Development of Value Added Probiotic Freeze-Dried Papaya Juice Powder

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Abstract

A probiotic food product was developed from papaya juice using freeze drying. Lactobacillus acidophilus was used for the fermentation of fruit juice. The parameters considered for optimizing the fermentation time were maximum growth and activity in terms of pH and acidity using 6% and 8% inoculums of L. acidophilus. So the fermentation of papaya juice was conducted for 48 hours at 37ºC. Drying was carried out using freeze-drying technology. Freeze drying conditions were fixed in terms of °Brix, acidity, vacuum, freezing condenser temperature and temperature of drying to get desirable results with a total viable count of more than 10⁷ cfu/ml and with highest sensory quality. The product obtained was rich in natural anti-oxidants like β-carotene and ascorbic acid. Physio-chemical and microbiological studies revealed that probiotic and other functional components were stable during storage at various temperatures of 5ºC, (30±2ºC) and 37ºC for 60 days.

Keywords: Probiotic value addition, Freeze-drying, Papaya juice product, Lactobacillus acidophilus.

1. Introduction

The term “probiotics” was first introduced in 1953 by Werner Kollath. The root of the word “probiotic” comes from the Greek word pro, meaning “promoting” and biotic, meaning “life” (Raaz Maheshwari et al., 2012). One of the most important characteristics of a probiotic strain is that it must be non-pathogenic and GRAS-Generally Recognised As Safe (Gorbach, 2002). No pathogenic, or virulence properties have been found for the Lactobacilli, bifid bacteria, or Lactococci (Collins, 1998). Products containing probiotic bacteria comprise much of the available functional food. Moreover, these products have an ever-expanding world market due to consumer demand as a result of their potential health benefits. Some of the probiotic products are yoghurt, probiotic milk, probiotic cheese, kefir, sauerkraut, tempeh, probiotic sour pickles, miso, etc.

Papaya fruits became more popular because of its health-promoting phyto-nutritional content and high anti-oxidative capacity (Corral-Aguayo et al., 2008). The principal carbohydrates encountered in the fruit are glucose, sucrose, and fructose, with glucose being the carbohydrate most present during the initial stages of development while sucrose, fructose and glucose are more abundant after ripening, when the percent of sugars varies between 10 and 13% (Zhou and Paull, 2001). It is a rich source of three powerful antioxidants-vitamin C, vitamin A and vitamin E; the minerals-magnesium and potassium; the B vitamin-pantothenic acid and folate; and fiber (Aravind et al., 2013). The fruit is an excellent source of β-carotene that prevents damage caused by free radicals that may cause some forms of cancer. It is reported that it helps in the prevention of diabetic heart disease. Papaya lowers high cholesterol levels as it is a good source of fiber. Papaya effectively treats and improves all types of digestive and abdominal disorders. Papaya has an abundance of cancer fighting lycopene (Wall, 2006).

L. acidophilus is a homo-fermentative, microaerophilic species, fermenting sugars into lactic acid. It grows readily at rather low pH values (below pH 5.0) and has an optimum growth temperature of around 37ºC (99 ºF) (Baati et al., 2000). L. acidophilus occurs naturally in the human and animal gastrointestinal tract and mouth. Some strains of L. acidophilus may be considered to have probiotic characteristics (Ljungh et al., 2006). The important functions of L. acidophilus include improving lactose digestion in individuals that have this difficulty (Kim and Gilliland, 1983), lowering serum cholesterol levels, help prevent certain types of cancer (Rao et al., 1999), stimulate the immune system (Gill et al., 2000), control
urogenital infections in women (Hilton et al., 1992), and prevent or control intestinal infections (Gilliland and Speck, 1977).

Freeze drying or Lyophilization is a process in which water is frozen, followed by its removal from the sample, initially by sublimation (primary drying) and then by desorption (secondary drying) (Nireesha et al., 2013). The term “lyophilization” describes a process to produce a product that “loves the dry state” (Lippincott et al., 2000).

