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# Survivability and Population Dynamics of Lactic Acid Bacteria (LAB) in Probioticated Sapota Juice (*Achras sapota* L.) and their Influence on Physicochemical Properties

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## ABSTRACT

**Background:** Sapota (*Achras sapota* L. or *Manilkara zapota* L.) is one of the major fruit crops grown in subtropical countries and it contains sugars, acids, protein, phenolics carotenoids, ascorbic acid, minerals and vitamins. Growing of lactic acid bacteria in fruit juices for health benefits and improving the nutritional and sensory attributes of the fruit juice is becoming more prominent in the present days. In recent years, consumer preference for non dairy food products has increased especially for lactose intolerance people. The present study is aimed to develop probioticated sapota fruit juice using probiotic bacteria like *Lactobacillus plantarum*, *Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus casei*.

**Methods:** Sapota fruit juice was prepared and inoculated with four different species of proven probiotic lactic acid bacteria (*Lactobacillus plantarum*, *Lactobacillus bulgaricus, Lactobacillus acidophilus* and *Lactobacillus casei*) and incubated at 30°C and 37°C for 72 hours. Population dynamics of lactic acid bacteria and their impact on physicochemical properties of probiotic sapota juice during fermentation at two different temperatures were studied. Sensory evaluation was also studied to know the overall acceptability of the probiotic fruit juice.

**Result:** The pH decreased and titratable acidity (TA) increased in all probiotic sapota juice samples incubated at 30°C and 37°C for 72 hours and the maximum titratable acidity was recorded by T4 (*Lactobacillus plantarum*) followed by T1 (*L. acidophilus*). All the three carbohydrates like glucose, fructose and sucrose present in sapota juice samples were utilized by the lactic acid bacteria during fermentation. Total phenolic concentration in the samples increased during fermentation. Viable cell counts in both samples kept at 30°C and 37°C gradually increased from 0 to 48 hours and then decreased. Sensory evaluation was conducted randomly for all the samples and no significant difference was recorded.

**Key words:** Fructose, Glucose, Probiotic lactic acid bacteria, pH, Sapota fruit juice, Sensory evaluation, Sucrose, Titratable acidity, Total phenol concentration, Viable cell counts.

## INTRODUCTION

The demand for nutraceutical functional foods has been significantly increasing due to nutritional and other health benefits (Kumar *et al*, 2015; Ranadheera *et al*., 2017; Dias *et al*., 2018). Due to increased preference for vegetarian foods and various problems associated with dairy products *i.e.,* lactose and casein intolerance, allergic to dairy products and cholesterol risks, consumers are prefer non dairy based probiotic functional foods like fruit juices (Sharma and Mishra, 2013). Probiotic cereals, fruits, vegetables, meat and meat based products are becoming popular through innovation and product development (Dey, 2018). In the present scenario, fruit juices with probiotic bacteria are gaining more importance (Kumar *et al*., 2015; Roberts *et al*., 2018) attributed to more health benefits (Hussain *et al*., 2016).

Probiotic bacteria are defined as ‘live microorganisms which when administered orally in adequate numbers for conferring health benefits on the host. Lactic acid bacteria (LAB), generally and widely from the genera *Lactobacillus* and *Bifidobacterium* constitute a maximum proportion of probiotic cultures as nutritional supplements, pharmaceuticals and functional foods (Mattila-Sandholm, *et al*., 2002; Del-Piano *et al*., 2006).

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Sapota (*Achras sapota* L. or *Manilkara zapota* L.) is a subtropical fruit crop grown in India, Mexico, Guatemala, Venezuela and other parts of the world. Sapota is rich in nutrients such as sugars, acids, protein, phenolics and other micro nutrients. (Tuorila *et al*., 2002; Betoret *et al.* 2003).

Many researchers have reported the fermentation of fruit juice with probiotic bacteria like *Lactobacillus plantarum, L. delbrueckii, L. bugaricus, L. acidophilus and L. casei* on different fruits *i.e.,* grape (Tabasco *et al*., 2011), peach (Pakbin *et al*., 2014), mango (Reddy *et al.,*2015), orange and apple (Ding and Shah, 2008) juice for probiotic beverage production. A few reports are available on probiotic sapota juice and hence, keeping in view of the health benefits and to add value addition to the sapota fruits, the present investigation was carried out to study the impact of different species of lactic acid bacteria (*Lactobacillus plantarum, L. bulgaricus, L. acidophilus, L. casei*) during fermentation and physico chemical properties of probioticated sapota juice.

