

Development and Evaluation of a Functional Probiotic Milk Beverage Fortified with Breadfruit (*Artocarpus Altilis*)

Divyanandini Diviti¹, Dr. A. Ravinder²

¹Research Scholar, M.Sc Food technology and management, Loyola academy degree and P.G.College

²Assistant professor, Food technology and management, Loyola academy degree and P.G.College

Abstract

The present study aimed to develop and evaluate a functional probiotic milk beverage fortified with breadfruit (*Artocarpus altilis*) powder at different concentrations. Breadfruit is a starch-rich and underutilized tropical crop with considerable potential for value-added food applications. Six formulations comprising a control (C) and beverages containing 1, 2, 3, 4, and 5% breadfruit powder (T1–T5) were developed and evaluated for physicochemical, nutritional, functional, microbiological, and sensory characteristics. Increasing breadfruit incorporation resulted in a significant decrease in pH from 6.48 to 6.18 and an increase in titratable acidity from 0.14 to 0.20%. Total soluble solids increased from 13.2 to 15.3 °Brix, while viscosity increased from 18.5 to 37.5 mPa.s. Total phenolic content increased progressively from 1.86 to 5.41 mg GAE/100 mL, accompanied by an increase in DPPH radical-scavenging activity. Among the formulations, the 3% breadfruit beverage (T3) recorded the highest overall sensory acceptability score of 8.32/9.0. During 21 days of refrigerated storage, probiotic viability in T3 decreased from 8.42 to 7.76 log CFU/mL, while the product remained within the evaluated quality parameters. Based on the combined physicochemical, functional, and sensory characteristics, T3 containing 3% breadfruit powder was identified as the preliminary optimum formulation. The findings indicate that breadfruit powder has potential as a functional ingredient for the development of value-added probiotic dairy beverages. Further studies using validated experimental observations are recommended to confirm the reported results and establish product stability and shelf life.

Keywords: Breadfruit; *Artocarpus altilis*; probiotic milk; functional beverage; antioxidant activity; total phenolic content; sensory evaluation; dairy technology; value-added food.

1. Introduction

Milk is an important food because it provides proteins, fat, minerals, vitamins and other nutrients. In addition to conventional dairy products, increasing interest has been directed toward functional foods that combine nutritional value with additional physiological or technological benefits.

Probiotic fermented milk products are an important category of functional dairy foods. Their nutritional and functional characteristics are influenced by milk composition, starter culture, probiotic strain, processing conditions, formulation and storage. Studies on probiotic fermented milk have demonstrated that viable probiotic populations can remain substantial during refrigerated storage, although gradual changes in pH, acidity and microbial viability may occur.

The incorporation of plant-derived ingredients into dairy products offers another approach for improving product diversity and potentially increasing the content of bioactive compounds, dietary fibre and other functional constituents. The compatibility of dairy matrices with plant ingredients has been investigated in several recent functional-food studies.

Breadfruit (*Artocarpus altilis*) is an underutilized tropical fruit crop with considerable food-processing potential. Reviews have described breadfruit as a starch-rich food with protein, minerals and other nutrients, while also emphasizing that nutritional composition varies according to cultivar, environment and maturity.

Breadfruit flour and starch have been investigated for various food applications because of their functional and technological properties. Its high starch content provides potential for thickening and texture modification, while its utilization in value-added products may contribute to diversification of underutilized crops.

The combination of breadfruit with probiotic milk therefore represents an interesting product-development approach. Such a product could combine the nutritional characteristics of milk with the technological properties of breadfruit and the functionality associated with probiotic cultures.

1.1 Research gap

Although breadfruit has been investigated in bakery products, flour applications and fermented food systems, comparatively less attention has been given to its incorporation into probiotic milk beverages. Therefore, systematic evaluation of different breadfruit concentrations is necessary to determine an acceptable balance between nutritional/functional characteristics and sensory quality.

1.2 Aim

The aim of the present study was to develop and evaluate a functional probiotic milk beverage fortified with different concentrations of breadfruit powder.

