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SCHOLARLY, PEER REVIEWED
Volume 25 No. 9
October 2025
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

Afr. J. Food Agric. Nutr. Dev. 2025; 25(9): 27795-27817 https://doi.org/10.18697/ajfand.146.26230

Date	Submitted	Accepted	Published
	11th August 2025	14th October 2025	6th November 2025

MICROBIAL, CHEMICAL AND SENSORY PROPERTIES OF PINEAPPLE AND BEETROOT JUICE BLEND FERMENTED USING MIXED PROBIOTIC LACTIC ACID BACTERIA CULTURES (*LACTOBACILLUS PLANTARUM* AND *L. RHAMNOSUS*)

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SCHOLARLY, PEER REVIEWED
Volume 25 No. 9
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ISSN 1684 5374

ABSTRACT

The rising demand for functional foods has increased interest in probiotic products beyond traditional dairy sources, particularly among individuals with lactose intolerance, dairy allergies, or those adhering to plant-based diets. Fruit juices such as pineapple and beetroot offer promising non-dairy alternatives for delivering probiotic cultures. This study aimed to analyze the microbial, physicochemical, nutritional, and sensory acceptability profiles of pineapple and beetroot juice blends fermented with a mixed culture of probiotic lactic acid bacteria (*Lactobacillus plantarum* and *Lactobacillus rhamnosus*). Juice blends were prepared in varying pineapple-to-beetroot ratios: 90:10 (P90:B10), 80:20 (P80:B20) and 70:30 (P70:B30), alongside 100% pineapple (control-P) and 100% beetroot (Br) juices. All blends were pasteurized and inoculated with the probiotic cultures and fermented for 28 days, except the control-P and 100% Br treatment, which underwent spontaneous fermentation. Lactic acid bacteria (LAB) count analysis showed that all inoculated blends maintained probiotic viability (>7 log CFU/mL) for up to 21 days, with the P90:B10 blend maintaining viability up to 28 days. Yeast or mold growth was not detected in the probiotic blends up to day 21, whereas the control-P and 100%Br treatment exceeded yeast and mold count limits by day 14. Physicochemical analysis for all treatments revealed a significant increase in titratable acidity, accompanied by a decrease in pH and sugar content over the fermentation period in the inoculated blends (P90:B10, P80:B20, P70:B30). Significant differences $P < 0.05$ in vitamin C content were observed between the control-P and all other treatments on day 1. Among the three blends, P90:B10 achieved the highest overall consumer acceptability. Fermenting pineapple-beetroot juice blends with *L. plantarum* and *L. rhamnosus* mixed culture is a feasible approach to producing a safe, palatable, and nutritionally beneficial non-dairy probiotic beverage. The P90:B10 blend was optimal in supporting probiotic viability and achieving desirable consumer sensory attributes and, therefore, would be recommended for future commercialization as a probiotic non-dairy product.

Key words: probiotics, lactic acid bacteria, pineapple-beetroot blend, fermentation, functional food

Citation: Thuku JK, Owaga EE and EN Wafula Microbial, Chemical and Sensory Properties of Pineapple and Beetroot Juice Blend Fermented Using Mixed Probiotic Lactic Acid Bacteria Cultures (*Lactobacillus plantarum* and *L. Rhamnosus*). *Afr. J. Food Agric. Nutr. Dev.* 2025; **25(9):** 27795-27817. https://doi.org/10.18697/ajfand.146.26230

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ISSN 1684 5374

INTRODUCTION

The use of probiotics in both dairy and non-dairy food products has gained increasing global attention due to growing awareness of their health benefits. Probiotics are live microorganisms that when administered in adequate amounts (≥7 log CFU/mL), confer health benefits to the host [1]. These benefits include improved gut health, enhanced immune response, prevention of gastrointestinal disorders and protection against respiratory and urinary tract infections [2]. Traditionally, dairy products such as yogurt and fermented milk have been the primary carriers of probiotics. However, with rising cases of lactose intolerance, milk allergies and the growing number of vegetarians and vegans, there is a pressing need for non-dairy alternatives that support probiotic delivery [3,4].

Pineapple is predominantly grown in the Central and Coastal regions of Kenya and is rich in dietary fiber, vitamins, minerals, antioxidants, and enzymes with anti-inflammatory and digestive benefits [5, 6]. Beetroot is commonly grown in Central Kenya and is valued for its antioxidant properties, betalain pigments (used as natural colorants) and therapeutic effects such as anti-inflammatory, antihypertensive and antidiabetic properties [7,8].

Over 50% of fruit production in Kenya is lost due to inadequate postharvest prevention and value addition strategies [9,10]. Therefore, there is a critical need to develop novel value- added products such as probiotic fruit juices that can enhance nutritional benefits, improve shelf life, and contribute to local economic empowerment.

Lactic acid bacteria (LAB) such as *Lactobacillus plantarum* and *Lactobacillus rhamnosus* are widely associated with health promoting properties [11,12,13]. Although these strains have been widely used in dairy applications, their incorporation into fruit-based probiotic beverages is gaining traction. Most existing research on probiotic products has focused on dairy matrices. Limited studies have examined the fermentation of blended pineapple and beetroot juices, particularly using whole fruit extractions and probiotic cultures. Therefore, this study addresses that gap by evaluating the potential of pineapple and beetroot juice blends as carriers for *L. plantarum* and *L. rhamnosus*. The present study aimed to develop and assess fermented juice blends in terms of probiotic viability, physicochemical attributes, and sensory acceptability.

MATERIALS AND METHODS

Sampling of pineapples and beet roots

Mature, high-quality pineapples of the Smooth Cayenne variety and red-colored beetroot were purchased locally from Thika town retail market, Kenya. The fruits

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were transported to the Food Processing Workshop at Dedan Kimathi University of Technology (DeKUT), refrigerated at 4°C to 7°C, while awaiting juice samples preparation.

