

Assessment of Lactic Acid Bacteria Growth Rate, Physicochemical Parameters, Vitamin Contents, and Antioxidant Potentials of Probiotic-Fortified Fruit Juices

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Received: March 11, 2026 | Review began: March 30, 2026 | Review ended: June 01, 2026 | Published: June 03, 2026

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Abstract

This study aimed to assess the impact of probiotic fortification on the microbiological qualities, growth rate of lactic acid bacteria (LAB), physicochemical properties, vitamin content, and antioxidant potential of fruit juices derived from watermelon, pineapple, and orange. Using standard analytical methods, both non-probiotic-fortified and probiotic-fortified juice samples were compared. Probiotic was achieved by inoculating juices with *Lactobacillus casei* and *Lactobacillus fermentum*, which significantly reduced coliform counts (from 7.20×10^3 to 4.00×10^2 colony forming unit [CFU]/mL) and fungal counts (from 2.40×10^2 to 4.00×10^1 CFU/mL) and enhanced LAB viability (from 0.4×10^4 to 1.8×10^4 CFU/mL), especially when both strains were combined. Probiotic-fortified juices demonstrated notable improvements in physicochemical parameters, including increased titratable acidity (0.20-0.56%), reduced pH (5.50-3.17), and decreased soluble solids (17.90-7.90% brix), indicative of successful fermentation. Orange juice with both probiotic strains contained the highest potassium content (234.75 mg/100 mL), while pineapple juice with both strains had the highest vitamin B12 content (17.80 mg/100 mL). Antioxidant potential, measured using 1,1-diphenyl-2-picrylhydrazyl and other radical-scavenging assays, was significantly higher in probiotic-enriched juices, with synergistic effects observed for multi-strain combinations. Organoleptic evaluation confirmed good acceptability of the functional beverages. These findings demonstrate that fortifying tropical fruit juices with probiotic LAB not only improves microbial safety and nutritional value but also enhances antioxidant properties, supporting the development of innovative, inclusive, non-dairy functional beverages suitable for health-conscious and lactose-intolerant individuals.

Categories: Food processing, Food security and sustainability, Food microbiology

Keywords: watermelon, pineapple, orange, fruit juice, probiotic, lactobacillus casei, lactobacillus fermentum

Introduction

Growing consumer demand for health-oriented products has accelerated the development of functional beverages that incorporate natural bioactive compounds in place of synthetic chemical preservatives [1]. Although chemical preservatives are widely employed in food systems, their consumption has been associated with potential health risks, prompting increased interest in safer natural alternatives [2,3]. Numerous natural preservatives derived from microorganisms, plants, and animals have been reported to exhibit bio-preservative properties. Among these, active microbial cultures, particularly lactic acid bacteria (LAB), are extensively utilized as starter cultures in probiotic fermentations due to their well-documented functional and health-promoting attributes [4]. LAB provide multiple advantages beyond their conventional role in fermentation when incorporated into fruit juices. Fruit juices present a promising matrix for probiotic delivery due to their nutrient density, absence of common dairy allergens, and widespread

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consumer acceptance [5,6]. However, the majority of existing literature has focused on probiotic fermentation in temperate-climate fruits or vegetables (such as apple, grape, and carrot), and comprehensive data on tropical fruit matrices remain limited. Watermelon (*Citrullus lanatus*), pineapple (*Ananas comosus*), and orange (*Citrus sinensis*) were selected for this study based on their unique scientific and nutritional contributions. Watermelon is a rich source of lycopene, citrulline, and phenolic compounds, with high water content and low acidity that can influence LAB metabolism and product palatability [7]. Pineapple is notable for its levels of vitamin C, manganese, and the enzyme bromelain, which may interact with probiotic activity and facilitate matrix breakdown [8]. Orange is globally recognized for its vitamin C and flavonoid content, in addition to its established consumption and health benefits [9]. These fruits also represent major dietary staples in tropical regions, making them highly relevant for local functional food development [5]. Scientifically, the selection of these fruits addresses critical knowledge gaps regarding the suitability and optimization of non-dairy, plant-based probiotic beverages in tropical contexts [5,6]. A distinguishing feature of this study is the use of *Lactobacillus casei* and *Lactobacillus fermentum*, both isolated from 'Omidun' (the supernatant of fermented maize, a traditional Nigerian product). These strains were chosen not only for their local dietary relevance but also for their proven probiotic potential, tolerance to acidic environments, and established ability to synthesize vitamins and promote mineral bioavailability [10]. Therefore, the objective of this study is not only to investigate the viability of LAB fortification in watermelon, pineapple, and orange juices but also to systematically evaluate the physicochemical parameters, vitamin content, and antioxidant potential of the fruit juices. By addressing these variables, this research seeks to provide foundational evidence for the development of innovative, non-dairy functional beverages tailored to consumer health trends and local agricultural resources.

Materials And Methods

Reagents used

de Man-Rogosa-Sharpe (MRS) agar, MRS broth, and peptone water were acquired from Merck Co. (Darmstadt, Germany). Folin-Ciocalteu, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ethanol, potassium persulfate, trichloroacetic acid, iron(II) sulphate, and butylated hydroxytoluene were purchased from Sigma-Aldrich (Steinheim, Germany). Hydrogen peroxide, lanthanum chloride, deoxyribose, and thiobarbituric acid were from Amresco (Ohio, USA).

Collection of fruits

Ripened fresh watermelon (*C. lanatus*), pineapple (*A. comosus*), and orange (*C. sinensis*) fruits at 80-90% maturity, exhibiting good visual and sensory quality and uniformity in size and appearance, were purchased from the Okitipupa local market, Ondo State, Nigeria.

Isolation and identification of probiotic strains

Isolation and identification of probiotic strains were performed according to the modified methodology, as described by Saboktakin-Rizi et al. [11]. A fermented maize slurry known as 'Omidun' was acquired from local market vendors in Okitipupa. A 5 mL sample of 'Omidun' was homogenized with 45 mL of peptone water, followed by serial dilutions. The bacteria isolated were identified through Gram staining, motility tests, spore assessments, citrate utilization, catalase activity, and sugar fermentation tests.

Production of probiotic-fortified fruit juices

The preparation of probiotic-fortified fruit juices was conducted according to the method described by Adebayo-Tayo et al. [5]. The watermelon, pineapple, and orange fruits were thoroughly washed with tap and distilled water to minimize microbial contamination and remove foreign particles. The fruits were then peeled, deseeded, cut into smaller pieces, and juiced using a Taisite juicer (FW100; Tianjin, China). The fruit juice samples were pasteurized at 70 °C for 10 min and allowed to cool before the introduction of probiotics. *L. casei* and *L. fermentum* were inoculated at a concentration of 1% (v/v) into 100 mL of the fruit juices. The samples containing both LAB were further introduced with 0.5% (v/v) each.

