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Fermentation of pineapple juice with *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13: Sensory and microbiological characteristics

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Abstract: Among the varieties of pineapples, honey pineapple is suitable to be processed as a probiotic beverage. The study aimed to evaluate the honey pineapple juice as a growth medium for *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 probiotic strain. The pineapple juice was fermented by adding a starter culture of *L. plantarum* Dad-13. The fermentation time (0, 4, 8, 12, 16, 20, and 24 h) was used as a variable. The number of *L. plantarum* Dad-13 cells increased significantly after fermentation ($P < 0.05$). In acidic pineapple juice *L. plantarum* Dad-13 can still grow more than 2 log cycles. After 16 h, the results showed the best characteristics with cell count (8.86 log CFU·mL^{-1}), pH (3.52), and titratable acidity (0.59%). To balance the sour taste, the 8% sucrose addition was the most preferred by the panellists ($n = 67$), with a 5.74 (slightly like) overall acceptance score. After 42 days, there was no significant decrease in cell viability. The number of cells on day 42 was 8.81 log CFU·mL^{-1} with significant changes in pH and titratable acidity. The study showed that honey pineapple juice is a suitable growth medium for *L. plantarum* Dad-13.

Keywords: beverage; fruit juice; lactic acid bacteria; probiotic; sucrose

Today, people's awareness of healthy living is increasing. This is followed by an increase in demand for functional food products that can bring health benefits (Shah et al. 2007; Panghal et al. 2018). One of the functional foods is the probiotic product. Probiotics are live microorganisms that can help provide health benefits when consumed in sufficient amounts (FAO and WHO 2002). Thus, the minimum count of viable probiotic cells to deliver health advantages should be 10^6 CFU·mL^{-1} (Zandi et al. 2016).

Lactiplantibacillus plantarum subps. *plantarum* Dad-13, previously known as *Lactobacillus plantar-*

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um Dad-13 (*L. plantarum* Dad-13), is an indigenous probiotic strain isolated through spontaneous fermentation of buffalo milk or *dadih* from West Sumatra, Indonesia. *L. plantarum* Dad-13 has been proven as a probiotic and has gone through a long research to identify its health benefits. Several investigations have been conducted to investigate the probiotic potential of *L. plantarum* Dad-13, such as gastrointestinal tract resistance and antibacterial activity (Rahayu et al. 2015). A safety assessment for L. *plantarum* Dad-13 was also carried out using Sprague Dawley rats, which revealed no organ and blood translocation in the treated rats, indicating that it was judged safe to be consumed (Rahayu et al. 2019).

The application of probiotic products is limited to dairy products. Even though there is currently an increasing trend of vegetarianism, avoiding cholesterol, and there are still animal protein allergies, one of the non-dairy products that have the potential to become a fermentation probiotic carrier is fruit juice (Kandylis et al. 2016; Silva et al. 2020).

Pineapple (*Ananas comosus* /L./ Merr) is a widely consumed and cultivated fruit in most tropical and subtropical countries (Debnath et al*.* 2021). It is the world's third most important fruit crop, after bananas and citruses (Rohbarch et al. 2003). Pineapple is an excellent source of vitamins B1, B6, and C (Sun et al. 2016). There are numerous pineapple cultivars worldwide, but the honey variety is the most favoured due to its sweet aroma. Honey pineapple is a pineapple variety from Indonesia which is cultivated in several areas such as Belik district, Pemalang, Indonesia. Honey pineapple has benefits due to its sweet taste and pleasant aroma in contrast to general pineapples. It has a sweeter taste because honey pineapple contains more simple sugars such as glucose, fructose, and sucrose (Sun et al. 2016). In addition, pineapple also has other supporting compounds such as vitamins, minerals, antioxidants, and other nutrients that will enrich the product's nutritive value (Debnath et al. 2021). These simple sugars and other nutrients are very suitable as substrates for the growth of lactic acid bacteria during fermentation.

In addition, the inoculation of *Lactobacillus* as a starter culture for fruit juice fermentation has a positive result (Nguyen et al. 2019). Various studies have also investigated the suitability of *Lactobacillus spp.* in different tropical fruit juices, such as lemon (Hashemi et al. 2017), cashew (Pereira et al. 2011), and guava (Andrade et al. 2019). In addition, there have been studies that show *L. plantarum* Dad-13 can grow well on a non-dairy medium such as sesame milk (Ulyatu et al. 2015a).