Preparation of pineapple and beetroot juice blends

Briefly, the crowns and leaves of the pineapple and beetroot were removed, and the fruits were then cleaned with sterile water. The whole fruits, including the peels, were sliced into smaller pieces, and grated. The pulp was extracted using a juice extractor for both fruits, weighed and allowed to settle for 5 min to reduce haziness and stabilize foaming [14]. The juice blends were prepared by mixing the extracted pulp in different ratios: pineapple to beetroot at 90%:10% (P90:B10), 80%:20% (P80:B20) and 70%:30% (P70:B30), as well as 100% pineapple juice (P-control) and 100% beetroot juice (Br). The blends were pasteurized at 85°C for 5 min and then cooled to 37°C. The blends were aseptically inoculated with a 0.02% inoculum and incubated at 37°C for 24 hours.

Preparation of LAB Starter cultures

The mixed culture of *L. plantarum* and *L. rhamnosus* was obtained from Dairy Consulting Africa Limited, Nairobi, Kenya, and stored at refrigeration temperatures of 4°C to 7°C in the Food Microbiology Laboratory at DeKUT. To assess the viability of the starter culture and determine the appropriate quantity to use for a given volume of juice, pure strains of *L. plantarum* and *L. rhamnosus* were reactivated by anaerobically subculturing in MRS Broth at 37°C for 10 min. Afterwards, serial dilutions and the spread plate technique were employed to determine the viable cell count. The cultures were then incubated at 37°C for 24 hours. To calculate the required quantity of viable LAB cells for inoculation, the formula $C_1V_1 = C_2V_2$ was used in calculating the precise volume of starter culture needed to achieve a desired final concentration of LAB in the total volume of the juice.

Where: C_1- Total Viable counts (MRS Broth), V_1-Volume of sample (for spread plate), C_2-Total viable count (expected -for juice), V_2-Volume of the Juice.

To ensure a consistent population of >7 log CFU/mL, 0.02% culture for inoculating the juice blends was used as the optimal percentage.

Fermentation of pineapple and beetroot juice blends

Lactic acid bacteria (LAB) count was enumerated on De Man, Rogosa, and Sharpe (MRS) agar, while yeast and mold count were determined using Rose Bengal Chloramphenicol agar [15]. Sterilized containers containing juice blends with varying pineapple to beetroot ratios (90%:10% (P90:B10), 80%:20% (P80:B20), 70%:30% (P70:B30), 100% pineapple juice (control- P), and 100% beetroot juice (Br)) were cooled to 37°C after pasteurization. The starter culture was added aseptically at a

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concentration of 0.02% and gently mixed. The mixtures were then incubated at 37°C for 24 hours [16]. After fermentation, the products were stored at 4°C to 7°C to arrest further proliferation of the probiotics. Aseptically drawn samples were collected after 24 hours of fermentation (day 1) and analyzed for LAB count, yeast and mold count, titratable acidity, pH, folate, vitamin C and Brix value. The samples were subsequently stored in a refrigerator at 4°C to 7°C. Additional samples were drawn at intervals on days 7, 14, 21, and 28 for further microbial and physicochemical analysis.

Analysis methods

Determination of LAB count

The viability of Lactic Acid Bacteria (LAB), specifically *L. plantarum* and *L. rhamnosus*, was analyzed using the spread plate technique [17]. Serial dilutions were made using sterile peptone water by transferring 1 ml of the previous dilution into 9 ml of sterile peptone water in sterile tubes. De Man, Rogosa, and Sharpe (MRS) agar media was prepared, autoclaved, and poured into plates to solidify. Using a sterile pipette (0.1 ml), inoculum from each test tube was transferred onto the agar plates and spread evenly using a sterile spreader. Each dilution was performed in duplicate.

The plates were incubated at 37°C for 24 hours, and the colony-forming units (CFUs) were counted. Plates with count between 25 and 250 colonies were selected for calculation. The LAB population per ml of the original sample was calculated using the following formula:

$$\text{Colony forming unit}\left(\frac{\text{CFU}}{0.1\text{ml}}\right) = \frac{\text{Number of colonies}}{\text{Volume of inoculum}} \times \text{Dilution factor}$$

Yeast and Mold count

Yeast and mold count were enumerated using the spread plate method using Rose Bengal Chloramphenicol agar [18]. Serial dilutions were made using sterile peptone water, and 0.1 ml from each dilution was pipetted onto the surface of the agar plates. The inoculum was spread evenly on the agar surface using a sterile spreader. Each dilution was performed in duplicate. The plates were incubated at 25°C for 7 days, while inverted to prevent condensation on the agar surface. Yeast and mold count were calculated using the following formula:

$$\text{Colony forming unit (CFU/0.1ml)} = \frac{\text{Number of colonies}}{\text{Volume of inoculum}} \times \text{Dilution factor}$$

Total titratable acidity (TTA)

Titratable acidity was measured and expressed as % acidity [19]. Briefly, 10 ml of each sample was placed in a conical flask, followed by the addition of 20 ml of

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distilled water. The sample was titrated dropwise with 0.1N NaOH until a pH of 8.0 was reached, and the burette reading was recorded.
TTA was calculated as follows:

$$\text{Total Titratable Acidity \%} = \frac{\text{Titer} \times \text{Normality of Alkali} \times \text{Volume made up} \times \text{Equivalent weight}}{\text{Volume of sample taken} \times \text{Volume of aliquot taken} \times 100}$$

pH

The pH of the samples was analyzed using an MP-6P pH meter (serial number MP04717) from Hach Company. The pH meter was calibrated using buffer solutions at pH 4 and pH 7.

Total soluble solids (TSS)

Total soluble solids (TSS) were determined using a hand-held refractometer model Number (HT113ATC Manufacturer US-HFLH) with a range of 0-32° Brix [20]. The percentage of soluble sugars (sugar content) was read directly from the refractometer scale.

Vitamin C analysis

The vitamin C content was determined using the High-Performance Liquid Chromatography (HPLC) method [22]. Each sample (2 ml) was extracted using metaphosphoric acid and diluted to a final volume of 30 ml. Calibration curves were prepared using ascorbic acid standard. High- Performance Liquid Chromatography (HPLC) analysis was performed using a Shimadzu 20A series system equipped with an SPD-M20A detector. The mobile phase consisted of 0.8% metaphosphoric acid, with a flow rate of 1.0 ml/min. Detection was carried out at a wavelength of 266 nm, and the column oven temperature was maintained at 30°C.