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Microbiological qualities of non-probiotic and probiotic-fortified fruit juices

The microbiological quality of both non-probiotic and probiotic-fortified fruit juices was evaluated using the pour plate technique according to the method described by Nnko and Mahadhy [12]. Approximately 25 mL of juice was mixed with 225 mL of sterile saline solution and subjected to serial dilution. Subsequently, 100 µL of the diluted samples was plated onto MacConkey agar and potato dextrose agar. The plates were incubated at 37 °C for 24 h and 48 h for the enumeration of coliforms and fungi, respectively.

Assessment of LAB growth rate in fruit juices

The growth rate of LAB in the probiotic-fortified fruit juices was evaluated using the pour plate technique. Briefly, 0.1 mL of each sample was inoculated onto MRS agar plates and incubated at 37 °C for 48 h. Viable cell counts were recorded and expressed as colony-forming units (CFU) [5].

Determination of physicochemical parameters of non-probiotic and probiotic-fortified fruit juices

The physicochemical parameters, including pH, total titratable acidity (TTA), and total soluble solids, of both non-probiotic and probiotic-fortified fruit juices were determined using standard methods [13]. The pH was measured using a handheld pH meter (Model 401). TTA was determined by titrating 20 mL of each fruit juice sample against 0.1 M NaOH using phenolphthalein as an indicator. The titre value obtained was used to calculate titratable acidity, expressed as percentage lactic acid. Total soluble solids were measured using a handheld refractometer (RX 1000, Atago Co., Ltd., Tokyo, Japan), calibrated with distilled water at 20 °C, and the readings were recorded as degrees Brix (°Brix).

Determination of mineral composition of non-probiotic and probiotic-fortified fruit juices

A 100 mL of each fruit juice sample was ashed in a muffle furnace at 600 °C for 5 h. The resulting ash was digested with 5 mL of hydrochloric acid (HCl) and 2 mL of 5% (v/v) lanthanum chloride solution, followed by boiling, filtration, and dilution to a standard volume with deionized water. The concentrations of zinc (Zn), iron (Fe), calcium (Ca), and phosphorus (P) were determined using Atomic Absorption Spectroscopy (Buck Scientific, Model 200, Inc., East Norwalk, Connecticut, USA) at their respective wavelengths. Sodium (Na) and potassium (K) contents were analyzed using a flame photometer (Jenway PFP 7, Staffordshire, UK). Calibration of the atomic absorption spectrophotometer was carried out using a mineral standard solution at 1,000 µg/mL in 1% (v/v) nitric acid [14].

Determination of vitamin C in non-probiotic and probiotic-fortified fruit juices

The concentration of vitamin C in both non-probiotic and probiotic-fortified fruit juice samples was determined through the standard titrimetric method using 2,6-dichloroindophenol. L-ascorbic acid at a concentration of 1 mg/mL served as the standard reference, and the results were expressed as mg/L of juice [15].

Determination of vitamin E in non-probiotic and probiotic-fortified fruit juices

Vitamin E content was determined following the colorimetric procedure described by Mohammed et al. [16]. Briefly, 1 mL of each sample was macerated with 20 mL of *n*-hexane in a test tube for 10 min and subsequently centrifuged for 10 min. The resulting solution was filtered, and 3 mL of the filtrate was transferred in duplicate into dry test tubes and evaporated to dryness in a boiling water bath. Saponification was performed by adding 2 mL of 0.5 N alcoholic potassium hydroxide and heating in a water bath for 30 min. Following saponification, 3 mL of *n*-hexane was added, and the mixture was vigorously shaken. The *n*-hexane layer was transferred into fresh test tubes and evaporated to dryness. The residue was reconstituted in 2 mL of ethanol, after which 1 mL of 0.2% (w/v) ferric chloride in ethanol and 1 mL of 0.5% (w/v) 2,2'-dipyridyl in ethanol were added sequentially. The volume was adjusted to 5 mL with ethanol, thoroughly mixed, and the absorbance was measured at 520 nm against a reagent blank.

Determination of vitamin B12 in non-probiotic and probiotic-fortified fruit juices

Vitamin B12 content was quantified according to the method described by Nguyen et al. [17]. The B-vitamin group was extracted using Takadiastase enzyme. Extracted samples (20 µL) were injected into a high-performance liquid chromatography system, and the quantification was achieved through chromatographic separation by comparison with

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certified vitamin B standard solutions.

Determination of bioactive compounds and antioxidant potential of non-probiotic and probiotic-fortified fruit juices

Determination of Total Phenol Content

Total phenolic content was determined using the Folin-Ciocalteu colorimetric assay with gallic acid as the reference standard [18]. Briefly, 50 μ L of the aqueous extract was transferred into a test tube, followed by the addition of 50 μ L of distilled water and 500 μ L of Folin-Ciocalteu reagent. The mixture was thoroughly mixed and allowed to stand for 3 min, after which 400 μ L of 7.5% (w/v) sodium carbonate solution was added. The reaction mixture was incubated in a water bath at 45 °C for 40 min, and the absorbance was measured at 765 nm. Total phenolic content was expressed as milligrams of gallic acid equivalents per millilitre of sample (mg GAE/mL), calculated from a gallic acid calibration curve.

Determination of Total Flavonoid Content

Total flavonoid content of the fruit juices was analyzed using the method outlined by Chang et al. [19]. Approximately 10 μ L of each fruit juice sample was combined with 250 μ L of distilled water, followed by the sequential addition of 10 μ L of 10% (w/v) aluminium chloride (AlCl_3) and 10 μ L of 1 M potassium acetate. The reaction mixture was incubated at 25 °C for 30 min, and the absorbance was measured at 415 nm. Quantification was carried out using a quercetin standard curve, and the results were expressed as milligrams of quercetin equivalent per millilitre of sample (mg QE/mL).

Ferric Reducing Antioxidant Power Assay

The ferric reducing antioxidant power (FRAP) of non-probiotic and probiotic-fortified fruit juices was determined using the method outlined by Benzie and Strain [20]. The FRAP reagent was prepared fresh by combining 25 mL of acetate buffer at 0.3 M (pH 3.6), 2.5 mL of 2,4,6-tripyridyl-*s*-triazine at a concentration of 10 mM in 40 mM HCl, and 2.5 mL of 20 mM ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$). To conduct the assay, 0.3 mL of the prepared reagent was added to 1 mL of juice extract along with 0.3 mL of deionized water. After the appropriate incubation period, optical density was recorded spectrophotometrically, and the antioxidant capacity was reported as micromoles of Trolox equivalent per millilitre of juice ($\mu\text{mol TE/mL}$).

