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Survival of putative *Lacticaseibacillus paracasei* C1I12 after supplementation of marang (*Artocarpus odoratissimus*) juice

Kriza Faye A. Calumba*, Carmina M. Demerey, Rovi Gem E. Villame, Zarryn D. Palangga and Jackie Lou J. Tagubase

Abstract

The demand for non-dairy functional beverages is increasing. Marang (*Artocarpus odoratissimus*) is an underutilized fruit in the Philippines. This study aimed to assess the survival of putative *Lacticaseibacillus paracasei* C1I12 strain previously isolated from *Nypa fruticans* after supplementation of marang juice. At the end of 30 days of storage at 4 °C, the viable cell count was significantly higher in the supplemented marang juice (8.17 log CFU/mL) compared to the raw marang juice (5.07 log CFU/mL) ($P < 0.05$). Cell counts in the juice with putative *L. paracasei* C1I12 were 7 log CFU/mL after 180 min in simulated gastric and intestinal fluids. Aerobic bacteria and coliforms were not detected in assessing the initial microbiological quality of the raw marang juice. The pH was 3.38 and 3.84 for the juice with putative *L. paracasei* C1I12 and the raw marang juice, respectively, while the total soluble solids reached 10.58°Brix and 14.00°Brix, respectively. This study shows that inoculation with the C1I12 strain ensured high cell counts in the marang juice after in vitro digestion. This is the first study demonstrating the potential of putative *L. paracasei* C1I12 in the production of a non-dairy marang beverage which can be further explored for functional food and probiotic applications.

Keywords *Artocarpus odoratissimus*, In vitro digestion, Lactic acid bacteria, *Lacticaseibacillus*

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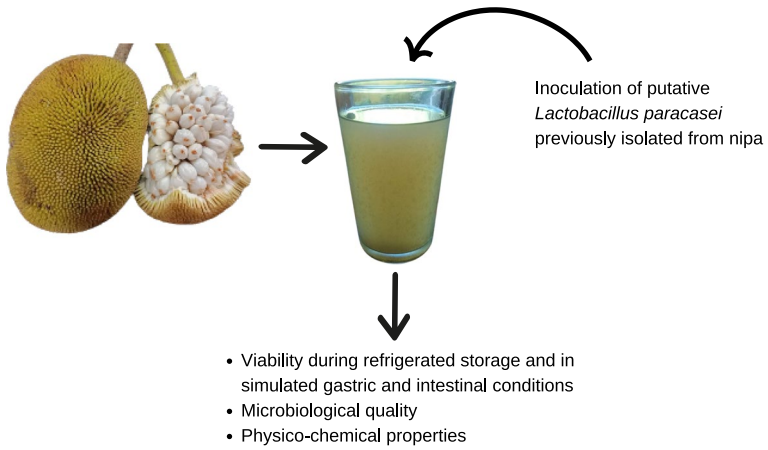
Graphical Abstract



Background

Functional foods are on the rise. Novel probiotic beverages with functional benefits, such as those from fruits, are continuously being developed (Lu et al. 2018). Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (Hill et al. 2014). These microorganisms must be available in sufficient amounts to exert probiotic activity (Ribeiro et al. 2020). As probiotics are exposed to the low pH of the stomach and bile salts, their viability in the gastrointestinal tract needs to be ensured to exert beneficial effects (Stasiak-Różańska et al. 2021). Among the common probiotic microorganisms, the most widely used are lactic acid bacteria (LAB), specifically *Lactobacillus* species, which are normally part of the human gastrointestinal tract (Gangiredla et al. 2018). *Lactobacilli* are particularly employed in probiotic products because of their innate resistance to acid, bile, and gastric enzymes (Kakelar et al. 2019). The general benefits of probiotics include their role in supporting a healthy gut microbiota, digestive tract, and immune system as justified by accumulated research evidence (Hill et al. 2014). These microorganisms can also aid in alleviating lactose intolerance and food allergies, as well as lowering blood cholesterol (Stasiak-Różańska et al. 2021). Bottari et al. (2020) and Sundararaman et al. (2020) analyzed the possible role of probiotics in modulating the gut microbiota as an approach to preventing and treating COVID-19.