Folate analysis

Folate analysis was done using HPLC method [23]. Sample preparation involved solid-phase extraction (SPE) to eliminate components that could interfere with vitamin detection. Five (5) ml of the sample were mixed with 20 ml of deionized water and homogenized at medium speed for 1 min. The homogenate was centrifuged at 14,000 revolutions per minute for 10 min. The stationary phase was activated by flushing with 10 ml of methanol followed by 10 ml of water adjusted to pH 4.2.

The detection wavelength was 282 nm. Identification and quantification of compounds were carried out by comparing retention times with those of known standards.

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Evaluation of sensory acceptability

Sensory analysis for consumer acceptability was conducted by 50 untrained panelists who granted and consented to participate. A 9-point hedonic scale, ranging from 1 (dislike extremely) to 9 (like extremely), was used to assess variables such as color, aroma, mouthfeel, taste, and overall acceptability.

Ethical considerations

Data collection for the study was authorized by the Dedan Kimathi University of Technology Scientific Ethics Review Committee (DeKUTSERC/CA/03/01) and National Commission for Science, Technology, and Innovations (NACOSTI) License Number NACOSTI /P/23/28949. Panelists gave their informed consent to willingly participate in the study, whereas details of every participant were kept private and confidential.

Data analysis

Statistical analysis was performed using GraphPad Prism software (version 8.01, La Jolla, CA, USA). Data were presented as the mean ± standard deviation (SD). The mean differences were compared using a two-way analysis of variance (ANOVA), and significant differences were established at $p < 0.05$ using Tukey's test.

RESULTS AND DISCUSSION

Growth dynamics of Lactic Acid Bacteria (LAB) of fermented juice blends

The viability threshold for probiotic efficacy is typically ≥7 log CFU/mL at the time of consumption. Figure 1 presents lactic acid bacteria (LAB) growth across the different juice blend treatments. On Day 1, The highest LAB count was observed for all the blends at 15.02 log CFU/mL, 14.98 log CFU/mL and 11.75 log CFU/mL for P90:B10, P80:B20 and P70:B30, respectively. These values were significantly higher than the control-P, which recorded only 3.26 log CFU/mL, indicating the strong influence of inoculated probiotic strains on bacterial proliferation. The low LAB count in the control-P and Br treatments can be attributed to the reliance on spontaneous fermentation, which depends on the indigenous LAB present in raw fruits. In contrast, commercial probiotic cultures, such as *Lactobacillus plantarum* and *Lactobacillus rhamnosus* are typically optimized for high viability and performance [24].

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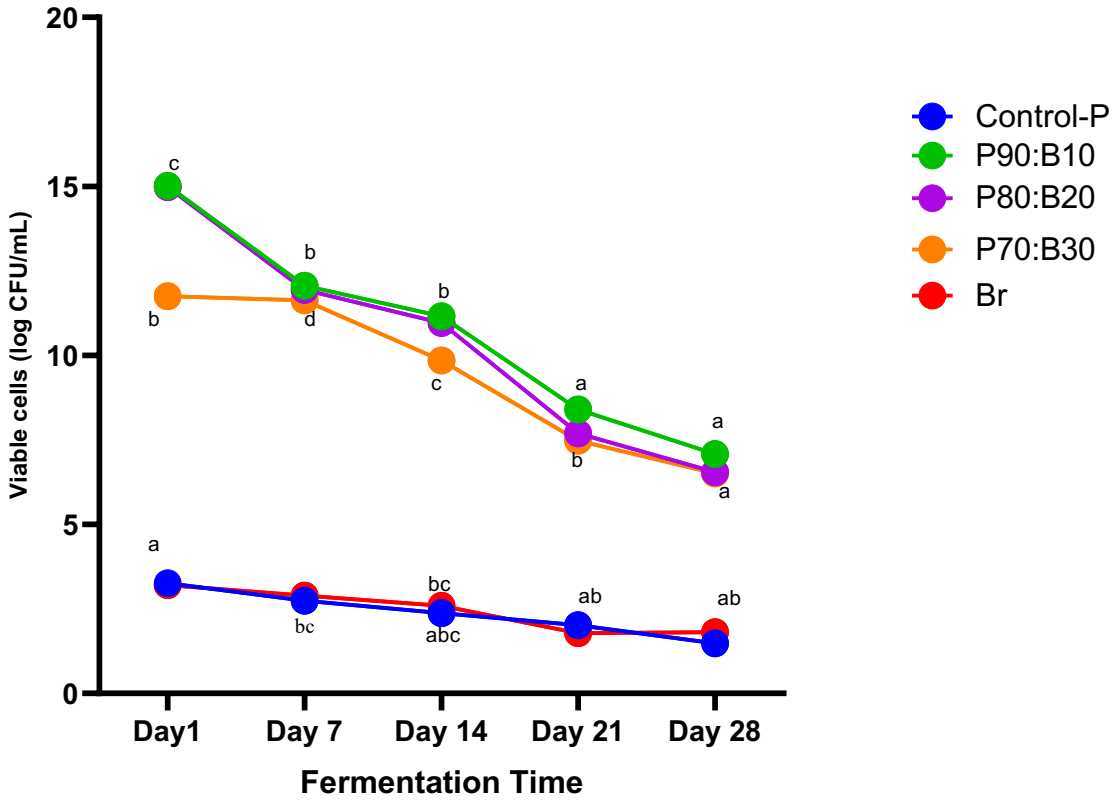

Figure 1: Lactic acid bacteria (LAB) count in fresh pineapple juice (control), fresh beetroot juice (naturally fermented), and various blends of pineapple and beetroot juice (inoculated)

LAB enumerations were conducted on days 1, 7, 14, 21, and 28, and all analyses were performed in duplicate. Means with different letters (a, ab, abc, b, bc, c and d) signifies statistical difference among treatments at $p < 0.05$.

Despite significant reduction in LAB count in all the blends (P90:B10), (P80:B20), (P70:B30), over the storage period, they retained their probiotic status up to day 21 after which the CFU/mL count reduced to below the threshold of ≥ 7; a similar trend had been reported in fermented beetroot based probiotic drink [16]. As fermentation progressed, sugar depletion and accumulation of organic acids such as lactic acid likely contributed to the reduced bacterial viability.

No significant differences were found between the control-P (pineapple only) and Br (beetroot only), which relied solely on natural fermentation despite substrate depletion over time [14].