## MATERIALS AND METHODS

### Strains and cultures

Probiotic strains (*Lactobacillus acidophilus* MTCC 10307, *Lactobacillus plantarum* MTCC 9511, *Lactobacillus casei* 1423, *Lactobacillus bulgaricus*) were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India. All lactic acid bacterial strains were maintained at -20°C in MRS medium (Himedia, India) containing 20% glycerol. The probiotic cultures were sub cultured on MRS agar (de Man Rogosa and Sharpe Agar,1960) for further use.

### Preparation of probiotic sapota juice

Healthy sapota fruits were purchased from local market and were cleaned. Sapota pulp was extracted by peeling the skin and removal of seeds and prepared the juice by mixing water and pulp in 1:1 ratio and 50 g of sugar was added per kilogram of pulp. Total soluble solids (TSS) were measured by hand refractometer and it was 12%. Sapota juice (150 ml) was filled in conical flasks and pasteurized to remove unfavourable microorganisms. Sapota juice was inoculated with one ml of probiotic culture (*Lactobacillus acidophilus* MTCC 10307, *Lactobacillus plantarum* MTCC 9511, *Lactobacillus casei* 1423, *Lactobacillus bulgaricus*) and allowed for fermentation at 30°C and 37°C. The samples were kept under cold storage for four weeks to know the effect of cold storage (4°C) on viable cell counts. All the treatments were maintained in triplicates.

### Physico chemical analysis during fermentation of sapota juice

The pH value of sapota juice samples were measured separately by a digital pH meter. The titratable acidity of the probiotic sapota fruit juice samples were determined by titrating against 0.1M NaOH with two drops of 0.1M phenolphthalein indicator. About 5 ml of juice was pipetted out into 50ml conical flask, to this 6-7 drops of indicator was added and titrated against 0.1M NaOH (50 ml) in burette till colour change noticed.

### Viable cell count

Viable cell counts (lactic acid bacteria) of all samples were analyzed by the standard plate count (SPC) method using MRS medium (Himedia, India) at 30°C after 48 hours of incubation.

### Estimation of carbohydrates

Quantitative analysis of carbohydrates like glucose, fructose and sucrose using HPLC (Shimadzu model LC6A) was done according to Casterline *et al*. 1999. Chromatograph equipped with a system controller, SCL6A, RID-10A RI detector, an integrator CR3A chromate pack and stainless steel LC-NH2 25 cm×4.6 mm column preceded by a Supel-guard column containing LC-NH2 packing (Supelcosil, 5 μm particle size). Sample extract (10 μL) was injected into the HPLC column. Isocratic separation of carbohydrates was done with a mobile phase consisting of acetonitrile: water [80:20 (v/v)] at a flow rate of 1 mL /min. The HPLC was calibrated by injecting 10 μL of a mixture of standard sugars *viz* (fructose, glucose, sucrose, maltose and lactose, Sigma Chemicals, USA); the concentration of each sugar ranged from 4.4 to 9.8 mg /ml.

### Total phenolic concentration (TPC)

Probioticated sapota juice samples were tested for total phenolic content (TPC) using Folin-Ciocalteu method, as described by Anand *et al*., 2007 by using spectrophotometric method. The absorbance against a prepared reagent blank was measured at 750 nm using a spectrophotometer (MODEL UV-10, Thermo Fisher Scientific, USA) and was expressed as milligrams of gallic acid equivalent per 100 ml).

### Sensory evaluation

The sensory characteristics of the probioticated fruit juices were evaluated with a 20-member panel (Peryam *et al*., 1957) The preferences for taste, aroma, flavour, colour and overall acceptability were determined using a 9-point hedonic scale. Sapota juice samples stored at refrigerated conditions for four weeks were randomly served for sensory evaluation. The mean scores of all the parameters were calculated and interpreted in the results.

### Statistical analysis

All treatments in the experiment were maintained in triplicates and the experimental data generated in lab studies was subjected to one-way ANOVA. The analysis of variance and interpretation of the data was done as per the statistical method given by Gomez and Gomez, 1984. Statistical analysis was conducted using Duncan's Multiple Range Test (DMRT) and means were separated by Duncan Multiple Range Test (DMRT).

