JEB®

Journal of Environmental Biology

p-ISSN: 0254-8704 • e-ISSN: 2394-0379 • CODEN: JEBIDP

Journal website : www.jeb.co.in ★ **E-mail :** editor@jeb.co.in

Triveni Enterprises (Educational Services Set-up)

Original Research

DOI : http://doi.org/10.22438/jeb/43/1/MRN-1940

Influence of probiotics on physico-chemical and organoleptic characteristics of sweet orange juice

H.W. Deshpande*, S.D. Katke and A. Poshadri

Department of Food Microbiology and Safety, Vasantrao Naik Marathwada Agricultural University (MAU), Parbhani- 431 402, India

***Corresponding Author Email :** hemantd22@gmail.com

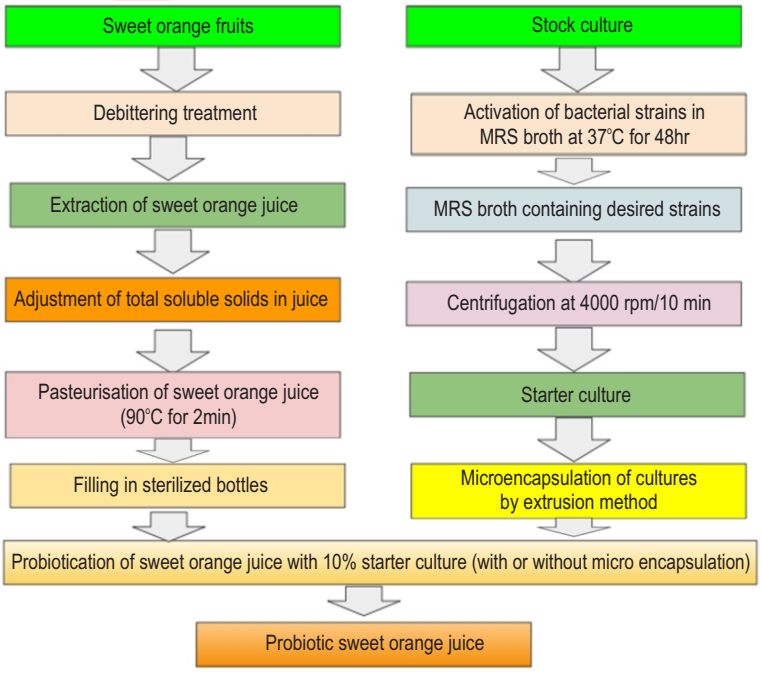
Received: 19.04.2021 *Revised*: 15.07.2021 *Accepted*: 09.09.2021

Abstract

Aim: The study was undertaken to evaluate the survival probiotic organisms and its influence on the physical, chemical, nutritional and sensory characteristics of sweet orange juice.

Methodology: Two samples of probiotic juice were prepared with 10 percent innoculum containing LAB strains (*Lactobacillus bulgaricus* and *Lactobacillus plantarum*). Sample A (without encapsulated strains) and Sample-B (with encapsulated strains) were prepared and incubated for 10hrs at 35°C. After incubation, the physico-chemical analysis of both the samples were analyzed for TSS, pH, acidity, total sugars, reducing sugars and ascorbic acid content.

Results: The results of TSS, pH, acidity, total sugars, reducing sugars and ascorbic acid content for sample –A and Sample –B were 11.4°Brix, 3.51, 0.82 percent, 6.1 percent, 1.5 percent, 4.6 percent, 40mgml^{-1} and 11.6° Brix, 3.68, 0.77 percent, 6.4 percent, 1.7 percent, 4.9 percent, 40 mg ml^{-1}, respectively. Sensory evaluation revealed that overall acceptance of probiotic juice containing encapsulated strains and free strains in the first week was 8.3 and 7.8, respectively. Even after 4 weeks of storage, the overall acceptance for juice with encapsulated strains was better than free strains with a score of 7.5 and 7.0 at the end of storage period.



Interpretation: The sweet orange juice with encapsulated strains has high viable cell count (10^{9}cfu ml^{-1}) even after 4 weeks of storage resulted in stable therapeutic probiotic sweet orange juice. It is further, suitable for commercial production of probiotic sweet orange juice with probiotic cultures.

Key words: LAB strains, *Lactobacillus bulgaricus*, Probiotic juice, Sensory analysis, Sweet orange

How to cite : Deshpande, H.W., S.D. Katke and A. Poshadri: Influence of probiotics on physico-chemical and organoleptic characteristics of sweet orange juice. *J. Environ. Biol.*, **43**, 170-176 (2022).

