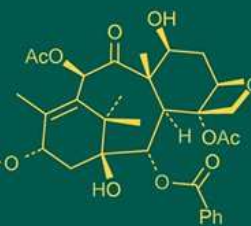
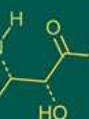
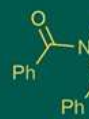
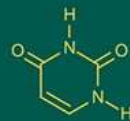
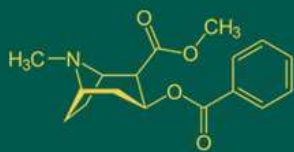


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Pingalkar Dayneshwar Trimbakrao
M.Tech Scholar, Department of Food Microbiology and Safety, College of Food Technology, VNMKV, Parbhani, Maharashtra, India

Dr. GM Macheawad
Associate Professor and Head, Department of Food Business Management, College of Food Technology, VNMKV, Parbhani, Maharashtra, India

Dr. Narendra Manikrao Deshmukh
Assistant Professor, Department of Food Chemistry and Nutrition, College of Food Technology, VNMKV, Parbhani, Maharashtra, India

Dr. Govind Balajirao Desai
Department of Food Microbiology and Safety, College of Food Technology, VNMKV, Parbhani, Maharashtra, India

Dr. Surendra Sadawarte
Associate Professor and Head, Department of Food Microbiology, College of Food Technology, VNMKV, Parbhani, Maharashtra, India

Harshada M Zoting
M.Tech Scholar, College of Food Technology, VNMKV, Parbhani, Maharashtra, India

Neha C Rathod
M.Tech Scholar, College of Food Technology, VNMKV, Parbhani, Maharashtra, India

Corresponding Author: Pingalkar Dayneshwar Trimbakrao
M.Tech Scholar, Department of Food Microbiology and Safety, College of Food Technology, VNMKV, Parbhani, Maharashtra, India

Development, optimization and comprehensive characterization of a probiotic-enriched mixed fruit juice beverage

Pingalkar Dayneshwar Trimbakrao, GM Macheawad, Narendra Manikrao Deshmukh, Govind Balajirao Desai, Surendra Sadawarte, Harshada M Zoting and Neha C Rathod

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Abstract

Plant-based functional drinks are rapidly gaining traction as consumer demand grows for non-dairy, health-promoting alternatives. However, sustaining live, beneficial bacteria in acidic fruit juices remains a major technical hurdle. In this study, we developed and evaluated a novel probiotic fruit beverage combining pineapple (*Ananas comosus*), apple (*Malus domestica*), and sapota (*Manilkara zapota*), naturally sweetened with jaggery. To protect the probiotic strains *Lactiplantibacillus plantarum* and *Lactiacaseibacillus casei* against matrix acidity, the cells were microencapsulated in a sodium alginate-guar gum matrix via ionic gelation before blending.

Keywords: Non-dairy probiotics; Microencapsulation; Mixed fruit beverage; *Lactiplantibacillus plantarum*; *Lactiacaseibacillus casei*; Storage stability; Functional foods

Introduction

The increasing scientific evidence linking dietary patterns with health outcomes has driven the development of functional foods and beverages designed to provide physiological benefits beyond basic nutritional requirements. Among these, probiotic beverages have attracted considerable attention as non-dairy functional food systems capable of delivering viable beneficial microorganisms together with naturally occurring nutrients and bioactive compounds (Granato *et al.*, 2010; Corbo *et al.*, 2014)^[12, 8]. Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2002; Hill *et al.*, 2014)^[10, 14].

Fruit juices represent promising matrices for probiotic delivery owing to their content of fermentable carbohydrates, vitamins, minerals, organic acids, phenolic compounds, and antioxidants. However, the inherent acidity, dissolved oxygen, organic acids, and storage conditions of fruit juices can adversely affect probiotic viability and functional stability (Ranadheera *et al.*, 2017)^[26]. *Lactiplantibacillus plantarum* and *Lactiacaseibacillus casei* have demonstrated considerable potential for incorporation into fruit-based beverages because of their adaptability to acidic food matrices and technological suitability for non-dairy applications (Perricone *et al.*, 2015)^[25]. Nevertheless, maintaining an adequate viable population throughout processing and refrigerated storage remains a major technological challenge. Microencapsulation using biocompatible hydrocolloids, particularly sodium alginate and guar gum, offers an effective approach for protecting probiotic cells against environmental stresses and improving their stability during storage and gastrointestinal transit (Anal & Singh, 2007; Burgain *et al.*, 2011)^[1, 4].