## RESULTS AND DISCUSSION

### Effect of lactic acid bacteria on physical parameters of probiotic sapota juice

The pH of samples decreased gradually in sapota juice supplemented with lactic acid bacteria during fermentation (Fig 1). Initial pH of the sapota juice sample was 5.69 and the pH decreased in all probiotic sapota juice samples incubated at 30°C and 37°C for 72 hours. At 30°C, T4 (*Lactobacillus plantarum*) recorded less pH (4.55, 3.85, 3.58 at 24, 48, 72 hours respectively) followed by T1 received with *L. acidophilus* (4.58, 4.11, 3.74 at 24, 48, 72 hours respectively). The lowest pH (5.43, 4.57, 4.41 at 24, 48, 72 hours respectively) was recorded by T3 (*L. casei*). At 37°C, treatment T4 recorded less pH (4.35, 3.52, 3.43 at 24, 48, 72 hours of incubation respectively) followed by T1 (4.76, 3.71, 3.65 at 24, 48, 72 hours of incubation respectively). The lowest pH was observed in T3 (*L. casei*).

All four probiotic cultures (*Lactobacillus acidophilus* MTCC 10307, *Lactobacillus plantarum* MTCC 9511, *Lactobacillus casei* 1423, *Lactobacillus bulgaricus*) used in the study were capable of utilizing easily available nutrients in the sapota juice for cell synthesis and cell survivability without any additional supplements. Reduction in pH levels of the sapota juice due to increase in organic acid production by effective consumption of variable sugars by the lactic acid bacteria during 72 hours of incubation at 30°C and 37°C (Kumar *et al.,* 2015). However, earlier findings reported that lower pH levels (2.5-3.7) of probiotic fruit juices can make the bacteria sensitive to stressful conditions (Sheehan *et al.,* 2007).

Hence, the sapota juice having a pH range of 5.0-5.5, was selected for preparation of a non-dairy probioticated drink. Fruit juice with *Lactobacillus acidophilus* recorded higher viable cell counts resulted in lowering of pH with more acid production. Probiotic fermentations with indigenous foods like fruit juice using lactic acid bacteria showed a decrease of pH and increase in acidity and the results are in accordance with other fruit juices like pomegranate (Mousavi *et al*., 2011), peach (Pakbin *et al* 2014), mango (Reddy *et al*., 2015) and apple juice (Roberts *et al*., 2018). Acid tolerance by lactic acid bacteria in probiotic juice sample is an important trait (Pakbin *et al*., 2014).

Titratable acidity was increased in probiotic sapota juice when incubated at 30°C and 37°C for 72 hours. Maximum titratable acidity was observed with *Lactobacillus plantarum* (0.74, 0.94, 1.16 at 30°C and 0.48, 0.56, 0.58 at 37°C at 24, 48, 72 hours of incubation respectively) followed by *Lactobacillus acidophilus* (0.54, 0.78, 0.99 at 30°C and 0.43, 0.45, 0.48 at 37°C at 24,48,72 hours of incubation respectively). Titratable acidity was less in the sapota juice supplemented with *Lactobacillus casei* (Fig 2). Increase in titratable acidity in the probiotic sapota juice may be attributed to the rapid consumption of available carbohydrates by the probiotic bacteria and releasing the end products in the medium. Some probiotic bacteria have potential ability to grow in the fruit juices and has to with stand the acidic environment.

### Sugar consumption and total phenolic concentration

Sapota juice samples have total sugar content of 15 g /100 mL /kg. Simultaneously, simple sugars were released from the juice due to saccharification, so that these simple sugars favours bacterial growth. The sugar levels in samples were presented in Fig 3. During fermentation, glucose levels in sapota juice samples, containing *Lactobacillus plantarum* (T4) recorded 11.83 gm followed by T1 (*L. acidophilus*), T2 (*L. bulgaricus*) and T3 (*L. casei*) (Fig 3). Similarly, fructose concentration in juice samples containing *L. plantarum* (T4) recorded 11.92 gm followed by T1 (*L. acidophilus*), T2 (*L. bulgaricus*) and T3 (*L. casei*) (Fig 3). The highest sucrose concentration (4.71 g) was recorded in the juice sample containing *L. plantarum* (T4) recorded 11.92 gm followed by T1 (*L. acidophilus*), T2 (*L. bulgaricus*) and T3 (*L. casei*). All three sugars concentration reduced after 72 hours of fermentation. The concentration of sucrose, fructose and glucose reduced significantly in sapota juice due to varied carbohydrate utilization by Lactobacilli and earlier findings reported that the metabolism of three carbohydrates varies with species to species of lactic acid bacteria and depends on the sugar substrate and fermentation time (Hou *et al*., 2000).