Introduction

Citrus is the third most important fruit crop after banana and mango in India. It is grown on 1.03 million ha area with 12.5 million tons production and 9.7 tons ha^{-1} productivity (NHB, 2018). Mandarins (*Citrus reticulata* Blanco) is the largest grown commercial citrus cultivar in India with 40.6% share, followed by sweet orange (*Citrus sinensis* L. Osbeck) with 25% area, acid lime and lemons (*Citrus aurantifolia* Swingle) with 25% area and others contribute 7% share. Sweet oranges (*Citrus sinensis* L. Osbeck.) are citrus fruits that belong to Rutaceae family. Sweet orange is a hybrid of *C. reticulate* (Mandarin) and *C. maxima* (Pumello). Sweet oranges are widely cultivated in tropical and sub tropical climates for its tasty juice and medicinal value. They are generally available from winter throughout summer with seasonal variations depending on the variety (Parle Milind *et al.,* 2012). Sweet oranges are an excellent source of vitamin C and sufficient amount of folacin, calcium, potassium, thiamine, niacin and magnesium and also a powerful natural antioxidant that builds the body immune system (Etebu and Nwauzoma, 2014).

Fresh juice of sweet orange is refreshing, thirst quenching and energizing drink that improves health and nutritional requirements. Fortification of foods and beverages with probiotics are growingly introduced into the functional food market (Zhang *et al.,* 2018).According to global market trends probiotic market will rise up to worth $46.55 billion by 2020, incorporating probiotics in different kind of food and beverage products to have beneficial therapeutic effect on human health (Patel, 2017).Traditionally, probiotics are used in yogurt and other fermented dairy products but nowadays, there is an increasing interest in non-dairy-based probiotic products (Espinoza and Navarro, 2010). The use of dairy products to deliver probiotics may cause some inconvenience for those with lactose intolerance and cholesterol problems. There is a genuine interest in the developing fruit juice based functional beverages with probiotics (Espinoza and Navarro, 2010). Probiotication of fruit juices is beneficial, as they are rich source of healthy nutrients such as antioxidants, vitamins, food fibers and minerals. Furthermore, fruits and vegetables do not contain any dairy allergens that might prevent usage by certain segments of the population (Luckow and Delahunty, 2004).

Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) defines probiotics as "live micro-organisms which when administered in adequate amounts confer a health benefit on the host". A number of studies have concluded that the number of cells required to affect the gastrointestinal environment ranges between 10^6 to 10^8 cfu ml^{-1} or cfu g^{-1} of the food item and this accepted dose is called as "the therapeutic minimum. Nonetheless, to boost beneficial health effects, microorganisms with probiotic claims must viable exposure to environmental factors, being able to colonize and continue the metabolic activity in the human gastrointestinal tract (Rodrigues *et al.,* 2020). It is essential that encapsulation of probiotic cell may enhance the viability of probiotic organisms under adverse environmental factors (Kim *et al.,* 2017) minimizing cell losses of encapsulated microorganisms in hydrocolloid based carrying material (Rodrigues *et al.,* 2017). In this sense, the selection of probiotic strains from a group of lactic acid bacteria (LAB), encapsulation of identified probiotic organisms, optimization of concentration of probiotic cells for probiotifcation of juice are vital for developing non-dairy based probiotic sweet orange beverage with acceptable sensory attributes. No studies have been conducted on the production of delayed bitterness free probiotic sweet orange juice and on sensory and phyico-chemical changes during storage. The core objective of this study was to produce probiotic sweet orange juice containing viable probiotic cells of *Lactobacillus bulgaricus* and *Lactobacillus plantarum* under refrigerated conditions. The survival of *Lactobacillus bulgaricus* and *Lactobacillus plantarum* and physico-chemical properties including acidity, pH, reducing sugars, total sugars, ascorbic acid content and sensory properties were evaluated during storage at weekly intervals up to 30 days.

Materials and Methods

Isolation and purification of lactic acid bacteria from curd and pickle weekly intervals for a period of 4 weeks: Curd and pickle samples were used for isolating probiotic LAB cultures. From each sample, 1: 10 serial dilution, 0.1ml from each dilution was sub-cultured aseptically on Man Rogosaand Sharpe (MRS) agar and incubated at 37°C for 24-48 hrs. Subsequently, isolated colonies were inoculated into MRS broth and incubated for 24 hrs. After vigorous growth of culture, it was again inoculated on MRS agar to identify pure culture by using Gram staining, biochemical tests and carbohydrate fermentation profile (FSSAI, 2016). The cultures of *Lactobacillus bulgaricus* and *Lactobacillus plantarum* were identified according to Bergey's Manual of Determinative Bacteriology and confirmed the identification by 16S rRNA multiplex PCR analysis (Mulamattathil *et al.,* 2014). After identification, *Lactobacillus bulgaricus* and *Lactobacillus plantarum* were cultured on MRS media slants and incubated at 37º C for 48 hrs and stored at 4°C for further use as stock culture. The stock cultures of *Lactobacillus bulgaricus* and *Lactobacillus plantarum* were individually propagated in MRS broth at 37°C for 48hr without agitation. Thirty millilitres of cell suspension were transferred into a centrifuge tube, and the cell pellets were collected by centrifugation (Thermo Fisher Scientific, USA) at 4000 rpm for 15 min, and were aseptically washed twice with sterile water. The biomass was taken as starter culture.