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) Antioxidant Activity

The ABTS assay for non-probiotic and probiotic-fortified fruit juice extracts was performed according to the method described by Re et al. [21]. The ABTS⁺ radical cation was generated by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate, and the mixture was stored in the dark at room temperature. The resulting ABTS working solution was subsequently diluted with methanol to obtain an absorbance of 0.700 ± 0.02 at 734 nm. Scavenging activity was expressed as micromoles of Trolox equivalents per millilitre of sample ($\mu\text{mol TE/mL}$).

DPPH Radical Scavenging Assay

The free radical neutralizing capacity of the fruit juice extracts was evaluated against DPPH radicals following the approach of Gyamfi et al. [22]. A volume of 100 μ L of each extract was introduced into 0.1 mM DPPH solution and thoroughly mixed. The resulting mixture was left to stand in the absence of light for 30 min, after which the optical density was recorded at 517 nm. A negative control comprising only DPPH solution in methanol without any extract was included, while pure methanol served as the blank reference.

Iron Chelation Activity

The capacity of fruit juice extracts to chelate ferrous iron was evaluated using iron(II) sulphate (FeSO_4) according to the method described by Puntel et al. [23]. Briefly, 150 mM FeSO_4 was added to a reaction mixture containing 168 μ L of 0.1 M Tris-HCl buffer (pH 7.4), 218 μ L of physiological saline (0.9% NaCl), and 100 μ L of sample extract. The reaction vessel was held at 27 °C for 5 min, after which 13 μ L of 0.25% (w/v) 1,10-phenanthroline was introduced, and the optical density was subsequently recorded at 510 nm using a spectrophotometer.

Hydroxyl Radical Scavenging Assay

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The ability of the extracts to inhibit $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ -induced oxidative degradation of deoxyribose was evaluated following the technique of Halliwell et al. [24]. A volume of 100 μL of each prepared extract was introduced into a reaction vessel containing 120 μL of 20 mM deoxyribose, 400 μL of 0.1 M phosphate buffer (pH 7.4), 40 μL of 20 mM hydrogen peroxide, and 40 μL of 500 μM FeSO_4 , with the total volume brought to 800 μL using distilled water. The reaction was allowed to proceed at 37 °C for 30 min, after which it was terminated by introducing 0.5 mL of 2.8% trichloroacetic acid, followed by 0.4 mL of 0.6% thiobarbituric acid. The resulting mixtures were then subjected to a boiling water bath for 20 min, and the absorbance was recorded at 532 nm using a spectrophotometer.

Organoleptic Properties of Non-Probiotic and Probiotic-Fortified Fruit Juices

The sensory assessment was conducted after the approval from the Research Committee on Sensory Evaluation and Consumer Protection (Ref: OAUSTECH/FST/2025-003). Organoleptic properties such as colour, taste, mouthfeel, characteristics flavour, and overall acceptability were evaluated using 15 semi-trained panelists using a 9-point hedonic scale, where a score of 9 denoted "like extremely" and 1 denoted "dislike extremely". Samples were presented to panelists in coded disposable cups under standardized conditions to minimize bias, and panelists were provided with water to rinse their palates between samples.

Statistical analysis

Data obtained during the experiment were subjected to One-way Analysis of Variance (ANOVA) using SPSS (Statistical Package for Social Sciences) version 22 software. The mean and standard deviation of the triplicate analyses were calculated. Significant differences ($p \leq 0.05$) between the mean values were compared by using the New Duncan Multiple Range Test.

Results And Discussion

Microbiological qualities and assessment of LAB growth rate in non-probiotic and probiotic-fortified fruit juices

The coliform count was higher (7.2×10^3 CFU/mL) in watermelon juice without LAB. The fungi count of 2.4×10^2 CFU/mL was obtained in non-probiotic juice from watermelon and orange (Table 1). This agrees with the report of Aneja et al. [25], who reported that non-probiotic fruit juices contain average level of spontaneously occurring microorganisms, in particular notable presence of fungi, mostly due to the sugar and acidity levels in the juices that support fungal growth. The inoculation of *L. casei* and *L. fermentum* reduced the microbial load of the probiotic fruit juices. This suggest that probiotic-fortified fruit juices are safer for consumption and may offer health benefits such as improved digestion and enhanced immune function due to the activity of *L. casei* and *L. fermentum* [26]. Figure 1 shows the LAB growth rate in non-probiotic and probiotic-fortified fruit juice. It was observed that fruit juices without LAB strains have no viability of LAB, indicating that the natural microbiota of the juice is not sufficient to sustain a probiotic population without the addition of specific probiotic strains [27]. Probiotic-fortified fruit juice inoculated with *L. fermentum* had higher growth rate than the fruit juice with *L. casei*. Adesulu-Dahunsi et al. [28] reported that *L. fermentum* is known for its robust fermentative capabilities and the ability to succeed in various plant-based substrates. It efficiently ferments sugars found in fruit juices, producing lactic acid and other beneficial metabolites that support gut health [29]. Hill et al. [30] reported that *L. casei* is more frequently linked with dairy environments where it flourishes in milk-based substrates, possibly its growth is not as vigorous in the probiotic-fortified fruit juices, as might be expected in more favourable environments like dairy products, and this could be supported by the findings reported in this study. The highest values were observed in probiotic-fortified fruit juices inoculated with the combined LAB strains. This suggests a synergistic interchange between these two strains, where their integrated presence leads to significantly enhanced growth compared to when they are cultured individually. Several studies have demonstrated that co-culturing different LAB strains can result in better nutrient usage, improved fermentation performance, metabolic activity, and enhanced growth rate [31,32].

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Samples	Coliform count ($\times 10^3$ CFU/mL)	Fungi count ($\times 10^2$ CFU/mL)
WCO	7.20 ± 0.30^a	2.40 ± 0.00^a
WAA	4.40 ± 0.01^c	1.80 ± 0.00^b
WBB	5.60 ± 0.00^b	1.00 ± 0.00^d
WAB	3.60 ± 0.01^d	0.80 ± 0.00^e
PCO	6.60 ± 0.04^a	2.00 ± 0.01^b
PAA	0.60 ± 0.00^k	0.40 ± 0.00^i
PBB	0.80 ± 0.00^j	1.00 ± 0.00^d
PAB	0.40 ± 0.00^l	0.60 ± 0.00^f
OCO	6.00 ± 0.30^b	2.40 ± 0.02^a
OAA	2.00 ± 0.01^f	1.40 ± 0.00^c
OBB	2.60 ± 0.03^e	1.60 ± 0.00^c
OAB	1.20 ± 0.00^i	0.40 ± 0.00^i

TABLE 1: Microbiological qualities of non-probiotic and probiotic-fortified fruit juices

All values are means $\pm$ standard deviation of duplicates. Values with different superscript within the same column are significantly different at $p \leq 0.05$.