While most probiotic products available in the market are dairy (Ribeiro et al. 2020), there is an increasing interest in non-dairy alternatives due to veganism, lactose intolerance, and high cholesterol in dairy products (Lu et al. 2018; Roberts et al. 2018). The emergence of vegan probiotic products and their health-promoting effects was explored by Pimentel et al. (2021). Fruit juices are increasingly studied as a carrier for probiotic microorganisms due to the nutritional benefits of fruits along with the health benefits of probiotics. Probiotication of sour cherry juice was explored (Perjéssy et al. 2021). In a recent study, passion fruit juice was fermented with probiotic *Lactobacilli* (Fonseca et al. 2022). Immobilized probiotic cells were also shown to survive in fermented apple juice as reported by Roberts et al. (2018). Another study describes the incorporation of probiotic *Lactobacillus* and *Bifidobacterium* strains in fermented pineapple juice (Nguyen et al. 2019). A probiotic beverage with banana, strawberry, and jucara was suitable for the viability of *Lactobacilli* (Ribeiro et al. 2020). Probiotics were also viable in star fruit juice (Lu et al. 2018). A fibrous superfruit abundant in Brazil, specifically Sapota-do-solimões, was used as a food matrix for the production of a probiotic and synbiotic juice (da Silva et al. 2022). Furthermore, probiotics inoculated in citrus juices manifested sufficient viable cell counts (Yuasa et al. 2021).

Moreover, LAB of plant origin have gained considerable attention due to their potential in creating sustainable food systems. LAB have been isolated from various plant materials, and these species are described to play a role in the sustainable development (Mota-Gutierrez & Cocolin 2021). LAB strains were previously isolated from *Nypa fruticans* (nipa), a mangrove found in many coastal areas in the Philippines. Studies have investigated the properties of bacterial strains from nipa, including their preservative effect on raw pork (Calumba et al. 2019), enzymatic

potential (Nkemnaso 2018), and potential on fermented coffee (Apriyani 2020). These strains isolated from nipa also show potential for probiotic food applications.

Marang (*Artocarpus odoratissimus*) is a tropical fruit grown in the Philippines with an annual average production of 12.20 thousand metric tons from 2018 to 2020. It must be noted that there were no imports and exports of marang in these years (Philippine Statistics Authority 2021). Marang pulp has a high nutritional content including macronutrients and key micronutrients such as calcium, phosphorus, iron, vitamin A, and ascorbic acid (Martinez & Perez 2016). Tang et al. (2013) previously analyzed the proximate composition of marang or 'tarap' in Brunei Darussalam, and found that the flesh contained 1.2 – 1.5 g/100 g crude protein and 0.8 – 1.3 g/100 g crude fiber, while fructose and potassium were found to be the most abundant sugar and mineral in the fruit flesh, respectively. Despite the nutritional benefits of marang, it still has low market value due to its short shelf life (Martinez & Perez 2016). This fruit can be further utilized, especially in functional food applications. Marang pulp was previously used in the development of juice, jam, jelly, ice cream, and concentrate (Sales et al. 2011). However, the suitability of this fruit as a matrix for potentially beneficial microorganisms has not yet been investigated.

The present study is the first to explore the potential of putative *Lacticaseibacillus paracasei* C1I12 previously isolated from nipa in the supplementation of marang juice. The inoculated raw marang juice was analyzed for in vitro viability during refrigerated storage and in simulated gastric and intestinal conditions. The aerobic and coliform counts, as well as pH and total soluble solids, were evaluated.

