

Article

# Probiotic Viability, Physicochemical and Sensory Properties of Probiotic Pineapple Juice

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**Abstract:** (1) Background: Probiotication is an important method in the food industry, and the use of probiotic dairy products has prevented lactose intolerant patients and vegetarians from their consumption. Hence, there is a need to incorporate probiotics in fruit juice without lactose; (2) Method: Probiotic viability, and physicochemical and sensory evaluation of stored probioticated pineapple juice using lactic acid bacteria (LAB) (*Pediococcus pentosaceus* LaG1, *Lactobacillus rhamnosus* GG, *Pediococcus pentosaceus* LBF2) as a single and mixed starter was investigated; (3) Results: There was an increase in the lactic acid production, and reduction in pH, vitamin C content, and colour during storage. At weeks 3 and 4, Propp2 and Pcontrol samples had the highest lactic acid content (317.9 mg/L and 160.34 mg/L). The vitamin C content ranged from 2.91–7.10 mg/100 g. There was a general reduction in total soluble solids during storage. The probiotic LAB were viable throughout the storage time ( $1.05\text{--}1.10 \times 10^9$  cfu/mL) in the juice samples. There was no significant difference in terms of taste, aroma, colour, or appearance during the time of storage; (4) Conclusion: The pineapple juice supported the viability, lactic acid production, vitamin C development, and the antagonistic potential of the probiotic candidate. This result is useful for the development of probiotic fruit juice as functional foods and nutraceuticals with health beneficial effect.

**Keywords:** probiotication; pineapple juice; lactic acid bacteria; sensory evaluation

## 1. Introduction

Probiotics are live microorganisms which, when consumed in large quantities, confer health benefits [1] to the consumer. The consumption of appreciable amounts of fermentative dairy products confers a health benefit by balancing intestinal microflora. The low viability of probiotic organisms is one of the most important problems in the processing and production of probiotic food supplements, because of their sensitivity to difficult conditions such as low pH in food and powerful enzymes of the stomach. A standardized probiotic food must contain a minimum of  $10^6$  CFU/g active and live organisms at the time of consumption [2].

Research on the production of functional foods which promote consumers' health has been on the increase in recent years. This development has brought a reduction in some of the diseases related to life style [3]. "Functional foods" refers to foods with health-promoting ingredients enriched beyond the normal traditional nutrients [4,5]. These foods contain a reasonable quantity of some bioactive components, such as probiotic, prebiotic, and symbiotic components [3].

For the most part, probiotics have been effectively incorporated in dairy products, but these products are known to contain a high amount of lactose, which places a limitation to lactose intolerant individuals, hence the need to find an alternative means of production that can make the product available for a larger population.

Sivudu et al. [6] reported the probiotic action of mixed watermelon and tomato juice using *Lactobacillus* strains.

The production of a probiotic mixture of black cherry and barberry juice by lactic acid bacteria (LAB) has been reported by Shahram et al. [7]. He concluded that a mixture with 0.2% whey powder could be considered as a suitable matrix for the growth of probiotic bacteria and functional beverage production.

Pineapple (*Ananas comosus*) is a tropical plant with edible multiple fruits; it is the most economically important plant in the *Bromeliaceae* family [8]. Pineapple can be consumed fresh, cooked, juiced, and preserved. The flesh and juice of the pineapple are used in cuisines around the world. It contains a high amount of vitamin C, magnesium, and calcium. The juice of the pineapple is served as a beverage, and it is also the main ingredient in cocktails [9]. The presence of a high amount of vitamin C and sugar makes it an essential medium for the cultivation of probiotics.

There are many dairy and cereal probiotic foods, but the demand for vegetable probiotic products from fruits and vegetables has increased, due to an increase in the population of vegetarians and lactose intolerant individuals who are allergic to dairy products [10–13]. Vegetables and fruits are good sources of vitamins, minerals, dietary fibers, antioxidants, and bioactive compounds which have a positive effect on some vital organs in the body [14]. Therefore, non-dairy items such as fruits and vegetables which lack some dairy allergens could be used [5]. The ability of fresh-cut pineapple to serve as a new carrier of probiotic LAB and the use of probiotic lactic acid bacteria for the production of multifunctional fresh-cut cantaloupe has been reported [15,16].

Therefore, this study aimed at the production and storage of probiotic pineapple juice using probiotic LAB (single and mixed culture) as starter, and determination of the physicochemical parameters, vitamin content, viability, antagonistic activity, and sensory evaluation of the samples.

## 2. Materials and Methods

### 2.1. Sample Collection and Laboratory Preparation of Pineapple Juice (*Ananas comosus*)

Pineapple was purchased from Bodija market, Favours Farm, and one other market in Ibadan, Oyo State, Nigeria. The fruit was kept at 4 °C for further use. The fruits were washed thoroughly with running tap water, rinsed with distilled water, peeled, and rinsed again with sterile distilled water. The pineapple juice was then extracted using a juice extractor. The extracted juice was pasteurized using a pasteurizer at a temperature of 93 °C for 48 s. After cooling, samples were stored at 4 °C before they were used for further analysis.