The utilization of complementary fruit matrices may further enhance the nutritional, physicochemical, antioxidant, and sensory attributes of probiotic beverages. Pineapple, apple, and sapota provide diverse profiles of carbohydrates, dietary fibre, vitamins, minerals, phenolic compounds, and antioxidant constituents, while their combination can contribute to desirable flavour, body, and overall acceptability (Boyer & Liu, 2004; Kute & Kadam, 2018; Sharma *et al.*, 2019)^[5, 20, 28]. Jaggery may serve as a natural sweetening agent while contributing characteristic flavour and additional nutritional constituents (Nath *et al.*, 2015)^[24].

Despite the established potential of probiotic fruit beverages, comparatively limited information is available regarding mixed fruit formulations based on pineapple, apple, and sapota incorporating encapsulated probiotic cultures. Therefore, the present investigation was undertaken to develop and comprehensively characterize a probiotic mixed fruit juice containing sodium alginate-guar gum encapsulated *Lactiplantibacillus plantarum* and *Lactiacaseibacillus casei*, with particular emphasis on physicochemical characteristics, nutritional and antioxidant attributes, probiotic viability, and sensory acceptability.

Materials and Methods

Fresh pineapple (*Ananas comosus*), apple (*Malus domestica*), and sapota (*Manilkara zapota*) fruits were procured from a local market and used for beverage preparation. Food-grade jaggery, sodium alginate, guar gum, and potable water were used as formulation ingredients. Freeze-dried cultures of *Lactiplantibacillus plantarum* and *Lactiacaseibacillus casei* were used as probiotic cultures.

Preparation of Mixed Fruit Juice

The fruits were sorted, washed, peeled where required, deseeded, and pulped separately under hygienic conditions. The individual fruit pulps/juices were blended in predetermined proportions to obtain the mixed fruit base. Jaggery was added as a natural sweetener, and the formulation was homogenized thoroughly.

Preparation of Probiotic Microcapsules

Probiotic cultures were individually suspended in sterile sodium alginate-guar gum solution and mixed uniformly. The probiotic-hydrocolloid mixture was subjected to ionic gelation using a sterile calcium chloride solution to form probiotic microcapsules. The capsules were collected, washed with sterile water, and incorporated into the prepared mixed fruit beverage following established microencapsulation procedures (Anal & Singh, 2007; Burgain *et al.*, 2011)^[1, 4].

Beverage Formulation

The mixed fruit juice was standardized for total soluble solids, acidity, and sweetness. Encapsulated *L. plantarum* and *L. casei* were incorporated at predetermined concentrations under aseptic conditions. The prepared beverage was filled into sterilized containers and stored under refrigerated conditions for subsequent evaluation.

Physicochemical Analysis

The beverage was analyzed for pH, titratable acidity, total soluble solids (°Brix), moisture, ash, protein, fat, and total sugars using standard analytical procedures. These parameters were determined to assess the physicochemical and nutritional quality of the developed beverage.

Microbiological and Probiotic Viability Analysis

Probiotic viability was determined by serial dilution and enumeration using selective microbiological media. Results were expressed as log CFU/mL. The viability of probiotic cultures was monitored during refrigerated storage to evaluate their stability in the fruit juice matrix (Ranadheera *et al.*, 2017)^[26].

Antioxidant Analysis

The antioxidant potential of the developed beverage was evaluated using an appropriate in-vitro radical-scavenging

assay. The results were expressed as percentage inhibition or equivalent antioxidant activity, as applicable

Sensory Evaluation

Sensory acceptability was evaluated using a trained/semi-trained panel employing a nine-point hedonic scale for colour, appearance, aroma, taste, mouthfeel, and overall acceptability. The sensory evaluation was conducted under controlled conditions.

Statistical Analysis

All analytical determinations were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation. The data were subjected to appropriate statistical analysis to determine significant differences among formulations at a suitable significance level.

Results and Discussion

Optimization of Probiotic-Enriched Mixed Fruit Juice Beverage through Sensory Evaluation

Sensory evaluation was carried out to determine the most acceptable formulation among the different treatment combinations of the probiotic-enriched mixed fruit juice beverage. The formulations were evaluated for color, flavor, taste, and overall acceptability using sensory scores. The results showed variation in the sensory responses among the treatments, indicating that the proportion and combination of ingredients influenced the consumer perception of the beverage.