Materials and methods

Microorganism and inoculum preparation

L. paracasei C1I12 was obtained from the culture collection of the Department of Food Science and Chemistry, University of the Philippines Mindanao, Philippines. This test organism was previously isolated from the sap of *Nypa fruticans* and was found to exhibit antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* (Farnazo 2013). Results from cultural, morphological, and biochemical characterization, including API CH 50 test, indicated that the isolate is identified as *L. paracasei*. This plant-derived strain was selected for the supplementation of marang juice based on preliminary screening in vitro experiments, with results describing the tolerance of the strain to low pH and simulated gastric and intestinal fluids.

Before analysis, the culture was grown in De Man, Rogosa, and Sharpe (MRS) broth. One mL of the culture was added to 9 mL MRS broth and incubated for 24 h at 37 °C. The bacterial suspension was used as an inoculum for 150 mL MRS broth which was incubated for another 24 h at 37 °C. The cells were harvested through centrifuging for 10 min at 7,500 rpm using a refrigerated benchtop centrifuge (Senova NovaFuge B115-20R) at a temperature of 4 °C and then washed using sterile distilled water. The pellets were then suspended in 45 mL sterile distilled water, wherein 15 mL was used as an inoculum (9.60 log CFU/mL) in each juice sample.

Preparation of raw marang juice and inoculation with putative *L. paracasei* C1I12

Marang fruits (Fig. 1) were purchased from local farmers in Davao City, Philippines. To obtain the marang concentrate, the white, juicy pulp of a mature fruit was separated from its flesh and seed. A matured marang fruit as emphasized by Sales et al. (2011) is a fruit harvested after 80–90 days of fruit setting. The pulp was then

Fig. 1 Marang fruit (Davao City, Philippines)

passed through a coarse sieve and cooked in sugar on medium heat for 15 min. The production of the concentrate followed the guidelines established by the Codex Stan 247–2005 (Food & Agriculture Organization 2005) wherein to guarantee a standard quality, the °Brix level after cooking the marang in sugar should be increased at least 50% greater than the °Brix value of the corresponding marang juice. The resulting concentrate (125 mL) was mixed with 375 mL of water to produce raw marang juice. The marang juice was then cooled down to 50 °C and stored under refrigerated conditions. After which, 15 mL of the concentrated cells were inoculated in 135 mL of marang juice, giving a cell concentration of 3.34 log CFU/mL in the juice initially. The inoculated marang juice was then stored in the refrigerator at 4 °C for 30 days. Raw marang juice with no inoculated *L. paracasei* C1I12 served as the control.

Simulation of gastric and intestinal conditions

The simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) solutions were prepared according to the method of Roberts et al. (2018) with some modifications. The SGF was obtained by dissolving 0.5 g of NaCl and 1.5 g of pepsin in 1.75 mL of 12 mol/L HCl. The solution was then diluted to 250 mL with sterile distilled water and the pH was adjusted to 3.0 and was passed through a 0.22-μm filter. After which, 1 mL of the sample was added to the test tubes with 9 mL of pre-warmed SGF and incubated at 37 °C under constant agitation. At 30, 120, and 180 min, samples were collected and the viability was assessed.

The SIF was prepared by dissolving 1.7 g of KH_2PO_4 in 62.5 mL of distilled water. Then, 19.25 mL of 0.2 mol/L NaOH was added and diluted using sterile distilled water to a volume of 250 mL. The pH of the solution was adjusted to 6.5. Then, the SIF solution was passed through a 0.22 μm filter. After which, 1 mL of the sample was added to the test tubes with 9 mL pre-warmed SIF and incubated at 37 °C under constant agitation. Samples were then taken at 30-, 120-, and 180-min intervals for viability assessment.

Cell viability

Cell viability was tested immediately upon inoculation and every 5 days during storage for 30 days. Serial dilutions were done with 1 mL of sample and 9 mL 0.85 g/100 mL NaCl solution. Using the pour plate technique, 0.1 mL was inoculated in MRS agar. The plates were incubated at 37 °C for 48 h under microaerophilic conditions. Colonies with clear zones were counted and the results were expressed as log CFU/mL of marang juice.