### 2.2. Culture Collection and Inocula Preparation

Probiotic *Pediococcus pentosaceus* LaG1, *Pediococcus pentosaceus* LBF2, and *Lactobacillus rhamnosus* GG were obtained from the culture collection of our previous work in the Department of Microbiology, University of Ibadan. The stock cultures were maintained on De Man Rogosa Sharpe (MRS) agar and stored at 4 °C. A 0.5 McFarland standard suspension with turbidity of  $1.5 \times 10^8$  cfu/mL [17] was used to standardize the inocula. The seed culture was grown in a 250 mL flask containing 50 mL of sterile MRS broth. Cells were harvested by centrifugation (10,000 g) for ten minutes and washed twice with sterile 0.1% peptone buffer [18]. The cultures were diluted with sterile distilled water, and the optical density of the bacterial suspension was measured using a spectrophotometer at 625 nm. The optical density of the bacterial suspension was compared with the optical density of 0.5 McFarland containing  $1.5 \times 10^8$  cfu/mL [17]. Serial dilution was done to obtain a  $10^7$  cfu/mL dilution of the probiotic LAB.

### 2.3. Production of the Probioticated Juice Samples

Probiotication of pasteurized pineapple juice samples was done by inoculating 100 mL of the sample with 1% (v/v) equivalent to 10 mL of the probiotic LAB starter culture (0.5 McFarland standard containing  $1.5 \times 10^8$  cfu/mL). The inoculated samples with the probiotic LAB

(*Pediococcus pentosaceus* LaG1, *Lactobacillus rhamnosus* GG, *Pediococcus pentosaceus* LBF2, and a mixed culture of the three probiotic LAB strains) was labelled as: Propp1 (juice sample inoculated with *Pediococcus pentosaceus* LaG1), Propp2 (juice sample inoculated with *Pediococcus pentosaceus* LBF2), ProRhamno (juice sample inoculated with *Lactobacillus rhamnosus* GG), Proconsortium (juice sample inoculated with *Pediococcus pentosaceus* LaG1, *Pediococcus pentosaceus* LBF2, and *Lactobacillus rhamnosus* GG), and Pcontrol (un-inoculated juice samples). The inoculated samples were incubated at 37 °C for 72 h and stored at 4 °C for four weeks. Weekly analysis, including total soluble solids, colour, and pH was conducted.

#### 2.4. Physicochemical Analysis

##### 2.4.1. Quantitative Estimation of Lactic Acid and pH

The production of lactic acid was determined by titrating 10 mL of the homogenized sample against 0.25 mol·L<sup>-1</sup> NaOH using 1 mL of phenolphthalein indicator (0.5% in 50% alcohol). Acid equivalent is the amount of NaOH consumed in mL, while each mL of NaOH is equivalent to 90.08 mg of lactic acid [19].

The pH of the probioticated juice samples stored at 4 °C for 1–4 weeks was determined using a pH meter (Titumum U9N model).

Colour assessment of the probioticated juice sample stored for different time intervals was done by using the colour meter. The colour parameters (Hunter L \*, a \*, and b \* value) were determined for each sample using a spectrophotometer—colorimeter (CM-2500D, Minolta, Japan) per the method of Maskan [20]. Total soluble solids (TSS) were determined using a hand refractometer (Erma, Tokyo, Japan) in terms of °Bx (°Brix) [20].

##### 2.4.2. Determination of Vitamin C (Ascorbic Acid)

Vitamin C (ascorbic acid) was determined by the titrimetric method as described by Mazumdar and Majumder [21] and James [22]. Ten millilitres of the probioticated juice samples was made up to 100 mL volume with 3% hydrogen phosphate (HPO<sub>3</sub>) and filtered. Ten millilitres of the filtrate was titrated against standard redox dye (2,6-dichlorophenol indophenol, DCPIPH). Changes in colour to pink as a result of the unavailability of an electron to reduce the DCPIPH indicate the end point. The ascorbic acid was calculated in mg/100 mL.

#### 2.5. Antagonistic Activity of Probioticated Substrate

The antagonistic activity of the probioticated substrate against some pathogenic bacteria (*Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus* sp., *L. monocytogenes*, *Klebsiella* sp., *B. subtilis*, *P. aeruginosa*, *E. faecalis*, and *Staphylococcus aureus*) was investigated using agar well diffusion. The test pathogens containing 2.5 × 10<sup>7</sup> cfu/mL was seeded on a sterile molten nutrient agar. After solidification, wells were bore on seeded agar plates, and the probioticated juice sample was introduced into the wells. The plates were first incubated at 4 °C for 60 min to allow the test material to diffuse in the agar, and were then incubated at 37 °C for 18 h. After incubation, the diameter of the clear zone was measured in centimetres from the centre of the well.

#### 2.6. Determination of the Survivability of the LAB Strain inside the Pineapple Juice

The survivability of the LAB strain in the probioticated juice samples was determined using a pour plate technique. The stored samples were pour plated at weekly intervals. Sample (1 mL) was inoculated on MRS agar plates, and the plates were incubated at 37 °C for 48 h. Viable counts were recorded by counting the visible colonies on the culture medium, and the number was multiplied by the reciprocal of the dilution factor and expressed as colony forming units (CFU).

### 2.7. Sensory Evaluation of Stored Probioticated Juice Samples

Coded samples of the probioticated juice samples were served to 10 trained panellists. The panellist was asked to rate the sample based on taste, aroma, colour, and appearance. Triplicate determinations were made per sample. The ratings were presented on a seven-point hedonic scale ranging from 1—extremely like, 7—extremely dislike. Obtained results were subjected to analysis of variance using one-way ANOVA, and the difference between means was separated using Duncan’s Multiple Ranged Test [23].

## 3. Results and Discussion

### 3.1. Acid Equivalent and Lactic Acid Production in the Stored Probioticated Juice Samples

Acid equivalent and the lactic acid production in the probioticated juice samples during storage is shown in Table 1. At week 1 of storage, the acid equivalent and lactic acid production ranged from 0.59–1.02 and 53.14–91.88 mg/mL, respectively. Propp1 had the highest acid content. At week 2 of storage, the acid equivalent and lactic acid production ranged from 1.96–3.90 and 177.55–351.31 mg/mL, respectively. Propp1 juice sample had the highest lactic acid. At weeks 3 and 4 of storage, the acid equivalent and lactic acid ranged from 2.49–3.53 and 224.29–317.90 mg/mL, and 1.08–1.78 and 97.28–160.34 mg/L, respectively, in which the Pcontrol had the highest values. There was a significant difference in the lactic acid production during the storage time.

