Journal of Animal & Plant Sciences, 33(3): 2023, Page: 644-654
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2023.3.0657

VIABILITY OF MICROENCAPSULATED PROBIOTICS (*LACTOBACILLUS REUTERI*) IN GUAVA JUICE

M. S. Javed[1], M. Amir*[2], A. Amjad[3], M. Shah[4], M. J. Anwar[2] and F. Ahmad*[6]

[1] Department of Food Safety and Quality Management, Faculty of Food Science and Nutrition, Bahauddin Zakariya University, Multan, Punjab-Pakistan
[2]Department of Food Science and Technology, Faculty of Food Science and Nutrition, Bahauddin Zakariya University, Multan, Punjab-Pakistan
[3]Department of Human Nutrition, Faculty of Food Science and Nutrition, Bahauddin Zakariya University, Multan, Punjab-Pakistan
[4] Department of Biochemistry, Bahauddin Zakariya University Multan, Punjab-Pakistan
[6]Institute of Technology, Estonian University of Life Sciences, Tartu-Estonia
*Corresponding author's email: amirm4984@gmail.com

ABSTRACT

Probiotics play a pivotal role to reduce gastrointestinal problems by exerting a drastic effect on various pathogenic microflora of the colon. *Lactobacillus reuteri* CECT-925 loaded beads were prepared by emulsion containing sodium alginate and sesame seed oil. Encapsulation was done by spraying emulsion into a 0.5% solution of calcium chloride. Microencapsulated probiotics incorporated guava juice was assessed for physicochemical analysis at the 15-day interval for 60 days. The juice was tested for probiotics viability, titratable acidity, pH, total soluble solids and organoleptic properties. In the control sample, viable counts of encapsulated probiotics were reduced from 7.68 to 1.96 log10 CFU/ml while in T_1, T_2 and T_3 the initial numbers 7.39, 7.7 and 7.87 were reduced to 5.97, 6.87 and 6.02 log10 CFU/ml respectively at the termination of the storage period. However, pH and sensory scores decreased while total soluble solids and titratable acidity increased. Results indicated that microencapsulation by sodium alginate in combination with sesame oil retained the viability of *Lactobacillus reuteri* > 90% in guava juice. The acceptability of the product was 82.04% till the end of the storage period.

Key words:

Published first online February 10, 2023 Published final June 18, 2023

INTRODUCTION

Evolutionary spin and industrial dawn influenced human behaviour approaching processed foods, leading to a shift in metabolic pathways. This catastrophic change in lifestyle pushed people towards several diseases including gastrointestinal complications which have a direct effect on vital organs like the heart, lungs, liver, kidney and colon (Pabolu *et al*., 2021; Boesveldt *et al*., 2018). Metastasis and proliferation of the cancerous cells were also linked with free radicals produced due to notorious pathogens in the large intestine and modern-day processing. One of the strategies to lower the risk of intoxication and free radical progression is the use of probiotics (Singh *et al*., 2020).

The viability of probiotics is affected by gastrointestinal transit conditions so there must be a mechanism to protect these microorganisms from the harsh environment of the stomach. Microencapsulation is a promising process to incorporate the cells into a coating matrix that guard probiotics against harsh gastrointestinal conditions and ensure controlled releases (Millette *et al*., 2013). Microencapsulation is a mechanical or physiochemical process that is the entrapment of any substance or living cells in a material like sodium alginate, guar gum, gum arabic and other gelling substances to produce smaller particles of diameters ranging from a few millimetres to a few nanometers (Iqbal *et al*., 2021).

Probiotics are living microbes and when present in the gastrointestinal tract in considerable amounts may benefit host health (Divya and Nampoothiri, 2015). To promote the beneficial effects of probiotics, these must survive the gastric conditions of high acid and bile and should reach the large intestine in amounts being capable of colonization (Markowiak and Śliżewska, 2017). For optimum health benefits, the minimum recommended dose of probiotics ranges from 10^6 to 10^7 colony forming units (CFU/g) (Flach *et al*., 2018).

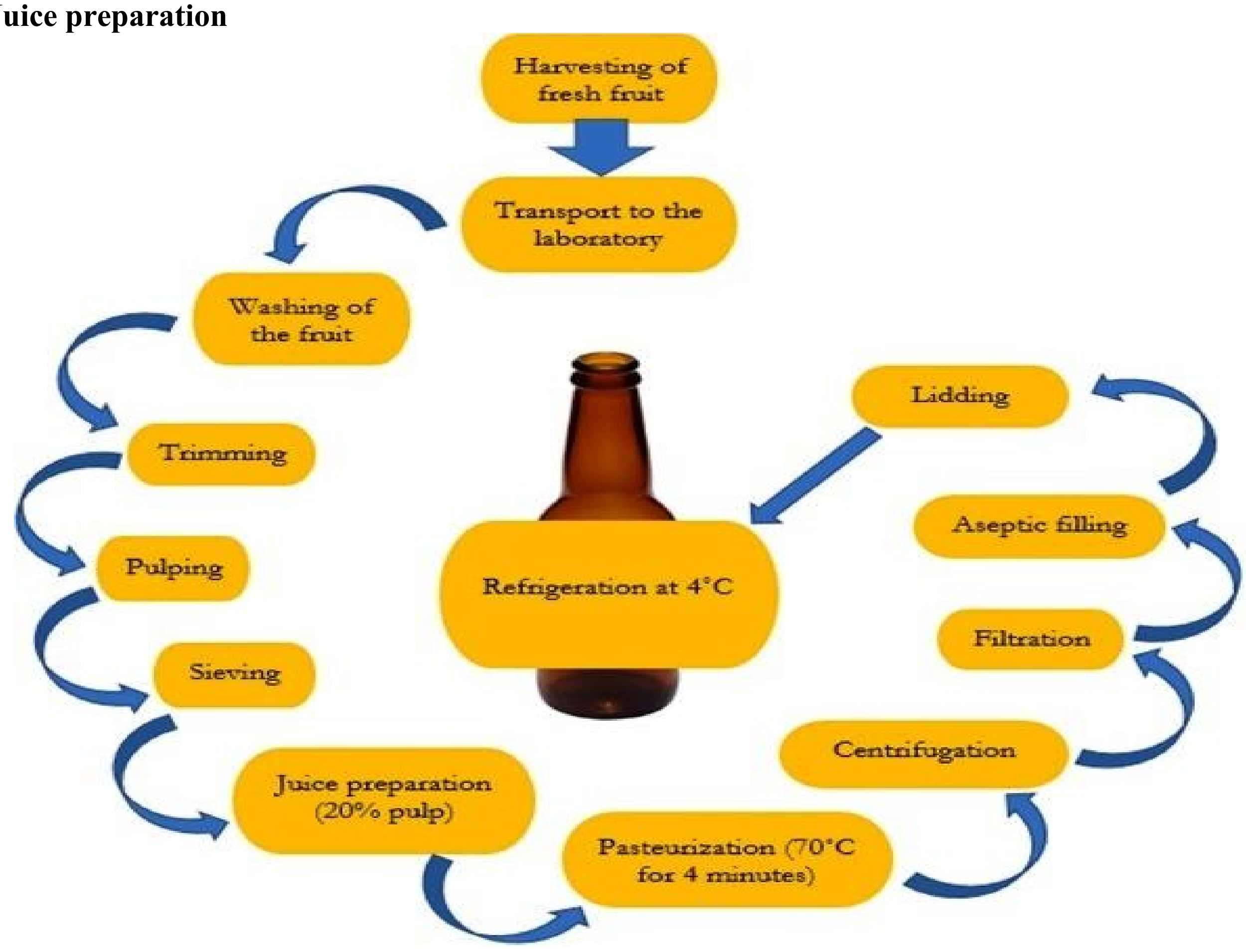
Fermentation and secondary metabolites by probiotics help to boost gut microflora that reduces lactose intolerance, liver toxicosis, act as an immunomodulator and lower body cholesterol (Jang *et al*., 2021). Probiotics are also linked with the mitigation of diarrhoea, antibiotic-associated diarrhoea, acute gastroenteritis, pouchitis and Crohn's disease (Domingo, 2017). The mixture of strains of probiotics is helpful in the treatment of *Helicobacter pylori* infection and inflammatory bowel disease (Bruno *et al*., 2018). Some of the nutraceuticals belonging to vitamin B are being produced by strains of lactic acid bacteria (Dianawati *et al*., 2016).