Various studies have evaluated the effect of adding probiotic starter cultures to pineapple juice fermentation (Nguyen et al. 2019; Palachum et al. 2021). However, there has been no research on fermenting pineapple juice with a local starter Dad-13 from Indonesia and the ability of this strain to grow and survive in the natural environment of acidic pineapple juice. Thus, in order to reduce dependence on imported starters and reduce the use of non-natural ingredients, *L. plantarum* Dad-13 was used as a local starter and this study used natural pineapple juice and did not adjust the pH with chemical compounds. Therefore, a study of pineapple juice fermentation with *L. plantarum* Dad-13 was conducted to evaluate the cell growth during fermentation and to determine its ability as a probiotic fermented beverage.

MATERIAL AND METHODS

The selected pineapple, the honey variety (Pemalang, Indonesia), mineral water brand Aqua (P.T. Tirta Investama, Klaten, Indonesia), and sucrose (P.T. Sugar Group Company, Lampung, Indonesia) were purchased from the local market, Yogyakarta, Indonesia. The starter powder of *L. plantarum* Dad-13 was provided by the Food and Nutrition Culture Collection (FNCC), Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta, Indonesia. De Man, Rogosa and Sharpe agar, calcium carbonate ($CaCO_3$) (Merck, Germany), and bacteriological agar (Oxoid, USA) were used for microbial analyses.

Pineapple juice preparation. The pineapple was peeled, and the pulp was cut into small pieces and blended for 5 min, using an HR2115 Philips electric blender (China), mixed with mineral water in an equal proportion of 1 : 1. The pineapple juice was filtered using a cotton filter cloth to obtain fine pineapple juice. The pineapple juice was pasteurised at 85 °C for 15 min and cooled until the ambient temperature was 30–33 °C.

Fermentation of pineapple juice using *L. plantarum* Dad-13 starter powder. Pineapple juice (100 mL) was inoculated with (0.1% *w*/*v*) probiotic powder of *L. plantarum* Dad-13 (2.10^{10} CFU·g^{-1}) or addition of the starter up to 6.6 log CFU·mL^{-1} at the initial stage. The fermentation process was carried out in sterile bottles for 24 h at 37 °C. Viable counts, pH, and total acidity were determined every 4 h during 24 h fermentation.

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Enumeration of bacterial population. The number of viable lactic acid bacteria cells was calculated using the pour plate method. One millilitre of a sample was diluted with nine millilitres of 0.85% NaCl solution. Then, a 10^{-1} dilution was achieved. The sample was serially diluted until the desired dilution (10^{-5} to 10^{-8}) was obtained. One mL of the sample was taken from the required dilution and placed in a sterile petri dish, and then 15 mL of sterile MRS (de Man, Rogosa, Sharpe) agar was poured. MRS medium contained 0.8% $CaCO_3$ and 1.5% agar. After incubation for 48 h at 37 °C, single colonies were counted.

pH measurements. Before taking measurements, the Apera ZenTest pH60S-Z pH meter (Ohio, USA) must be calibrated with a buffer solution pH (4.0 and 7.0).

Titratable acidity. The sample was titrated with 0.1 N NaOH solution (Merck, Germany). A phenolphthalein (PP) indicator was used to identify the endpoint of the titration (Equation 1).

$$\text{Titratable acidity (\%)} = \frac{\text{volume NaOH}(\text{mL}) \times \text{N NaOH} \times \text{MW lactic acid}}{\text{volume sample}(\text{mL}) \times 1000} \times 100\% \quad (1)$$

where: MW – molecular weight.

Sensory evaluation. Sensory evaluation of probiotic fermented pineapple juice was carried out using a sensory test on colour, aroma, taste, viscosity, aftertaste, and overall acceptance. The test was performed by 67 untrained panellists. The addition of sucrose aims at a sweet taste. The formulation was carried out by adding 0, 4, and 8% sucrose into pineapple juice before fermentation. Fermentation was carried out at 37 °C for 16 h (the best fermentation time). Samples of the fermented beverages were refrigerated (4 °C) immediately after preparation and evaluated by the panellists within 48 h. The panellists received the beverage samples (± 25 mL) in plastic cups at 4 °C with 3-digit identifying codes in random order. In between samples, they rinsed their mouths with water.