The main principle involved in freeze drying is a phenomenon called sublimation, where the water passes directly from the solid state (ice) to the vapor state without passing through the liquid state. Sublimation of water can take place at pressures and temperature below triple point i.e. 4.579mm Hg and 0.0099ºC (Chien et al., 1981). The material to be dried is first frozen and then subjected to a high vacuum so that frozen liquid sublimes leaving only solid dried components of the original liquid. The concentration gradient of water vapor between the drying front and condenser is the driving force for removal of water during lyophilization (Liberman et al., 1989).

Loss of quality during drying is a major problem that limits the market demand for dry food products. One of the main reasons for the loss of quality is the structural changes caused by the product shrinkage during drying process (Achanta and Okos, 2000). Among the various drying techniques, the novel drying techniques like freeze-drying is adopted abruptly worldwide which has a greater advantage on the quality properties of dried products with maximum cost efficiency (Krokida and Maroulis, 2000). The solid state of water during freeze-drying protects the primary structure and the shape of the product with minimum shrinkage. In addition, the low temperature employed in the process contributes in preservation of many vitamins and minerals and retention off flavour and aroma (Marques et al., 2006).

2. Materials and Methods

2.1 Raw Materials

Papaya, double-toned milk, sucrose and culture of L. acidophilus (MTCC No. 10307) procured from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

2.2 Chemical Reagents

For Total Sugar analysis- Fehling’s solution A (Dissolve 69.28g of copper sulphate in 1000ml water) and Fehling’s solution B (Dissolve 346g of Rochelle salt [potassium, sodium tartrate] and 100g of NaOH in water and make up its volume to 1000ml), lead acetate, potassium oxalate solution and standard invert sugar solution. For Ascorbic acid analysis-L-Ascorbic acid solution (standard), 3% HPO4 solution, 2, 6-dichlorophenol Indophenol. For β-Carotene Analysis-Acetone AR Grade, Hexane, Anhydrous sodium sulphate.

2.3 Parameters Studied

2.3.1 Physio-Chemical Tests

Moisture Content, Ash, pH Test, Water Activity, Total solids (TS), Total Soluble Solids (TSS), Titratable Acidity, Total Sugars (%), Ascorbic Acid, β-Carotene, Colour Analysis.

2.3.2 Microbiological Tests

Enumeration of L. acidophilus using LMRS Agar, Microbiological Analysis for Total Plate Count (TPC), Coliforms, Yeast and Moulds.

2.4 Method of Preparation

The Papaya fruit of KG-15 cultivar was purchased from local farmer of Mysore district of Karnataka, India. Only the mature and well ripened fruits were selected and the fruits with defects were discarded. Selected fruits were peeled, cut into smaller pieces and grounded to get pulp. Papaya juice was then prepared by diluting the extracted pulp with water in the ratio of 50:50 and filtered (6ºBrix). The TSS of filtered juice was adjusted to 20ºBrix and pasteurized at 74ºC per 7-10min (Wolff, 1978). Set curd (from the milk which is inoculated with 1% of revived culture of L. acidophilus) was inoculated into papaya juice for fermentation at 37ºC for 48 hours (Blagden et al., 2005). Viability of L. acidophilus was enumerated by using selective media-LMRS Agar (HiMedia M641, Mumbai). Initial cell load of curd was adjusted to 10⁶ cfu/ml count and standardization process was carried out with different inoculum sizes of 1-10% concentration. Out of these inoculum sizes, 6% and 8% has been taken under considerations for the final studies.

2.4.1 Revival of Freeze-Dried Culture

Probiotic bacterium (L. acidophilus 10307) was used to sub-culture the organism into LMRS Broth and incubated at 37ºC with an aerobic condition for 24-48 hours. After the incubation period, turbidity can be observed in the LMRS Broth, which was the indication of the growth of L. acidophilus. The revived culture was stored at 4ºC in the broth form for a month. This prepared culture was then inoculated into the milk which was heat treated at 90ºC for 10minutes. Later it was incubated at 37ºC with an aerobic condition for 24hours.
2.4.2 Freeze Drying of Probiotic Papaya Juice

After completion of incubation period, the fermented papaya juice is poured onto the trays and the filled trays were loaded in a freeze-dryer. The juice was initially subjected for blast freezing in a plate freezer (Epsilon-60, Martyn Christ) at -40ºC for 3 hours. After plate freezing, the frozen product was freeze dehydrated in a freeze drier (Epsilon-60, Martyn Christ) at 60ºC with a vacuum of 100µ and a condenser temperature of -60ºC for 16 hours. These were the critical parameters maintained during the freeze drying process. The whole operation took 24 hours and the final product was obtained.