The viability threshold for probiotic efficacy is typically ≥7 log CFU/mL at the time of consumption [24]. On Day 1, all probiotic-enriched blends exceeded this threshold

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with P90:B10 maintaining probiotic status throughout the 28 days, with a final count of 7.08 log CFU/mL. P80:B20 remained viable until Day 21 (7.69 log CFU/mL), dropping to 6.55 log CFU/mL on Day 28. Similarly, P70:B30 retained probiotic viability up to Day 21 (7.48 log CFU/mL) but declined to 6.51 log CFU/ml by Day 28. In contrast, the control-P and Br never exceeded 3.26 log CFU/ml, failing to meet the probiotic threshold at any point throughout the storage period.

Among all treatments, the P90:B10 blend consistently supported the highest LAB growth throughout the storage period. This can be attributed to two key factors: (i) the higher proportion of pineapple which has naturally higher sugar content (°Brix), providing an ideal substrate for probiotic metabolism, and (ii) the inclusion of unpeeled pineapple, which increased fiber content. Pineapple peel is rich in prebiotic molecules that stimulate probiotic activity. Similar findings on the positive impact of fruit peels including pineapple on the growth of *L. plantarum* and *L. rhamnosus* had been reported by Akter *et al.* [26].

Yeast and mold count of fermented juice blends

Yeast and mold growth were not detected in all the treatments, that is, P90:B10, P80:B20, P70:B30, 100% pineapple (control-P) and 100% beetroot (Br) juices on Day 1 after inoculation and incubation. However, in the control-P and Br (beetroot-only, naturally fermented) treatments, yeast and mold growth appeared from day 7 and increased progressively, reaching the highest count of 6.98 log CFU/mL by Day 28. In contrast, the inoculated juice blends (P90:B10, P80:B20, and P70:B30) showed no detectable yeast or mold growth during the first 14 days of storage. Figure 2 illustrates the trends in yeast and mold count across the different treatments from day 1 to Day 28.

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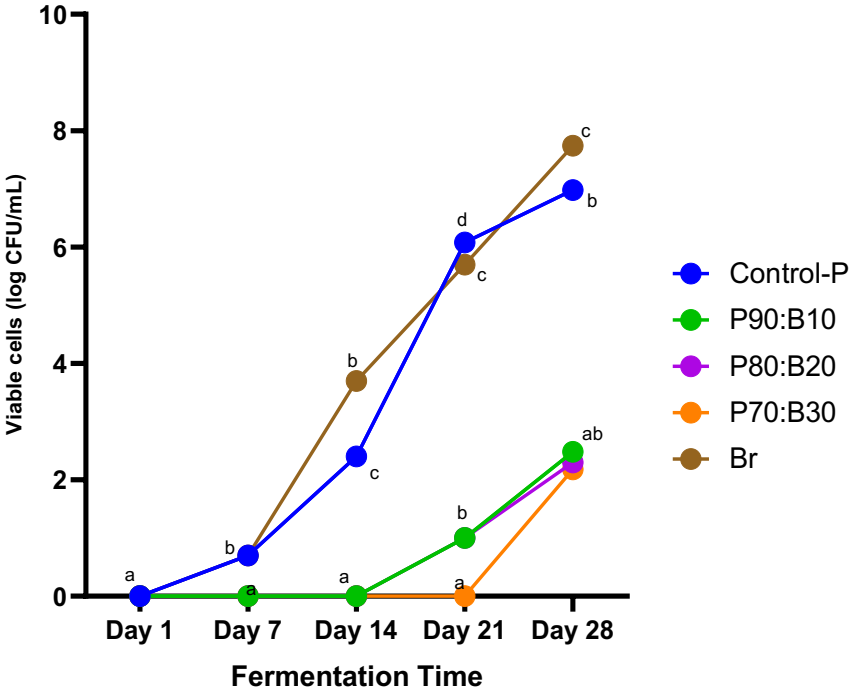

Figure 2: Yeast and mold count in fresh pineapple juice (control), fresh beetroot juice (naturally fermented), and various inoculated blends of pineapple and beetroot juice

Yeast and mold count were conducted on days 1, 7, 14, 21, and 28, with all tests performed in duplicate. Means with different letters (a, ab, b, c and d) signifies statistical difference among treatments at $p < 0.05$.

Significant differences ($p < 0.05$) were observed in yeast and mold count between the control-P and all inoculated treatments on Day 28. The local recommended limit for yeast and mold in fruit juices is <1 log CFU/mL (equivalent to <10 CFU/mL) [27]. The control-P and Br exceeded this limit as early as day 14, while the inoculated treatments remained within acceptable limits until Day 21. By Day 28, however, all treatments, including the inoculated blends, had yeast and mold count exceeding the threshold thus indicating potential spoilage.

The delayed onset of microbial growth in the inoculated blends can be attributed to the antimicrobial activity of lactic acid bacteria (LAB), which produce bacteriocins and organic acids that inhibit the proliferation of spoilage organisms. This preservative effect was contributed by *Lactobacillus plantarum*, which is recognized as a potent bacteriocin producer capable of suppressing yeast and mold growth [11]. Furthermore, the production of organic acids by LAB contributes to a lower pH

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environment, creating unfavorable conditions for spoilage and pathogenic microorganisms [27].

Total titratable acidity of fermented juice blends

As shown in Figure 3, there were significant differences ($p < 0.05$) in acidity among the various treatments on Day 1. The control -P exhibited the lowest acidity among the inoculated blends, with a value of 0.40%, compared to P90:B10 (0.80%), P80:B20 (0.74%) and P70:B30 (0.61%). Notably, the naturally fermented beetroot juice (Br) had an even lower initial acidity (0.32%) than the control-P, indicating limited early microbial activity due to reliance on indigenous microflora.