Table 1: Sensory Evaluation Scores for Color, Flavor, Taste, and Overall Acceptability of Different Treatments

Treatment	Color	Flavor	Taste	Overall Acceptability
T ₁	7.10	7.00	7.20	7.05
T ₂	7.80	7.60	7.70	7.65
T ₃	8.60	8.40	8.50	8.44
T ₄	7.30	7.10	7.25	7.20

Among the four formulations, T₃ obtained the highest scores for all evaluated sensory attributes. The formulation recorded scores of 8.60 for color, 8.40 for flavor, and 8.50 for taste, resulting in the highest overall acceptability score of 8.44. In comparison, T₂ showed an overall acceptability score of 7.65, while T₁ and T₄ received scores of 7.05 and 7.20, respectively. Based on these results, T₃ was selected as the optimized formulation for further evaluation.

The higher sensory acceptance of T₃ may be associated with the combined sensory characteristics of pineapple, apple, and sapota juices. The blending of these fruits likely contributed to a harmonious combination of sweetness, acidity, characteristic fruit aroma, and attractive appearance. The relatively high color score (8.60) suggests that the visual characteristics of the formulation were well received by the sensory panel. Similarly, the high flavor and taste scores indicate good acceptance of the beverage's overall flavor profile.

The findings are consistent with previous research demonstrating that sensory attributes such as color, flavor, taste, and overall acceptability are important parameters in the development and optimization of fruit-based probiotic beverages. Studies on probiotic fruit beverages have also reported that differences in formulation can influence consumer acceptance and that an optimized fruit blend can

provide desirable sensory characteristics. Therefore, based on the sensory evaluation results obtained in the present study, T₃ was considered the most acceptable formulation and was selected for subsequent physicochemical, microbiological, and/or storage studies.

Physical Characteristics of the Optimized Beverage

The physical properties of the optimized probiotic beverage (T₃) were evaluated and compared with those of the control sample. The results indicated differences in pH, total soluble solids (TSS), and viscosity between the two samples.

Table 2: Physicochemical Properties of Control and Optimized (T₃) Treatment

Parameter	Control	T ₃ (Optimized)
pH	4.05	4.25
TSS (°Brix)	16.0	18.0
Viscosity (cP)	22.8	25.6

The optimized formulation (T₃) exhibited a pH of 4.25 compared with 4.05 in the control. The relatively higher pH indicates a slight difference in the acid balance of the optimized formulation. Such variation may be associated with differences in the proportions of the fruit ingredients and other components used in the formulation.

The TSS of T₃ was 18.0 °Brix, which was higher than that of the control (16.0 °Brix). This increase may be attributed to the contribution of soluble solids from the fruit juices and jaggery used in the formulation. Higher TSS generally reflects a greater concentration of soluble components, including sugars, and may consequently influence the sweetness and overall body of the beverage.

A higher viscosity was also observed in T₃ (25.6 cP) compared with the control (22.8 cP). This increase may be related to the presence of naturally occurring pectic substances and soluble polysaccharides contributed by the fruit ingredients. These components can increase the thickness of fruit-based beverages and contribute to a fuller mouthfeel. The observed differences in viscosity may therefore have contributed to the favorable sensory characteristics of the optimized formulation.

Overall, the optimized T₃ formulation showed higher TSS and viscosity than the control, together with a slightly higher pH. The combined physicochemical characteristics of T₃ may have contributed to its desirable sensory performance and supported its selection as the optimized probiotic beverage formulation.

Chemical Characterization of the Optimized Beverage

The chemical characteristics of the optimized probiotic beverage (T₃) were evaluated in comparison with the control sample, and the results are presented in Table X. The parameters assessed included titratable acidity, reducing sugars, total sugars, protein, and fat content.

Table 3: Comparative Chemical Composition of Control and T₃ Treatment

Parameter	Control	T ₃
Titrateable Acidity (%)	0.40	0.54
Reducing Sugars (%)	8.2	7.3
Total Sugars (%)	13.17	12.62
Protein (%)	0.30	0.34
Fat (%)	0.2	0.19

The titratable acidity of the optimized formulation increased from 0.40% in the control to 0.54% in T₃. This increase in acidity may be associated with the metabolic activity of the probiotic microorganisms during fermentation, particularly the conversion of available fermentable carbohydrates into organic acids. Similar increases in titratable acidity have been reported during the probiotic fermentation of fruit-based beverages, where organic acid production is an important consequence of microbial metabolism.