Sugar analysis. Sugar contents were determined using the HPLC method described by Matsuyama et al. (1992).

Storage of probiotic fermented pineapple juice. These parameters were used to make probiotic fermented pineapple juice: initial pH of 4.10; initially viable cells of 6.62 log CFU·mL^{-1} of *L. plantarum* Dad-13 with additional sucrose 8% (the best sensory test results) and fermentation at 37 °C for 16 h (the best fermentation time result). After fermentation, cold storage was carried out at 4 °C; cell viability, pH, and titratable acidity were measured at 7-day intervals for 42 days.

Statistical analysis. Rstudio statistics software (Version 2021.09.0 Build 351 Ghost Orchid) was used for assessing the experimental data. Results are presented as mean values ± standard deviation. Data were analysed by one-way ANOVA and Duncan's test with a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Pineapple juice fermentation. Figure 1 shows the growth curve of lactic acid bacteria. During the fermentation process, there was an increase in total lactic

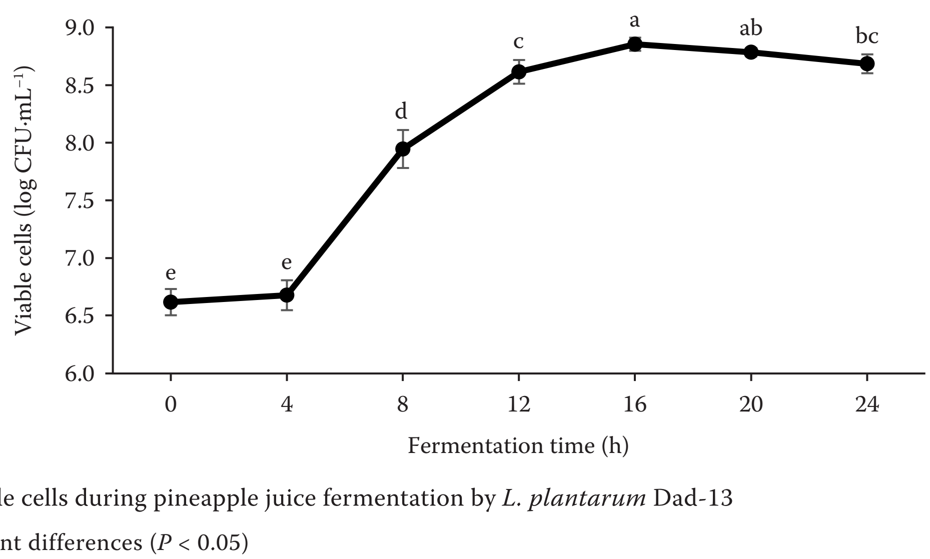

Figure 1. Viable cells during pineapple juice fermentation by *L. plantarum* Dad-13

a–e – significant differences ($P < 0.05$)

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acid bacteria with 6.62 log CFU·mL^{-1} at 0 h of fermentation to 8.69 log CFU·mL^{-1} at 24 h of fermentation. Until 24 h fermentation, the total increase in lactic acid bacteria during the fermentation process was 31.27%. However, the highest increase in total lactic acid bacteria was at 16 h of fermentation.

At the beginning of fermentation, there is a lag phase for up to 4 h. In this condition, the cells adapted to the pineapple juice environment, and the growth was only 0.02 log CFU·mL^{-1}. Then, the population increased drastically, or exponential phase, between the 4 h to the maximum amount of growth at the 16 h of fermentation (8.86 log CFU·mL^{-1}) or an increase of 2.24 log CFU·mL^{-1} (33.8%).