**Table 1.** Acid equivalence and lactic acid (mg/mL) development during storage of the probioticated juice samples.

	Week 1		Week 2		Week 3		Week 4	
	Acid Equivalent	Lactic Acid	Acid Equivalent	Lactic Acid	Acid Equivalent	Lactic Acid	Acid Equivalent	Lactic Acid
ProRhamno	0.82	73.86	3.49	314.37	2.69	242.31	1.08	97.28
Proconsortium	0.98	88.27	1.96	176.55	3.53	317.98	1.77	159.44
Pcontrol	0.59	53.14	1.96	176.55	2.49	224.29	1.78	160.34
Propp1	1.02	91.88	3.90	351.31	2.69	242.31	1.77	159.44
Propp2	0.78	70.26	1.96	176.55	3.53	317.9	1.57	141.42

Means with the same superscript letters across the rows are not significantly different from each other at 5% level of probability. KEY: Propp2—Pineapple with *Pediococcus pentosaceus* LaG1, ProRhamno—Pineapple with *Lactobacillus rhamnosus* GG, Propp1—Pineapple with *Pediococcus pentosaceus* LBF2, Proconsortium—Pineapple with Consortium (*Pediococcus pentosaceus* LaG1, *Pediococcus pentosaceus* LBF2, and *Lactobacillus rhamnosus* GG).

The increase in the acid equivalent and a concomitant increase in lactic acid during probiotication and storage may be due to the metabolic activity of the probiotic LAB. The increase in lactic acid will inhibit the activity of the spoilage microorganisms. This agrees with the report of Gaanappriya et al. [24]. Karovicova and Kohajdona [25] reported that lactic acid fermentation can be suppressed by butyric bacteria activity in a slowly acidified medium. Moraru et al. [14] reported that changes in the pH of the medium and lactic acid development are due to the production of organic acid by LAB culture.

### 3.2. pH of the Stored Probioticated Juice Samples

The pH of the probioticated juice sample is shown in Table 2. There was a significant difference in the pH during storage. The highest pH was recorded at week 4 after storage for all samples. There was a reduction in pH after week 1 of storage. For the Proconsortium juice sample, the pH ranged from 4.99–5.54. During storage, the lowest pH (4.99) was recorded in the Proconsortium juice sample at week 3.

**Table 2.** pH of the probioticated pineapple juice sample stored at different time intervals (weeks).

Juice Code	pH					
	Storage Time (Weeks)					
	1	2	3	4	Mean	S.E.
ProRhamno	5.12 <sup>b</sup>	5.05 <sup>c</sup>	5.06 <sup>c</sup>	5.30 <sup>a</sup>	5.13 <sup>c</sup>	0.10
Proconsortium	5.48 <sup>b</sup>	5.00 <sup>c</sup>	4.99 <sup>c</sup>	5.54 <sup>a</sup>	5.25 <sup>a,b</sup>	0.27
Pcontrol	5.16 <sup>b,c</sup>	5.14 <sup>c</sup>	5.12 <sup>d</sup>	5.90 <sup>a</sup>	5.33 <sup>a</sup>	1.71
Propp1	5.07 <sup>c</sup>	5.09 <sup>b</sup>	5.06 <sup>c</sup>	5.35 <sup>a</sup>	5.13 <sup>c</sup>	0.13
Propp2	5.26 <sup>b</sup>	5.03 <sup>c</sup>	5.00 <sup>d</sup>	5.35 <sup>a</sup>	5.14 <sup>c</sup>	0.16

Means with the same superscript letters across the rows are not significantly different from each other at 5% level of probability. KEY: Propp2—Pineapple with *Pediococcus pentosaceus* LaG1, ProRhamno—Pineapple with *Lactobacillus rhamnosus* GG, Propp1—*Pediococcus pentosaceus* LBF2, Proconsortium—Pineapple with Consortium (*Pediococcus pentosaceus* LaG1, *Pediococcus pentosaceus* LBF2, and *Lactobacillus rhamnosus* GG).

Reduction in pH during fermentation and storage of the probioticated juice sample is of paramount importance for maintaining the quality of the probioticated juice samples. This agrees with the report of Viander et al. [26] that pH reduction is of great importance for the quality of the end product. Changes in the pH and concomitant increase in lactic acid development during fermentation and storage of the probioticated juice sample may be due to the metabolic activity of the probiotic LAB.

### 3.3. Colour Assessment of the Stored Probioticated Juice Samples

Colour assessment of the probioticated juice samples during storage is shown in Table 3. There was a significant difference in the colour assessment using a colour meter. The colour ranged from 35.28–59.92. The best colour was recorded in Propp2 juice sample at week 3, and the lowest in Procontrol juice sample at week 4 after storage.

**Table 3.** Colour assessment of the probioticated pineapple juice sample stored at different time intervals (weeks) using colour meter.