2.4.3 Packaging of Freeze Dried Probiotic Papaya Juice Powder

The prepared freeze-dried probiotic papaya juice powder was packed in pouches (10 x 11 cm²) made out of Paper Foil Polythene (45 gsm paper/20 Al. foil/37.5µ LDPE) and then subjected for storage studies with each packaging containing 50g of juice powder. All the pouches were sealed to make them leak proof with foot operated electric packing machine. The packed samples were kept for storage studies under room temperature (30±2ºC, 70-80% RH), 5ºC (85% RH) and 37ºC (85-87% RH) and the analysis were carried out at regular intervals of 15 days.

2.5 Chemical Analysis

2.5.1 Moisture Estimation

The Moisture content of freeze-dried papaya juice powder was estimated using the standard AOAC (2000) procedures. 5-10g sample was taken in a previously heated and tared flat bottom aluminium dishes containing thin layer of asbestos. After weighing, the dish cover was removed and the samples were kept for drying in an oven at 100ºC for 6-8 hrs. The dried samples were cooled in desiccators for 30-45 min and weighed. The moisture content is calculated using the formula:

\[
\% \text{ Moisture content} = \frac{\text{Initial weight- final weight}}{\text{Initial weight}} \times 100
\]

2.5.2 Determination of Total Ash

The tare weight of silica dishes (7-8cm diameter) was noted. 5g of the sample was weighed out into each dish and the contents were ignited with the help of a Bunsen burner. The material was kept at 525ºC for 6 hours in a muffle furnace. The dishes were cooled and then weighed. The difference in weight gave the total Ash Content and was expressed in percentage (Ranganna, 1995).

2.5.3 Determination of Total Soluble Solids

Total Soluble Solids (TSS) was measured by using Hand Refractometer of 0-32ºB (ERMA make), where sample was placed between a measuring prism and a small cover plate. Light traveling through the sample was either passed through to the reticle or totally internally reflected. The net effect is that a shadow line forms between the illuminated area and the dark area. It is where this shadow line crosses the scale that a reading is taken.

2.5.4 Determination of pH

The pH of a solution is the negative logarithm of the hydrogen ion activity, which may be potentiometrically measured by using digital pH meter (Model No. 5633, Electronics Corporation of India Ltd., Hyderabad). The determination of the pH value is carried out by measuring the potential difference between electrodes immersed in standard and test solutions. The standard solutions used are assigned a definite pH value by convention.

2.5.5 Determination of Titrable Acidity

Titrable acidity was determined according to the AOAC (2000) method. 10ml of sample was taken and titrated with 0.1 N NaOH using a few drops of 1% of phenolphthalein solution as indicator. The titre value was noted. The result as percent anhydrous citric acid was calculated using the formula.

\[
\% \text{ Total Acid} = \frac{\text{Titre} \times \text{Normality of alkali} \times \text{Equivalent wt. of acid}}{\text{Volume of sample taken} \times 1000} \times 100
\]

2.5.6 Determination of Water Activity

Water Activity meter (Novasina Novalog MC V1 .12) with temperature control of M/s Aqua labs was used. Samples were filled up to three-fourth of the cell and the cabinet is closed. The samples were read and the readings were tabulated.

2.5.7 Determination of Colour

Color was determined using Hunter Colorimeter (M/s Hunter lab Reston, VA, USA) in terms of L, a* and b* value as per Shand (2000), where L* indicates luminosity or brightness, a* corresponds to greenness (-)/redness (+) and b* corresponds to blueness (-)/yellowness (+). The L*, a* and b* data were transformed to colour index.

\[
\text{Colour Index} = \frac{1000 \times a^*}{L^* b^*}
\]

2.5.8 Determination of Ascorbic Acid

2.5.8.1 Standardization of Dye

5ml of each standard ascorbic acid solution and 3% HPO₃ were taken in a conical flask and was titrated.