Encapsulation of probiotic organisms: Microencapsulation of probiotic bacteria was performed by extrusion technique. The hydrocolloid coating material was prepared by using a combination of sodium alginate and guar gum at 1 and 0.8% (w/v) respectively. For probiotification of 100 ml sweet orange juice, 10 ml of inoculum (5 ml each of *L. bulgaricus* and *L. plantarum*) was mixed in 20 ml of polymer solution. Probiotic cultures and polymer solution were mixed properly and passed through a syringe in the form of droplets into 0.3M calcium chloride solution. Interaction between the two solutions led to formation of beads (2-5mm) and the resulting beads were then stored in 0.1% peptone solution at 4°C (Poshadri and Aparna, 2010).

Standardization of activated charcoal and lye treatment for prevention of delayed bitterness in juice: The bitterness

caused by limonin is referred to as delayed bitterness, since it is not detected in fresh juice but develops gradually and slowly during storage or with heat treatment. Therefore, prevention of delayed bitterness in mosambi juice was done by using a combination of processes like activated charcoal treatment and lye peeling. The first adsorbent used in food industry for debittering of juices was activated carbon which reduced the bitterness effectively. However, this method is no longer in use as it results in loss of many vital components such as vitamins and carotenoids along with reduction in bitter components. Hence, an attempt was made to prevent the bitterness by dipping the peeled fruits in activated charcoal solution instead of treating the juice to adsorb the bitter precursors from the surface and core of the fruit. The second method opted for debittering was lye treatment of peeled fruits. Lye peeling of segments reduces the bitterness in Kinnow juice due to removal of white papery segment walls (Sandhu and Singh, 2001). The treatments were standardized based on organoleptic evaluation.

Juice extraction and probiotification of sweet orange juice: Peeled fruits were dipped in 1% activated charcoal solution and allowed to stand for 1hr. The fruits were then lye peeled by dipping it in boiling lye solution for 2 min to remove the albedo section which is the major contributor of limonin precursors during juice extraction. After lye treatment, the fruits were washed thoroughly in running tap water and the remaining alkali was neutralised by dipping in citric acid solution for 1min and washed again. Juice was extracted without pressing the seeds and subsequently the juice was filtered using a strainer and the filtered juice was collected in a dispenser. The juice was pasteurized at 90°C for 30 to 60 sec and packed hot in sterilized glass bottles. Sweet orange juice with the final TSS of 12°Brix, inoculum level at 10% and incubation period of 10hr finalized through sensory evaluation by blending different TSS content juice with range of inoculum levels (6, 8 and 10%) and incubation period. For preparing sample A (without encapsulated cultures), the starter culture was added to the juice at 10% inoculum level (5 per cent each of *L. bulgaricus* and *L. plantarum*) and incubated at 37°C for 10hr. The probiotic juice was then stored at refrigerated conditions (4°C). For the preparation of sample B with encapsulated strains, inoculum at 10 per cent of the final juice was encapsulated and the beads were aseptically added to 100ml pasteurized fruit juice and incubated at 37°C for 10 hrs. The probiotic juice was stored at refrigerated conditions (4°C).

Physico-chemical characteristics of probiotic sweet orange juice: Total soluble solids (°Brix) and pH of juice and the developed probiotic Sweet orange juice were measured with the help of digital pocket refractometer and pH meter, respectively. Acidity was determined by titrating against 0.1 N NaOH and expressed as percentage of citric acid (Datta Mazumdar *et al.,* 2012). Ascorbic acid content, reducing sugars, non reducing sugars and total sugars were determined using approved AOAC methods (2019).

Sensory evaluation of probiotic sweet orange juice samples: Probiotic sweet orange juice samples (A and B) was evaluated for sensory characteristics like appearance, color, taste, flavor and overall acceptability post incubation period and also during storage at refrigerated conditions. Sensory evaluation was conducted in laboratory by a 25 panel of semi trained judges which comprised of postgraduate students and academic staff members of College of Food Technology, V.N.M.K.V., Parbhani. Samples were scored based on a nine point hedonic scale. Judges were asked to rate the product on 9 point Hedonic scale with corresponding descriptive terms ranging from 9 'like extremely' to 'dislike extremely (Meilgaard *et al.,* 1999).