WCO, Watermelon juice alone; WAA, Watermelon juice + *Lactobacillus casei*; WBB, Watermelon juice + *Lactobacillus fermentum*; WAB, Watermelon juice + *Lactobacillus casei* + *Lactobacillus fermentum*; PCO, Pineapple without strain (control); PAA, Pineapple + *Lactobacillus casei*; PBB, Pineapple + *Lactobacillus fermentum*; PAB, Pineapple + *Lactobacillus fermentum* + *Lactobacillus casei*; OCO, Orange without strain (control); OAA, Orange juice + *Lactobacillus casei*; OBB, Orange juice + *Lactobacillus fermentum*; OAB, Orange juice + *Lactobacillus casei* + *Lactobacillus fermentum*

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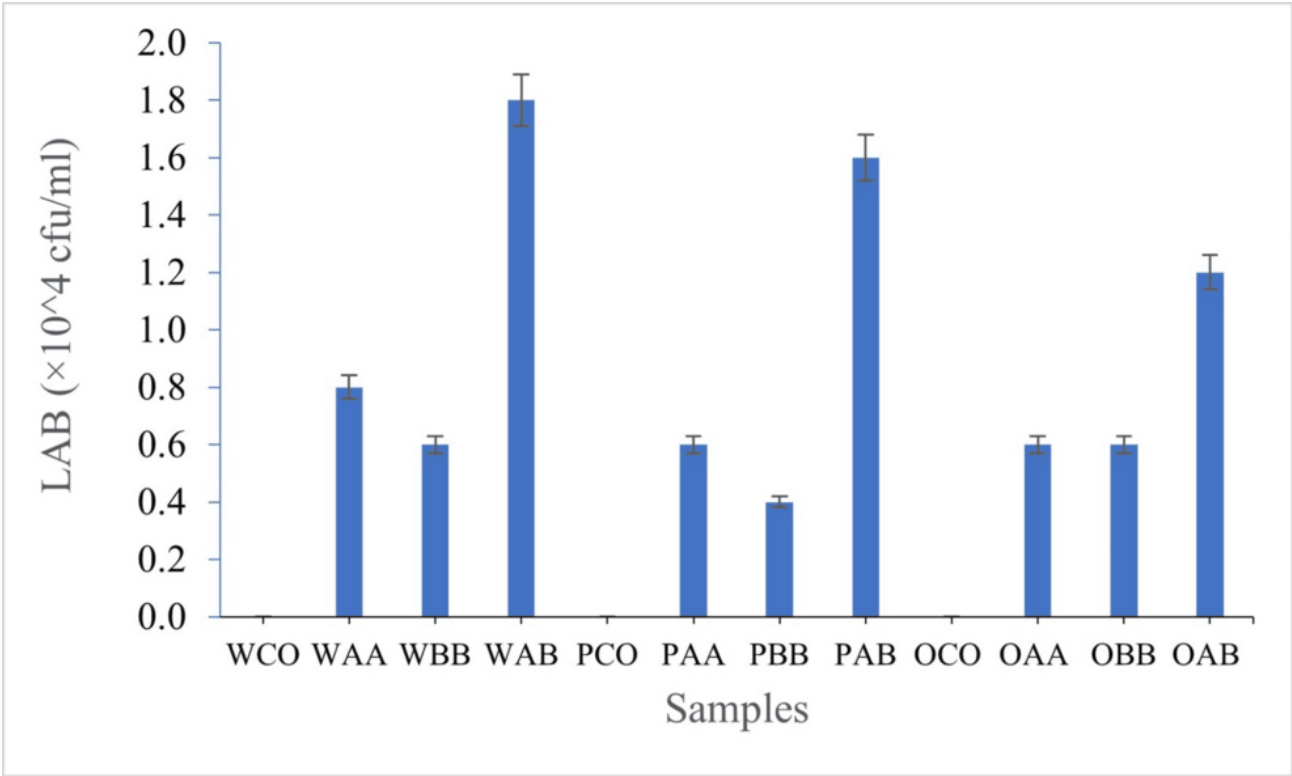


FIGURE 1: Growth rate (CFU/mL) of lactic acid bacteria (LAB) in non-probiotic and probiotic-fortified fruit juices

WCO, Watermelon juice alone; WAA, Watermelon juice + *Lactobacillus casei*; WBB, Watermelon juice + *Lactobacillus fermentum*; WAB, Watermelon juice + *Lactobacillus casei* + *Lactobacillus fermentum*; PCO, Pineapple without strain (control); PAA, Pineapple + *Lactobacillus casei*; PBB, Pineapple + *Lactobacillus fermentum*; PAB, Pineapple + *Lactobacillus fermentum* + *Lactobacillus casei*; OCO, Orange without strain (control); OAA, Orange juice + *Lactobacillus casei*; OBB, Orange juice + *Lactobacillus fermentum*; OAB, Orange juice + *Lactobacillus casei* + *Lactobacillus fermentum*

Physicochemical properties of non-probiotic and probiotic-fortified fruit juices

The physicochemical properties of non-probiotic and probiotic-fortified fruit juices are presented in Table 2. The TTA of the probiotic-fortified fruit juices ranged from 0.31-0.34% in watermelon juice, 0.47-0.61% in pineapple juice, and 0.46-0.57% in orange juice. The pH values of the probiotic-fortified juices were lower than those of the non-probiotic samples, ranging from 3.90-4.10 in watermelon juice, 3.20-3.65 in pineapple juice, and 3.17-3.54 in orange juice. The results further showed that inoculation with *L. casei* and *L. fermentum* strains in the probiotic-fortified fruit juices led to a decrease in pH values, corresponding to an increase in TTA. This finding agrees with that of AdebayoTayo and Akpeji [33], who studied the probiotic viability and physicochemical and sensory properties of probiotic pineapple juice. Similar observations were also reported by Adebayo-Tayo et al. [5], who inoculated mango juice with *L. casei* UK 318. The reduction in pH alongside the increase in TTA supports the findings that pH and TTA are inversely proportional [34]. The increase in TTA observed in the probiotic-fortified fruit juices, particularly in samples containing a combination of the two strains, is a positive indicator of successful fermentation. This increase may be attributed to the production of organic acids, such as lactic acid, by probiotics during fermentation [35,36]. The total soluble solids (7.10-17.90 °Brix) decreased in the probiotic-fortified fruit juice samples compared to the control samples. The *Lactobacillus* strains were able to ferment sugars in the fruit juices, thereby reducing the total soluble solids. Similar decreases in total soluble solids have been reported by Rahman et al. [27] and Kumar et al. [37] in probiotic-fortified fruit juices inoculated with *Lactobacillus* strains. This

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reduction can be attributed to the metabolic activities of LAB, which utilized sugars during the fermentation process [38]. These findings suggest that probiotic-fortified fruit juices may provide a less sweet alternative, potentially appealing health-conscious consumers [39].