Juice Code	Colour					
	Storage Time (Weeks)					
	1	2	3	4	Mean	S.E.
ProRhamno	55.08 <sup>b</sup>	55.56 <sup>b</sup>	56.37 <sup>a,b</sup>	57.54 <sup>a</sup>	56.21 <sup>a</sup>	0.48
Proconsortium	53.47 <sup>d</sup>	54.56 <sup>c</sup>	57.22 <sup>b</sup>	59.08 <sup>a</sup>	56.08 <sup>a</sup>	0.29
Pcontrol	53.66 <sup>b</sup>	54.03 <sup>b</sup>	56.92 <sup>a</sup>	35.28 <sup>c</sup>	49.97 <sup>b</sup>	0.07
Propp1	54.82 <sup>d</sup>	56.00 <sup>c</sup>	58.47 <sup>b</sup>	59.34 <sup>a</sup>	55.0 <sup>a</sup>	0.16
Propp2	53.14 <sup>b</sup>	53.49 <sup>b</sup>	55.91 <sup>a</sup>	57.46 <sup>a</sup>	57.16 <sup>a</sup>	0.48

Means with the same superscript letters across the rows are not significantly different from each other at 5% level of probability. KEY: Propp2—Pineapple with *Pediococcus pentosaceus* LaG1, ProRhamno—Pineapple with *Lactobacillus rhamnosus* GG, Propp1—*Pediococcus pentosaceus* LBF2, Proconsortium—Pineapple with Consortium (*Pediococcus pentosaceus* LaG1, *Pediococcus pentosaceus* LBF2, and *Lactobacillus rhamnosus* GG).

### 3.4. The Total Soluble Solids of the Stored Probioticated Juice Samples

The TSS assessment of the probioticated pineapple juice samples during storage is shown in Table 4. There were significant differences ( $p \leq 0.05$ ) in the TSS in all the probioticated juice samples. The lowest TSS was recorded in the fourth week in all the probioticated juice samples assessed.

**Table 4.** Total suspended solids (TSS) of the probioticated pineapple juice sample stored at different time interval (weeks).

Juice Sample Code	TSS						
	Storage Time (Weeks)					Mean	S.E.
	1	2	3	4			
ProRhamno	15.25 <sup>a</sup>	14.00 <sup>b</sup>	12.25 <sup>b</sup>	9.20 <sup>c</sup>	12.68 <sup>c,d</sup>	2.37	
Proconsortium	17.25 <sup>a</sup>	14.00 <sup>b</sup>	12.00 <sup>c</sup>	9.75 <sup>d</sup>	13.25 <sup>b,c</sup>	2.88	
Pcontrol	20.25 <sup>a</sup>	17.00 <sup>b</sup>	17.00 <sup>c</sup>	15.00 <sup>d</sup>	17.31 <sup>a</sup>	1.97	
Propp1	16.25 <sup>a</sup>	14.00 <sup>b</sup>	12.25 <sup>c</sup>	9.20 <sup>d</sup>	12.56 <sup>d</sup>	2.37	
Propp2	17.75 <sup>a</sup>	15.00 <sup>b</sup>	12.00 <sup>c</sup>	9.00 <sup>d</sup>	12.68 <sup>c,d</sup>	3.42	

Means with the same superscript letters across the rows are not significantly different from each other at 5% level of probability. KEY: Propp2—Pineapple with *Pediococcus pentosaceus* LaG1, ProRhamno—Pineapple with *Lactobacillus rhamnosus* GG, Propp1—*Pediococcus pentosaceus* LBF2, ProConsortium—Pineapple with Consortium (*Pediococcus pentosaceus* LaG1, *Pediococcus pentosaceus* LBF2, and *Lactobacillus rhamnosus* GG).

The reduction in the TSS during fermentation and storage may be due to the utilization of sugars and other metabolic activity by the probiotic LAB in the probiotic juice samples. This agrees with the report of Kumar et al. [27], who observed a similar reduction in fruit juice with *Lactobacillus casei*.

### 3.5. The Vitamin C Content of the Stored Probioticated Juice Samples

The vitamin C content of the probioticated juice samples during storage is shown in Table 5. For Pcontrol and Proconsortium juice samples, it ranged from 4.52–4.77 mg/100 g and 4.65–5.34 mg/100 g, and there was no significant difference in the vitamin content during storage. For Propp1, Propp2, and ProRhamno, the vitamin content ranged from 3.07–7.01 mg/100 g, 2.95–6.10 mg/100 g and 2.91–4.91 mg/100 g respectively. The Propp1 juice sample had the highest vitamin content (7.01 mg/100 g) after 4 weeks of storage.

**Table 5.** Weekly vitamin C analysis of probioticated pineapple juice sample (weeks).

Weeks	Vitamin C Composition (mg/100 g)				
	Pcontrol	Proconsortium	Propp1	Propp2	ProRhamno
1	4.77 <sup>a</sup>	4.65 <sup>a</sup>	3.07 <sup>c</sup>	2.95 <sup>d</sup>	2.91 <sup>c</sup>
2	4.69 <sup>a</sup>	4.88 <sup>a</sup>	4.40 <sup>b</sup>	3.26 <sup>c</sup>	3.24 <sup>c</sup>
3	4.52 <sup>a</sup>	4.88 <sup>a</sup>	6.43 <sup>a</sup>	5.43 <sup>b</sup>	4.27 <sup>b</sup>
4	4.77 <sup>a</sup>	5.34 <sup>a</sup>	7.01 <sup>a</sup>	6.10 <sup>a</sup>	4.91 <sup>a</sup>
S.E.	0.07	0.12	0.48	0.41	0.25

Means with the same superscript letters across the rows are not significantly different from each other at 5% level of probability. KEY: Propp2—Pineapple with *Pediococcus pentosaceus* LaG1, ProRhamno—Pineapple with *Lactobacillus rhamnosus* GG, Propp1—*Pediococcus pentosaceus* LBF2, ProConsortium—Pineapple with Consortium (*Pediococcus pentosaceus* LaG1, *Pediococcus pentosaceus* LBF2, and *Lactobacillus rhamnosus* GG).