In this study, it was shown that under conditions of acidic pineapple juice (pH 4.11), the probiotic *L. plantarum* Dad-13 could still grow for more than 2 log cycles. During 4–12 h fermentation, exponential conditions indicate that the cells have adapted to environmental conditions and grow by consuming simple sugars. The exponential phase is characterised by a period of exponential growth − the most rapid growth possible under the conditions present in the batch system. During exponential growth, the rate of an increase of cells in the culture is proportional to the number of cells present at any particular time. In general, glucose (53.1 g·L^{-1}), fructose (52.5 g·L^{-1}), and sucrose (43.5 g·L^{-1}) are the major sugars in pineapples (Sun et al. 2016; Palachum et al. 2021). Sugar metabolism provides energy for cell growth and metabolic activity (Ulyatu et al. 2015a). This study presents that honey pineapple juice can be used as a suitable growth medium for *L. plantarum* Dad-13 without additional carbon sources, nutrients, and pH setting. This study is in line with Fitrotin et al. (2015) about fermented sesame milk using *L. plantarum* Dad-13. The study revealed that the *L. plantarum* Dad-13 could grow well on non-dairy media. The results are in line with other studies (Nguyen et al. 2019; Palachum et al. 2021), which confirmed that pineapple juice is a suitable growth medium for probiotics. The selection of the best fermentation time was based on the highest number of lactic acid viable cells, which is 16 h of fermentation.

The concentration of glucose and fructose in the fermentation of pineapple juice with *L. plantarum* Dad-13 is shown in Figure 2. There was a decrease in fructose and glucose because probiotics consumed them for growth and metabolism activity. Consumption of glucose > fructose for *L. plantarum* Dad-13 in the pineapple juice medium was observed.

These results explain that glucose in pineapple juice is sufficient for cell growth and lactid acid bacteria (LAB) metabolic activity. Glucose is metabolised by *L. plantarum* Dad-13 utilising glucose in pineapple juice brought first by the phosphotransferase system (PTS) into cells and then oxidised into energy for cell growth or metabolic activity. The concentration of sucrose was not measured because we referred to Ulyatu et al. (2015b), who explained that sucrose did not have a significant effect on the growth of *L. plantarum* Dad-13 cells.

Besides the carbon source, naturally, LAB needs nitrogen and other nutrients for growth and metabolism activity which are already present in pineapples. Leucine, valine, cysteine, or methionine are all necessary amino acids for *L. plantarum* (Teusink and

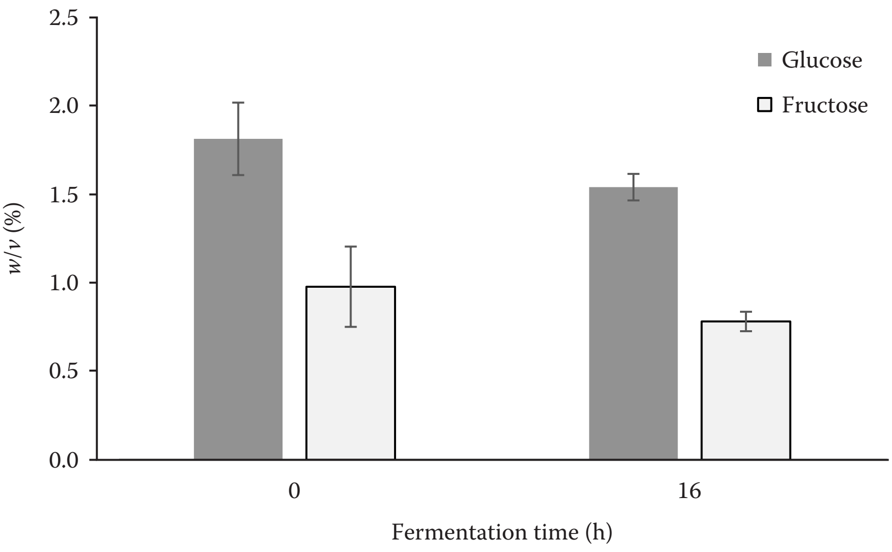

Figure 2. Concentration of glucose and fructose in pineapple juice before and after fermentation