Total phenolic concentration (TPC) increased significantly in probioticated sapota juice during 72 hours of fermentation. Sapota juice recorded TPC of 145 mg GAE/ 100 ml in 72 hours of fermentation (Fig 4). In sapota juice, TPC increased progressively with increase in fermentation time. The presence of total phenolic content (TPC) in probioticated sapota juice may be due to formation of

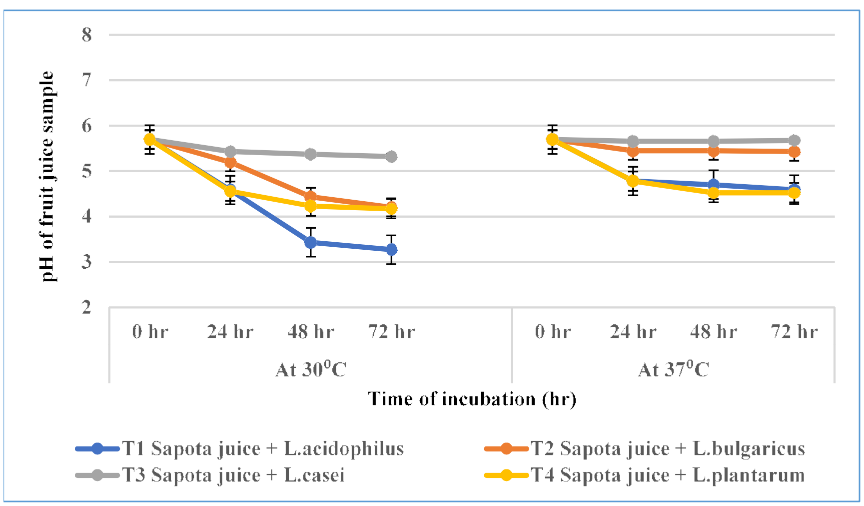


**Fig 1:** Effect of incubation temperature on pH of the sapota fruit juice inoculated with lactic acid bacterial cultures at different intervals.

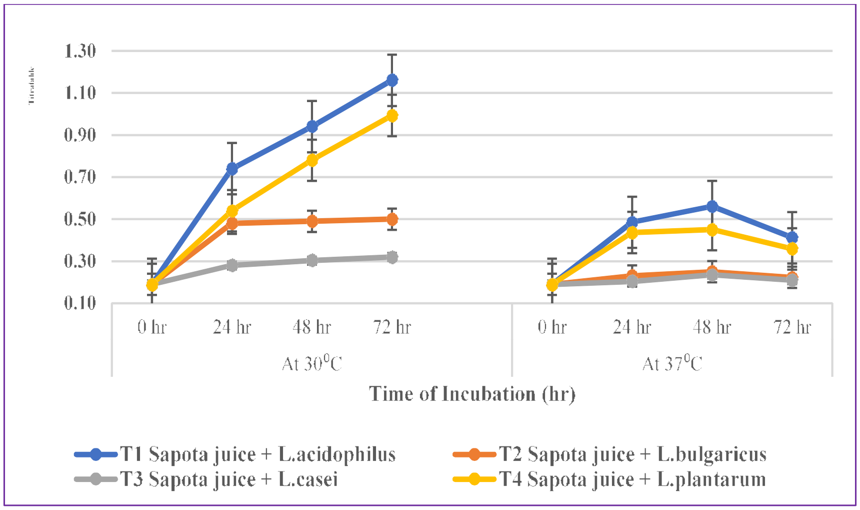


**Fig 2:** Effect of incubation temperatures on titratable acidity of sapota juice inoculated with lactic acid bacteria cultures.