Samples	pH	Total titratable acidity (%)	Total soluble solid (% brix)
WCO	5.50±0.04 ^a	0.20±0.00 ^e	7.90±0.10 ^c
WAA	4.10±0.10 ^b	0.32±0.04 ^d	7.30±0.10 ^d
WBB	4.09±0.00 ^b	0.31±0.04 ^d	7.10±0.10 ^d
WAB	3.90 ±0.40 ^c	0.34 ±0.07 ^c	7.70 ±0.10 ^d
PCO	4.10± 0.10 ^b	0.38± 0.00 ^c	17.90 ± 0.10 ^a
PAA	3.64 ± 0.05 ^d	0.47 ± 0.00 ^b	17.70 ± 0.10 ^a
PBB	3.65 ± 0.02 ^d	0.48± 0.05 ^b	17.50 ± 0.10 ^a
PAB	3.20 ± 0.01 ^e	0.61 ±0.02 ^a	17.30 ± 0.10 ^a
OCO	3.90±0.03 ^c	0.36±0.04 ^c	10.10±0.10 ^b
OAA	3.54±0.00 ^d	0.46±0.10 ^b	9.90±0.10 ^b
OBB	3.56±0.01 ^d	0.46±0.07 ^b	9.85±0.15 ^b
OAB	3.17±0.00 ^e	0.57±0.05 ^a	9.70±0.10 ^b

TABLE 2: Physicochemical properties of non-probiotic and probiotic-fortified fruit juices

All values are means ± standard deviation of duplicates. Values with different superscript within the same column are significantly different at $p \leq 0.05$.

WCO, Watermelon juice alone; WAA, Watermelon juice + *Lactobacillus casei*; WBB, Watermelon juice + *Lactobacillus fermentum*; WAB, Watermelon juice + *Lactobacillus casei* + *Lactobacillus fermentum*; PCO, Pineapple without strain (control); PAA, Pineapple + *Lactobacillus casei*; PBB, Pineapple + *Lactobacillus fermentum*; PAB, Pineapple + *Lactobacillus fermentum* + *Lactobacillus casei*; OCO, Orange without strain (control); OAA, Orange juice + *Lactobacillus casei*; OBB, Orange juice + *Lactobacillus fermentum*; OAB, Orange juice + *Lactobacillus casei* + *Lactobacillus fermentum*

Mineral composition of non-probiotic and probiotic-fortified fruit juices

The mineral composition of non-probiotic and probiotic-fortified fruit juices is presented in Table 3. It was observed that probiotic fortification led to increased concentrations of potassium (80.10-234.75 mg/100 mL), phosphorus (69.95-137.90 mg/100 mL), calcium (11.95-51.15 mg/100 mL), magnesium (23.55-60.75 mg/100 mL), and zinc (3.45-9.30 mg/100 mL) in the fruit juices, particularly in samples containing both *L. casei* and *L. fermentum* strains. The observed elevation in

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mineral content, particularly potassium, may be attributed to several mechanisms associated with probiotic fermentation. LAB are known to produce organic acids (such as lactic and acetic acids) during fermentation, which can chelate minerals and promote their release from the fruit matrix, thereby increasing their bio-accessibility and measurable concentration in the juice [35,40]. Furthermore, enzymatic activities induced by probiotic strains, including pectinases, cellulases, and proteases, can further degrade cell walls and intracellular compartments, facilitating the liberation of minerals such as potassium, magnesium, and calcium into the juice [36,40]. Recent studies have reported that fermentation with specific LAB strains may improve mineral solubility and reduce the presence of anti-nutritional factors that otherwise inhibit mineral absorption [40,41]. The significance of potassium enrichment is particularly noteworthy, as potassium plays a vital role in fluid balance, nerve transmission, and cardiovascular health; its increased presence in functional beverages aligns with dietary recommendations for hypertension and cardiovascular disease management [42]. Similarly, the elevation of magnesium, calcium, and zinc may contribute to improved metabolic, bone, and immune health attributes of the juices [43,44]. These findings are consistent with recent reports on the ability of probiotic fermentation to enhance the nutritional profile of plant-based beverages [40,45]. However, it should be noted that the extent of mineral release may vary depending on the fruit matrix, fermentation conditions, and specific probiotic strains employed.

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Samples	Potassium	Phosphorous	Sodium	Calcium	Iron	Zinc	Magnesium
WCO	80.10±1.80 ^h	122.60±3.10 ^b	0.11±0.00 ^d	11.95±0.15 ^j	2.20±0.01 ^e	3.45±0.05 ^e	28.45±0.65 ^d
WAA	91.25±0.45 ^g	125.75±0.05 ^b	0.08±0.00 ^e	13.75±0.05 ⁱ	5.58±0.00 ^b	5.50±0.00 ^b	32.85±1.05 ^c
WBB	89.55±1.25 ^g	126.75±1.05 ^b	0.07±0.00 ^e	12.85±0.05 ⁱ	5.15±0.05 ^b	4.85±0.05 ^c	33.00±0.20 ^b
WAB	104.50±3.40 ^g	137.90±0.00 ^a	0.06±0.00 ^e	15.30±0.10 ^f	8.05±0.00 ^a	6.50±0.10 ^b	38.70±0.60 ^c
PCO	154.85±1.55 ^f	77.75 ± 0.00 ^c	0.30±0.00 ^a	30.60±0.10 ^e	1.85±0.00 ^e	5.34±0.01 ^c	45.65±0.25 ^b
PAA	162.40± 0.90 ^f	88.35 ± 0.00 ^c	0.22±0.01 ^b	35.55±0.05 ^d	3.30±0.00 ^d	7.79±0.00 ^a	56.75±0.45 ^a
PBB	176.00±1.70 ^e	88.65 ± 2.00 ^c	0.20±0.00 ^b	34.45±0.05 ^d	3.20±0.10 ^d	8.88±0.03 ^a	57.95±0.25 ^a
PAB	201.65±0.85 ^d	90.00 ± 0.10 ^c	0.16±0.01 ^c	38.55±0.05 ^c	5.10±0.40 ^b	9.30±0.00 ^a	60.75±0.35 ^a
OCO	202.20±0.50 ^d	69.95±1.10 ^e	0.23±0.02 ^b	41.25±0.35 ^c	0.80±0.00 ^f	3.95±0.15 ^d	23.55±0.65 ^f
OAA	212.30±1.00 ^c	74.95±0.02 ^e	0.20±0.01 ^b	46.80±0.10 ^b	1.95±0.05 ^e	4.20±0.00 ^d	25.15±0.35 ^e
OBb	220.45±0.05 ^b	77.75±3.05 ^d	0.20±0.01 ^b	47.25±0.05 ^a	2.05±0.00 ^e	4.85±0.05 ^c	24.35±0.05 ^e
OAB	234.75±0.85 ^a	88.45±1.02 ^c	0.17±0.01 ^c	51.15±2.20 ^a	4.20±0.10 ^c	6.45±0.05 ^b	28.15±1.35 ^d