Survival of probiotic bacteria: To determine the viability of encapsulated probiotics in juice, enumeration was done at weekly intervals by releasing the entrapped strains from the microcapsules following the method of Sheu and Marshall (1993). One gram of micro-encapsulated beads was added in test tubes containing 10 ml of depolymerization solution and incubated at 37°C for 10 min. The mixture was vortexed at high speed for breaking the polymer formed and releasing the encapsulated culture into the buffer. The number of probiotic bacterial cells in juice were enumerated using MRS media at 37°C for 24-48 hr. Enumeration was done by pour-plate method. The population was recorded for every enumeration and expressed in cfu ml^{-1}.

Microbial analysis of probiotic juice: Microbial analysis was conducted to determine the total plate counts (TPC) on plate count agar and for enumeration of yeast and moulds on potato dextrose agar and incubated at 37°C for 48 hr for TPC and at 25°C for 48 hr for yeast and moulds, respectively. Colonies were counted and expressed as colony forming units (cfu) per gram (Harrigan and Mc.Cance,1966). The presence of coliforms in high numbers (CC >2 log10 CFU ml^{-1}) indicate the contamination of juice and consumption resulting in food borne diseases. Following pour plate technique, 1 ml of aliquots were inoculated onViolet Red Bile agar and incubated at 35°C for 24 hr. As *Coliform* gives red pink colonies on VRB agar so it was used for examination. Red colonies surrounded by a zone of precipitate are reported as "presumptive coliforms in cfu ml^{-1}.

Statistical analysis: The experimental data were analyzed by ANOVA followed by Duncan's Multiple Range Test ($P < 0.05$) to determine the significant difference among samples. The data were analyzed according to user's guide of statistical analysis system (SAS, 1996).

Results and Discussion

The perusal of data showed that during 10 hrs of incubation, free strains in probiotic juice reduced the TSS to 11.4°Brix (sample A) and the encapsulated strains reduced it to 11.6°Brix (Sample B) along with reduction in pH of the juices by both without and with encapsulated strains leading to increase in acidity (Table 1). This reduction in pH may due to utilization of sugars present in juice by the probiotics to produce organic acids (Afzaal *et al.,* 2020). Similarly, decrease in the pH of carrot juice was observed due to the addition of *Lactobacillus acidophilus* (Shigematsu *et al.* (2018). The final °Brix, pH and total sugars of sample B were higher than those of sample A. These results are also in agreement with the reports of Ding and Shah (2008). The percent acidity of both the samples A and B increased to 0.82 and

0.77, respectively. The total sugars of both the samples also reduced to 6.1 in sample A and 6.4 in sample B. These values could have been lower if the TSS was not adjusted to 12°Brix. The prepared probiotic juices showed a decline in ascorbic acid content which may be attributed to treatments and processing conditions before and after juice extraction. The ascorbic content of both the samples decreased to 40 mg $100ml^{-1}$.

The results of sensory evaluation revealed that the taste score of sample A (8.4) was at par with sample B (8.6) but B was tastier than control sample. Considering the overall acceptability of sample A and B concluded that there was no significant difference in sensory characteristics of freshly prepared probiotic juice sample A and B after 10 hrs of incubation (Table 2). However, considering the higher sensory score in sample B, it can be concluded that encapsulation of LAB strains may have prevented excess utilization of sugars, controlling the pH and percent acidity production at optimum level resulting in better acceptability of the sample. Although juice containing beads is a new product, consumers compared the sample with the commercial juices containing juice sacs, and thus found it to be highly acceptable. The mean sensory score of freshly prepared probiotic juice samples is presented in Fig. 1. The sensory evaluation of probiotic sweet orange juice was also performed at weekly intervals for a period of 4 weeks to examine the acceptance and the scores are presented in Table 3. The overall sensory acceptability of sweet orange juice with or without encapsulated probiotic cells presented average scores between 8 (liked very much) and 7 (liked moderately) with no significant influence ($P > 0.05$) of storage time (Table 3). In case of sweet orange juice without encapsulated probiotic cells (Sample-A) that presented the lowest acceptance by the panelist at 30 days ($P < 0.05$). The overall assessment showed that probiotification of sweet orange juice with encapsulated LAB cells had average overall acceptance scores between 7.5 to 8.0, meaning "liked it a lot", with a higher preference by panelists in the end of 4^{th} week ($P < 0.05$). In this way, we can say that the production of delayed bitterness tastes free sweet orange juice, as well as addition of encapsulated probiotic cells of *Lactobacillus bulgaricus* and *Lactobacillus plantarum*, contributed positively to the formulation of better acceptable probiotic sweet orange juice.