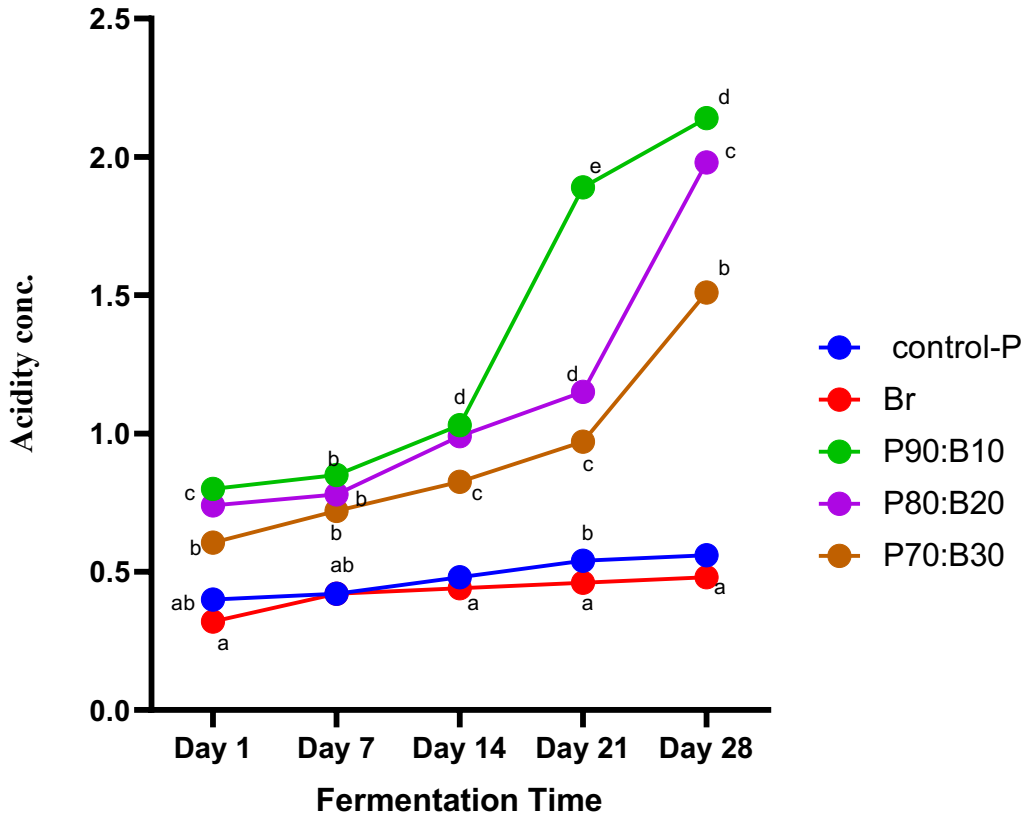

Figure 3: Total titratable acidity in fresh pineapple juice, fresh beetroot juice (naturally fermented), and inoculated blends of pineapple and beetroot juice at varying ratios

Analyses were conducted on days 1, 7, 14, 21, and 28, with each test performed in triplicate. Means with different letters (a, ab, b, c, d and e) signifies statistical difference among treatments at $p < 0.05$.

By Day 28, treatment P90:B10 recorded the highest acidity at 2.14%, followed by P80:B20 and P70:B30, while Br and the control-P exhibited lower final acidity levels.

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The overall trend of increasing acidity across treatments (Br < control-P < P70:B30 < P80:B20 < P90:B10) demonstrates the progressive fermentation activity and correlates with higher initial LAB populations in the inoculated blends.

Previously reported studies had observed similar trends in probiotic mango and carrot juices, where increasing acidity during storage was associated with ongoing LAB fermentation [28].

pH of fermented juice blends

As illustrated in Figure 4, there were significant differences ($p < 0.05$) in pH between the control and all other treatments on Day 1. However, no significant difference was observed between the P80:B20 and P70:B30 blends on the same day, suggesting comparable initial acidification levels in these formulations. The highest initial pH was recorded in the naturally fermented beetroot juice (Br) at 5.53, indicating minimal early fermentation activity due to the absence of an added starter culture.

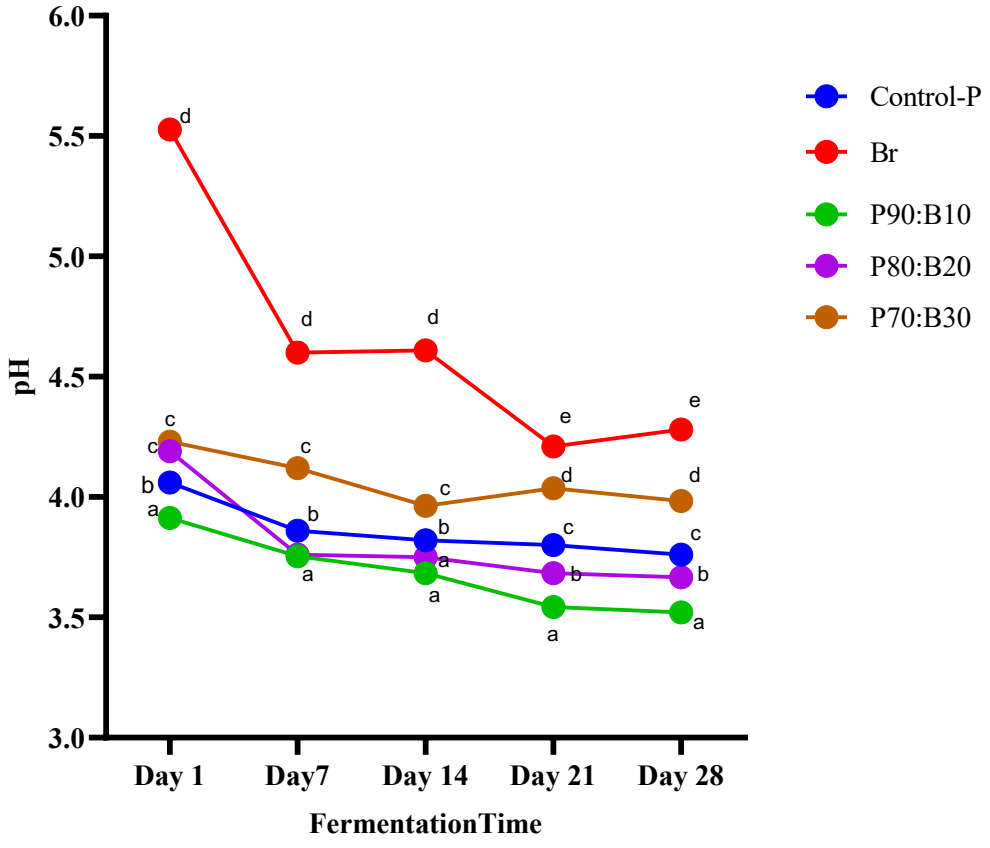

Figure 4: pH of fresh pineapple juice (control), fresh beetroot juice (naturally fermented), and inoculated blends of pineapple and beetroot juice at varying ratios

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pH analyses were conducted on days 1, 7, 14, 21, and 28, with each test performed in triplicate. Means with different letters (a, b, c, d and e) signifies statistical difference among treatments at $p < 0.05$.