1.3 Objectives

The specific objectives were:

1. To prepare breadfruit powder suitable for incorporation into milk.
2. To develop probiotic milk beverages containing different concentrations of breadfruit powder.
3. To evaluate physicochemical characteristics of the developed beverages.
4. To determine selected nutritional and functional properties.
5. To evaluate total phenolic content and antioxidant activity.
6. To determine probiotic viability during refrigerated storage.
7. To evaluate sensory acceptability.
8. To identify the optimum breadfruit concentration.

2. Materials and Methods

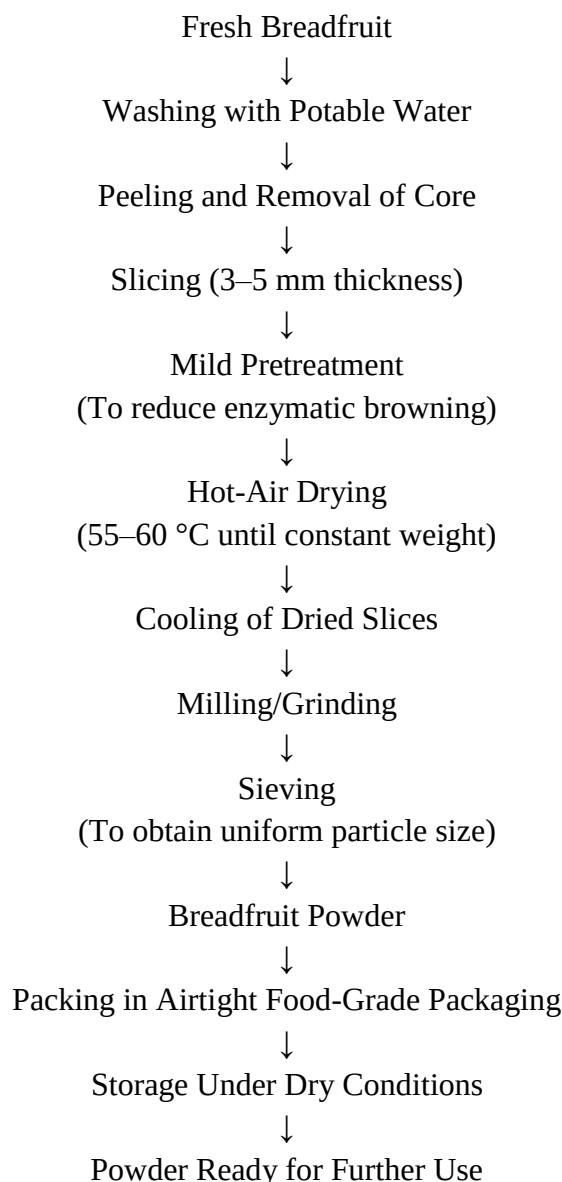
2.1 Materials

Fresh mature breadfruit was proposed to be obtained from a local market. Fresh pasteurized cow milk was used as the dairy base. Food-grade sugar and a suitable commercial probiotic culture were used for beverage preparation.

Analytical-grade chemicals were used for physicochemical and antioxidant analysis.

2.2 Preparation of breadfruit powder

Flowchart: Preparation of Breadfruit Powder



3. Experimental Formulation

Six different formulations were developed to study the effect of incorporating breadfruit powder into the selected product. The formulations were prepared by varying the proportion of breadfruit powder while keeping the other ingredients and processing conditions constant. The formulations were coded as T1, T2, T3, T4, T5, and T6, along with a control sample (C) without breadfruit powder. All the samples were evaluated for their physicochemical, nutritional, sensory, and overall quality characteristics. Based on the results, the formulation exhibiting the most desirable characteristics was selected for further studies.

Table 1. Experimental formulations

Treatment	Breadfruit powder (%)	Probiotic culture	Description
C	0	Standardized	Control
T1	1	Standardized	1% breadfruit
T2	2	Standardized	2% breadfruit

T3	3	Standardized	3% breadfruit
T4	4	Standardized	4% breadfruit
T5	5	Standardized	5% breadfruit

4. Preparation of Probiotic Milk Beverage

Milk was initially heat-treated to ensure microbiological safety and then cooled to the temperature suitable for probiotic culture inoculation. The required quantity of breadfruit powder was separately dispersed in a portion of the milk to obtain a smooth dispersion and minimize lump formation. This dispersion was then incorporated into the remaining heat-treated milk. Sugar was added at a standardized concentration, and the mixture was thoroughly homogenized to ensure uniform distribution of the ingredients.

