However, in synbiotic juice the counts were maintained for justifying the probiotic properties. In a study by Shukla et al. (2013) it was revealed that the initial total viable count of the whey-pineapple beverage was 3.8×10⁷ cfu/ml which decreased to 1.8×10⁷ at refrigerated storage. Although the viability of *Lactobacillus acidophilus* population decreased, the viable count of the probiotic beverage did not fall below 10⁶ cfu/ml. Also during storage at 30 ± 1°C, the total viable count first increased to 9.5×10⁸ (in 48 hr.) and then gradually declined to 2.9×10⁷cfu/ml after 120 hr.
Figure 5-10: Lactobacillus Count of Probiotic juice and Synbiotic juices

Figure 5, 6, 7 Probiotic juice (**L. Acidophilus**) – **0th Day, 3rd Day, 6th Day**
Figure 8, 9, 10 Synbiotic juice (**L. Acidophilus**) – **0th Day, 3rd Day, 6th Day**

**Sensory:** The highest score for overall acceptability was 7.70 for control juice followed by 7.13 for synbiotic juice and 6.35 for probiotic juice on 0 day. There was no significant difference observed. On 6th day, the highest score for overall acceptability was for 7.75 of control juice followed 7.00 of synbiotic juice. There was no significant difference observed. Shukla et al. (2013) represented that the pineapple-whey based probiotic beverage did not show sensory differences for the first two weeks at refrigerated storage, but after the second week, difference was perceived in color and flavor. According to the panelists, it was determined that the main descriptors that characterized the product were acidity and sweetness, with acidity being the attribute responsible for the sensory difference perceived by the panelists. Even though a slight acidification was detected by the sensory panels, they agreed that the beverage was acceptable for a period of 24 d at 5±1°C and 48 hr at 30±1°C.

**Color Analysis:** It was revealed that the retention of color was observed in all the juices during storage.
Conclusion
The present study concludes that the addition of probiotic strains and prebiotic source to the orange juice was found to be acceptable as control orange juice. It can be served as a functional food. Synbiotic juice incorporated with L. Acidophilus was found to be better in terms of probiotic property and antioxidants. The viable count of the synbiotic juices did not fall below 10^6 cfu/ml and retained its probiotic property. Hence, it could be served as a health beverage for the vegetarian consumers who are allergic to dairy products.

References