By Day 28, significant differences in pH were observed across all treatments. The decline in pH over time was attributed to the metabolic activity of lactic acid bacteria (LAB), which actively converted fermentable sugars into lactic acid and other organic acids. This biochemical transformation led to acid accumulation, thereby lowering the pH across the storage period. pH reduction in blueberry jam fermented with LAB has been reported by de Oliveira *et al*. [19]. The progressive decrease in pH highlights the efficiency of LAB in acid production and further confirms successful fermentation.

Total soluble solids (TSS) of fermented juice blends

As shown in Figure 5, there were significant differences ($p < 0.05$) in Total Soluble Solids (TSS) between the control-P and all other treatments on day 1. The control-P sample recorded the highest initial TSS at 13.35°Brix, followed by Br (12.37°Brix), P90:B10 (11.98°Brix), P80:B20 (11.63°Brix), and P70:B30 (11.41°Brix). This trend reflects the influence of pineapple concentration on sugar content. Pineapple juice inherently contains more fermentable sugars than beetroot juice thereby accounting for the observed gradient in TSS across the blended treatments.

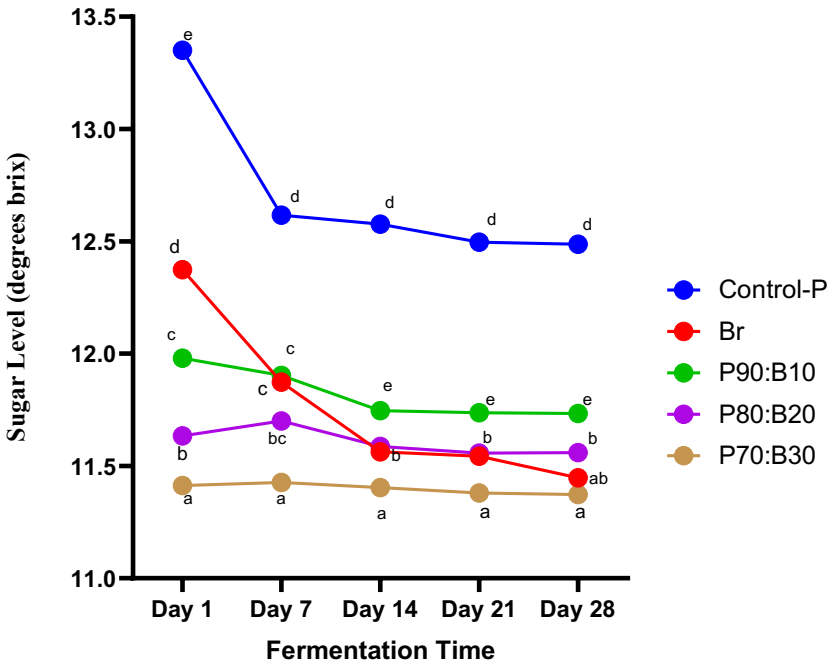

Figure 5: Brix levels of fresh pineapple juice (control), fresh beetroot juice (Br) (naturally fermented), and inoculated blends of pineapple and beetroot juice at different ratios

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Analyses were performed on days 1, 7, 14, 21, and 28, with each test conducted in triplicate. Means with different letters (a, ab, b, bc, c, d and e) signifies statistical difference among treatments at $p < 0.05$.

Throughout the fermentation and storage period, a consistent reduction in TSS was observed in all probiotic treatments. For instance, in the P90:B10 blend, TSS decreased gradually from 11.98°Brix on Day 1 to 11.90, 11.75, 11.74, and 11.73°Brix on Days 7, 14, 21, and 28, respectively. These reductions reflect the metabolic activity of LAB, which utilize sugars as substrates during fermentation, leading to the formation of organic acids and other fermentation metabolites.

However, it is noteworthy that from Day 21 to Day 28, the TSS levels remained relatively stable across all treatments. This plateau suggests a decline in LAB metabolic activity over time, possibly due to substrate depletion and increased acidity that may have inhibited further bacterial growth. Similar trend in TSS of pineapple juice fermented with *Pediococcus pentosaceus* and *Lactobacillus rhamnosus* strains have been reported in previous studies [14]. The observed decrease in TSS and its eventual stabilization toward the end of the storage period provides critical insights into the shelf life and probiotic activity dynamics in fruit juice blends during fermentation and storage.

Vitamin C content of fermented juice blends

As illustrated in Figure 6, there were significant differences ($p < 0.05$) in vitamin C content between the control-P and all other treatments on Day 1. The control-P sample recorded the highest vitamin C content on Day 1 at 13.82 mg/100 mL. However, on the same day, no significant differences were observed between the Br and P70:B30 treatments. Vitamin C content in pineapple juice was generally higher than in beetroot juice, which explains the higher levels observed in P90:B10 compared to P70:B30. A consistent decline in vitamin C content was noted across all treatments from Day 1 to Day 28.