Vitamin C is a water-soluble antioxidant found in fruits and vegetables [28,29]. It prevents tissue damage [30,31]. Vitamin C is routinely prescribed in the hospital to aid recovery from several ailments and diseases, such as cold, cough, influenza, sores, wounds, gingivitis, skin diseases, diarrhoea, malaria, and bacterial infections [32].

Packed fruit juices have been reported as one of the best sources of fluids containing sugars, vitamins, and minerals [33,34]. The role of fruits in the human diet and as a food supplement cannot be overemphasized. Due to their chemical composition, including carbohydrates, proteins, vitamins, and minerals such as Ca, Mg, K, Zn, and Fe, they are considered as healthy food supplements [35,36].

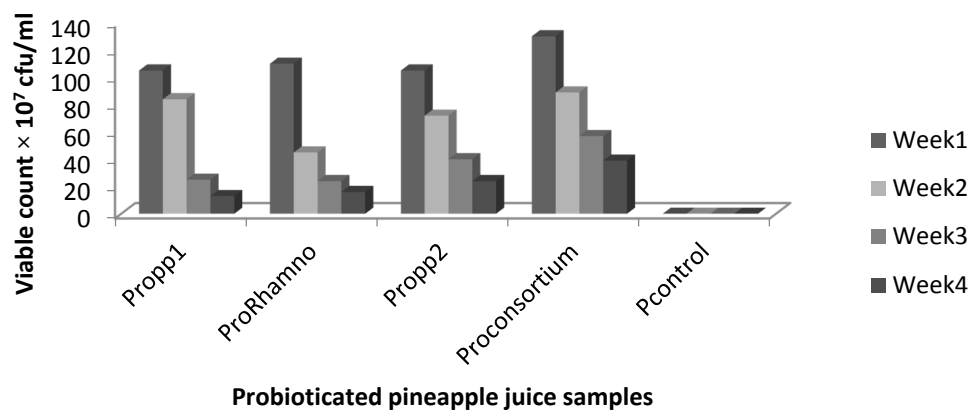
### 3.6. Viability of the Probiotic LAB in the Stored Probioticated Juice Samples

The viability of the probiotic LAB in the probioticated juice sample is shown in Figure 1. It ranged from  $1.05\text{--}1.10 \times 10^9$  cfu/mL. There was a general reduction in viability during storage time. After week 4, Proconsortium had the highest viable LAB ( $1.30 \times 10^7$ ), followed by Propp2, and the least was recorded in Propp1. At the first week of storage, the highest count was recorded in Proconsortium. The probiotic LAB used in this study were viable throughout the storage time. The probioticated juice prepared using the consortium had the highest viability after the 4th week of storage at 4 °C.

A general reduction in the viability during storage from week 1 to week 4 may be as a result of a reduction in the sugar level in the juice samples, due to the metabolism of the probiotic LAB, an accumulation of organic acid as some metabolites, and storage temperature [37]. Yanez et al. [38] reported that an increase in acidity as a result of the fermentation process can reduce the survivability and viability of the probiotic bacteria. Shah [39] equally reported that the viability of probiotic organisms is dependent on factors such as oxygen level in the product, oxygen permeation of the package, fermentation time, and storage temperature.

A high viable count ( $>10^7$  cfu/mL) after 4 weeks of storage is important for maximum health benefits. This agrees with the report of Anita et al. [24], who said that probioticated pineapple juice could be considered as a probiotic beverage without any nutrient supplementation.

Lourens-Hattingh and Viljoen [40] reported that for effective transfer of the probiotic effect to consumers for maximum health benefits, no less than a million viable cells/mL of the probiotic product have to be present.



**Figure 1.** Viability of probiotic LAB in the probioticated pineapple juice samples. KEY: Propp2—Pineapple with *Pediococcus pentosaceus* LaG1, ProRhamno—Pineapple with *Lactobacillus rhamnosus* GG, Propp1—*Pediococcus pentosaceus* LBF2, ProConsortium—Pineapple with Consortium (*Pediococcus pentosaceus* LaG1, *Pediococcus pentosaceus* LBF2, and *Lactobacillus rhamnosus* GG).

The probiotic culture should be able to multiply to reach high cell counts in the fermented product and possess a high acid tolerance to ensure high viable cell numbers during storage. The viability of probiotic organisms is dependent on many factors, such as the level of oxygen in products, oxygen permeation of the package, fermentation time, and storage temperature [37]. Furthermore, it is also affected by lactic acid produced during production and cold storage.

### 3.7. Antagonistic Activity of the Probioticated Pineapple Juice Samples

Antagonistic activity of the probioticated juice against some pathogens is shown in Table 6. Proconsortium juice samples had the best antagonistic activity against all the test pathogens. The highest activity was recorded against *E. coli* and *Pseudomonas* sp., with a zone of inhibition of 14 mm. The least activity was against *Klebsiella* sp. (10 mm). Propp1 and Propp2 juice samples had

antagonistic activity against three of the test pathogens, with a zone of inhibition ranging 8–19 mm and 10–14 mm.

**Table 6.** Antagonistic activity of the probioticated pineapple juice against some pathogenic microorganisms.

	<i>Staph. aureus</i>	<i>E. coli</i>	<i>Bacillus sp.</i>	<i>Pseudomonas sp.</i>	<i>Klebsiella sp.</i>
ProRhamno	0	12	0	17	10
Proconsortium	11	14	13	14	10
Pcontrol	0	0	0	0	0
Propp1	0	14	8	19	0
Propp2	0	10	0	11	14

KEY: Propp2—Pineapple with *Pediococcus pentosaceus* LaG1, ProRhamno—Pineapple with *Lactobacillus rhamnosus* GG, Propp1—*Pediococcus pentosaceus* LBF2, ProConsortium—Pineapple with Consortium (*Pediococcus pentosaceus* LaG1, *Pediococcus pentosaceus* LBF2, and *Lactobacillus rhamnosus* GG).