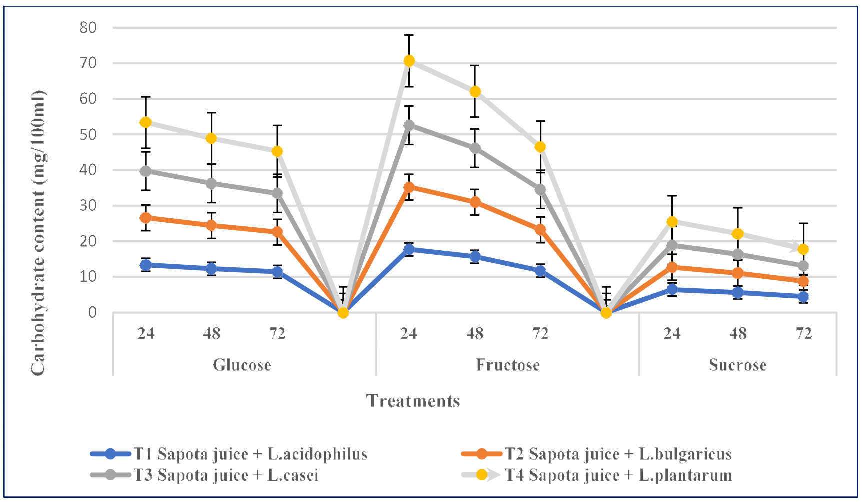


**Fig 3:** Sugar contents of sapota juice prepared using probiotics cultures and different sugars at time intervals.

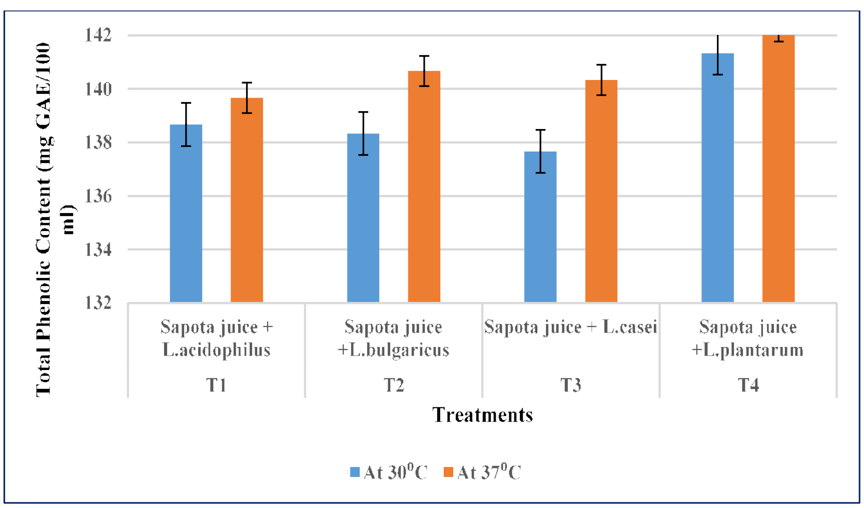


**Fig 4:** Estimation of Total Phenolic concentration in probioticated sapota juice after 72 hours of fermentation.

ascorbic acids, carotenoids and other phenolics during fermentation, which have been reported to have many beneficial health properties. The presence of total phenolics in sapota juice samples were in accordance with an earlier report on raw or non probioticated sapota juice (Anand *et al.,* 2007). DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was significantly higher in probioticated sapota fruit juices than in non probioticated fruit juices. Probioticated juices had higher levels of organic acids, which might have formed during fermentation.

**Viable cell counts at different time intervals**

The probiotic sapota juice were kept for 72 hours of incubation at 30°C and 37°C to know the effect of different incubation temperatures on probiotic bacteria (viable cell counts). Increase in viable cell counts were recorded in both samples incubated at 30°C and 37°C from the time of incubation (0 to 48 hours) and gradually decreased after 72 hours of incubation (Fig 5).

Less number of viable cells were recorded in the samples after 72 hours of fermentation and this decline in viable count may be due to depletion of available nutrients, low pH and oxygen levels and autolysis of cells (Daneshi *et al.,* 2013; Sharma and Mishra, 2013). Better health benefits can be derived only when a significant number of viable cells are present in the finished product and the ability of probiotic bacteria to with stand the stressful conditions like low pH levels a beneficial trait to maintain optimum population in the probiotic fruit juice. The Lactobacillus *plantarum* and *L. acidophilus* culture survived and grew well at lower pH levels in sapota juice compared to *L. bulgaricus* and *L. casei*. An earlier study with fruit juices indicated the growth of *Lactobacillus plantarum*, which resulted in a viable count of $8.0\times10^8$ CFU/mL after 72 hours of fermentation (Mousavi *et al*., 2011).