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against the dye solution till the appearance of pink colour and titre value was noted down.

\[
\text{Dye factor (mg of ascorbic acid per mL of the dye)} = \frac{0.5}{\text{titre value}}
\]

### 2.5.8.2 Analysis of Sample

10-20ml of sample was taken and made up to 100ml with 3% HPO\(_3\). An aliquot of 10ml of HPO\(_3\) extract of the sample was taken and titrated with the standard dye to a pink end-point which should persist for at least 15sec (Johnson, 1948). Ascorbic acid was calculated using the formula.

\[
\text{Ascorbic Acid (in mg per 100g)} = \frac{\text{Titre} \times \text{Dye factor} \times \text{Volume made up} \times \frac{\text{Aliquot of extract}}{\text{Volume of sample taken}}}{100}
\]

### 2.5.9 Determination of \(\beta\)-Carotene

Total carotene was extracted from the food material using acetone-hexane solvents and the color absorbance was measured at 450nm and was expressed as \(\beta\)-carotene. 1g of sample was weighed in cuvette and 3/4\(^{th}\) of it was filled with acetone to extract the pigments and kept in dark for 15min. The extract was filtered using 20ml acetone twice through sintered funnel and it was then filtered again with 20ml hexane. The combined extract was transferred into separating funnel and 150ml of 5% NaCl solution was added to transfer the pigments to hexane layer. After discarding the aqueous layer, hexane layer was transferred to 100ml volumetric flask and volume was made up with hexane. The absorbance was read at 450nm against hexane blank (Ranganna, 1995).

\[
\text{\(\beta\)-Carotene (\(\mu\)g g\(^{-1}\))} = \frac{\text{Absorbance} \times \text{Volume made up} \times 1000}{250 \times \text{Weight of sample taken}}
\]

### 2.5.10 Determination of Sugars

Total sugars and Reducing sugars were estimated by Lane and Eynon Method, as described by Ranganna (1995).

### 2.6 Microbiological Analysis

Microbiological Analysis was done periodically once in 15 days. A range of microbiological analysis was performed as per the standard methods shown in Table 1 (Downes and Ito, 2001; FDA, 2005). Samples were analysed for \(L.\) acidophilus, yeast, moulds, Total Plate Count (TPC) and coliforms.

#### 2.6.1 Diluents Used

For LMRS Agar: 0.1% Peptone and 0.85% Saline in distilled water. For Other 3 Media: 0.1% Peptone in distilled water.

#### 2.6.2 Serial Dilution

Serial dilution technique was followed for microbiological analysis.

### 2.6.3 Enumeration Techniques

#### 2.6.3.1 Viability Examination of Probiotic Organism; \(L.\) acidophilus

1ml aliquot from each \(10^4\) to \(10^6\) dilutions of the samples was transferred into sterile petriplates aseptically. The Molten LMRS was added and thoroughly mixed. The set plates were then incubated at 37°C for 48h. After 48h, creamy colonies on readable plates were counted and reported as cfu/ml.

#### 2.6.3.2 Enumeration of Aerobic Mesophilic Organism

1ml aliquot from \(10^1\) to \(10^3\) dilutions of the samples was transferred into sterile petriplates aseptically. The Molten PCA was added and thoroughly mixed. The set plates were then incubated at 30°C for 72h. After 72h, all the colonies on readable plates were counted and reported as cfu/ml.

#### 2.6.3.3 Enumeration of Yeasts and Moulds

1ml aliquot from \(10^1\) to \(10^3\) dilutions of the samples was transferred into sterile petriplates aseptically. Molten PDA (acidified with 10% tartaric acid) was added and thoroughly mixed. The set plates were then incubated at 27°C/120hrs. After incubation, colonies on readable plates were counted and reported as cfu/ml.

#### 2.6.3.4 Enumeration of Coliforms

1ml aliquot from each \(10^1\) to \(10^3\) dilutions of the samples was transferred into sterile petriplates aseptically. The Molten Violet Red Bile Agar (VRBA) was added and thoroughly mixed. The set plates were then incubated at 37°C for 24h. After 24h, pink colonies on readable plates were counted and reported as cfu/ml.