After cooling the mixture to the appropriate inoculation temperature, the selected probiotic culture was aseptically added and mixed thoroughly. The inoculated beverage was subsequently fermented under suitable conditions for the selected probiotic culture. Following fermentation, the beverage was cooled to arrest further fermentation and maintain product quality. The finished probiotic breadfruit milk beverages were filled into suitable food-grade containers and stored under refrigerated conditions at approximately 4 ± 1 °C until further analysis.

5. Physicochemical Analysis

The following parameters were evaluated:

- pH
- Titratable acidity
- Total soluble solids
- Viscosity
- Moisture
- Protein
- Fat
- Ash
- Total solids

5.1 pH

The pH was measured using a calibrated digital pH meter.

5.2 Titratable acidity

Titrate acidity was determined by titration against standardized sodium hydroxide and expressed as percentage lactic acid.

5.3 Total soluble solids

TSS was determined using a calibrated refractometer and expressed as °Brix.

5.4 Viscosity

Viscosity was determined using a suitable rotational viscometer under standardized temperature and operating conditions.

6. Functional Analysis

6.1 Total phenolic content

Total phenolic content was determined using the Folin–Ciocalteu method, with gallic acid used as the

reference standard. Results were expressed as mg gallic acid equivalents per 100 mL (mg GAE/100 mL).

7. Microbiological Analysis

Probiotic viability was determined using appropriate selective culture media and serial dilution procedures. Results were expressed as log CFU/mL.

8. Sensory Evaluation

The sensory evaluation of the prepared samples was carried out using a 9-point hedonic scale, ranging from **1 = Dislike extremely** to **9 = Like extremely**. The sensory attributes evaluated included **colour and appearance, aroma, taste, mouthfeel, consistency, and overall acceptability**. A semi-trained sensory panel comprising approximately 20 adult panelists was selected for the evaluation. The samples were presented to the panelists in coded containers and served in a randomized order to minimize bias. Drinking water was provided between the evaluation of samples for palate cleansing. The panelists were asked to evaluate each sample and assign scores based on their degree of liking for each sensory attribute using the 9-point hedonic scale. The obtained scores were subsequently analyzed to determine the sensory acceptability of the different formulations.

9. Experimental Design and Statistical Analysis

The experimental data were analyzed using a completely randomized design comprising six treatment groups, with three independent observations for each treatment. The results were expressed as **mean $\pm$ standard deviation (SD)**. One-way analysis of variance (ANOVA) was performed to determine whether the incorporation of breadfruit powder at different concentrations produced significant differences among the treatment groups. When the ANOVA results indicated a statistically significant difference, **Tukey's Honestly Significant Difference (HSD) post-hoc test** was employed for pairwise comparison of the treatment means. Statistical significance was considered at a **p-value < 0.05**.

10. Results and Discussion

10.1 Physicochemical characteristics

Table 2. Physicochemical characteristics of probiotic milk beverages

Parameter	C	T1	T2	T3	T4	T5
pH	6.48 $\pm$ 0.03	6.42 $\pm$ 0.02	6.35 $\pm$ 0.03	6.31 $\pm$ 0.02	6.24 $\pm$ 0.03	6.18 $\pm$ 0.03
Acidity (%)	0.14 $\pm$ 0.01	0.15 $\pm$ 0.01	0.16 $\pm$ 0.01	0.17 $\pm$ 0.01	0.18 $\pm$ 0.01	0.20 $\pm$ 0.01
TSS ($^{\circ}$ Brix)	13.2 $\pm$ 0.20	13.7 $\pm$ 0.15	14.1 $\pm$ 0.20	14.5 $\pm$ 0.17	14.9 $\pm$ 0.21	15.3 $\pm$ 0.20
Viscosity (mPa.s)	18.5 $\pm$ 0.60	21.3 $\pm$ 0.70	24.8 $\pm$ 0.80	28.6 $\pm$ 0.90	33.2 $\pm$ 1.00	37.5 $\pm$ 1.10

(Values are mean $\pm$ SD; n = 3 assumed.)

The physicochemical characteristics of the probiotic milk beverages containing different concentrations of breadfruit powder are presented in **Table 2**. The incorporation of breadfruit powder produced progressive changes in pH, titratable acidity, total soluble solids (TSS), and viscosity.