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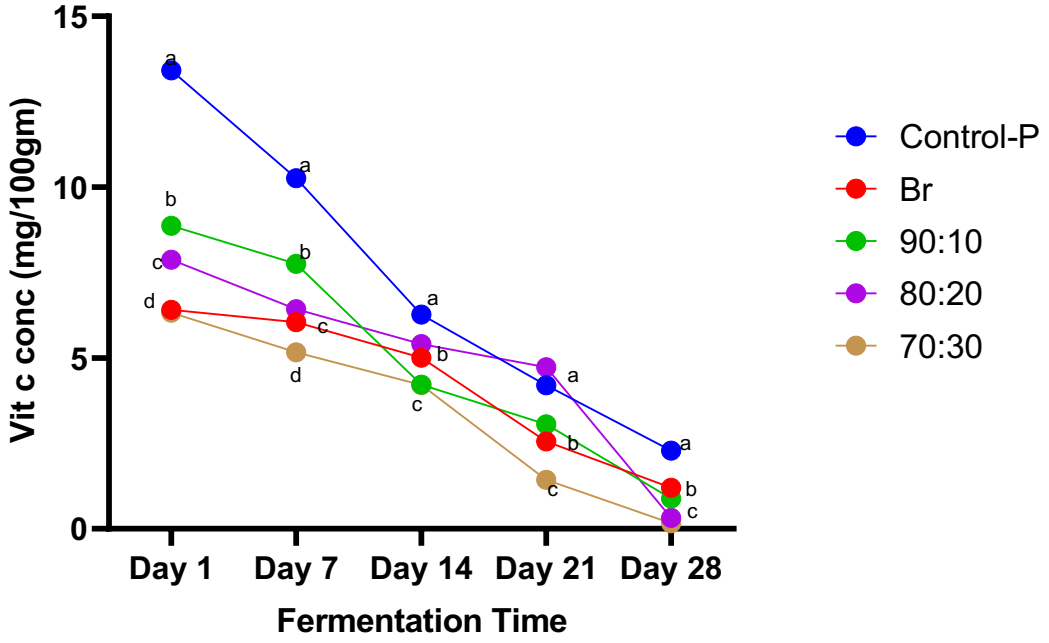

Figure 6: Vitamin C content in fresh pineapple juice (control), fresh beetroot juice (Br) (naturally fermented), and inoculated blends of pineapple and beetroot juice at different ratios

Analyses were conducted on Days 1, 7, 14, 21, and 28, with each test performed in triplicate. Means with different letters (a, b, c and d) signifies statistical difference among treatments at $p < 0.05$.

The decline in vitamin C content over the 28-day storage period is attributed to its sensitivity to time and environmental factors [21]. Vitamin C is known to be heat-labile and susceptible to oxidation, particularly in the presence of light and air [29]. Vitamin C is a vital antioxidant that helps neutralize free radicals. However, its instability during storage underscores the need for proper handling and packaging to preserve its nutritional value [30]. The recommended daily intake (RDI) of vitamin C is 45 mg/day for both men and women [31]. Based on the vitamin C content measured on Day 1, a 500 mL serving provides 123.15% of the RDI for the control sample, 71.22% for Br, 98.62% for P90:B10, 87.56% for P80:B20, and 70.32% for P70:B30.

Folate content of fermented juice blends

Folate content in beetroot juice was higher than in pineapple juice. As the proportion of beetroot increased from P90:B10 to P70:B30, the folate content of the juice blends also increased. The P70:B30 treatment recorded the highest folate concentration among all treatments.

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SCHOLARLY, PEER REVIEWED
Volume 25 No. 9
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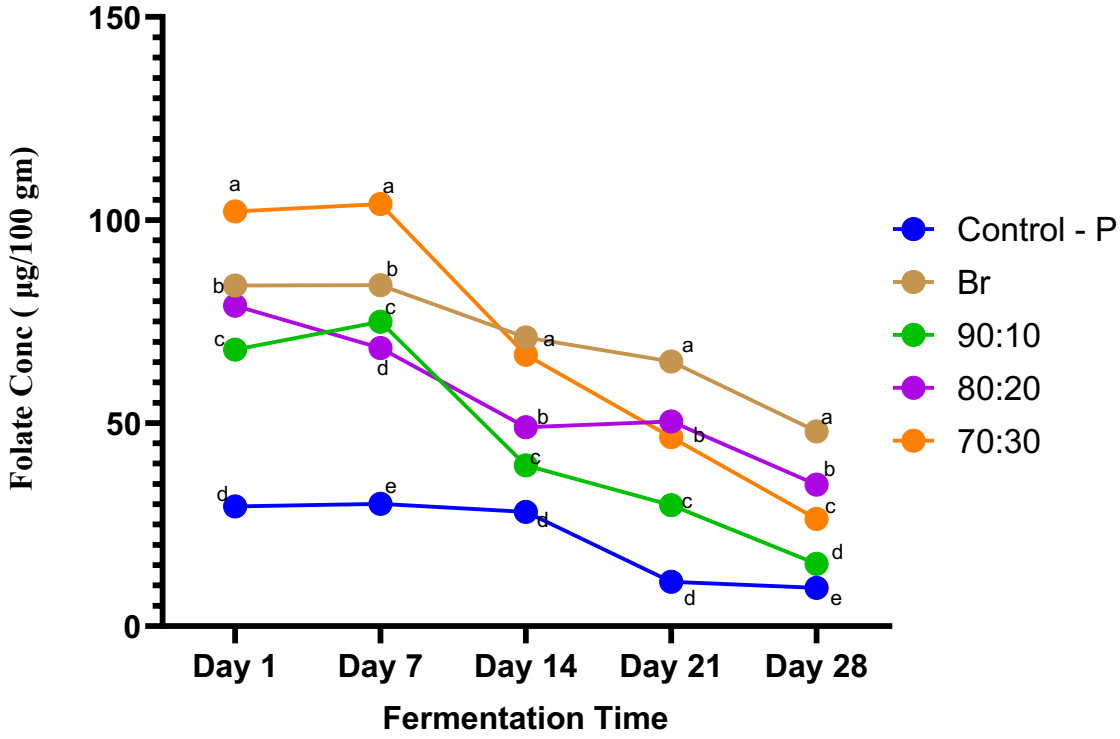

Figure 7: Folate content in fresh pineapple juice (control), fresh beetroot juice (Br) (naturally fermented), and inoculated blends of pineapple and beetroot juice at different ratios

Analyses were performed on Days 1, 7, 14, 21, and 28, with each test conducted in triplicate. Means with different letters (a, b, c, d and e) signifies statistical difference among treatments at $p < 0.05$.

On Day 1, significant differences were observed between the control-P (28.28 µg/100 mL) and the other treatments: Br (83.91 µg/100 mL), P90:B10 (66.32 µg/100 mL), P80:B20 (79.67 µg/100 mL), and P70:B30 (102.33 µg/100 mL). The mixture P70:B30 yielded more folate than the beetroot because the combination of beet root juice and pineapple juice provides environment (fermentation), nutrients and folate precursors which makes folate more accessible. A decline in folate levels from Day 1 to Day 28 was observed across all treatments. This reduction is likely attributed to the sensitivity of folate to light exposure during storage [32]. The recommended daily intake (RDI) of folate is 320 µg/day for both men and women [31]. On average, a 500 mL serving on Day 1 provided 14.74%, 41.95%, 34.04%, 39.46%, and 51.07% of the RDI for the Control, Br, P90:B10, P80:B20, and P70:B30 treatments, respectively.