The viability in simulated gastric and intestinal conditions was evaluated on samples taken every 15 days for 30 days of refrigerated storage. For the in vitro viability in SGF and SIF, the results were obtained following the above procedure and expressed as log CFU/mL of marang juice.

Aerobic and coliform count determination

The initial microbiological quality of the marang juice was assessed following the procedure of Roberts et al. (2018). One mL of the marang juice was serially diluted in test tubes containing 9 mL of 0.1 g/100 mL NaCl solution. Then, 1 mL of the diluted marang juice was inoculated separately onto different 3 M Petrifilms to conduct aerobic plate counts. The Petrifilms were incubated at 37 °C for 48 h. A similar procedure was followed for the coliform count. Both determinations were also obtained at the end of the storage period. The results were expressed as log CFU/mL of marang juice.

pH and total soluble solids determination

The pH and total soluble solids were evaluated every 5 days for 30 days at refrigerated storage. The total soluble solids were determined using a digital refractometer (China) and expressed in °Brix. The pH was measured with a pH meter (Eutech, UK).

Statistical analysis

Data from triplicate experiments were statistically analyzed using SPSS version 27.0. Cell viability results were presented as means of two repetitions, while pH and °Brix results were presented as means of three repetitions. An independent *t*-test was used to determine significant differences between the samples, whereas a one-way analysis of variance (ANOVA) was used to determine significant differences within each sample across storage times at 0.05 level of significance.

Results and discussion

Cell viability in marang juice during 30 days of storage at 4 °C

The viable cell counts in marang juice supplemented with putative *L. paracasei* C1I12 were generally significantly higher (Table 1). The cell viability in marang juice throughout refrigerated storage was around 8 log CFU/mL, which is higher than that required (7 log CFU/mL) for the shelf life of a probiotic product (Alcine Chan et al. 2019; Mokhtari et al. 2019; Pakbin et al. 2014). However, it must be noted that this also accounts for bacteria present in raw marang juice, and the viable count in the supplemented juice at Day 0 was one log cycle lower than the amount of C1I12 cells inoculated (9.60 log CFU/mL). This could be due to competition between

Table 1 Viable cell counts in marang juice supplemented with putative *L. paracasei* C1I12 and raw marang juice during 30 days of storage at 4 ºC

Sample	Viable cell counts during refrigerated storage for 30 days (log CFU/mL)						
	0	5	10	15	20	25	30
Marang juice supplemented with putative *L. paracasei* C1I12	8.08^{aA}	8.20^{aA}	8.21^{aA}	8.15^{aA}	7.96^{aA}	8.18^{aA}	8.17^{aA}
Raw marang juice	4.74^{bA}	5.29^{bABC}	5.50^{bBC}	5.72^{aABC}	5.75^{bB}	5.97^{bBC}	5.07^{bAC}

Values represent means of two determinations. a-b Means with different letters within a column are significantly different ($P < 0.05$). A-C Means with different letters within a row are significantly different ($P < 0.05$)

putative *L. paracasei* C1I12 and the inherent bacteria for the same resources in the matrix, as explained in other studies (Toscano et al. 2017; Zampieri et al. 2023). Nonetheless, the results suggest that the added bacteria might have utilized the sugars naturally present in marang. In a related study, probiotic viability just after fermentation and before cold storage signified metabolization of sugars by a probiotic strain (da Silva et al. 2022). Moreover, this inference can be corroborated by the generally decreasing trend in pH (Fig. 2a) and total soluble solids (Fig. 2b), suggesting fermentation, hence, the production of lactic acid from the utilization of sugars. Despite the relatively stable viability during storage (Table 1), Rokka and Rantamaki (2010) explained that probiotic bacteria may still be metabolically active even if they are non-cultivable using the plate count method. The consistently viable counts obtained were not observed in other studies wherein probiotic strains incorporated in several fruit juices had decreasing viability throughout storage (Fonseca et al. 2022; Nematollahi et al. 2016). The slight increase in viability in the raw marang juice until Day 25 can be attributed to the high protein and fiber contents of the marang which could protect cells from acidic stress brought by the low pH (Perricone et al. 2015). The