Maintaining the viable cells and the activity of probiotic bacteria till the end of shelf life are two important criteria to

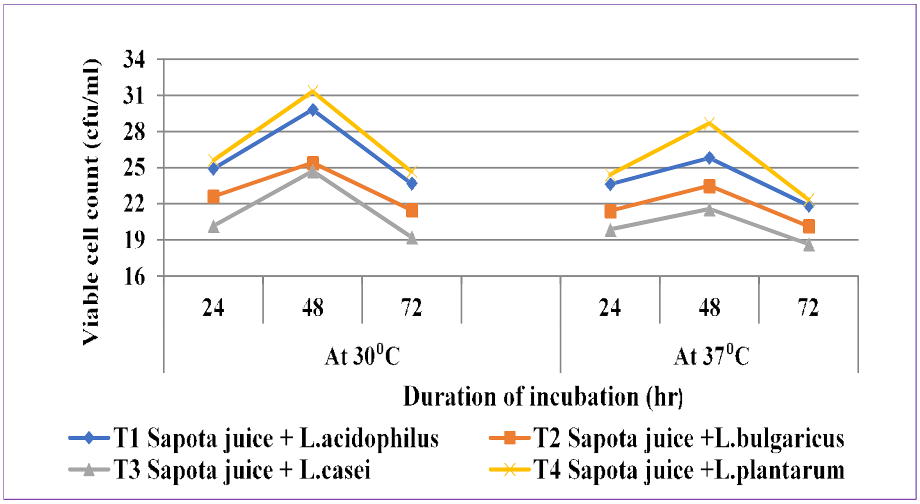


**Fig 5:** Effect of cold storage (4°C) on lactic acid bacterial population in sapota juice incubated at different temperatures at different weeks intervals.

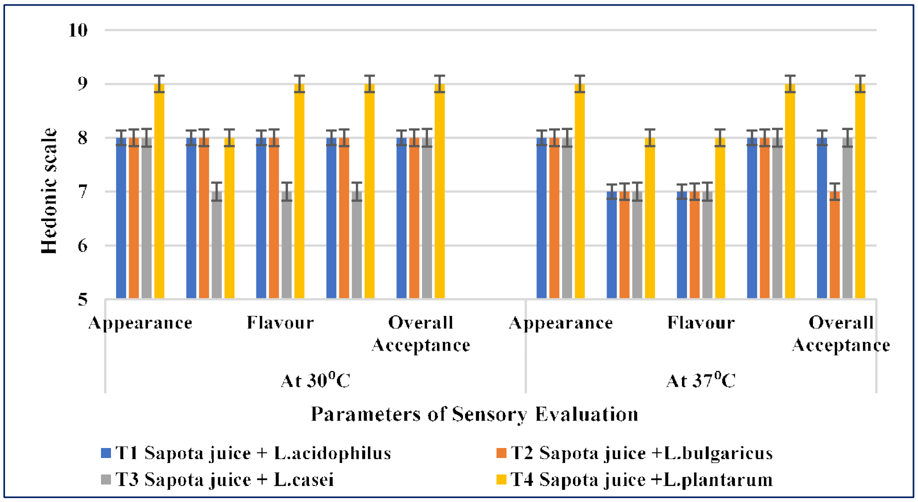


**Fig 6:** Sensory evaluation of probioticated and non probioticated sapota fruit juice.

be fulfilled in juices, where low pH represents a drawback. Several strains of *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Lactobacillus casei* can grow in fruit matrices due to their tolerance to acidic environments. The health benefit of probiotics mainly relies upon their concentration in foods, as well as on their ability to survive to adverse conditions of the gastrointestinal tract. Minimum recommended probiotic population should be at least greater than $10^7$ CFU/ mL at the end of shelf life to confer the health benefits (Nualkaekul and Charalampopoulos, 2011, Carbo *et al.,* 2014, Tripathi *et al.,* 2014).

### Sensory evaluation

Probioticated sapota juices had good sensory scores. No significant difference was found between the treatments pertaining to sensory scores of probioticated sapota juice samples (Fig 6) and the influence of fermentation on juice texture, taste, flavour and overall acceptance was insignificant. Some of the workers reported that the probiotics did not affect the overall acceptance of fruit juices *i.e.,* pineapple juice containing *Lactobacillus reuteri* (Perricone *et al*., 2014) apple juice containing *Lactobacillus casei* (Ellendersen *et al*., 2012). The studies reveal that the fruit juices can be used for fermentation with probiotic bacteria for health benefits especially to lactose intolerant people and those who are allergic to milk based products.

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### Conflict of interest

Authors declare that there is no conflict of interest.

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