The ability of the probioticated juice samples to inhibit pathogenic organisms may be due to the production of bioactive metabolites such as lactic acid, hydrogen peroxide, diacetyls, and other organic acids and secondary metabolites [24]. This result agrees with the work of Soleimani et al. [41], who found that antimicrobial substances produced by LAB have an inhibitory effect on some pathogenic microorganisms.

### 3.8. Sensory Evaluation of the Stored Probioticated Juice Samples

The sensory evaluation of the probioticated juice samples during storage is shown in Table 7. There was no significant difference in taste, aroma, colour, and appearance of the probioticated juice samples during storage.

**Table 7.** Sensory evaluation (Taste, Aroma, Colour, Appearance) of the probioticated pineapple juice sample at different storage intervals (weeks).

Juice Sample Code	Storage Time (Weeks)															
	Taste				Aroma				Colour				Appearance			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1. ProRhano	2.0 <sup>a</sup>	2.0 <sup>a</sup>	2.0 <sup>b</sup>	4.0 <sup>b</sup>	2.0 <sup>a</sup>	2.0 <sup>a</sup>	2.0 <sup>b</sup>	2.0 <sup>b</sup>	2.0 <sup>a</sup>	2.0 <sup>a</sup>	3.0 <sup>b</sup>	3.0 <sup>b</sup>	2.0 <sup>a</sup>	2.0 <sup>a</sup>	2.0 <sup>b</sup>	3.0 <sup>b</sup>
2. Proconsortium	2.0 <sup>a</sup>	2.0 <sup>a</sup>	2.0 <sup>b</sup>	2.0 <sup>c</sup>	2.0 <sup>a</sup>	2.0 <sup>a</sup>	2.0 <sup>b</sup>	2.0 <sup>b</sup>	2.0 <sup>a</sup>	2.0 <sup>a</sup>	2.0 <sup>b</sup>	2.0 <sup>b</sup>	2.0 <sup>a</sup>	2.0 <sup>a</sup>	2.0 <sup>b</sup>	2.0 <sup>b</sup>
3. Propp1	2.0 <sup>a</sup>	2.0 <sup>a</sup>	2.0 <sup>b</sup>	2.0 <sup>c</sup>	2.0 <sup>a</sup>	2.0 <sup>a</sup>	2.0 <sup>b</sup>	2.0 <sup>b</sup>	2.0 <sup>a</sup>	2.0 <sup>a</sup>	3.0 <sup>b</sup>	3.0 <sup>b</sup>	2.0 <sup>a</sup>	2.0 <sup>a</sup>	2.0 <sup>b</sup>	2.0 <sup>b</sup>
4. Propp2	2.0 <sup>a</sup>	2.0 <sup>a</sup>	2.0 <sup>b</sup>	2.0 <sup>c</sup>	2.0 <sup>a</sup>	2.0 <sup>a</sup>	2.0 <sup>b</sup>	2.0 <sup>b</sup>	2.0 <sup>a</sup>	2.0 <sup>a</sup>	2.0 <sup>b</sup>	3.0 <sup>b</sup>	2.0 <sup>a</sup>	2.0 <sup>a</sup>	2.0 <sup>b</sup>	3.0 <sup>b</sup>
5. Pcontrol	2.0 <sup>a</sup>	2.0 <sup>a</sup>	6.0 <sup>a</sup>	7.0 <sup>a</sup>	2.0 <sup>a</sup>	2.0 <sup>a</sup>	6.0 <sup>a</sup>	7.0 <sup>a</sup>	2.0 <sup>a</sup>	2.0 <sup>a</sup>	7.0 <sup>a</sup>	7.0 <sup>a</sup>	2.0 <sup>a</sup>	2.0 <sup>a</sup>	7.0 <sup>a</sup>	7.0 <sup>a</sup>
S.E	0.0	0.0	0.25	0.28	0.0	0.0	0.24	0.28	0.0	0.0	0.28	0.28	0.0	0.0	0.28	0.28

Scoring system: 1—Extremely liked; 2—Strongly like; 3—Liked; 4—Moderately liked; 5—Dislike; 6—Strongly disliked; 7—Extremely disliked. KEY: Propp2—Pineapple with *Pediococcus pentosaceus* LaG1, ProRhamno—Pineapple with *Lactobacillus rhamnosus* GG, Propp1—*Pediococcus pentosaceus* LBF2, ProConsortium—Pineapple with Consortium (*Pediococcus pentosaceus* LaG1, *Pediococcus pentosaceus* LBF2, and *Lactobacillus rhamnosus* GG).

The result of the hedonic scale assessment of the probioticated juice in terms of taste, aroma, colour, and appearance shows that the probioticated samples were preferred by all the panellists. The Proconsortium juice sample had the highest preferred during the 4th week of storage, which may be as a result of the consortium or mixed starter on the sample. This result agrees with the work of Ho et al. [42], who reported the impact of a mixed culture of *Lactobacillus brevis* and *Pediococcus pentosaceus* on the sensorial quality of “Nem Chua”, a Vietnamese fermented meat product. Luckow and Delahunty [5] reported that the sensory characteristics of probiotic blackcurrant juice was perfumery and dairy in odour and sour and savoury in flavour.