The delivery of encapsulated probiotics has remained a question for a long and many scientists tried different food systems for an efficient approach to the colon. Fruit juices are one of them because these are the natural reservoir of bioactive and nutraceuticals and can transfer encapsulated cells more quickly than solid foods (Terpou *et al*., 2019). Guava fruit contains 275% daily value of vitamin C and 9% of potassium. It is also comprised of > 5% dietary fibre of the flesh which is an added advantage for probiotics (Suwanwong and Boonpangrak, 2021). Phenolics can stimulate the human immune response to identify and kill cancer cells and prevent the angiogenesis required for cancer progression. They also reduce the adhesion and pervasiveness of tumour cells, thus decreasing their metastasis (George *et al*., 2021). So, there is a need to supplement the diet with fruit juices along with suitable probiotics to reduce the risk of non-communicable diseases and to promote the health of the individuals. The purpose of this study was to assess the survival of encapsulated cells in guava juice for 8 weeks because guava is rich in vitamin C and other phytochemicals possessing antioxidant activity and have the potential to defend against free radicals and reactive oxygen species to prevent degenerative and chronic disorders.

MATERIALS AND METHODS

Sample procurement: Fresh, white-fleshed Guava fruits were harvested from a town near Multan, the city of Pakistan and brought to the fruit and vegetable processing laboratory of the Institute of Food Science and Nutrition, Baha Uddin Zakariya University Multan, Pakistan. After pretreatments pulping and juice preparation was done at a 20% pulp ratio, under hygienic conditions to prevent any sort of contamination.

Juice preparation



Chemicals Used: Calcium chloride CAS 10043-52-4 anhydrous powder, MRS (Man, Rogosa, Sharpe) broth and food grade sodium alginate W-201502 were purchased from Merk (Germany). Other analytical grade chemicals for juice physicochemical testing were procured from Sigma Aldrich USA. *Lactobacillus reuteri* CECT-925 strain was procured from Sigma Aldrich USA in lyophilized form and stored at -80˚C.

Sterilization of the glassware: For activation of lyophilized cells, MRS broth and glassware were sterilized in a FALC vertical autoclave ATV600-60 LT by wrapping in a paper towel. Standard operating conditions of autoclave i.e., 121˚C and 15psi were kept for 15 minutes following the method as described by Wang *et al.* (2016).

Inoculation and incubation: Inoculum of the lyophilized bacterial culture was prepared by using MRS broth. Streaking of the inoculum was done under a UV chamber in pre-sterilized Petri dishes in which MRS agar was used as culture media for the growth of bacterial cells. Incubation of the plates was done at 37°C in an incubator (memmert IN750) and *Lactobacillus reuteri* propagation was done three times to enhance the viability of lyophilized cells following the method of Bekhit *et al.* (2016) with slide modification.

Formation of microbeads: To prepare microbeads, 1.5g of sodium alginate was taken in a beaker which was filled to mark using deionized water. Mixing was done by magnetic stirrer (WENSAR, LMMS-300) along with the hot plate. Emulsification efficiency of the sodium alginate solution was promoted by adding 2-3ml of sesame oil. The solution was sterilized at 121°C for 15 minutes and cooled to palm heat. Pre-harvested cells from the centrifugation process were added to the solution under controlled conditions and mixed well. In a micro-syringe having stainless steel needle, 10ml of the probiotic-containing emulsion was loaded and sprayed over 0.5% solution of calcium chloride under controlled conditions which resulted in the formation of microbeads. These beads were collected, and moisture content was reduced by dehydration to 16%, by modifying the procedure as explained by Laelorspoen *et al.* (2014).

Guava juices pasteurization: Guava juice pasteurization was done in 250ml pre-sterilized glass bottles capped with cotton plugs in the water bath at 70°C for 4 minutes, then the juice was cooled below 10°C and stored for further analysis by modifying the method of Ding and Shah, (2008).

Treatment plan (Probiotics = log10 CFU/ml).

Treatments	Guava pulp %	Free cells	Encapsulated cells
T_0	20	7.68	No
T_1	20	No	7.39
T_2	20	No	7.7
T_3	20	No	7.87

Preparation of probiotic juice: According to the method of Ying *et al*., (2013), microencapsulated *Lactobacillus reuteri* cells were incorporated into guava juices under sterile conditions at 10°C by maintaining a 3.5 pH.

Efficiency of encapsulation: The encapsulation efficiency of the probiotic cells was assessed by taking 25ml of sample from the juice. In 225ml of 1% sodium citrate solution, 25ml juice was added at 25˚C. Sodium citrate solution containing juice sample was stirred at 100rpm for 10minutes. After the disintegration of beads, the juice sample was assessed by pour plate and streak method by incubating at 37˚C for 48 hours. Colony-forming units were counted by colony counter (Mokhtari *et al*., 2017).

Following mathematical expression was used for calculating encapsulation efficiency.

$$E \quad ef \quad y = \frac{N\ o\ c\ b\ s - N\ o\ c\ a\ s}{N\ o\ c\ b\ s} \times 100$$

TSS: Hand-held refractometer (IR-B32) Laxco Inc. was used to measure total soluble solid of guava juice at ambient temperature in ˚Brix as per the guidelines described by the manual AOAC, (2015).

pH: Before pH measurement, calibration of the digital pH meter (Model Ino-Lab720 Germany) was done by buffer solution of pH 4 and 9. From the juice, 10ml was pipetted to a 50ml beaker and readings were taken thrice, dipping the electrode in the sample. During consecutive readings electrode of the pH meter was washed using distilled water and cleaned with tissue paper as per the protocol of Fisk *et al.* (2008).