TABLE 3: Mineral composition (mg/100 mL) of non-probiotic and probiotic-fortified fruit juices

All values are means ± standard deviation of duplicates. Values with different superscript within the same column are significantly different at $p \leq 0.05$.

WCO, Watermelon juice alone; WAA, Watermelon juice + *Lactobacillus casei*; WBB, Watermelon juice + *Lactobacillus fermentum*; WAB, Watermelon juice + *Lactobacillus casei* + *Lactobacillus fermentum*; PCO, Pineapple without strain (control); PAA, Pineapple + *Lactobacillus casei*; PBB, Pineapple + *Lactobacillus fermentum*; PAB, Pineapple + *Lactobacillus fermentum* + *Lactobacillus casei*; OCO, Orange without strain (control); OAA, Orange juice + *Lactobacillus casei*; OBb, Orange juice + *Lactobacillus fermentum*; OAB, Orange juice + *Lactobacillus casei* + *Lactobacillus fermentum*

Vitamin content of non-probiotic and probiotic-fortified fruit juices

Table 4 presents the vitamin content of non-probiotic and probiotic-fortified fruit juices. The vitamin E content ranged from 1.65-1.85 mg/100 mL in watermelon juice, 8.65-15.55 mg/100 mL in pineapple juice, and 4.75-7.90 mg/100 mL in orange juice, with significant differences ($p \leq 0.05$) observed among the samples. It was observed that the inoculation of *L. casei* and *L. fermentum* strains reduced the vitamin E content of the probiotic-fortified watermelon juice. This reduction could be due to the oxidative environment created during the fermentation process, which may degrade vitamin E [40]. Feng and Wang [46] reported that the oxidative stability of vitamin E can be compromised in the presence

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of specific probiotics, such as certain *Lactobacillus* strains, due to their metabolic activity, which can lead to the production of reactive oxygen species during fermentation. The inoculation of *L. casei* and *L. fermentum* strains contributed to an increase in vitamin B12 in all the probiotic-fortified fruit juices. The significant increase in vitamin B12 content in the probiotic-fortified fruit juices, especially in fruit juice from pineapple with both LAB strains, is a promising outcome, as it suggests that probiotics can enhance the synthesis or bioavailability of vitamin B12. This finding aligns with the report of Palachum et al. [45] who demonstrated that certain probiotic strains, particularly, *Lactobacillus* genus, can synthesize vitamin B12 in fermented foods. Inoculation of *L. casei* and *L. fermentum* strains increased the vitamin C content in probiotic-fortified pineapple juice up to 60.20 mg/100 mL. There was reduction in vitamin C content of probiotic-fortified orange juice from 19.20 to 10.30 mg/100 mL, and in probiotic-fortified watermelon juice from 19.20 to 10.30 mg/100 mL. Reduction in vitamin C content of probiotic-fortified watermelon juice is in consistent with the findings of Markowiak-Kopeć and Śliżewska [47], who reported that the metabolic activity of probiotics, particularly in acidic environments, can lead to the degradation of vitamin C due to increased enzymatic activity and lower pH levels during fermentation. Vitamin C has been known to support skin health and wound healing, offering additional functional benefits [48].

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Samples	Vitamin E	Vitamin B12	Vitamin C
WCO	1.85±0.00 ⁱ	4.20±0.10 ^f	19.20±0.00 ^d
WAA	1.76±0.00 ⁱ	6.85±0.15 ^e	14.10±0.00 ^e
WBB	1.68±0.00 ^j	7.15±0.05 ^e	13.15±0.05 ^e
WAB	1.65±0.01 ^j	8.10±0.25 ^d	10.30±0.06 ^f
PCO	8.65 ± 0.05 ^c	11.05 ± 0.75 ^c	43.30 ± 0.50 ^c
PAA	9.80 ± 0.00 ^c	14.85 ± 0.95 ^b	56.75 ± 0.05 ^b
PBB	10.15 ± 0.05 ^b	15.35 ± 0.55 ^b	57.25 ± 0.05 ^b
PAB	15.55 ± 0.05 ^a	17.80 ± 0.60 ^a	60.20 ± 0.10 ^b
OCO	4.75±0.05 ^f	7.65±0.15 ^e	70.81±0.20 ^a
OAA	6.85±0.05 ^d	9.30±0.10 ^d	63.30±0.40 ^b
OBB	6.75±0.05 ^d	10.00±0.30 ^c	65.00±0.50 ^b
OAB	7.90±0.05 ^c	14.00±0.12 ^b	41.00±0.20 ^c

TABLE 4: Vitamin content (mg/100 mL) of non-probiotic and probiotic-fortified fruit juices

All values are means ± standard deviation of duplicates. Values with different superscript within the same column are significantly different at $p \leq 0.05$.