#### 4. Conclusions

In conclusion, the probiotic pineapple juice supported the growth and viability of probiotic LAB. During storage, the probiotic LAB candidate grew and had significant pH development and lactic acid production, increased vitamin C content, reduction in total soluble solids, and no significant changes in the sensory parameters. The stored juice had an antagonistic effect on some pathogens. The results obtained in this study will be useful for the development of probiotic fruit juice with health beneficial effects. This could serve as a nutraceutical with health benefits for vegetarians, lactose intolerant people, and those who are allergic to milk products. Further work is necessary to check the biological evaluation of the products and the production of probioticated juice using more LAB consortium and effect of immobilization on the viability and functionality.

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#### References

1. Food and Agriculture Organization (FAO); World Health Organization (WHO). *Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria*; Working Group Report; Food and Agriculture Organization of the United Nations and World Health Organization: Cordoba, Argentina, 2001.
2. Food and Agriculture Organization (FAO); World Health Organization (WHO). *Joint FAO/WHO Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food*; Food and Agricultural Organization of the United Nations: London, ON, Canada, 2002.
3. Moussavi, Z.E.; Mousavi, S.M.; Razavi, S.; Emam\_Djomehand, Z.; Kiani, H. Fermentation of pomegranate juice by probiotic lactic acid bacteria. *World J. Microbiol. Biotechnol.* **2011**, *27*, 123–128. [[CrossRef](#)]
4. Nosrati, R.; Hashemiravan, M.; Talebi, M. Fermentation of vegetable juice by probiotic bacteria. *Int. J. Biosci.* **2014**, *4*, 171–180.
5. Luckow, T.; Delahunty, C. Which juice is ‘healthier’? A consumer study of probiotic non-dairy juice drinks. *Food Qual. Preference* **2004**, *15*, 751–759. [[CrossRef](#)]
6. Sivudu, S.N.; Umamahesh, K.; Reddy, O.V.S. A Comparative study on Probiotication of mixed Watermelon and Tomato juice by using Probiotic strains of *Lactobacilli*. *Int. J. Curr. Microbiol. Appl. Sci.* **2014**, *3*, 977–984.
7. Shahram, S.; Mahnaz, H.; Rezvan, P.J. Production of Probiotic mixture of Barberry and Black cherry juice by lactic acid bacteria. *Bull. Environ. Pharmacol. Life Sci.* **2014**, *3*, 53–61.
8. D’Eeckenbrugge, C.; Leal, G.F. Morphology, anatomy, and taxonomy. In *The Pineapple: Botany, Production, and Uses*; Bartholomew, D.P., Paull, R.E., Rohrbach, K.G., Eds.; CABI Publishing: Wallingford, UK, 2004; p. 21.
9. Sheehan, V.M.; Ross, P.; Fitzgerald, G.F. Assessing the acid tolerance and the technological robustness of probiotic cultures for fortification in fruit juices. *Innov. Food Emerg. Technol.* **2007**, *8*, 279–284. [[CrossRef](#)]
10. Von Mollendorff, J.W. Characterization of Bacteriocins Produced by Lactic Acid Bacteria from Fermented Beverages and Optimization of Starter Cultures. Master’s Thesis, University of Stellenbosch, Stellenbosch, South Africa, 2008.
11. Pereira, A.L.F.; Maciel, T.C.; Rodrigues, S. Probiotic beverage from cashew apple juice fermented with *Lactobacillus casei*. *Food Res. Int.* **2011**, *44*, 1276–1283. [[CrossRef](#)]
12. Roble, C.; Auty, M.A.E.; Brunton, N.; Gormley, R.T.; Butler, F. Evaluation of fresh-cut apple slices enriched with probiotic bacteria. *Innov. Food Emerg. Technol.* **2010**, *11*, 203–209.
13. Helland, M.H.; Wicklund, T.; Narvhus, J.A. Growth and metabolism of selected strains of probiotic bacteria, in maize porridge with added malted barley. *J. Food Microbiol.* **2004**, *91*, 305–313. [[CrossRef](#)] [[PubMed](#)]
14. Moraru, D.; Blanca, I.; Segal, R. Probiotic vegetable juices. *Food Technol.* **2007**, *4*, 87–91.