Titratable Acidity: For the determination of titratable acidity, 0.1N NaOH solution was prepared by taking 0.4g in a volumetric flask and making the final volume 100ml. In a 250ml beaker, 10ml from juice was taken and diluted to 100ml

using distilled water. Phenolphthalein indicator approximately 2-3 drops were also added to the sample and titrated till light pink endpoint (AOAC, 2015).

Following mathematical expression was used to calculate the acidity percentage.

$$\% A = \frac{\frac{1}{10} \times e\ .w\ \ o\ \ a\ \ \times N\ \ \ \ o\ \ b\ \ \times ti}{w\ .\ o\ \ sc}$$

Consumer acceptance and purchase intention test: Different sensory parameters of juice were evaluated by 20 members (10 trained) on a 9-point scale at the Institute of Food Science and Nutrition Bahauddin Zakariya University Multan, Pakistan to verify the homogeneity of the results. All the panellists above the age of 20 years were selected on their ethical consent. Before observation participants were provided with water and record sheets for numerical computation, under white light, in individual cabins to maximize reproductivity (Grizotto *et al*., 2010). Samples were coded to maintain the secrecy of the product composition. All the panellists were given 20ml of juice from each sample. For evaluating purchase intention, a five-point scale was designed to represent 1 "certainly would not buy" and 5 "certainly would buy" (Meilgaard *et al*., 2006).

Statistical analysis: Data from the study were analyzed by using statistics 10 Analytical Software 2105 Miller Landing Rd Tallahassee, FL 32312 USA. A complete randomized design was applied to interpret the data in combination with factorial arrangements. Significance level was determined by the analysis of various (ANOVA) (Steel, 1997).

RESULTS AND DISCUSSION

Encapsulation protects probiotics to prevent them from harsh conditions, minimally affecting the juice physicochemical and sensory parameters. In this research two-way investigation was done to analyze the effect of juice on the viability of microencapsulated probiotics and that of probiotics on quality characteristics of juice.

Effect of sodium alginate beads and storage on pH of juice: The effect of sodium alginate beads and storage on the pH of juice was significant during the entire period of storage. A linear decrease in pH can be seen in Table 1. In the control group having free cells, pH varies from 3.763 to 3.343. While this drop was less in treatments containing microencapsulated *lactobacillus reuteri* (CECT-925) strain. In T_1 and $T_{3,}$ pH decreased from 3.749 and 3.751 to 3.364 and 3.406 respectively but this decrease was less prominent in T_2 in which pH changed from 3.742 to 3.569 showing more stability of the juice under refrigerated conditions. A decrease in pH may be associated with organic acid breakdown, the Tricarboxylic acid (TCA) cycle and fluctuations in temperature during storage. Ying *et al.* (2013) observed a similar trend in reducing pH value influencing the sensory characteristics of juice. The decreasing pattern was similar irrespective of the microencapsulated probiotics present. The trends reported by Gandomi et al. (2016) also showed that the pH of apple juice containing inulin encapsulated probiotics decreased during storage.

Effect of sodium alginate beads and storage on titratable acidity (%) of the juice: The titratable acidity of the juice was measured against ascorbic acid. Table 2. indicate that a significant effect of treatments was observed. Highest TA was seen in T_0 followed by the $T_{1,}$ T_2 and T_3 after 60 days of storage. The result indicates that the rate of increase of TA reduced with the increased probiotics concentration. TA of the juice increased during storage, because of the breakdown of sugars under anaerobic conditions and citric acid added (Rashid *et al*., 2018). During storage, invertase may convert acids into sugars and salts (Hashem *et al*., 2014). Similar results were observed by Zaman *et al.* (2016) in mango-pineapple juice blend RTS.

Effect of sodium alginate beads and storage on total soluble solids (°Brix) of juice: Primarily total soluble solids are sugars; glucose, fructose, and sucrose. They serve as an indicator of juice quality. It can be shown from figure 3. that TSS were 12.57 in control on the initial day, which increased to 13.15˚Brix on the 60th day. This increase was less as compared to T_1 and T_2 due to free cells. However, TSS concentration increased slightly more in T_3 i.e., 12.49 to 13.71 ˚Brix. This increase was might be due to the conversion of polysaccharides into simpler sugars providing a sweeter taste. Hanif *et al.* (2016) suggested a similar trend for an increase in TSS of the juice. TSS of the guava juice increased during storage due to acid hydrolysis of polysaccharides (Pandey, 2014). Oliveira et al. (2018) reported that pectinases are responsible for the increased TSS of the juice. But there may be an adverse effect of total soluble solids of juice when stored for longer periods. The TSS starts decreasing due to the hydrolysis of polysaccharides into simpler sugar affecting the juice sensory parameters.

Effect of sodium alginate beads and storage on the colour of juice: Colour is the basic sensory character and is perceived first on the consumer acceptance test. It is evident from figure 1. that colour of the guava juice containing free and encapsulated probiotics on a hedonic scale decreased during storage. A significant decrease was observed in the control sample. Although this observed trend was less prominent in T_1 and T_3. In T_2 sensory colour was more stable than other treatments. The reduction in the colour value of the juice was might be due to a change in pH. According to Bal *et al.* (2014) increase in acidity of the juice is also responsible for initiating hydrolytic reactions, caramelization and Millard reaction leading to browning in juice. In another study, Szczepańska *et al.* (2020) suggested that when polyphenol contents present in the juices react with enzymes, change the colour of the natural products. There was no effect of storage and encapsulation matrix on the physicochemical properties of grape juice except colour and titratable acidity (Mokhtari et al., 2017)

Table 1: Effect of probiotics on pH of Guava juice with the progression of storage

Treatments	Days				
	0	15	30	45	60
T_0	3.76±0.12abc	3.64±0.11abc	3.53±0.15a-d	3.42±0.13bcd	3.343±0.14d
T_1	3.749±0.19a	3.68±0.22ab	3.56±0.18a-d	3.468±0.23a-d	3.364±0.11cd
T_2	3.742±0.14a	3.69±0.18ab	3.66±0.26abc	3.619±0.25a-d	3.569±0.18a-d
T_3	3.751±0.17a	3.52±0.11a-d	3.48±0.21a-d	3.416±0.12bcd	3.406±0.24bcd

Table 2: Effect of probiotics on titratable acidity (%) of Guava juice with the progression of storage.

Treatments	Days				
	0	15	30	45	60
T_0	$0.41±0.012^{b\text{-}f}$	$0.418±0.022^{a\text{-}d}$	$0.425±0.018^{abc}$	$0.431±0.015^{ab}$	$0.442±0.019^{a}$
T_1	$0.394±0.015^{d\text{-}h}$	$0.409±0.013^{b\text{-}f}$	$0.416±0.016^{a\text{-}e}$	$0.424±0.017^{abc}$	$0.433±0.022^{ab}$
T_2	$0.389±0.019^{e\text{-}h}$	$0.398±0.015^{c\text{-}g}$	$0.411±0.021^{b\text{-}f}$	$0.417±0.011^{a\text{-}e}$	$0.419±0.01^{4a\text{-}d}$
T_3	$0.374 ± 0.011^{h}$	$0.378± 0.017^{gh}$	$0.385±0.026^{fgh}$	$0.393±0.022^{d\text{-}h}$	$0.406±0.012^{b\text{-}g}$

Table 3: Effect of probiotics on total soluble solids (°Brix) of Guava juice with the progression of storage.