A reduction in reducing sugars was observed in T₃, with the value decreasing from 8.20% in the control to 7.30% in the optimized beverage. Similarly, total sugar content decreased from 13.17% to 12.62%. These changes may be attributed to the utilization of readily available carbohydrates by the probiotic microorganisms as substrates for growth and metabolism. During fermentation, sugars can be metabolized into organic acids and other microbial metabolites, thereby reducing the concentration of residual sugars in the beverage. The observed increase in titratable acidity together with the reduction in sugar content is consistent with the metabolic activity generally associated with probiotic fermentation of fruit juices.

The protein content showed a slight increase from 0.30% in the control to 0.34% in T₃. This difference may be related to the composition of the optimized formulation and changes occurring during fermentation. Although microbial biomass formation may contribute to changes in measured protein content, this interpretation should be considered cautiously unless supported by measurements of microbial biomass or nitrogen fractions.

The fat content was very low in both samples, measuring 0.24% in the control and 0.19% in T₃. The slightly lower fat content observed in the optimized beverage indicates that fat was present only in a minor proportion and was unlikely to be a major contributor to the nutritional or sensory characteristics of the formulation.

Overall, the chemical characterization revealed that the probiotic fermentation process was associated with an increase in titratable acidity and a reduction in both reducing and total sugars. These changes suggest active utilization of fermentable carbohydrates during fermentation and are in agreement with observations reported for other probiotic fruit-based beverages. The optimized T₃ formulation therefore demonstrated distinct chemical characteristics compared with the control, while retaining low fat content and a slightly higher protein concentration.

Nutraceutical Properties of the Probiotic Beverage

The nutraceutical characteristics of the optimized probiotic beverage (T₃) were evaluated in comparison with the control sample. Vitamin C content, antioxidant activity, and crude fiber were considered important nutritional parameters for assessing the functional potential of the beverage. The results are presented in Table

Table 4: Comparative Nutritional and Antioxidant Properties of Control and T₃ Treatment

Parameter	Control	T ₃
Vitamin C (mg/100 g)	15.2	22.5
Antioxidant Activity (%)	52.40	54.37
Crude Fiber (%)	1.20	2.10

The vitamin C content increased from 15.2 mg/100 g in the control to 22.5 mg/100 g in the optimized formulation. This

represents an increase of approximately 48%. The higher vitamin C content in T₃ can primarily be associated with the contribution of the selected fruits, particularly pineapple and other fruit components incorporated into the formulation. Vitamin C is an important water-soluble antioxidant and contributes to the overall nutritional value of fruit-based beverages.

A marked improvement in antioxidant activity was also observed in the optimized beverage. The antioxidant activity increased from 38.60% in the control to 54.37% in T₃, corresponding to an increase of approximately 41%. This enhancement may be related to the combined contribution of antioxidant compounds naturally present in the fruits, including phenolic compounds and vitamin C. In addition, fermentation can modify the availability or composition of certain bioactive compounds through microbial metabolic activity. However, the specific contribution of probiotic metabolites to the observed antioxidant activity would require further biochemical characterization.

The crude fiber content increased from 1.20% in the control to 2.10% in T₃, representing an increase of approximately 75%. This difference may be attributed to the greater contribution of fruit-derived structural components, including dietary fiber and other insoluble or soluble plant materials, in the optimized formulation. The higher fiber content may further contribute to the nutritional value of the beverage.

Overall, the optimized probiotic formulation exhibited higher vitamin C content, antioxidant activity, and crude fiber compared with the control. These improvements indicate that the incorporation of selected fruits and probiotic fermentation may enhance the nutraceutical characteristics of the beverage. The results therefore support the potential of T₃ as a functional beverage with improved nutritional and bioactive properties.

Storage Stability of the Probiotic Beverage

The storage stability of the optimized probiotic beverage (T₃) was evaluated over a period of 21 days by monitoring changes in pH, titratable acidity, and total soluble solids (TSS). The results are presented in Table

Table 5: Changes in pH, Acidity, and TSS during 21 Days of Storage

Parameter	Day 0	Day 07	Day 14	Day 21
pH	4.05	3.98	3.91	3.84
Acidity (%)	0.54	0.57	0.60	0.64
TSS (°Brix)	18.0	18.10	18.20	18.30

A gradual decline in pH was observed during storage, with the value decreasing from 4.05 on day 0 to 3.84 on day 21. In contrast, titratable acidity increased progressively from 0.54% to 0.64% over the same storage period. The inverse relationship between pH and titratable acidity indicates a gradual increase in the acidic character of the beverage during storage. Such changes are commonly observed in probiotic fruit beverages and may be associated with the continued metabolic activity of viable microorganisms and the formation or accumulation of organic acids during storage.