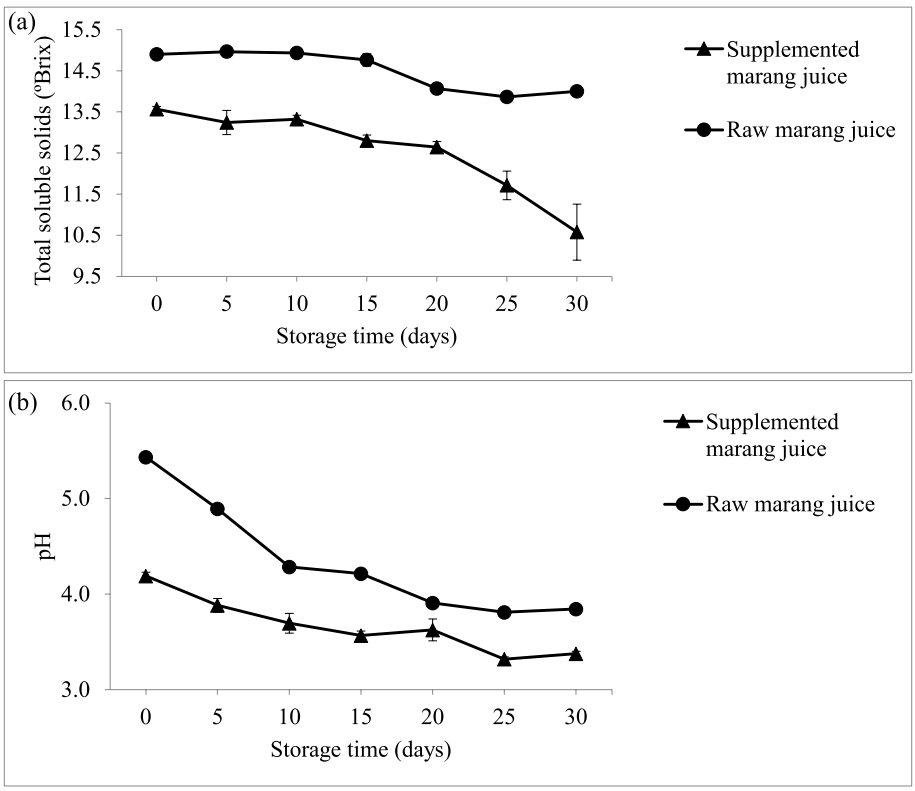


Fig. 2 pH (**a**) and total soluble solids (**b**) of supplemented and raw marang juice during 30 days of storage at 4 ºC. Values represent mean ± SD of three determinations. The vertical bars are standard errors of the mean (± SD)

results indicate that supplementation with putative *L. paracasei* C1I12 ensured high cell counts in the marang juice.

The slight increase in viability observed in some sampling times in this study was also reported by other researchers. In one study wherein fruit juice pH was adjusted to 3.0, a native strain *L. casei* T4 showed a significant increase in live cell counts during 28 days of storage at 4 °C, labeling it as a leading probiotic (Nematollahi et al. 2016). Higher viable cell counts of *L. casei* were also observed in cashew apple juice until 21 days of refrigerated storage (Pereira et al. 2011). The bacteria present in the juice inoculated with putative *L. paracasei* C1I12 maintained their survival with 8 log CFU/mL viability at the end of shelf life, which can also be explained by their high resistance to acid stress even at the end of refrigerated storage (da Silva et al. 2022). However, it must be noted that the viability varies on the strain along with the nature of the matrix (Nguyen et al. 2019). Moreover, as this is the first study to investigate the viability of LAB in raw juice, the results provide directions for future research to further assess the probiotic potential of indigenous LAB in the fruit. Nonetheless, the high protein and fiber content of marang as reported in previous studies further support the use of this fruit as a vector for beneficial bacteria.