Treatments	Days				
	0	15	30	45	60
T_0	$12.57±0.31^{gh}$	$12.71±0.27^{fgh}$	$12.98±0.26^{c\text{-}f}$	$13.11±0.16^{b\text{-}e}$	$13.15±0.25^{b\text{-}e}$
T_1	$12.6±0.23^{gh}$	$12.83±0.18^{d\text{-}h}$	$13.11±0.22^{b\text{-}e}$	$13.27±0.27^{bc}$	$13.39±0.19^{ab}$
T_2	$12.54±0.19^{gh}$	$12.67±0.24^{fgh}$	$12.9±0.28^{c\text{-}g}$	$13.18±0.14^{bcd}$	$13.39±0.13^{ab}$
T_3	$12.49±0.14^{h}$	$12.8±0.35^{e\text{-}h}$	$13.2±0.11^{bcd}$	$13.42±0.30^{ab}$	$13.71±0.10^{a}$

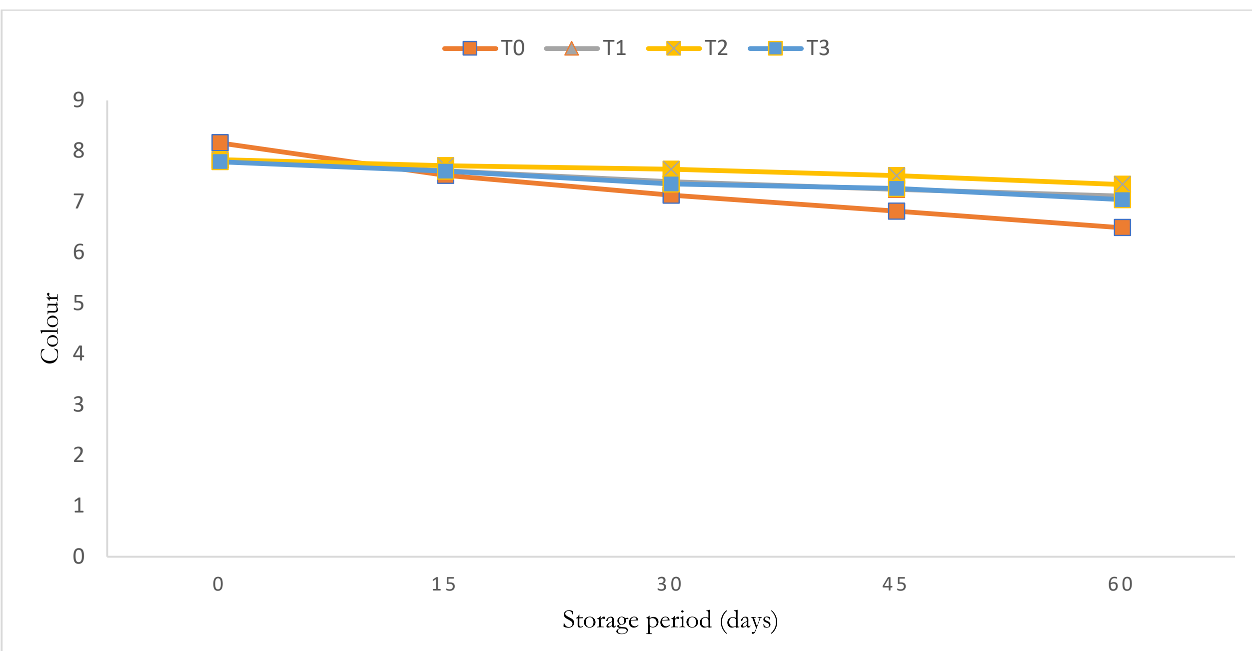


Figure 1: Effect of probiotics on the colour of Guava juice with the progression of storage

Effect of sodium alginate beads and storage on the taste of juice: Figure 2. indicated that with advancement in the storage period, the taste of the juice reduced. In T_0 value was 8.42 at the initial level which reduced to 6.44 at the end of storage. Like T_0 decreasing pattern was prominent in T_1 and T_3 but found less in T_2 from 8.18 to 7.61. A decrease in taste was might be due to development in acidity, sugar breakdown, caramelization and Millard reaction. Wurlitzer *et al.* (2019) also found that the more acidic taste observed by the panellists was due to the hydrolysis of polysaccharides thus decreasing the acceptability of camu-camu pulp. Bal *et al.* (2014) suggested a similar effect for guava nectar.

Effect of sodium alginate beads and storage on the mouthfeel of juice: During storage of juice there observed a non-significant effect on the mouthfeel. Figure 6. indicate that sensory scores in control decreased from 8.5 to 6.50 during 60 days of storage which was more prominent than T_1 and T_2 and comparable to T_3 showing a decline of 17.75%, which may be due to the increased number of microcapsules. This decrease may be associated with the breakdown of pectic substances and an increase in total soluble solids. Otero, (2019) reported that an increase in viscosity may affect mouthfeel. Nonetheless, the decreasing pattern was also linked with the findings of Rashid *et al.* (2018).

Effect of sodium alginate beads and storage on the flavour of juice: A combination of taste and odour is termed flavour. There was a non-significant effect of storage on the flavour of the juice. Figure 4. show that in control having free cells of probiotics sensory score of flavour decreased from 8 to 5.13 during 60 days of storage shifting to the unacceptable range. A similar trend was also observed in T_2. However, a comparable decline was found in T_1 and T_3 from 7.83 to 7.19 and 7.83 to 6.5 respectively. According to Otero, (2019) this decreasing trend was due to physicochemical reactions and sodium alginate beads present in the juice. However, flavour score may decrease due to caramelization and fermentation (Bal *et al*., 2014).

Effect of sodium alginate beads and storage on overall acceptability of juice: Based on consumer preference and purchase intention test, overall acceptability results were non-significant. Figure 5. indicate that overall acceptability reduced during storage. Control having free cells of probiotics showed a decrease of 8.3 to 4.79. Control was rejected by the panellist at the end of storage. However, a decline in T_3 was also observed but not comparable to control. The reduction in overall acceptability score in T_1 and T_2 was 8.13 and 8 to 7.42 and 7.51 respectively. This drop was might be due to more beads in T_3, caramelization, Millard reaction and other oxidative changes during storage. Similar changes were found by Bal *et al.* (2014) in guava nectar. Rashid *et al.* (2018) associated this declining trend with physicochemical changes during storage in guava RTS. A linear decrease in guava juice overall acceptability was also observed by Pandey, (2014).