Probiotification of sweet orange juice (B) with encapsulated probiotic cells of *L. bulgaricus* and *L. plantarum* had significantly ($p<0.05$) affected the sensory properties (appearance, flavor, taste, and general perception) of the products as compared to sample–A and control. The TSS concentration declined from an initial value of 11.6 to 10.7°Bx during a storage period of 4 weeks. The changes in pH during storage was 3.68, 3.65, 3.64, 3.59 and 3.59 on the day of preparation, first week, second week, third week and fourth week after production, respectively. LAB cultures may have utilized carbohydrates and produced small amounts of organic acids thus lowering the pH of the product during storage. The changes in pH and growth of probiotic culture are much related to each other. Shukla *et al.* (2013) also reported a decrease in the pH of probiotic beverage from Whey and pineapple juice after 28 days of storage. Ding and Shah (2008) concluded that probiotic strains reduced the pH of juice during storage regardless of whether they are in free or encapsulated form. It was also observed that the titratable acidity of sample increased during storage which may be attributed to increase in acids due to breaking down of sugars to acids by LAB cultures. The acidity values of beverage sample significantly increased from 0.77 per cent on the day of

Table 1: Physico-chemical properties of standardized probiotic sweet orange juice samples

Properties	Sample A	Sample B
TSS (°Brix)	12.0±0.4*	12.0±0.3*
% Acidity	0.82±0.02	0.77±0.05
pH	3.51±0.02	3.68±0.04
Total Sugars (%)	6.1±0.3	6.4±0.4
Reducing Sugars (%)	1.5±0.1	1.7±0.1
Non Reducing Sugars (%)	4.6±0.3	4.9±0.5
Ascorbic Acid (mg $100ml^{-1}$)	40±2	40±1

*TSS of extracted juice was adjusted to12°Brix before inoculation of strains; Sample A= without encapsulated strains ; B = with encapsulated strains

Table 2: Mean sensory score of freshly prepared probiotic juice samples

Sample	Color	Taste	Flavor	Overall Acceptability
Control	8.6±0.2	8.3±0.1	8.1±0.3	8.2±0.1
A	8.3±0.3	8.4±0.4	8.5±0.2	8.4±0.3
B	8.5±0.2	8.6±0.1	8.5±0.2	8.5±0.3
SE	0.13176	0.06455	0.05528	0.02357
CD @ 1%	0.5443	0.26665	0.22835	0.09737

*Each value is average of 25 determinations

Table 3: Mean sensory scores of Sample A and B during storage

Time in Weeks	Sample A	Sample B
0	8.0±0.3	8.4±0.2
1	7.8±0.1	8.3±0.3
2	7.7±0.6	8.0±0.3
3	7.3±0.2	7.9±0.1
4	7.0±0.1	7.5±0.4
SE	0.07169	0.06455
CD @ 1%	0.29613	0.26665

*Each value is the average of 25 determinations

Table 4: Chemical changes in sweet orange juice with encapsulated probiotic cells during storage

Time (weeks)	TSS (°Brix)	pH	% Acidity (Lactic acid)	Ascorbic acid (mg 100 ml^{-1})	Total Sugars (%)
0	11.6	3.68±0.02	0.77±0.01	40±2	6.4±0.2
1	11.3	3.65±0.05	0.79±0.04	39±4	6.4±0.1
2	11.1	3.64±0.03	0.93±0.03	37±2	6.2±0.3
3	11.0	3.59±0.01	1.01±0.01	34±3	6.0±0.2
4	10.7	3.59±0.06	1.03±0.01	33±1	5.8±0.1

Table 5: Probiotic cell viability cfu ml^{-1} and other microbes in sweet orange juice Sample-B with encapsulated probiotic cells

Time (weeks)	Viability of Probiotic LAB cultures	Total Plate Count (cfu ml^{-1})x10^8	Yeast & Mould count (cfu ml^{-1})x10^3	Coliform count
0	$3.0x10^9$	$2.5x10^8$	Absent	Absent
1	$3.1x10^9$	$2.9x10^8$	Absent	Absent
2	$4.7x10^9$	$3.9x10^8$	$1.6x10^3$	Absent
3	$2.6x10^9$	$5.1x10^8$	$1.2x10^3$	Absent
4	$1.5x10^9$	$4.8x10^8$	$1.0x10^3$	Absent

preparation to 1.03 per cent in the fourth week of storage (Table 4). As anticipated, pH decreased and acidity increased during the first fermentation. Similar results for total acidity (0.5 to 1.7%) and pH (6.3 to 4.1) were reported at the end of fermentation in probiotic juice of prickly pears (Sandeep *et al.*, 2017).