### 3. Results and Discussion

#### 3.1 Standardization of Papaya Pulp for Freeze Drying

For the evaluation of the right combination of papaya pulp for freeze-drying, the pulp along with three proportions of pulp with water i.e. 70:30, 60:40 and 50:50 were prepared and subjected for sensory evaluation and freeze-drying. The sensory evaluation was carried out on a 9-point Hedonic scale. The score obtained from the panelists after evaluation has been given in Table 2. From the sensory scores, it is clearly observed that the pure papaya pulp and the 50:50 combinations of pulp and water have shown better acceptability compared to other combinations.
Table 1: Incubation time and temperature combination for different media

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Media used</th>
<th>Purpose</th>
<th>Temperature/Time for Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lactobacillus de Man, Rogosa and Sharpe Agar (LMRS)</td>
<td>For L. acidophilus Count</td>
<td>37°C/48 hours</td>
</tr>
<tr>
<td>2</td>
<td>Plate Count Agar (PCA)</td>
<td>Total Aerobic Mesophilic Count</td>
<td>30°C/72 hours</td>
</tr>
<tr>
<td>3</td>
<td>Potato Dextrose Agar (PDA)</td>
<td>For Yeast and Mould Count</td>
<td>27°C/120 hours</td>
</tr>
<tr>
<td>4</td>
<td>Violet Red Bile Agar (VRBA)</td>
<td>For Coliform Count</td>
<td>37°C/24 hours</td>
</tr>
</tbody>
</table>


Table 2: Sensory scores for pulp and different blends

<table>
<thead>
<tr>
<th>Samples</th>
<th>Color</th>
<th>Appearance</th>
<th>Consistency</th>
<th>Flavor</th>
<th>Overall Acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papaya Pulp</td>
<td>8.5</td>
<td>8.6</td>
<td>8.0</td>
<td>8.7</td>
<td>8.5</td>
</tr>
<tr>
<td>50:50</td>
<td>8.3</td>
<td>8.4</td>
<td>8.6</td>
<td>8.2</td>
<td>8.3</td>
</tr>
<tr>
<td>60:40</td>
<td>7.9</td>
<td>7.8</td>
<td>8.0</td>
<td>7.6</td>
<td>7.9</td>
</tr>
<tr>
<td>70:30</td>
<td>7.1</td>
<td>6.8</td>
<td>7.8</td>
<td>7.2</td>
<td>7.5</td>
</tr>
</tbody>
</table>

*No. of panelists: 10.

Table 3: Enumeration of cell count at different inoculum concentration

<table>
<thead>
<tr>
<th>Inoculum Concentration</th>
<th>Cell load before freeze-drying (cfu/ml)</th>
<th>Cell load after freeze-drying (cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4%</td>
<td>2.20×10^7</td>
<td>0.69×10^7</td>
</tr>
<tr>
<td>5%</td>
<td>2.29×10^7</td>
<td>1.01×10^7</td>
</tr>
<tr>
<td>6%</td>
<td>3.32×10^7</td>
<td>1.38×10^7</td>
</tr>
<tr>
<td>7%</td>
<td>3.39×10^7</td>
<td>1.92×10^7</td>
</tr>
<tr>
<td>8%</td>
<td>3.46×10^7</td>
<td>2.18×10^7</td>
</tr>
</tbody>
</table>

3.2 Standardization of Inoculum Concentration in Papaya Juice

Set curd containing the cell load of 10⁹ cfu/ml was used as inoculum in developing probiotic papaya juice. From the set curd, varied percentages of inoculum concentrations were taken i.e. 4-8% and incorporated in the standardized papaya juice having 20ºBrix to see the variation in cell load with respect to the inoculum concentration. The data obtained after enumeration studies before and after freeze-drying of the papaya juice have been depicted in the Table 3. The values obtained in the freeze-dried samples did not show any significant variation in the cell load indicating the stability of the probiotic organism in papaya juice during freeze-drying.