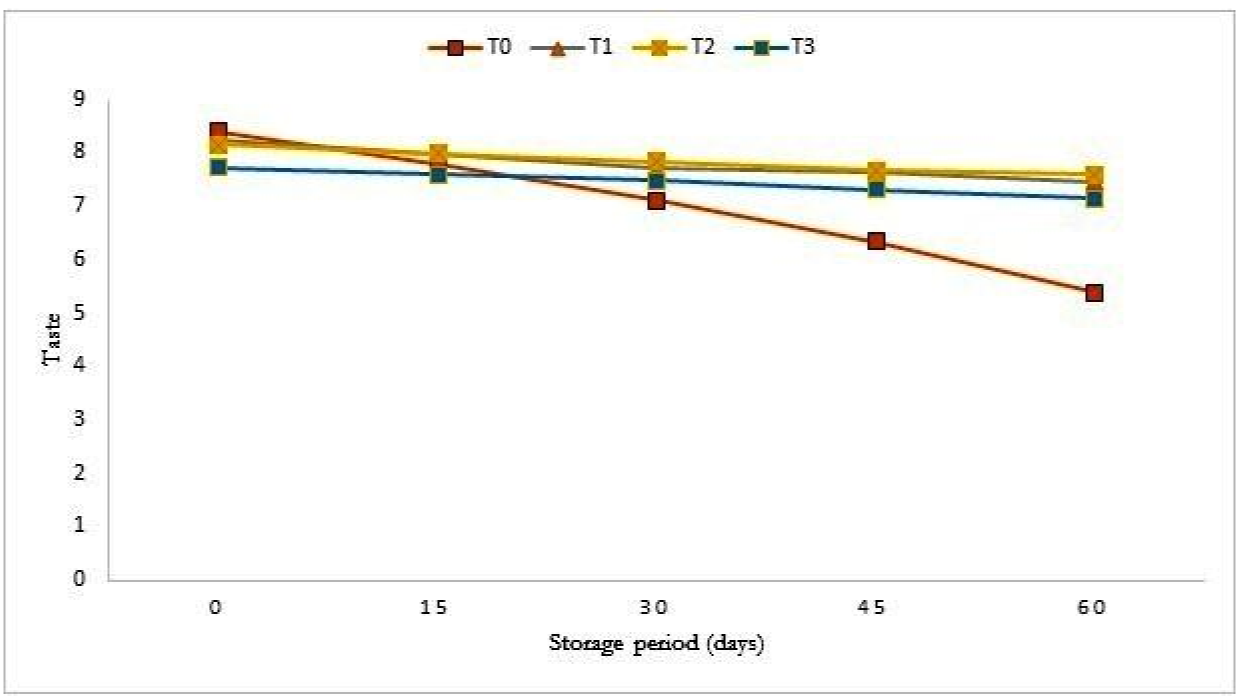


Figure 2: Effect of probiotics on taste of Guava juice with the progression of storage

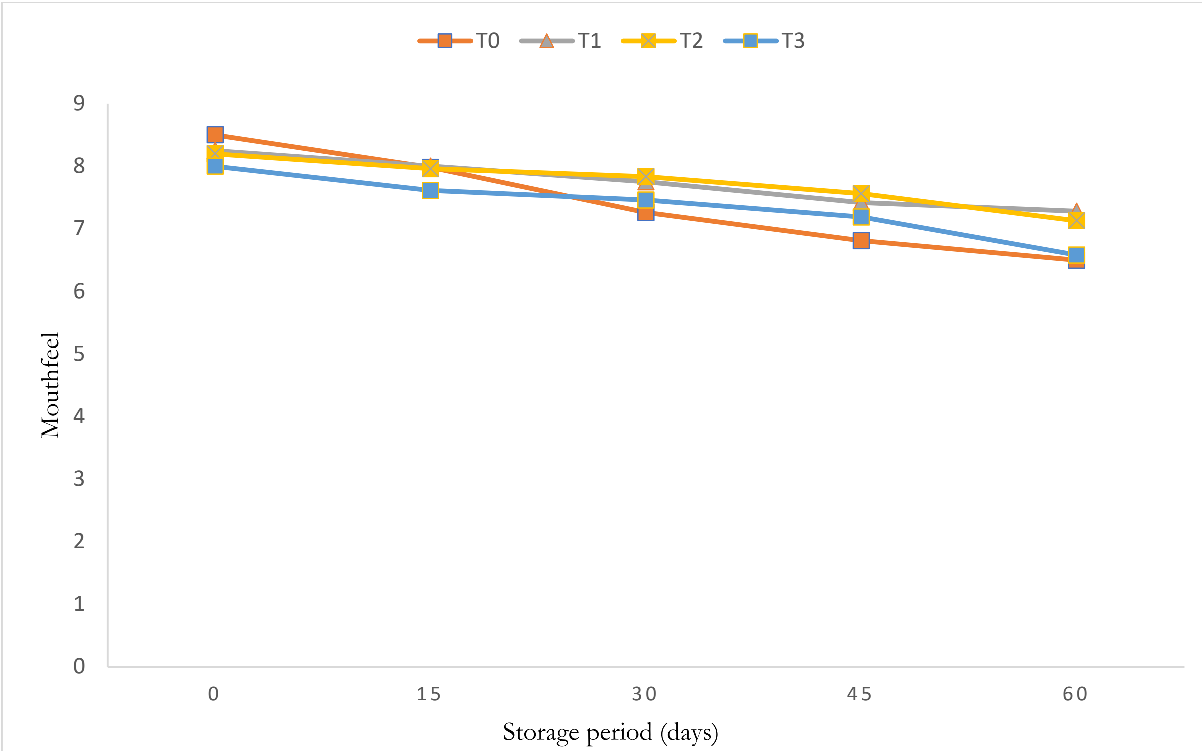


Figure 3: Effect of probiotics on the mouthfeel of Guava juice with the progression of storage