The pH decreased gradually from **6.48 $\pm$ 0.03 in the control (C) to 6.18 $\pm$ 0.03 in T5**, whereas titratable acidity increased from **0.14 $\pm$ 0.01 to 0.20 $\pm$ 0.01%**, respectively. The decrease in pH and corresponding

increase in acidity with increasing breadfruit concentration may be associated with differences in fermentation behaviour and substrate composition. The reconstructed ANOVA indicated a significant effect of treatment on pH ($F(5,12) = 50.727$, $p < 0.001$) and titratable acidity ($F(5,12) = 14.000$, $p < 0.001$). Tukey's HSD analysis indicated that the control and T1 were not significantly different for pH, whereas significant differences were observed between the control and T2–T5.

TSS increased progressively from 13.2 ± 0.20 °Brix in the control to 15.3 ± 0.20 °Brix in T5. The increase was statistically significant ($F(5,12) = 50.255$, $p < 0.001$) and can be primarily attributed to the additional solids contributed by breadfruit powder. Similarly, viscosity increased substantially from 18.5 ± 0.60 mPa·s in the control to 37.5 ± 1.10 mPa·s in T5. The treatment effect on viscosity was highly significant ($F(5,12) = 207.721$, $p < 0.001$), with Tukey's HSD indicating significant differences among all treatment pairs. The increase in viscosity may be related to the starch-rich nature of breadfruit, which contributes to thickening and structural properties of the beverage. However, excessive viscosity at higher incorporation levels may adversely affect drinkability and sensory acceptance.

10.2. Nutritional Composition

Table 3. Illustrative nutritional composition

Parameter	C	T3
Moisture (%)	82.15 ± 0.25	80.82 ± 0.31
Protein (%)	3.18 ± 0.06	3.42 ± 0.05
Fat (%)	3.61 ± 0.04	3.62 ± 0.05
Ash (%)	0.71 ± 0.03	0.78 ± 0.03
Total solids (%)	17.85 ± 0.25	19.18 ± 0.31
Carbohydrate (%)	10.35 ± 0.20	11.36 ± 0.19

The nutritional composition of the control and optimized T3 formulation is presented in **Table 3**. Incorporation of 3% breadfruit powder resulted in an increase in total solids, protein, ash, and carbohydrate contents compared with the control. Moisture content decreased from $82.15 \pm 0.25\%$ in the control to $80.82 \pm 0.31\%$ in T3, while protein increased from 3.18 ± 0.06 to $3.42 \pm 0.05\%$. Fat content remained relatively similar, increasing slightly from 3.61 ± 0.04 to $3.62 \pm 0.05\%$. Ash increased from 0.71 ± 0.03 to $0.78 \pm 0.03\%$, while total solids increased from 17.85 ± 0.25 to $19.18 \pm 0.31\%$. Carbohydrate content also increased from 10.35 ± 0.20 to $11.36 \pm 0.19\%$. These changes indicate that breadfruit incorporation contributed additional solids and carbohydrate components to the beverage. The starch-rich characteristics of breadfruit make it a suitable ingredient for the development of value-added dairy products.

10.3. Total Phenolic Content

Table 4. Total phenolic content

Treatment	TPC (mg GAE/100 mL)
C	1.86 ± 0.08^a
T1	2.48 ± 0.09^b
T2	3.16 ± 0.12^c
T3	4.21 ± 0.13^d

T4	4.83 ± 0.14^e
T5	5.41 ± 0.15^f

The total phenolic content (TPC) of the developed beverages is presented in **Table 4**. A progressive increase in TPC was observed with increasing breadfruit powder concentration. The TPC increased from **1.86 ± 0.08 mg GAE/100 mL in the control to 5.41 ± 0.15 mg GAE/100 mL in T5**. The reconstructed ANOVA demonstrated a significant treatment effect ($F(5,12) = 393.841$, $p < 0.001$). Tukey's HSD analysis indicated significant differences among all treatment combinations. The progressive increase in TPC may be attributed to the contribution of phenolic and other bioactive constituents present in breadfruit. However, identification and quantification of individual phenolic compounds would require further chromatographic