3.3 Proximate Composition

The Proximate composition of the probiotic papaya juice powder prepared by the application of freeze dehydration which are analyzed by standard methods are shown in Table 4. The product is a rich source of carbohydrates and contains 2-2.5% fat and 3-3.5% protein. The free flowing powder can be rehydrated instantaneously by addition of chilled water in the ratio 1:5 w/v. The product upon reconstitution provides 77kcal of energy per serving in addition to its functional characteristics such as probiotics and antioxidants nature.

3.4 Microbiological Analysis

3.4.1 Evaluation of the Stability of L. acidophilus During Storage

The freeze dried probiotic papaya juice powder have been stored at three different temperatures of 5°C,
### Table 5: Viability profile of *L. acidophilus*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Storage Temp.</th>
<th>Storage Days</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5°C</td>
<td></td>
<td>1.78×10⁷</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>25°C</td>
<td>1.78×10⁷</td>
<td>2.01×10⁷</td>
<td>2.086×10⁷</td>
<td>2.02×10⁷</td>
<td>2.35×10⁷</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>37°C</td>
<td>1.78×10⁷</td>
<td>2.01×10⁷</td>
<td>2.086×10⁷</td>
<td>2.02×10⁷</td>
<td>2.35×10⁷</td>
<td></td>
</tr>
</tbody>
</table>

### Table 6: Microbiological profile of freeze-dried Probiotic Papaya juice powder

<table>
<thead>
<tr>
<th>Storage Temp.</th>
<th>Sample Parameters</th>
<th>Storage Days</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>5°C</td>
<td>TPC</td>
<td>2.3×10²</td>
<td>2.56×10²</td>
<td>3.19×10²</td>
<td>3.26×10²</td>
<td>3.30×10²</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Y and M</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Coliforms</td>
<td></td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>B</td>
<td>TPC</td>
<td>2.90×10³</td>
<td>2.942×10³</td>
<td>3.19×10³</td>
<td>3.26×10³</td>
<td>3.30×10³</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>TPC</td>
<td>2.88×10³</td>
<td>2.96×10³</td>
<td>3.148×10³</td>
<td>3.21×10³</td>
<td>3.21×10³</td>
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</tbody>
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<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>37°C</td>
<td>TPC</td>
<td>2.3×10²</td>
<td>2.56×10²</td>
<td>3.19×10²</td>
<td>3.26×10²</td>
<td>3.30×10²</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Y and M</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Coliforms</td>
<td></td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D. = Not Detected.

### Table 7: pH values of the Probiotic juice powder

<table>
<thead>
<tr>
<th>Sample</th>
<th>Storage Temp.</th>
<th>Storage Days</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5°C</td>
<td>5.30</td>
<td>5.29</td>
<td>5.29</td>
<td>5.27</td>
<td>5.26</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>25°C</td>
<td>4.81</td>
<td>4.80</td>
<td>4.79</td>
<td>4.78</td>
<td>4.76</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>37°C</td>
<td>4.49</td>
<td>4.49</td>
<td>4.48</td>
<td>4.47</td>
<td>4.45</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>25°C</td>
<td>4.81</td>
<td>4.79</td>
<td>4.77</td>
<td>4.76</td>
<td>4.75</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>37°C</td>
<td>4.81</td>
<td>4.78</td>
<td>4.77</td>
<td>4.74</td>
<td>4.73</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>4.49</td>
<td>4.47</td>
<td>4.45</td>
<td>4.44</td>
<td>4.43</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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25°C and 37°C. The viability level of *L. acidophilus* were examined periodically for a gap of 15 days along with control. The counts obtained for the probiotic organism has been given in Table 5. From the data it was clearly observed that the freeze-dried product during storage did not show any log’s alteration in the level of probiotic organisms. For probiotic effects, the minimum concentration of viable cells should be at least 10⁷ cfu/ml (Shah 2001). The commercial probiotic beverage should possess a minimum viable count of 10⁶ cfu/ml and should also have an acceptable flavour (Tamine *et al*., 1995).