WCO, Watermelon juice alone; WAA, Watermelon juice + *Lactobacillus casei*; WBB, Watermelon juice + *Lactobacillus fermentum*; WAB, Watermelon juice + *Lactobacillus casei* + *Lactobacillus fermentum*; PCO, Pineapple without strain (control); PAA, Pineapple + *Lactobacillus casei*; PBB, Pineapple + *Lactobacillus fermentum*; PAB, Pineapple + *Lactobacillus fermentum* + *Lactobacillus casei*; OCO, Orange without strain (control); OAA, Orange juice + *Lactobacillus casei*; OBB, Orange juice + *Lactobacillus fermentum*; OAB, Orange juice + *Lactobacillus casei* + *Lactobacillus fermentum*

Bioactive compounds and antioxidant potential of non-probiotic and probiotic-fortified fruit juices

The bioactive compounds and antioxidant potential of non-probiotic and probiotic-fortified fruit juice samples are presented in Table 5. The phenolic and flavonoid contents increased in fruit juice containing the two LAB. Highest phenolic content of 0.79 mg GAE/mL was obtained in probiotic-fortified pineapple juice containing *L. casei* and *L. fermentum*. Orange juice with *L. casei* or *L. fermentum* had similar phenolic contents of 0.75 and 0.77 mg GAE/mL, respectively. The addition of *Lactobacillus* spp. improved the antioxidant activity of the fruit juice. These findings are consistent with previous studies, which report that *Lactobacillus* strains enhanced the antioxidant properties of fruit juices [15,27,49]. The enhanced antioxidant properties of the probiotic-fortified juice samples suggest that probiotic

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fermentation can augment the juice's ability to neutralize free radicals. This is significant because oxidative stress, caused by an imbalance between free radicals and antioxidants, is connected to various chronic diseases, including neurodegenerative disorders, cancer, and cardiovascular diseases [50]. The superior antioxidant capacity of the probiotic-fortified juice samples containing the combined strains indicates that combining multiple probiotic strains may have a synergistic effect, leading to greater health benefits compared to using a single strain [51]. Antioxidants play a vital role as health-protecting agents, especially in foodstuffs, as they respond to scavenging free radicals and delay and prevent food spoilage.

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Samples	Phenol (mg GAE/mL)	Flavonoid (mg QE/mL)	FRAP (μmol TE/mL)	DPPH (%)	ABTS (μmol TE/mL)	Fe ²⁺ chelation (%)	OH (%)
WCO	0.24±0.00 ⁱ	0.14 ±0.00 ^e	0.42 ±0.01 ⁱ	29.06 ±0.98 ^e	1.23 ±0.00 ^k	30.85 ±0.45 ^d	37.43±0.85 ^e
WAA	0.39±0.00 ^f	0.17 ±0.00 ^d	0.94 ±0.02 ^f	42.60 ± 1.20 ^c	1.82 ±0.00 ^j	40.03 ±0.80 ^b	53.70±0.40 ^c
WBB	0.41 ±0.00 ^{ef}	0.19 ±0.00 ^d	0.96 ±0.02 ^f	48.15 ±0.65 ^b	1.78 ±0.25 ^j	39.50 ±0.60 ^b	52.25±0.45 ^c
WAB	0.44 ±0.00 ^e	0.27 ±0.11 ^b	1.05 ±0.02 ^f	50.35 ±0.55 ^b	1.93 ±0.00 ⁱ	47.55 ±0.65 ^a	57.30±0.40 ^b
PCO	0.44 ± 0.00 ^e	0.23 ± 0.02 ^c	0.84± 0.02 ⁱ	40.85± 0.55 ^d	2.40 ± 0.10 ^f	31.20 ± 1.10 ^d	35.00±0.20 ^e
PAA	0.49 ± 0.00 ^e	0.22 ± 0.02 ^c	1.06±0.01 ^f	44.70 ± 0.80 ^c	2.75 ±0.05 ^e	37.00 ± 0.60 ^c	43.05±0.25 ^d
PBB	0.54 ± 0.00 ^d	0.24 ± 0.02 ^c	1.08± 0.01 ^f	47.75 ± 0.50 ^b	2.75 ±0.05 ^e	40.76 ± 0.45 ^b	44.05±0.25 ^d
PAB	0.57 ± 0.00 ^d	0.27 ± 0.02 ^b	1.13± 0.02 ^e	58.75 ± 0.55 ^a	2.95 ± 0.05 ^e	46.80 ± 0.30 ^a	52.60±0.70 ^c
OCO	0.63±0.01 ^c	0.28±0.00 ^b	1.38±0.02 ^d	40.55±1.75 ^d	3.14±0.02 ^d	30.10±0.30 ^d	43.15±0.35 ^d
OAA	0.75±0.01 ^b	0.32±0.01 ^b	1.69±0.05 ^c	47.25±0.55 ^b	3.38±0.03 ^b	39.55±0.75 ^b	48.05±0.25 ^c
OBB	0.77±0.01 ^b	0.36±0.02 ^a	1.77±0.05 ^b	57.65±0.75 ^a	3.28±0.02 ^c	37.75±0.35 ^b	52.55±0.75 ^c
OAB	0.79±0.01 ^a	0.36±0.00 ^a	1.83±0.06 ^a	61.15±0.75 ^a	3.49±0.02 ^a	48.75±0.55 ^a	61.20±0.40 ^a

TABLE 5: Bioactive compounds and antioxidant potential of non-probiotic and probiotic-fortified fruit juices

All values are means ± standard deviation of duplicates. Values with different superscript within the same column are significantly different at $p \leq 0.05$.

WCO, Watermelon juice alone; WAA, Watermelon juice + *Lactobacillus casei*; WBB, Watermelon juice + *Lactobacillus fermentum*; WAB, Watermelon juice + *Lactobacillus casei* + *Lactobacillus fermentum*; PCO, Pineapple without strain (control); PAA, Pineapple + *Lactobacillus casei*; PBB, Pineapple + *Lactobacillus fermentum*; PAB, Pineapple + *Lactobacillus fermentum* + *Lactobacillus casei*; OCO, Orange without strain (control); OAA, Orange juice + *Lactobacillus casei*; OBB, Orange juice + *Lactobacillus fermentum*; OAB, Orange juice + *Lactobacillus fermentum* + *Lactobacillus casei*; FRAP, ferric reducing antioxidant power; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; OH, hydroxyl radical; ABTS, 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); GAE, Gallic Acid Equivalent; QE, Quercetin Equivalent; TE, Trolox Equivalent

Organoleptic properties of non-probiotic and probiotic-fortified fruit juices

The organoleptic properties of non-probiotic and probiotic-fortified fruit juices are presented in Table 6. The result revealed that inoculation of *L. casei* and *L. fermentum* strains did not significantly affect the colour of the probiotic-fortified fruit juices. This is a positive finding as colour is a critical factor in consumer perception, often serving as a visual cue for freshness and quality. The color of a beverage is one of the first attributes noticed by consumers and plays an

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important role in their initial perception and acceptance of the product [52]. Similar trends were observed in the aroma, mouthfeel, and characteristics flavour of the probiotic-fortified fruit juices. The sensory scores showed that all the probiotic-fortified fruit juice samples were accepted by the panelists. The wide acceptance of probiotic-fortified fruit juices by consumers established the importance of *L. casei* and *L. fermentum*, which have been inoculated into fruit juices.