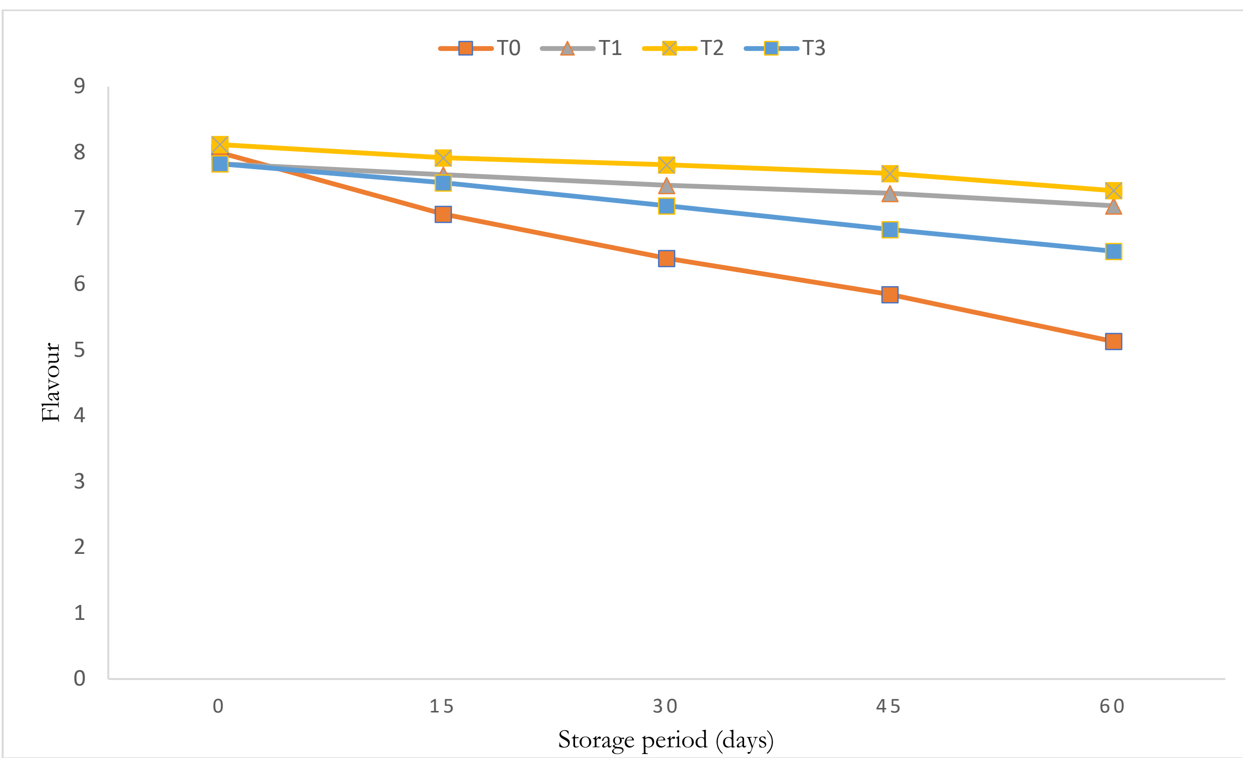


Figure 4: Effect of probiotics on the flavour of Guava juice with the progression of storage

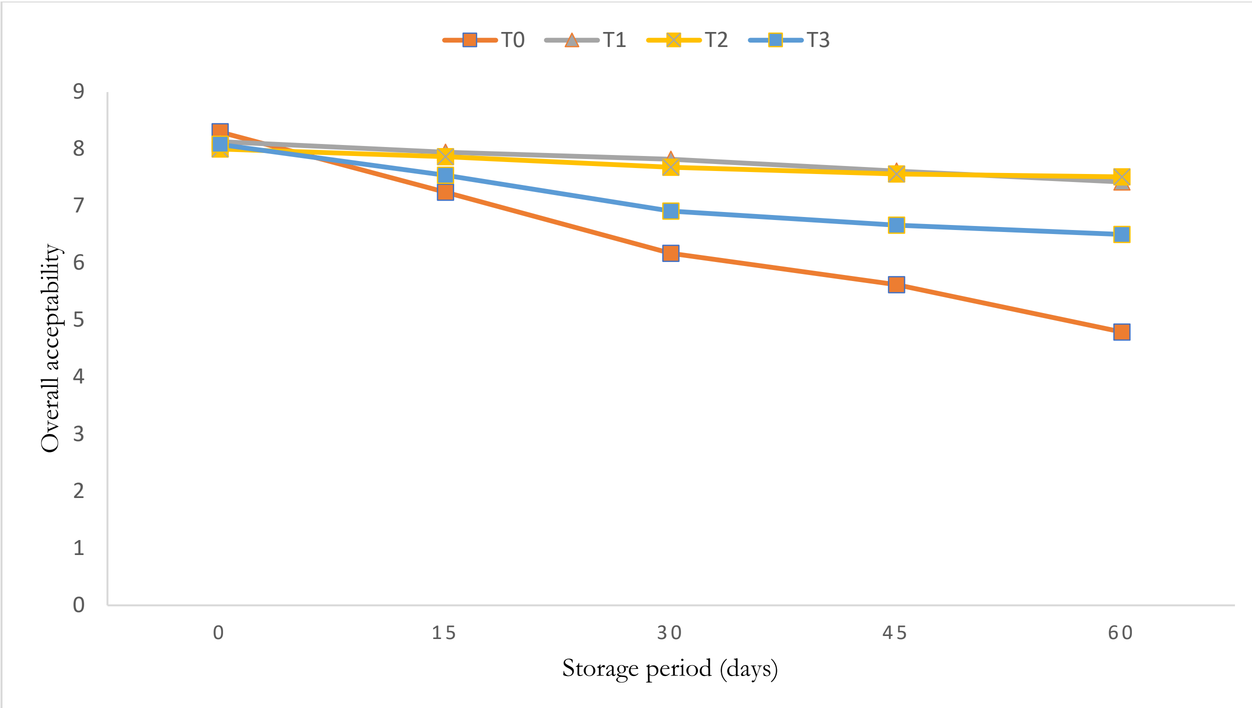


Figure 5: Effect of probiotics on overall acceptability of Guava juice with the progression of storage.

Effect of storage on the viability of probiotics: The survival rate of microencapsulated and free probiotics cells inoculated into guava juice is shown in Table 4. During the storage period of 60 days, free cells rapidly lost their viability by about 74.47%. The reduction in the number of viable cells was from 7.68 to 1.96 log CFU/ml, possibly due to the low pH and acidic environment of the juice. Encapsulated cells showed more resistance to harsh conditions owing to less viability loss. In T_1 bacterial cells were reduced from 7.39 to 5.97 log CFU/ml, about 19.21%. The declining rate in T_2 and T_3 was 18.05 and 23.50% respectively having $\leq$ 6 log CFU/ml of the bacteria after 60 days of storage. Silva *et al.* (2021) reported that microencapsulated probiotics by the coacervation method can survive for 63 days. The survival rate of microencapsulated probiotics found by Bora *et al.* (2019) was 91% in orange juice. The viability of microencapsulated probiotics by vibration technology was 91% in orange juice while 100% in pineapple juice (Olivares *et al*., 2019). This promising technique can assure the delivery of bacterial cells to the human gastrointestinal system by reducing complications due to notorious pathogens.

Table 4: Effect of Guava juice and storage on the viability of microencapsulated probiotics.