The TSS showed a slight increase from 18.00 °Brix on day 0 to 18.30 °Brix on day 21. The relatively small change in TSS suggests that the soluble-solids content of the beverage remained comparatively stable throughout the storage period. Variations in TSS during storage may result from changes in soluble sugars and other dissolved components; however, the observed increase was small and may also reflect normal analytical variation.

Overall, the optimized probiotic beverage exhibited gradual changes in pH and acidity during the 21-day storage period, while TSS remained relatively stable. The decrease in pH from 4.05 to 3.84 and corresponding increase in acidity from 0.54% to 0.64% suggest progressive acid development during storage. Similar changes in pH and titratable acidity have been reported during refrigerated storage of probiotic fruit beverages. Therefore, monitoring these parameters is important for assessing the physicochemical stability and sensory quality of probiotic beverages during storage.

Microbial Quality and Probiotic Viability During Storage:

The microbial quality and probiotic viability of the optimized beverage (T₃) were monitored during refrigerated storage for 21 days. The probiotic count, yeast and mold, and coliform status were assessed at regular intervals, and the results are presented in Table

Table 6: Changes in Probiotic Count and Microbiological Quality during 21 Days of Storage

Parameter	Initial	Day 07	Day 14	Day 21
Probiotic Count (CFU/g)	1.6×10^8	2.1×10^8	2.4×10^8	2.8×10^8
Yeast & Mold	Absent	Absent	Absent	Absent
Coliforms	Absent	Absent	Absent	Absent

The probiotic count showed a gradual increase during the storage period, rising from 1.6×10^8 CFU/g at the beginning of storage to 2.1×10^8 , 2.4×10^8 , and 2.8×10^8 CFU/g on days 7, 14, and 21, respectively. This progressive increase indicates that the probiotic microorganisms remained viable under the storage conditions and were able to maintain their population throughout the 21-day period. The increase may be associated with the availability of residual fermentable nutrients in the beverage, which could support continued microbial activity during refrigerated storage.

Overall, the optimized probiotic beverage demonstrated good microbial stability during the 21-day storage period. The maintenance and progressive increase of probiotic counts, together with the absence of detectable yeast, mold,

and coliforms, indicate that the formulation provided favorable conditions for probiotic survival while maintaining microbiological quality. Similar studies on probiotic fruit beverages have emphasized the importance of monitoring viable probiotic counts alongside undesirable microorganisms during storage.

Conclusion

The present study demonstrated the successful development and optimization of a non-dairy probiotic beverage based on a mixed fruit blend of pineapple (*Ananas comosus*), apple (*Malus domestica*), and sapota (*Manilkara zapota*), with jaggery serving as a natural sweetening ingredient. The beverage was further fortified with microencapsulated

probiotic cultures, *Lactiplantibacillus plantarum* and *Lactiacaseibacillus casei*. Based on the sensory, physicochemical, nutritional, and microbiological evaluations, the optimized formulation (T₃) showed promising characteristics for development as a functional beverage. Overall, the findings suggest that the combination of mixed fruits, jaggery, and microencapsulated probiotic cultures can be used to develop a promising non-dairy functional beverage. The use of an alginate-guar gum encapsulation system may have contributed to maintaining probiotic viability in the acidic fruit matrix. Nevertheless, direct encapsulation efficiency and probiotic protection should be quantified separately to establish the effectiveness of the encapsulation process. Further research is required to establish the functional and commercial potential of the developed beverage. Future studies may focus on:

- **Gastrointestinal survival:** Evaluation of probiotic survival under simulated gastric and intestinal conditions and, subsequently, through appropriate in-vivo studies.
- **Probiotic functionality:** Assessment of strain-specific probiotic properties, including adhesion, tolerance to gastrointestinal conditions, and potential health-promoting effects.
- **Extended shelf-life studies:** Investigation of physicochemical, microbiological, sensory, and probiotic stability over longer storage periods.
- **Storage optimization:** Evaluation of different storage temperatures and development of predictive shelf-life models under real-world distribution conditions.
- **Encapsulation optimization:** Further optimization of alginate-guar gum concentration, encapsulation efficiency, particle characteristics, and probiotic release behavior.
- **Scale-up and process development:** Optimization of fermentation, encapsulation, filling, and packaging processes for pilot- and industrial-scale production.
- **Consumer and economic assessment:** Large-scale consumer acceptability studies, packaging evaluation, cost analysis, and market feasibility assessment.

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