During storage, the ascorbic acid content of probiotic sweet orange juice with encapsulated LAB culture was reduced (40 mg $100ml^{-1}$ to 33 mg $100ml^{-1}$). Sandeep *et al.* (2017) reported decrease in ascorbic acid content of the juice from (10 mg $100ml^{-1}$ to 6 mg $100ml^{-1}$) in the lacto-juice of prickly pears fermented with probiotic *Lactobacillus fermentum* - ATCC 9338 culture. Further, it was reported that lactic acid fermentation resulted in decrease of ascorbic acid content in fermented camel and goat milk (Bahobail *et al.,* 2014). The total sugars showed a decline from 6.4 percent at initial to 5.8 percent at the end of storage period.

The viability of probiotics during storage is of paramount importance because for a probiotic food to confer health benefit, the number of cells should be > 10^7cfu ml^{-1} or gm at the time of consumption. To determine the viability of encapsulated probiotics in the juice, the enumeration will be done at weekly intervals by releasing the entrapped strains from the microcapsules following the method of Sheu and Marshall (1993). The viable cell counts enumerated in Table 5 indicated that the number of probiotic bacteria's increased from an initial number of 3.0 x 10^9 to 4.7 x 10^9 during second week of storage. However, viable counts of probiotic bacteria decreased after third and fourth week of storage at 4°C. The colony forming unit count of sweet orange juice presented satisfactory values for the beverage to be considered a probiotic juice, in other words, probiotic cell count equal or at above $1.5x10^9$ CFU ml^{-1} (Galvão *et al.,* 2020). A rapid reduction was observed in non-encapsulated bacterial cells dried apple snack in contrast to the cells encapsulated with sodium alginate and carrageenan (Afzaal *et al.,* 2020). Tootoonchi *et al.* (2015) reported that the number of *Lactobacillus acidophilus* and *Lactobacillus casei* bacteria in orange juice after 4 weeks of storage at 4°C was more than 10^8 and 10^7 bacteria per ml of juice, suggesting that the capsules

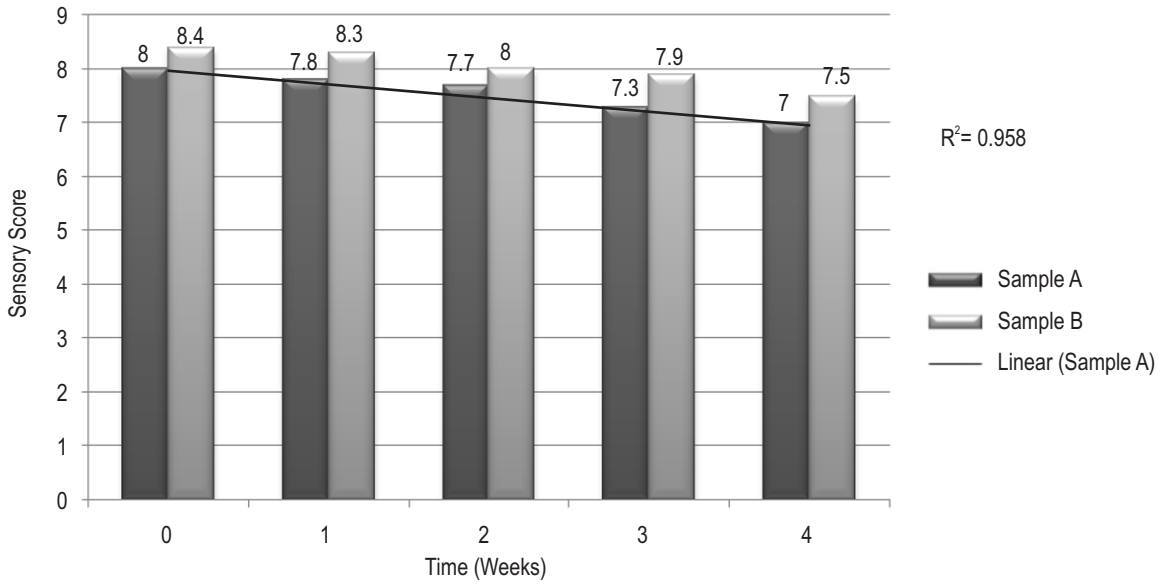


Fig. 1: Sensory score (overall acceptance) of probiotic sweet orange juice samples A and B during storage.

containing lactobacillus and acidophilus could preserve the required durability during their containment in orange juice.

Moreover in a study, Ding and Shah (2008) studied the survival of 8 strains of probiotic bacteria in their free and encapsulated forms in orange juice and showed that the number of probiotics had rapidly reduced up to 4 weeks of storage in 4°C in orange and apple juices and had lost their ability to survive after 5 weeks of storage whereas bacteria encapsulated in fruit juice had survived after 6 weeks of storage. They reported that probiotics encapsulated in orange juice and apple juice are more durable than free cells. However, the main factors for loss of viability of probiotic organisms have been attributed to the decrease in the pH of the medium and accumulation of organic acids as a result of growth and fermentation (Yoon *et al.,* 2006).