Treatments	Days				
	0	15	30	45	60
T_0	7.68 ± 0.24^{ab}	6.39 ± 0.16^{gh}	4.56 ± 0.15^{j}	3.39 ± 0.19^{k}	1.96 ± 0.20^{l}
T_1	7.39 ± 0.21^{bc}	7.13 ± 0.22^{cde}	6.72 ± 0.27^{fg}	6.24 ± 0.26^{hi}	5.97 ± 0.18^{i}
T_2	7.7 ± 0.20^{ab}	7.41 ± 0.19^{bc}	7.14 ± 0.25^{cde}	6.97 ± 0.11^{def}	6.87 ± 0.21^{ef}
T_3	7.87 ± 0.25^{a}	7.29 ± 0.28^{cd}	6.98 ± 0.13^{def}	6.51 ± 0.14^{gh}	6.02 ± 0.13^{i}

Purchase intention test: The purchase intention of the consumer was influenced significantly during 60 days of the storage period. The control sample was rejected by the consumer at the end of storage while the sample having 7.39 ± 0.21 encapsulated cells showed that 74.36% of the individuals were interested to buy it. The acceptance of T_3 to the consumer was at the borderline suggesting its instability and increased viscosity on the 60^{th} day of storage. The acceptance percentage of T_2 was 82.04 highlighting the maximum market potential of the microencapsulated beads supplemented guava juice.

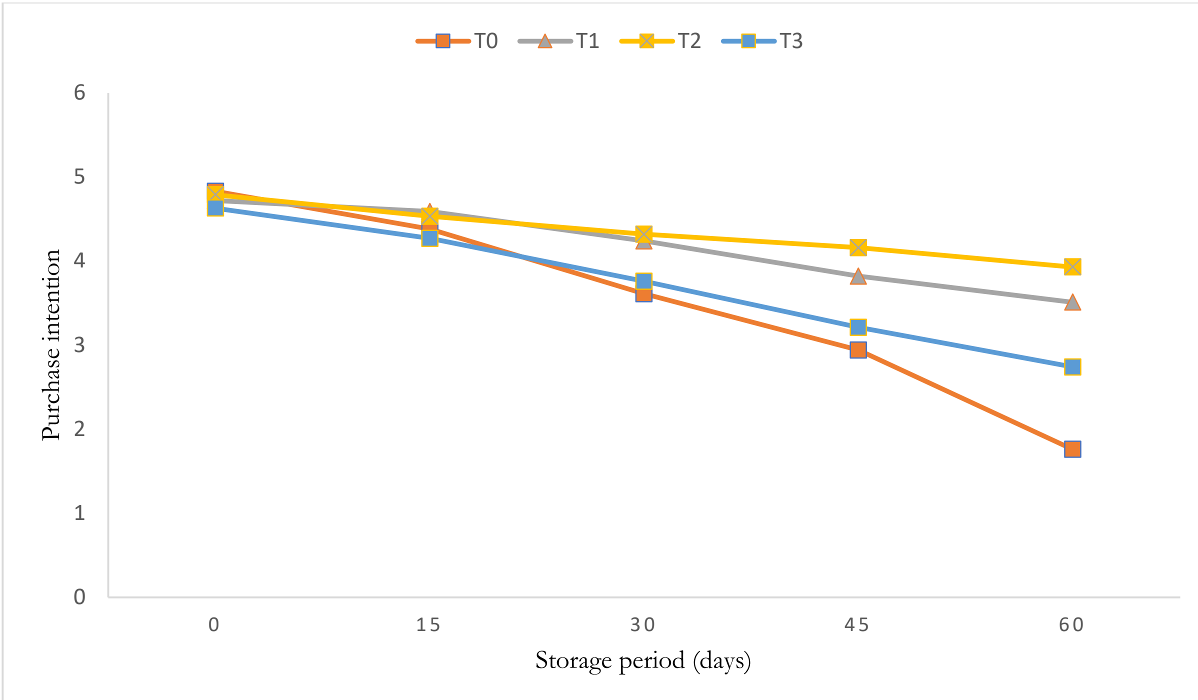


Figure 6: Purchase intention of the panellists after 60 days of storage.

Conclusion: Microencapsulation using sodium alginate increase the viability of probiotic cells and provide stability to the juice during a storage period of 60 days. In the control sample viability of free probiotic cells was 25.53% and in microencapsulated cells viability was 89.22% leaving the probiotic count to ≈ 10^6 logs CFU/ml. The result suggested that sodium alginate do not affect the quality parameters of Guava juice when stored at lower temperature i.e., 4°C. Increased viability is linked with the promising health benefits of probiotics when delivered to the gastrointestinal system in a safe and approved manner.

REFERENCES

Pabolu, S., A. Dudekula, and C.S. Pitchumoni (2021). Gastrointestinal Manifestations of Non-GI Disorders. J. geriat. gastroenterol. 2117-2166.

Boesveldt, S.N. Bobowski, K, McCrickerd, I., Maître, C. Sulmont-Rossé and C.G. Forde (2018). The changing role of the senses in food choice and food intake across the lifespan. Food Qual. Prefer. 68: 80-89.

Singh, B., G.Mal, D. Sharma, R. Sharma, C.P. Antony, and R.S. Kalra (2020). Gastrointestinal biotransformation of phytochemicals: Towards futuristic dietary therapeutics and functional foods. Trends Food Sci Technol. 106: 64-77

Divya, J.B. and K.M. Nampoothiri (2015). Encapsulated Lactococcus lactis with enhanced gastrointestinal survival for the development of folate enriched functional foods. Bioresour. technol. 188: 226-230.

Markowiak, P. and K. Śliżewska (2017). Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients. 9(9): 1021.

Flach, J., M.B. van der Waal, M. van den Nieuwboer, E. Claassen and O.F. Larsen (2018). The underexposed role of food matrices in probiotic products: Reviewing the relationship between carrier matrices and product parameters. Crit. Rev. Food Sci. Nutr. 58(15): 2570-2584.

Jang, W.J., C.E. Kim, M.H. Jeon, S.J. Lee, J.M. Lee, E.W. Lee, and M.T. Hasan (2021). Characterization of Pediococcus acidilactici FS2 isolated from Korean traditional fermented seafood and its blood cholesterol reduction effect in mice. J. Funct. Foods 87: 104847.

Domingo, J.J. (2017). Review of the role of probiotics in gastrointestinal diseases in adults. Gastroenterol. Hepatol. 40(6): 417-29.

Bruno, G., G. Rocco, P. Zaccari, B. Porowska, M.T. Mascellino, and C. Severi (2018). Helicobacter pylori infection and gastric dysbiosis: can probiotics administration be useful to treat this condition?. Can. J. Infect. Dis. Med. Microbiol. 2018:7

Dianawati, D., V. Mishra and N.P. Shah (2016). Survival of microencapsulated probiotic bacteria after processing and during storage: a review. Crit. Rev. Food Sci. Nutr. 56(10): 1685-1716.

Millette, M., A. Nguyen, K.M. Amine and M. Lacroix (2013). Gastrointestinal survival of bacteria in commercial probiotic products. Int. J. Probiotics Prebiotics. 8(4): 149.

Iqbal, R., A. Liaqat, M. F. Jahangir Chughtai, S. Tanweer, S. Tehseen, S. Ahsan,... and A. Khaliq (2021). Microencapsulation: a pragmatic approach towards delivery of probiotics in gut. J Microencapsul, 38:437-458.

Terpou, A., A. Papadaki, I.K. Lappa, V. Kachrimanidou, L.A. Bosnea and N. Kopsahelis (2019). Probiotics in food systems: Significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. Nutrients. 11(7): 1591.