Samples	Colour	Taste	Mouthfeel	Characteristics flavour	General acceptability
WCO	7.15 ± 0.87 ^a	4.15 ± 0.01 ^c	6.45 ± 0.02 ^b	5.15 ± 0.05 ^c	6.00 ± 0.05 ^c
WAA	6.95 ± 1.23 ^a	3.70 ± 0.00 ^c	6.40 ± 0.01 ^b	5.00 ± 0.01 ^c	5.80 ± 0.01 ^c
WBB	7.25 ± 0.91 ^a	3.75 ± 0.00 ^c	6.10 ± 0.05 ^b	4.95 ± 0.07 ^c	5.70 ± 0.19 ^c
WAB	7.00 ± 0.97 ^a	4.25 ± 0.00 ^c	6.00 ± 0.07 ^b	4.70 ± 0.12 ^c	5.65 ± 0.06 ^c
PCO	8.45 ± 0.68 ^a	8.10 ± 0.05 ^a	7.40 ± 0.05 ^a	7.80 ± 0.19 ^a	8.20 ± 0.12 ^a
PAA	8.25 ± 0.63 ^a	7.55 ± 0.09 ^a	7.30 ± 0.10 ^a	7.75 ± 0.16 ^a	8.10 ± 0.04 ^a
PBB	8.25 ± 0.63 ^a	7.65 ± 0.08 ^a	7.35 ± 0.03 ^a	7.75 ± 0.01 ^a	7.90 ± 0.17 ^{ab}
PAB	8.25 ± 0.91 ^a	7.55 ± 0.03 ^a	7.15 ± 0.03 ^a	7.70 ± 0.16 ^a	7.65 ± 0.04 ^b
OCO	6.95 ± 0.83 ^a	6.65 ± 0.01 ^b	7.10 ± 0.09 ^a	6.85 ± 0.09 ^b	6.70 ± 0.10 ^b
OAA	6.80 ± 1.11 ^a	6.35 ± 0.04 ^b	7.00 ± 0.03 ^a	6.90 ± 0.05 ^b	6.70 ± 0.18 ^b
OBB	6.95 ± 0.10 ^a	6.25 ± 0.01 ^b	6.95 ± 0.09 ^a	6.80 ± 0.11 ^b	6.70 ± 0.08 ^b
OAB	6.65 ± 0.88 ^a	6.35 ± 0.04 ^b	7.15 ± 0.03 ^a	6.80 ± 0.09 ^b	6.80 ± 0.03 ^b

TABLE 6: Organoleptic properties of non-probiotic and probiotic-fortified fruit juices

All values are means ± standard deviation of duplicates. Values with different superscript within the same column are significantly different at $p \leq 0.05$.

WCO, Watermelon juice alone; WAA, Watermelon juice + *Lactobacillus casei*; WBB, Watermelon juice + *Lactobacillus fermentum*; WAB, Watermelon juice + *Lactobacillus casei* + *Lactobacillus fermentum*; PCO, Pineapple without strain (control); PAA, Pineapple + *Lactobacillus casei*; PBB, Pineapple + *Lactobacillus fermentum*; PAB, Pineapple + *Lactobacillus fermentum* + *Lactobacillus casei*; OCO, Orange without strain (control); OAA, Orange juice + *Lactobacillus casei*; OBB, Orange juice + *Lactobacillus fermentum*; OAB, Orange juice + *Lactobacillus casei* + *Lactobacillus fermentum*

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Conclusions

This study successfully isolated *L. casei* and *L. fermentum* from 'Omidun' (ogi supernatant) and incorporated them into watermelon, pineapple, and orange juices to develop probiotic beverages through fermentation. The probiotic-fortified fruit juices exhibited increased titratable acidity, reduced pH, and enhanced levels of potassium, calcium, and magnesium, as well as significantly improved antioxidant activity (as measured by DPPH and other radical-scavenging assays), particularly in samples with combined *Lactobacillus* strains. The results indicate that both *L. casei* and *L. fermentum* are suitable candidates for the production of functional fruit juices. Based on these findings, watermelon, pineapple, and orange juices offer a promising non-dairy alternative for delivering probiotics to lactose-intolerant individuals, while also providing a variety of nutrients, including vitamins, minerals, and antioxidants, that contribute to their functional beverage status. However, this study was limited to three fruit substrates and two probiotic strains, with all experiments conducted under laboratory conditions that may not fully reflect commercial processing and storage environments. Future research should explore a wider range of fruit matrices, additional probiotic strains, long-term storage stability, and industrial-scale feasibility. These findings have important implications for the food industry, providing a scientific foundation for the development of innovative, non-dairy functional beverages tailored to health-conscious, lactose-intolerant, and vegetarian consumers. Continued research in this area will contribute to the diversification of functional food products and support public health through the creation of nutrient-enriched, microbiologically safe, and antioxidant-rich beverages.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Adeyanmola O. Faturoti, Clement O. Ogidi, Oluwatoyin A. Oladeji

Acquisition, analysis, or interpretation of data: Adeyanmola O. Faturoti, Clement O. Ogidi, Oluwatoyin A. Oladeji

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Critical review of the manuscript for important intellectual content: Adeyanmola O. Faturoti, Clement O. Ogidi, Oluwatoyin A. Oladeji

Supervision: Adeyanmola O. Faturoti, Clement O. Ogidi, Oluwatoyin A. Oladeji

Disclosures

Plant subjects: All authors have confirmed that this study did not involve any herbarium specimen. **Human subjects:** Consent was obtained or waived by all participants in this study. Research Committee on Sensory Evaluation and Consumer Protection issued approval OAUSTECH/FST/2025-003. Ethical approval for the organoleptic properties for this study was obtained from the Research Committee on Sensory Evaluation and Consumer Protection, Department of Food Science and Technology, Olusegun Agagu University. The committee approved the organoleptic properties of probioticated fruit juices used in this research titled "Assessment of Lactic Acid Bacteria Growth Rate, Physicochemical Parameters, Vitamin Contents, and Antioxidant Potentials of Probiotic-Fortified Fruit Juices" (Approval Number: OAUSTECH/FST/2025-003). **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

How to cite this article:

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Data Availability Statements

The datasets (and/or code) supporting this study are available from the corresponding author upon reasonable request.

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