The accepted probiotic juice sample B was assessed for total plate count, yeast and mould count and *Coliform* growth during storage period and the results observed are presented in Table 5. Sweet orange juice sample-B with encapsulated probiotic cells was free off *Coliform* and *E. coli* when the samples were fresh and throughout the storage period of 4 weeks at refrigerator temperature (4°C) as result of good hygienic and sanitary conditions, during the preparation of juice. Yeast and mold was not detected until second week, while the total plate count was 2.1 $x10^8$cfu ml^{-1} in the first week, which further increased to 5.1$x10^8$ cfu ml^{-1} in the third week and then decreased to 4.8$x10^8$ cfu ml^{-1} in the fourth week. Staniszewski and Kordowska (2021) reported that yeast counts were strongly correlated with LAB count. Co-metabolism between yeast and LAB and *Bifidobacterium bifidum* may exist where bacteria provides acid environment, which selects the growth of yeast, that in turn provide vitamins and other growth factors to the bacteria. Railany *et al.* (2020) clarified Cerrado cashew juice and supplemented with probiotic Sacharomyces boulardii culture and reported that the chemical composition of fruit juice is also responsible for the maintenance of probiotic viability during storage.

The progressive decrease in yeast and mould count may be due to resultant increase in acidity during storage. The development and production of sweet orange juice containing probiotic microorganism using encapsulation technology was successful. Probiotics of LAB strains were added in encapsulated and free form. When stored for 30 days at 4°C, sweet orange juice with encapsulated strains showed more viability than that of free form and organoleptically more acceptable and reduced the bitterness of juice which indicates the use of sweet orange juice as an effective probiotic carrier.

The results of the study show that microencapsulation has a vital role in enhancing the viability and stability of probiotics in acid environment of juice. Further, the results also reveal that the development of sweet orange juice containing probiotic cells is an attractive approach to market the juice as nutritious and functional beverage.

Acknowledgments

Authors are thankful to Vasantrao Naik Marathwada Agricultural University, Parbani for providing financial support for conducting the research work effectively. We also express special thanks to the practicing farmers of sweet orange.

Add-on Information

Authors' contribution: H.W. Deshpande: Project concept and encapsulation of Probiotics; **S.D. Katke:** Sweet orange juice extraction, Probiotification of juice; **A. Poshadri:** Microbial and sensory evaluation.

Research content: The research content of manuscript is original and has not been published elsewhere.

Ethical approval: Not applicable

Conflict of interest: The authors declare that there is no conflict of interest.

Data from other sources: Not applicable

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology*.

References

Afzaal, M., S. Farhan, S. Hussain, A. M. Abdellatif, M.S. Alamri, A. Ahmad, H. Ateeq, T. Tufail and M. Hussain: Survival and storage stability of encapsulated probiotic under simulated digestion conditions and on dried apple snacks. *Food Sci Nutri.,* **8**, 5392-5401 (2020).

AOAC: Official Methods of Analysis. 21st Edn., Rockville, Maryland, US (2019).

Bahobail, A.A. and A.A. Abdallah: Effect of fermentation process on the improvement of nutrition value of camel milk. *Int. J. Multidisci. Curr. Res.,* **2**, 78-81 (2014).

Datta Mazumdar, S., A. Poshadri, P.S. Rao, C.R. Reddy and B.V.S. Reddy: Innovative use of sweet sorghum juice in the beverage industry. *Int. Food Res. J.,* **19**, 1361-1366 (2012)

Ding, W.K. and N.P. Shah: Survival of free and microencapsulated probiotic bacteria in orange and apple juices. *Int. Food Res. J.,* **15**, 219-232 (2008).

Espinoza-Rivera, Y. and Y. Navarro-Gallardo: Non-dairy probiotic products. *Food Microbiol.,* **27**, 1–11(2010).

Etebu, E. and A.B. Nwauzoma: A review on sweet orange (*Citrus Sinensis* Osbeck): Health, diseases, and management. *American J. Res. Comm.,* **2**, 33-70 (2014).

Food Safety and Standards Authority of India. Manual on method of microbiological testing. Available online: https:// old. fssai. gov. in/ Portals/0/Pdf/Microbiological_Testing_Foods_Draft_ Manual_06_09_2016.pdf(accessed on 18 February, 2020).

Galvão, A.M.M.T., S. Rodrigues and F.A.N. Fernande: Probiotic dried apple snacks: Development of probiotic coating and shelf-life studies. *J. Food Proc. Pres.,* **44**, 14974 (2020).