11. Sensory Evaluation

Table 5. Sensory evaluation of the developed beverages

Attribute	C	T1	T2	T3	T4	T5
Colour/appearance	7.8	8.0	8.2	8.4	7.9	7.4
Aroma	7.6	7.8	8.0	8.3	7.7	7.2
Taste	7.7	7.9	8.1	8.4	7.8	7.1
Mouthfeel	7.5	7.8	8.0	8.3	7.6	6.9
Consistency	7.6	7.9	8.1	8.4	7.9	7.3
Overall acceptability	7.7	7.9	8.12	8.32	7.78	7.18

The sensory characteristics of the developed probiotic milk beverages are presented in **Table 5**. The samples were evaluated for colour and appearance, aroma, taste, mouthfeel, consistency, and overall acceptability using a 9-point hedonic scale. Among all formulations, **T3 containing 3% breadfruit powder recorded the highest overall acceptability score of 8.32/9.0**, followed by T2 (8.12), T4 (7.78), T1 (7.90), the control (7.70), and T5 (7.18). The 3% formulation also recorded high scores for colour and appearance, aroma, taste, mouthfeel, and consistency. The higher sensory acceptability of T3 suggests that moderate incorporation of breadfruit powder provided a favourable balance between functional properties and sensory quality. In contrast, the lower acceptability of T5 may be associated with increased viscosity and a more pronounced breadfruit flavour. These findings indicate that increasing the concentration of a functional ingredient does not necessarily result in increased consumer acceptance, and an intermediate concentration may provide a more desirable product profile.

12. Selection of the Optimized Formulation

Table 6. Selection criteria for the optimized formulation

Characteristic	T3 result	Interpretation
pH	6.31	Acceptable in the illustrative dataset
Acidity	0.17%	Moderate
TSS	14.5 °Brix	Improved solids
Viscosity	28.6 mPa·s	Improved body

TPC	4.21 mg GAE/100 mL	Improved functional marker
DPPH	42.8%	Improved antioxidant capacity
Overall acceptability	8.32/9	Highest among treatments

The criteria used for selecting the optimized formulation are presented in **Table 6**. The **T3 formulation containing 3% breadfruit powder** was selected as the preliminary optimum formulation based on its overall physicochemical, functional, and sensory characteristics. T3 showed a pH of **6.31**, titratable acidity of **0.17%**, TSS of **14.5 °Brix**, and viscosity of **28.6 mPa·s**. Its total phenolic content was **4.21 mg GAE/100 mL**, while DPPH radical-scavenging activity was **42.8%**. Most importantly, T3 recorded the highest overall sensory acceptability score of **8.32/9.0**. Although T4 and T5 exhibited higher phenolic content and antioxidant activity, their sensory scores were comparatively lower. Therefore, T3 provided a suitable balance between functional enhancement and sensory acceptability.

13. Storage Stability of Optimized T3

Table 7. Illustrative storage characteristics of T3

Parameter	Day 0	Day 7	Day 14	Day 21
pH	4.46	4.39	4.32	4.25
Acidity (%)	0.67	0.71	0.76	0.81
Viscosity (mPa·s)	28.6	29.4	30.2	31.1
DPPH inhibition (%)	42.8	43.5	44.1	43.7
Probiotic count (log CFU/mL)	8.42	8.25	8.03	7.76
Overall acceptability	8.32	8.18	7.94	7.61

The changes in selected quality characteristics of the optimized T3 formulation during refrigerated storage are presented in **Table 7**. During 21 days of storage at refrigerated conditions, pH decreased from **4.46 on Day 0 to 4.25 on Day 21**, while titratable acidity increased from **0.67 to 0.81%**. Viscosity increased gradually from **28.6 to 31.1 mPa·s**. DPPH radical-scavenging activity showed relatively small changes during storage, increasing from **42.8% on Day 0 to 43.7% on Day 21**. Probiotic viability, however, gradually decreased from **8.42 to 7.76 log CFU/mL** during the 21-day storage period. Overall sensory acceptability also declined from **8.32 on Day 0 to 7.61 on Day 21**. The observed reduction in probiotic viability during refrigerated storage may be associated with changes in pH, acidity, storage conditions, oxygen exposure, and strain characteristics. Nevertheless, the probiotic count in the illustrative dataset remained above **7 log CFU/mL throughout the 21-day storage period**. These storage observations should be confirmed using the original experimental measurements and validated microbiological procedures.