Suwanwong, Y. and S. Boonpangrak (2021). Phytochemical contents, antioxidant activity, and anticancer activity of three common guava cultivars in Thailand. Eur. J. Integr. Med. 42: 101290.

George, B.P., R. Chandran, and H. Abrahamse (2021). Role of Phytochemicals in Cancer Chemoprevention: Insights. Antioxidants. 10: 1455.

Wang, Y., J. Zhou, X. Xia, Y. Zhao and W. Shao (2016). Probiotic potential of Lactobacillus paracasei FM-LP-4 isolated from Xinjiang camel milk yoghurt. Int. Dairy J. 62: 28-34.

Bekhit, M., L. Sánchez-González, G.B. Messaoud and S. Desobry (2016). Encapsulation of Lactococcus lactis subsp. lactis on alginate/pectin composite microbeads: Effect of matrix composition on bacterial survival and nisin release. J. Food Eng. 180: 1-9.

Laelorspoen, N., S. Wongsasulak, T. Yoovidhya and S. Devahastin (2014). Microencapsulation of Lactobacillus acidophilus in zein–alginate core–shell microcapsules via electrospraying. J. Funct. Foods. 7: 342-349.

Ding, W.K. and N.P. Shah (2008). Survival of free and microencapsulated probiotic bacteria in orange and apple juices. Int. Food Res. J. 15(2): 219-232.

Ying, D., S. Schwander, R. Weerakkody, L. Sanguansri, C. Gantenbein-Demarchi and M.A. Augustin (2013). Microencapsulated Lactobacillus rhamnosus GG in whey protein and resistant starch matrices: Probiotic survival in fruit juice. J. Funct. Foods. 5(1): 98-105.

Mokhtari, S., M. Khomeiri, S.M. Jafari, Y. Maghsoudlou and M. Ghorbani (2017). Descriptive analysis of bacterial profile, physicochemical and sensory characteristics of grape juice containing Saccharomyces cerevisiae cell wall-coated probiotic microcapsules during storage. Int. J. Food Sci. Technol. 52(4): 1042-1048.

AOAC. (2015). Official Method of Analysis of Analytical Chemists, 14th edition. Washington DC.

Fisk, C.L., A.M. Silver, B.C. Strik and Y. Zhao (2008). Postharvest quality of hardy kiwifruit (Actinidia arguta 'Ananasnaya') associated with packaging and storage conditions. Postharvest Biol. Technol. 47(3): 338-345.

Grizotto, R.K., C.R. Rufi, E.A. Yamada and E. Vicente (2010). Evaluation of the quality of a molded sweet biscuit enriched with okara flour. Food Sci. Technol. 30: 270-275.

Meilgaard, M.C., B.T. Carr and G.V. Civille (2006). Sensory evaluation techniques. CRC press.

Steel, R.G. (1997). Pinciples and procedures of statistics a biometrical approach.

Ying, D., S. Schwander, R. Weerakkody, L. Sanguansri, C. Gantenbein-Demarchi and M.A. Augustin (2013). Microencapsulated Lactobacillus rhamnosus GG in whey protein and resistant starch matrices: Probiotic survival in fruit juice. J. Funct. Foods. 5(1): 98-105.

Rashid, R., A. Bhat, A. Dayal, M. Sood and S. Sharma (2018). Studies on storage stability of guava RTS. Pharma Innov. 7(5, Part D): 230.

Hashem, H.A., A.M. Sharaf, S.A. Amira and G.E. Ibrahim (2014). Changes in physico-chemical quality and volatile compounds of orange-carrot juice blends during storage. Food Sci. Qual. Manag. 33: 21-35.

Kamarul, Zaman A.A., R. Shamsudin and N. Mohd Adzahan (2016). Effect of blending ratio on quality of fresh pineapple (Ananas comosus L.) and mango (Mangifera indica L.) juice blends. Int. Food Res. J. 23.

Mohd-Hanif, H.A., R. Shamsudin and N.M. Adzahan (2016). Effects of UVC irradiation and thermal treatment on the physico-chemical properties and microbial reduction of clear and turbid tamarind juice. Int. Food Res. J. 23: S107.

Pandey, A.K. (2004). Study about the storage stability of guava beverages. Progress. Horticult. 36: 142-145.

Bal, L.M., T. Ahmad, A.K. Senapati and P.S. Pandit (2014). Evaluation of quality attributes during storage of guava nectar cv. Lalit from different pulp and TSS ratio. J. Food. Process. Technol. 5(5).

Szczepańska, J., F.J. Barba, S. Skąpska and K. Marszałek (2020). High pressure processing of carrot juice: Effect of static and multi-pulsed pressure on the polyphenolic profile, oxidoreductases activity and colour. Food chem. 307: 125549.

Wurlitzer, N.J., A.P. Dionísio, J.R. Lima, D. dos Santos Garruti, I.M. da Silva Araújo, R.F. da Rocha and J.L. Maia (2019). Tropical fruit juice: Effect of thermal treatment and storage time on sensory and functional properties. J. food sci. technol. 56(12): 5184-93.

Otero, L. (2019). Hyperbaric storage at room temperature for fruit juice preservation. Beverages.5(3): 49.

da Silva, T.M., V.S. Pinto, V.R. Soares, D. Marotz, A.J. Cichoski, L.Q. Zepka, E.J. Lopes, C.D. da Silva and C.R. de Menezes (2021). Viability of microencapsulated Lactobacillus acidophilus by complex coacervation associated with enzymatic crosslinking under application in different fruit juices. Food Res. Int. 141: 110190.

Bora, A.F., X. Li, Y. Zhu and L. Du (2019). Improved viability of microencapsulated probiotics in a freeze-dried banana powder during storage and under simulated gastrointestinal tract. Probiotics Antimicrob. Proteins. 11(4): 1330-1339.

Olivares, A., C. Soto, E. Caballero and C. Altamirano (2019). Survival of microencapsulated Lactobacillus casei (prepared by vibration technology) in fruit juice during cold storage. Electron. J. Biotechnol. 42: 42-48.

de Oliveira, R.L., J.L. Dias, O.S. da Silva and T.S. Porto (2018). Immobilization of pectinase from Aspergillus aculeatus in alginate beads and clarification of apple and umbu juices in a packed bed reactor. Food Bioprod. Process. 109: 9-18.

Gandomi, H., S. Abbaszadeh, A. Misaghi, S. Bokaie, and N. Noori (2016). Effect of chitosan-alginate encapsulation with inulin on survival of Lactobacillus rhamnosus GG during apple juice storage and under simulated gastrointestinal conditions. LWT- Food Sci. Technol. 69: 365-371.

Mokhtari, S., M. Khomeiri, S.M. Jafari, Y. Maghsoudlou, and M. Ghorbani (2017). Descriptive analysis of bacterial profile, physicochemical and sensory characteristics of grape juice containing Saccharomyces cerevisiae cell wall-coated probiotic microcapsules during storage. Int. J. Food Sci. 52: 1042-1048.