Kim, J.U., B. Kim, H.M. Shahbaz, S.H. Lee, D. Park and J. Park: Encapsulation of probiotic *Lactobacillus acidophilus* by ionic gelation with electrostatic extrusion for enhancement of survival under simulated gastric conditions and during refrigerated storage. *Int. J. Food Sci. Tech.,* **52**, 519-530 (2017).

Luckow, T. and C. Delahunty: Which juice is healthier? A consumer study of probiotic non-dairy juice drinks. *Food Quali. Pref.,* **15**, 751-759 (2004).

Meilgaard, M., G.V. Civille and B.T.Carr: Sensory evaluation techniques. 3rd Edn., CRC Press, Boca Raton (1999).

Mulamattathil, S.G., B. Carlos, M. Moses and N.A. Collins: Isolation of environmental bacteria from surface and drinking water in Mafikeng, South Africa, and characterization using their antibiotic resistance profiles. *J. Pathog.,* **371208**, 1-11 (2014).

NHB..http://nhb.gov.in/statistics/State_Level/2018-19(1st%20Adv).pdf. (2018).

Poshadri, A. and K. Aparna: Microencapsulation technology: A review. *J. Res. ANGRAU.,* **38**, 86-102 (2010). A.O.A.C., Association of Official Analytical Chemists Official journal 17th Edn., Maryland: Gaithersburg, MD (2000).

Parle Milind and Chaturvedi Dev: Orange: Range of benefits. *Int. Res. J. Phar.,* **3**, 59-63 (2012).

Patel, A.R.: Probiotic fruit and vegetable juices- recent advances and future perspective. *Int. Food Res. J.,* **24**, 1850-1857(2017).

Railany, V. S., C.S. Daiane, A. S. Cláudia, G. O. Josemar, B. A. Adrielle, M. L. Thayanara, G. S. Fabiano, B. E. Mariana: Quality parameters and sensorial profile of clarified "Cerrado" cashew juice supplemented with Sacharomyces boulardii and different sweeteners. *LWT - Food Sci. Tech.*,**128**, 109319 (2020).

Rodrigues, F.J., M.F. Cedran, J.L. Bicas and H.H. Sato: Encapsulated probiotic cells: Relevant techniques, natural sources as encapsulating materials and food applications - A narrative review. *Food Res. Int.,* **137**, 109682 (2020).

Rodrigues, F. J., M.H. Omura, M.F. Cedran, R.F. Dekker, A.M. Barbosa-Dekker and S. Garcia: Effect of natural polymers on the survival of *Lactobacillus casei* encapsulated in alginate microspheres. *J. Microencap.,* **34**, 431-439 (2017)

Sandeep, K.P., K.B. Sunil, X.Q. Witness, Sudharshan Sekar, T.N. Derek, H.M. Nanjundaswamy, R.C. Ray and K. Eugenie: Quality enhancement of prickly pears (*Opuntia* sp.) juice through probiotic fermentation using *Lactobacillus fermentum* - ATCC 9338, *LWT - Food Sci. Tech.*, **75**, 453-459 (2017).

Sandhu, K.S. and N. Singh: Studies on factors affecting the physicochemical and organoleptic properties of kinnow juice. *J. Food Sci. Technol.,* **38**, 266-269 (2001).

Sheu, T.Y and R.T. Marshall: Microentrapment of *Lactobacilli* in calcium alginate gels. *J. Food Sci.,* **54**, 557-561 (1993).

Shigematsu, E., C. Dorta, F.J. Rodrigues, M.F. Cedran, J.A. Giannoni, M. Oshiiwa and M.A. Mauro: Edible coating with probiotic as a quality factor for minimally processed carrots. *J. Food Sci. Tech.,* **55**, 3712–3720 (2018).

Shukla, M., Y.K. Jha and S. Admassu: Development of probiotic beverage from whey and pineapple juice. *J. Food Process. Technol.,* **4,** 1-4 (2013).

Staniszewski, A., M. Kordowska-Wiater: Probiotic and Potentially Probiotic Yeasts— Characteristics and Food Application. *Foods.*, **10**,1-33 (2021).

Toontoochi, P., J. Hesari, M. Moradi and F. Mehrnoosh: Survival of encapsulated *Lactobacillus acidophilus* LA5, *Lactobacillus casei* 431 encapsulated in orange juice stored in refrigerator temperature. *Int. J. Bio. Pharma. Allied Sci.,* **4**, 268-276 (2015).

Yoon, K.Y., E.E. Woodams and Y.D. Hang: Production of probiotic cabbage juice by lactic acid bacteria. *Bio. Reso. Techn.,* **97**, 1427-1430 (2006).

Zhang, L., X.D. Chen, R.M. Boom and M.A.I. Schutyser: Survival of encapsulated *Lactobacillus plantarum* during isothermal heating and bread baking. *LWT - Food Sci. Tech.,* **93**, 396-404 (2018).