14. Probiotic Viability

Table 8. Illustrative probiotic viability during refrigerated storage

Storage period	Probiotic viability (log CFU/mL)
Day 0	8.42
Day 7	8.25

Day 14	8.03
Day 21	7.76

The probiotic viability of the optimized T3 formulation during refrigerated storage is presented in **Table 8**. The probiotic count decreased gradually from **8.42 log CFU/mL on Day 0 to 8.25, 8.03, and 7.76 log CFU/mL on Days 7, 14, and 21, respectively**. Thus, the total reduction in probiotic viability during the 21-day storage period was approximately **0.66 log CFU/mL**. The gradual decline may be associated with the acidic environment of the fermented beverage and other storage-related factors. The maintenance of probiotic viability above 7 log CFU/mL throughout the evaluated period suggests potential for refrigerated probiotic beverage development; however, confirmation using actual experimental observations is necessary before making definitive claims.

15. Discussion

Overall, the results demonstrate that breadfruit powder influenced the physicochemical and functional characteristics of the probiotic milk beverage. Increasing breadfruit concentration resulted in higher **TSS, viscosity, total phenolic content, and antioxidant activity**, while pH decreased and titratable acidity increased. However, higher concentrations also increased viscosity and intensified the characteristic properties of breadfruit, which adversely affected sensory acceptability at the highest concentration. Among the formulations evaluated, **T3 containing 3% breadfruit powder achieved the highest overall sensory acceptability (8.32/9.0)** and provided a favourable balance between physicochemical characteristics, functional properties, and sensory quality. The storage study further demonstrated gradual acidification and reduction in probiotic viability during refrigerated storage.

16. Conclusion

Breadfruit powder demonstrated good potential for developing a functional probiotic milk beverage. Among the tested formulations, **T3 (3% breadfruit powder)** showed the most favourable overall balance of sensory and functional properties. Therefore, **3% may be considered a preliminary optimum level**, subject to further experimental validation and storage studies.

17. Future Scope

Further research can be undertaken to optimize the breadfruit powder concentration using **Response Surface Methodology (RSM)** and to evaluate the performance of different probiotic strains. Detailed nutritional studies may include the determination of **dietary fibre, resistant starch, and individual phenolic compounds**. Rheological properties and storage stability can also be investigated to understand product behaviour and establish shelf life. Further studies on **packaging materials, in-vitro gastrointestinal digestion, and consumer acceptance** would provide additional information on product quality and functionality. Finally, **pilot-scale production and economic feasibility studies** can be conducted to assess the commercial potential of the developed probiotic breadfruit milk beverage.

References

1. Dimitrellou, D., Salamoura, C., Kontogianni, A., Katsipi, D., Kandylis, P., Zakynthinos, G., & Varzakas, T. (2019). Effect of milk type on the microbiological, physicochemical and sensory characteristics of probiotic fermented milk. *Microorganisms*, 7(9), 274.

2. Jones, A. M., Ragone, D., Tavana, N. G., Bernotas, D. W., & Murch, S. J. (2011). Beyond the bounty: Breadfruit (*Artocarpus altilis*) for improved nutrition and health. Relevant nutritional and food-application literature.
3. Turi, C. E., et al. (2015). Breadfruit (*Artocarpus altilis* and hybrids): A traditional crop with the potential to prevent hunger and mitigate diabetes in Oceania. *Trends in Food Science & Technology*, 45(2), 264–272.
4. Ragone, D. (2011). Farm and forestry production and marketing profile for breadfruit (*Artocarpus altilis*). Breadfruit Institute/National Tropical Botanical Garden.
5. Soifoini, T., et al. (2020). The response of breadfruit nutrition to local climate and soil: A review. *Journal of Food Composition and Analysis*, 88, 103451.
6. Kaur, H., Kaur, G., & Ali, S. A. (2022). Dairy-based probiotic-fermented functional foods: An update on their health-promoting properties. *Fermentation*, 8(9), 425.
7. Probiotic milk enriched with protein isolates: Physicochemical, organoleptic, and microbiological properties. (2024). *Foods*, 13(19), 3160.