15. Russo, P.; Valeria de Chiara, M.L.; Vernile, A.; Amodio, M.L.; Arena, M.P.; Capozzi, V.; Massa, S.; Spano, G. Fresh-cut pineapple as a new carrier to drive probiotic lactic acid bacteria. *BioMed Res. Int.* **2014**, *1*–9. [[CrossRef](#)]
16. Russo, P.; Pena, N.; Valeria de Chiara, M.L.; Amodio, M.L.; Colelli, G.; Spano, G. Probiotic lactic acid bacteria for the production of multifunctional fresh-cut cantaloupe. *Food Res. Int.* **2015**, *762*–772. [[CrossRef](#)]
17. Ashrafi, F. *Practical Microbiology*; First Published Compilation; Ahsan Press: Tehran, Iran, 2002.
18. Mokarram, R.R.; Mortazavi, S.A.; HabibiNajafi, M.B.; Shahidi, F. The influence of multi stage alginate coating on survivability of potential probiotic bacteria in simulated gastric and intestinal juice. *Food Res. Int.* **2009**, *42*, 1040–1045. [[CrossRef](#)]
19. Spicher, G.; Stephen, H. *Hanbuch Sauerteig: Biologie, Biochemie, Technologies*; Handbuch Sauerteig; B. Behrs Verlag, Hamburg, 1982.
20. Varakumar, S.; Kumar, Y.S.; Reddy, O.V.S. Carotenoid composition of mango (*Mangifera indica* L.) wine and its antioxidant activity. *J. Food Biochem.* **2011**, *35*, 1538–1547. [[CrossRef](#)]
21. Mazumdar, B.C.; Majumder, K. *Methods on Physico-Chemical Analysis of Fruits*; University College of Agriculture, Calcutta University: Kolkata, India, 2003; pp. 108–109.
22. James, C.S. *Analytical Chemistry of Food*; Seale-Hayne Faculty of Agriculture, Food and Land Use, Department of Agriculture and Food Studies, University of Plymouth: Plymouth, UK, 1995; Volume 1, pp. 96–97.
23. Duncan, P.B. New Multiple Range and Multiple F-Tests Biometrics. *Biometrics* **1955**, *11*, 1–42. [[CrossRef](#)]
24. Gaanappriya, M.; Guhankumar, P.; Kiruththica, V.; Santhiya, N.; Anita, S. Probiotication of fruit juices by *Lactobacillus acidophilus*. *Int. J. Adv. Biotechnol. Res.* **2013**, *4*, 72–77.
25. Karovičová, J.; Kohajdová, Z.; Šimko, P.; Lukáčová, D. Using of capillary isotachopheresis for determination of biogenic amines and D-isocitric acid in food products. *Nahrung* **2003**, *47*, 188–190. [[CrossRef](#)] [[PubMed](#)]
26. Viander, B.; Maki, M.; Palva, A. Impact of low salt concentration, salt quality on natural large scale sauerkraut fermentation. *Food Microbiol.* **2003**, *20*, 391–395. [[CrossRef](#)]
27. Kumar, B.V.; Manneppula, S.; Obulam, V.S.R. Physicochemical analysis of fresh and probioticated juices with *Lactobacillus casei*. *Int. J. Appl. Sci. Biotechnol.* **2013**, *1*, 127–131. [[CrossRef](#)]
28. Szeto, Y.; Tomlinson, B.; Benzie, I.F. Total antioxidant and ascorbic acid content of fresh fruits. *Br. J. Nutr.* **2002**, *87*, 55. [[CrossRef](#)] [[PubMed](#)]
29. Iqbal, K.; Khan, A.; Khattak, M.A.K. Biological significant of ascorbic acid in human. *Pak. J. Nutr.* **2004**, *3*, 5.
30. Parviainen, M.T. *Encyclopedia of Analytical Science*; Townsend, A., Ed.; Academic Press: London, UK, 1995; Volume 9.
31. Xu, D.P.; Wahburn, M.P.; Sun, G.P.; Wells, W.W. Purification and characterization of a glutathione dependent dehydroascorbate reductase from human erythrocytes. *Biochem. Biophys. Res. Commun.* **1996**, *221*, 117. [[CrossRef](#)] [[PubMed](#)]
32. Romay, A.; Armesto, J.; Remirez, D.; Gonzales, R.; Ledon, N.; Garcia, I. Antioxidant and anti-inflammatory properties of C-phycoyanin from blue-green algae. *Infamm. Res.* **1998**, *47*, 36. [[CrossRef](#)] [[PubMed](#)]
33. Ogunlesi, M.; Okiei, W.; Azeez, L.; Obakachi, V.; Osunsanmi, M.; Nkenchor, G. Vitamin C Contents of Tropical Vegetables and Foods Determined by Voltammetric and Titrimetric Methods and Their Relevance to the Medicinal Uses of the Plants. *Int. J. Electrochem. Sci.* **2010**, *5*, 105–115.
34. United State Department Of Agriculture (USDA). *Household food security in the United states*; United State Department of Agriculture: Washington, DC, USA, 2003.
35. Dosumu, O.; Oluwaniyi, O.; Awolola, G.; Okunola, M.O. Stability Studies and Mineral Concentration of Some Nigerian Packed Fruit Juices, Concentrate and Local Beverages. *Afr. J. Food Sci.* **2009**, *3*, 82–85.
36. Okwu, D.E.; Emenike, I.N. Evaluation of the Phytonutrients and Vitamin Content of Citrus Fruits. *Int. J. Mol. Med. Adv. Sci.* **2006**, *2*, 1–6.
37. Yoon, K.; Woodams, E.; Hang, Y. Probiotication of tomato juice by lactic acid bacteria. *J. Microbiol.* **2004**, *42*, 315–318. [[PubMed](#)]
38. Yanez, R.; Marques, S.; Gírio, F.M.; Roseiro, J.C. The effect of acid stress on lactate production and growth kinetics in *Lactobacillus rhamnosus* cultures. *Process Biochem.* **2008**, *43*, 356–361. [[CrossRef](#)]
39. Shah, N. Functional foods from probiotics and prebiotics. *Food Technol.* **2001**, *55*, 46–53.
40. Lourens-Hattingh, A.; Viljoen, B.C. Yoghurt as probiotic carrier food. *Int. Dairy J.* **2001**, *1*, 1–17. [[CrossRef](#)]

41. Soleimani, N.A.; Kermanshahi, R.K.; Yakhchali, B.; Sattari, T.N. Antagonistic activity of probiotic *Lactobacilli* against *Staphylococcus aureus* isolated from bovine mastitis. *Afr. J. Microbiol. Res.* **2010**, *4*, 2169–2173.
42. Ho, T.N.T.; Nguyen, N.T.; Deschamps, A.; Hadj Sassi, A.; Urdaci, M.; Caubet, R. The impact of *Lactobacillus brevis* and *Pediococcus pentosaceus* on the sensorial quality of “nem chua” a Vietnamese fermented meat product. *Int. Food Res. J.* **2009**, *16*, 71–81.



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