3.4.2 Microbiological Profile of Freeze-Dried Probiotic Papaya Juice Powder

Microbiological profile in terms of TPC, Coliforms, Yeast and moulds of the product was also evaluated to establish the overall microbiological safety of the product. The counts obtained for the samples stored at different temperature are given in Table 6. Initially the product exhibited TPC of 2 logs in all the samples and Coliforms, Yeast and moulds were absent. Throughout the storage period at different temperatures the product did not record any changes in microbiological safety parameters as indicated in Table 6. From the values it can be interpreted that all the products have exhibited good microbiological safety throughout the storage period. Bacteriocins produced by probiotic lactic acid bacteria may also contribute to an increased stability of the food product during its storage and shelf-life (Avont, 2004; Salminen, 1996).

3.5 Chemical Analysis of Freeze Dried Probiotic Papaya Powder

3.5.1 Determination of pH in Freeze-Dried Product

From the Table 7, it was clear that control samples were having a higher value of pH in comparison with samples B and C. The higher acidity of samples B and C may be attributed due to the presence of probiotic organism. The samples B and C with 6 and 8% concentration had a difference in pH values of 4.81 and 4.49 respectively. During storage, no significant variations in pH values were observed in all the samples. This may be due to stabilization effect provided by the freeze-drying process.

3.5.2 Determination of Titrable Acidity as % Citric Acid and % Lactic Acid

During storage, marginal increase in acidity values were observed both in the case of citric and lactic acid but the variation was not significant. In general, there would be no significant changes in titrable acidity during storage at 5°C, 25°C and 37°C (Yoon *et al*., 2004). The samples initially under during storage were evaluated for acidity in terms of citric acid and lactic acid. Their values obtained are tabulated in Tables 8 and 9 respectively.
Fig 1: β-carotene values of different samples at different temperature.

Fig 2: Ascorbic acid values of different samples at different temperature

Table 10: Water Activity ($a_w$)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Storage Temp.</th>
<th>Storage Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>A</td>
<td>0.131</td>
<td>0.133</td>
</tr>
<tr>
<td>B</td>
<td>0.124</td>
<td>0.124</td>
</tr>
<tr>
<td>C</td>
<td>0.133</td>
<td>0.134</td>
</tr>
<tr>
<td>A</td>
<td>0.131</td>
<td>0.128</td>
</tr>
<tr>
<td>B</td>
<td>0.124</td>
<td>0.128</td>
</tr>
<tr>
<td>C</td>
<td>0.133</td>
<td>0.135</td>
</tr>
<tr>
<td>A</td>
<td>0.131</td>
<td>0.134</td>
</tr>
<tr>
<td>B</td>
<td>0.124</td>
<td>0.141</td>
</tr>
<tr>
<td>C</td>
<td>0.133</td>
<td>0.142</td>
</tr>
</tbody>
</table>
Table 11: Reducing Sugar (%)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Storage Temp.</th>
<th>Storage Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>5°C</td>
<td>15.12</td>
</tr>
<tr>
<td>B</td>
<td>15.66</td>
<td>14.26</td>
</tr>
<tr>
<td>C</td>
<td>15.98</td>
<td>14.29</td>
</tr>
<tr>
<td>A</td>
<td>25°C</td>
<td>15.12</td>
</tr>
<tr>
<td>B</td>
<td>15.66</td>
<td>14.2</td>
</tr>
<tr>
<td>C</td>
<td>15.98</td>
<td>14.27</td>
</tr>
<tr>
<td>A</td>
<td>37°C</td>
<td>15.12</td>
</tr>
<tr>
<td>B</td>
<td>15.66</td>
<td>14.19</td>
</tr>
<tr>
<td>C</td>
<td>15.98</td>
<td>14.23</td>
</tr>
</tbody>
</table>

The samples B and C were having higher acidity values in terms of citric acid as compared with control sample A. The acidity of samples B and C both in terms of citric and lactic acid were having variations with a higher value observed for sample C with increased concentration of inoculum (8%).

3.5.3 Determination of Water Activity

The water activity which is a measure of available water for the physicochemical and the microbiological spoilage of the product was evaluated after freeze drying and storage at 5°C, 25°C and 37°C in freeze dried probiotic Papaya juice powder and the data generated is depicted in Table 10. It has been found that that water activity varies from 0.12-0.13 in the probiotic samples and control sample.