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Sensory acceptability for fermented juice blends

Sensory evaluation was conducted to assess consumer preference for color, aroma, mouthfeel, taste and overall acceptability of the juice blends and the results are illustrated in Figure 8.

Color was most preferred in the P80:B20 treatment, which received the highest score of 7.71. This was followed by P70:B30 at 7.46, P90:B10 at 6.91, and the control at 6.00. All treatments retained the characteristic deep red hue of beetroot, suggesting the presence of beetroot. The higher preference for P80:B20 and P70:B30 could be attributed to the balanced color intensity resulting from optimal blending of pineapple and beetroot juices.

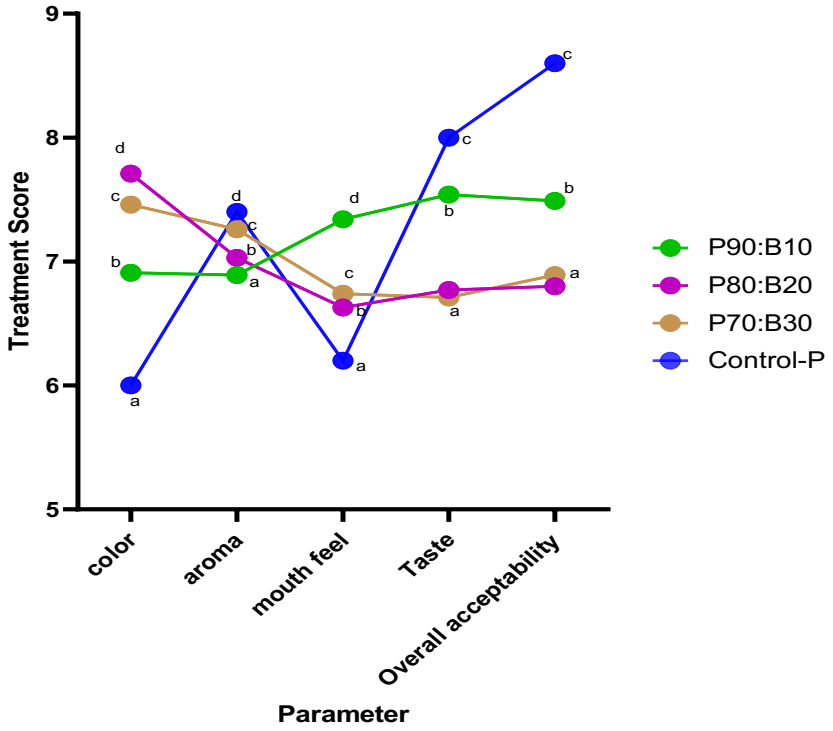

Figure 8: Sensory acceptance test results based on a 9-point hedonic scale, with scores provided by 50 untrained panelists

The scale ranged from 1 = dislike extremely, and 9 = like extremely. Means with different letters (a, b, c and d) signifies statistical difference among treatments at $p < 0.05$.

In terms of aroma, the control sample received the highest rating at 7.40, followed closely by P70:B30 (7.26), P80:B20 (7.03), and P90:B10 (6.89). The control's high rating may be due to its fresh pineapple scent, unaltered by fermentation or beetroot's earthy aroma. Blends with higher beetroot ratios appeared to reduce

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aroma appeal slightly, though scores remained within an acceptable range. For mouthfeel, P90:B10 was the most preferred with a score of 7.34, followed by P70:B30 (6.74), P80:B20 (6.63), and the control (6.20). The smoothness and slightly viscous texture of P90:B10 may have enhanced consumer perception, while the control relatively lower rating might be attributed to the absence of fermentation-related texture improvements.

Regarding taste, the control sample again led with the highest score of 8.00, followed by P90:B10 at 7.54. P80:B20 (6.77) and P70:B30 (6.71) received lower ratings. This suggests that while fermentation contributed to enhanced probiotic properties, it may have also introduced sourness that was less preferred by some panelists. The P90:B10 blend, with the highest pineapple content, retained more of the natural sweetness, leading to higher acceptability. In terms of overall acceptability, the control was most favored, scoring 8.60. This was followed by P90:B10 (7.49), P70:B30 (6.89), and P80:B20 (6.80). Despite the control higher scores in taste and aroma, the P90:B10 blend demonstrated good consumer acceptance, likely due to its balanced sensory profile and higher probiotic viability. These results underscore the importance of optimizing fruit blend ratios to achieve both functional and sensory appeal. The P90:B10 formulation stood out as a strong probiotic drink with a good appealing taste, balancing sensory quality with microbial efficacy.

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

Probiotic lactic acid bacteria (LAB) count exceeding 7 log CFU/mL were achieved up to 21 days in the fermented pineapple and beetroot juice blends. The physicochemical results showed significant differences in pH and total titratable acidity throughout fermentation, which correlated with the activity of LAB in the juices. Yeast and mold count were within the recommended threshold in the juice blends, confirming the product's safety. The juice blends were also rich in Vitamin C and folate which is highly recommended for both males and females as per the FAO/WHO guidelines on Recommended Dietary Intake (RDI). Sensory acceptability evaluation revealed that the fermented juice blends were generally well accepted, with notable differences in sensory attributes such as color, aroma, mouthfeel, taste, and overall acceptability. Based on these findings, the shelf life of the probiotic beverage was determined to be 21 days. The results of this study demonstrated that the combination of pineapple and beetroot juice held substantial potential for the development of a fruit -based probiotic beverage. Further research is recommended to explore additional ratios of pineapple juice to beetroot juice, such as 60%:40% and 50%:50%, and further studies on expanded but targeted sensory acceptability evaluation among individuals from specific consumer groups including vegetarians, individuals with lactose intolerance, diabetics, and young children.

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ISSN 1684 5374

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the technical support of the laboratory technologists at the Institute of Food Bioresources Technology, Dedan Kimathi University of Technology.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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