Cell viability in marang juice during exposure to simulated gastric and intestinal fluids

Tables 2 and 3 present the cell viability in the marang juice supplemented with putative *L. paracasei* C1I12 as well as in the control under SGF and SIF, respectively. The counts in the inoculated juice were significantly higher at all sampling times. The viability of the bacteria in marang juice remained high (7.10 log CFU/mL) after 30 days of exposure to simulated gastric conditions, signifying that the cells present could survive the low pH in in vitro digestion. *Lactobacilli* must survive a pH of at least 3.0 in the stomach, and the gastric acid resistance described in the present results agrees with that obtained in another study (Calumba et al. 2021). LAB in passion fruit also kept viable numbers even at pH 2.0 in simulated gastric conditions (Fonseca et al. 2022). *Lactobacilli* can tolerate and adjust to acidic conditions in the cytoplasm (da Silva et al. 2022), and the novel strain used in this study has acid-tolerant properties.

Similar values were obtained for the marang juice supplemented with C1I12 cells in SIF conditions at the end of storage (7.31 log CFU/mL). According to Cook et al. (2012), the intestinal fluid has a pH of 6.0 to 7.0, which is milder than that of gastric fluid. The decline in viability after exposure to SIF may be due to the digestive enzymes and bile acids present (Yao et al. 2018, 2020). Nonetheless, the cell viability in marang juice remained above 7

Table 2 Viable cell counts in marang juice supplemented with putative *L. paracasei* C1I12 and raw marang juice after exposure to simulated gastric fluid (pH 3.0) during 30 days of storage at 4 °C

Sample	Viable cell counts during refrigerated storage for 30 days (log CFU/mL)								
	Day 0			Day 15			Day 30		
	0 min	120 min	180 min	0 min	120 min	180 min	0 min	120 min	180 min
Marang juice supplemented with putative *L. paracasei* C1I12	7.50^{aA}	7.16^{aA}	7.37^{aA}	7.42^{aA}	7.28^{aA}	7.31^{aA}	7.22^{aA}	7.04^{aB}	7.10^{aAB}
Raw marang juice	3.77^{bA}	3.59^{bA}	3.74^{bA}	3.87^{bA}	4.84^{bB}	3.71^{bC}	4.33^{bA}	4.27^{bA}	4.07^{bA}

Values represent means of two determinations. a-b Means with different letters within a column are significantly different ($P < 0.05$). A-C Means with different letters within a row are significantly different ($P < 0.05$)

Table 3 Viable cell counts in marang juice supplemented with putative *L. paracasei* C1I12 and raw marang juice after exposure to simulated intestinal fluid (pH 6.5) during 30 days of storage at 4 °C

Sample	Viable cell counts during refrigerated storage for 30 days (log CFU/mL)								
	Day 0			Day 15			Day 30		
	0 min	120 min	180 min	0 min	120 min	180 min	0 min	120 min	180 min
Marang juice supplemented with putative *L. paracasei* C1I12	7.34^{aA}	7.22^{aA}	7.39^{aA}	7.30^{aA}	7.20^{aA}	7.14^{aA}	7.35^{aA}	7.14^{aB}	7.31^{aAB}
Raw marang juice	3.74^{bA}	3.88^{bA}	3.93^{bA}	4.00^{bA}	5.21^{bB}	5.01^{bC}	4.44^{bA}	4.52^{bAB}	4.66^{bB}

Values represent means of two determinations. a-b Means with different letters within a column are significantly different ($P < 0.05$). A-C Means with different letters within a row are significantly different ($P < 0.05$)

Table 4 Microbiological quality in terms of aerobic count and total coliform count of raw marang juice initially and after 30 days of storage at 4 °C

Day	Aerobic count (log CFU/mL)	Coliform count (log CFU/mL)
0	ND	ND
30	4.41	ND

* *ND* Not detected

log CFU/mL during SIF exposure. Sufficient numbers of LAB were still present even after 3 h of exposure to SIF.