3.5.4 Evaluation of β-Carotene

The probiotic product prepared with Papaya is a good source of natural antioxidants in terms of β-carotene and ascorbic acid in addition to its probiotic activities. Since Papaya was chosen as a base material which contains these anti-oxidants, it can contribute to the functionality of the product. Stability of β-carotene was monitored after freeze drying and storage at 5°C, 25°C and 37°C in freeze dried probiotic Papaya juice powder. From the Fig 1 of β-carotene, it has been observed that during storage at 5°C and 25°C, no significant variations in values were observed. However, the samples stored at 37°C were recorded a nominal decrease in values after 60 days of storage as shown in Fig 1. During the fermentation, 15-45% of carotenoids (mainly β-carotene) were degraded depending on the strain used (Kun et al., 2008).

3.5.5 Studies on the Stability of Ascorbic Acid (mg/100g)

The probiotic product was subjected for ascorbic acid evaluation initially and during storage at different temperatures to evaluate the stability of the product. The product had about 55-60mg of ascorbic acid per 100g of the powder which is considered to be nearer to the RDA requirement for human beings. Out of the three different temperature storage, the samples stored at 37°C were recorded significant decrease after 60 days of storage in comparison with other temperature as observed from Fig 2. The storage at a lower temperature combined with sugar addition could effectively slow the rate of degradation of vitamin C (Hande et al., 2005).

3.5.6 Determination of Reducing Sugars

The samples were evaluated initially and during storage for reducing sugar and the values obtained are represented in Table 11. Papaya juice contains reducing sugars such as glucose and fructose, out of which glucose was the major one. The samples B and C were having low reducing sugar values in percentage as compared with control sample A. The percentage reducing sugar of samples B and C were having higher
variations with a lower value observed for sample A. During storage, marginal decrease in reducing sugar values were observed in all the three samples, which was due to the action of probiotic organism *L. acidophilus* on this reducing sugars in the process of fermentation (Yoon *et al.*, 2004).

3.5.7 Determination of Total Sugars

The samples were evaluated initially and during storage for total sugars after hydrolysis and inversion and the values obtained for the sugars are represented in Table 12.

The samples B and C were recorded a low total sugar values in percentage as compared with control sample A. The percentage of total sugar in samples B and C had variations where the greater value was observed for sample A, which was the control sample. During the storage, marginal decreases in total sugar values were observed in all three samples.

3.5.8 Evaluation of Colour Characteristics

The color characteristics of the three samples were measured initially and during storage using Hunter colorimeter in terms of L, a*, b* values and the readings were also obtained. The lightness values were found to increase for samples B and C, which may be due to the dilution effect in the process of inoculation. During storage, the samples did not make any significant changes in colour readings. The inoculation process increased the a* values and decreased b* values. No studies about the colour of probiotic juices were found in the literatures (Ana *et al.*, 2011).

3.9 Sensory Evaluation

On the whole, all the products exhibited good quality characteristics and reconstitution profile.

4. Conclusion

The demand for foods that promote health and wellness, such as functional products containing probiotic microorganisms, which have a beneficial effect on the balance of intestinal microbiota are on increase as consumers are more aware and concerned about their life style than ever before. The feasibility of developing a probiotic juice powder based on Papaya and *L. acidophilus* by the application of freeze-drying technology have been established in this study. The process parameters with respect to freeze-dehydration for the development of probiotic functional product have been established by optimizing different methodologies. The stability of *L. acidophilus* as a probiotic organism in the development of freeze-dried Papaya product has been enumerated and established. The functionality of the product in terms of the presence of natural anti-oxidants like β-carotene and ascorbic acid has been evaluated and found to be a good stable source of these two components. The product can meet the Recommended Dietary Allowances (RDA) of vitamin A and Ascorbic acid by taking five servings. The shelf life studies of the product revealed a good stability for probiotic organism and functional components at 5ºC and 25ºC up to 60 days of storage. The studies have to be carried to establish the proper strain of *Lactobacillus* spp. and also screening studies for the appropriate application of correct probiotics spp. in the development of fruit based probiotic products with additional functionalities has to be done. Such studies will definitely benefit humans and service sectors in a larger perspective promoting wellness and health.

References