Aerobic and coliform counts of raw marang juice

The microbiological quality of the raw marang juice in terms of aerobic count and coliform count is presented in Table 4. Aerobic bacteria and coliforms were not detected on Day 0. According to the FDA, the aerobic plate count measures the level of aerobic microorganisms in a product (Maturin & Peeler 2001). The aerobic count is also used to indicate the effectiveness of a hazard analysis of a critical control point system (Hong et al. 2008). After 30 days of storage at 4 °C, several colonies were detected in the raw marang juice, which can be attributed to the indigenous lactic acid bacteria present in the marang. While no existing literature discusses the presence of LAB in marang, a similar study isolated putative LAB from another *Artocarpus* fruit (Panthavee et al. 2017), and this could be enumerated in the aerobic count films. On the other hand, coliforms are an indicator of fecal contamination (Feng et al. 2020). No coliform count was detected in this study, signifying the absence of fecal contamination.

pH and total soluble solids of marang juice with putative *L. paracasei* C1I12 during 30 days of storage at 4 °C

Figure 2a shows the change in pH during 30 days of refrigerated storage, where the final values were 3.38 and 3.84 for the supplemented marang juice and the control, respectively. The pH of the former was significantly lower, which can be attributed to higher viable counts (Table 1) and consequently more acid produced by the bacteria present. The decrease in pH in both juices throughout the storage period can be attributed to the constant capacity of the lactic acid bacteria for acid production during storage, which is mainly observed among *Lactobacilli* (Soares et al. 2019). The pH values of the marang juice supplemented with *L. paracasei* C1I12 significantly differed during storage, except during day 10, 15, and 20. After 20 days, the pH dropped significantly, describing the production of lactic acid as abovementioned. It was previously reported that the viability of probiotic bacteria in a given matrix can lead to the release of lactic acid (Pakbin et al. 2014). A similar decreasing trend in pH is reported in other studies during both fermentation and storage periods (Lu et al. 2018; Mousavi et al. 2011; Pakbin et al. 2014). Moreover, the low pH of the marang juice is optimal for inhibiting competing microorganisms as *Lactobacilli* can lower the pH of food substrates to 3.50 (Steinkraus 1992). The pH reduction is also important for maintaining the quality of the end product (Tayo & Akpeji 2016).

The decreasing trend in total soluble solids shows the sugar consumption by the bacteria in marang juice during storage at 4 °C (Fig. 2b). Supplementation with putative *L. paracasei* C1I12 resulted in significantly lower total soluble solids compared to the control. This trend is similarly reported in other studies (Lu et al. 2018; Pakbin et al. 2014; Tayo & Akpeji 2016). The significant drop in total soluble solids after Day 20 shows the same trend as in the pH (Fig. 2a), signifying possible exhaustion of the sugars present and leading to lactic acid production. Moreover, in terms of final °Brix, the supplemented marang juice is comparable to peach, whereas the raw marang juice has a °Brix value that is similar to that of cocoa pulp (Food & Agriculture Organization 2005).

Conclusions

This is the first study demonstrating the potential of utilizing putative *Lacticaseibacillus paracasei* C1I12 isolated from *Nypa fruticans* in the supplementation of marang (*Artocarpus odoratissimus*) juice. Raw, unpasteurized marang juice has potentially probiotic bacteria, and the supplementation with *Lactobacillus* allowed the bacteria to maintain viable counts above 8 log CFU/mL until 30 days of storage at 4 °C. Further research is needed to investigate the molecular characteristics and safety of the novel isolate, broad probiotic characterization of the strain, and consequently the organoleptic properties and consumer acceptability of the supplemented marang juice.

Abbreviations

LAB Lactic acid bacteria
SGF Simulated gastric fluid
SGF Simulated intestinal fluid

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Not applicable.

Authors' contributions
KFC, RGV, and JLT designed the study. KFC, CD, ZP, and RGV performed the experiments and obtained the data. CD and KFC analyzed and interpreted the results. KFC and CD wrote the article. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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