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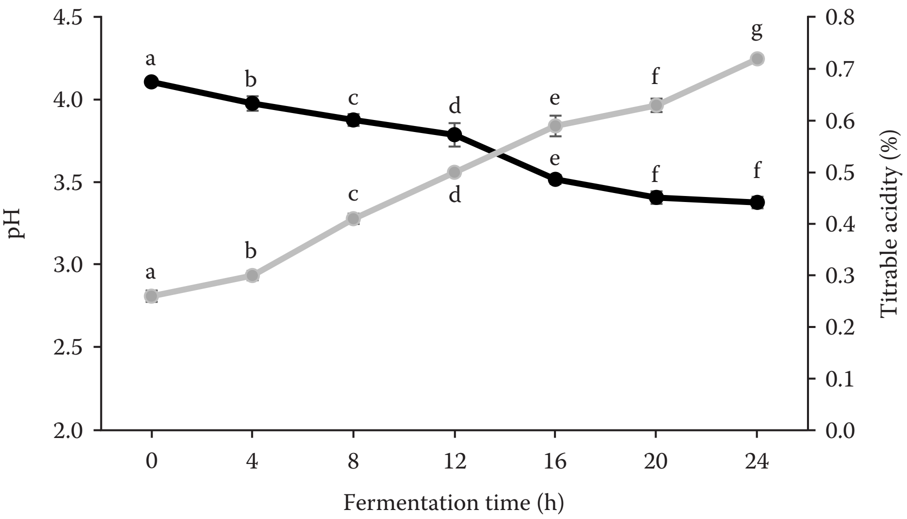

Figure 3. pH and % titratable acidity during pineapple juice fermentation by *L. plantarum* Dad-13

a–g – significant differences ($P < 0.05$)

Molenaar 2017). Furthermore, LAB requires vitamin B like thiamine and niacin (Sun et al. 2016; Palachum et al. 2021). Additionally, pineapple contains several minerals, including Fe, Zn, and Mn (Debnath et al. 2021), which LAB need for the enzymatic processes (Endo and Dicks 2014). As a result, LAB might grow well.

The LAB used the nutrients in the pineapple juice for growth and their metabolic processes to produce acids. Figure 3 shows acid production during fermentation with pH and titratable acidity. The initial pH was 4.11 and 0.26% for titratable acidity. The pH decreased, and titratable acidity significantly increased, illustrating how the acid was produced during fermentation. At 16, 20, and 24 h of incubation, the pH decreased to 3.52, 3.41, and 3.38, respectively, while the titratable acidity increased to 0.59, 0.63, and 0.72%, respectively.

The results are in line with Wardani et al. (2017), who explained that there was a significant decrease in pH and an increase in titratable acidity in milk fermented by *L. plantarum* Dad-13. Hashemi et al. (2017) and Ulyatu et al. (2015a) also explained that lactic acid bacteria consume glucose and fructose during fermentation and convert them to acids, especially lactic acid through a homofermentative pathway.

After 16 h, the fermentation enters the stationary phase, caused by the cell environment not being ideal for growth. At 20 h of fermentation, the pH was in the range of 3.36, and there was a decrease in the number of cells by 0.07 log CFU·mL^{-1} from the peak of 16 h of fermentation. This indicates that the longer the fermentation time, the more acidic the environmental condition is, so the cell resistance and population decrease. This is proved by the 24h fermentation; the number of cells decreases to 0.17 log CFU·mL^{-1} compared to the 16h fermentation. At 24h of fermentation, the sample pH was 3.38. This result is in line with Charalampopoulos et al. (2002) regarding the growth of *L. plantarum* which will stop when its pH reaches 3.40. *Lactobacillus plantarum* can maintain proton (H+) and the difference in concentrations inside and outside the cell despite large amounts of lactate and proton. This indicates that pH < 3.40 inhibited the growth of *L. plantarum* Dad-13 in pineapple juice.

Sensory test. Sixty-seven panellists scored the sensory test of the three beverages, as presented in Figure 4. The beverages were no added sucrose 0% (429), sucrose 4% (735), and sucrose 8% (276). The attributes tested were colour, aroma, viscosity, taste, aftertaste, and overall acceptance. All fermented products had a yellow, turbid appearance. They all had a slightly sweet odour and a sour, sweet taste. There was a significant difference ($P < 0.05$) in taste and overall acceptance compared to all attributes. These data indicate that the preference for probiotic pineapple juice beverages comes from the taste attribute.

The panellists evaluated sucrose addition with higher scores than non-sucrose addition. Adding 8% (*w*/*v*) sucrose was the most preferred, with an overall acceptance of 5.74 ± 0.89^{a} (slightly like). Moreover, if we compare with the overall acceptance of 0% and 4% sucrose, it is 4.2 ± 1.44^{c} (neutral) and 5.17 ± 1.27^{b} (slightly like).

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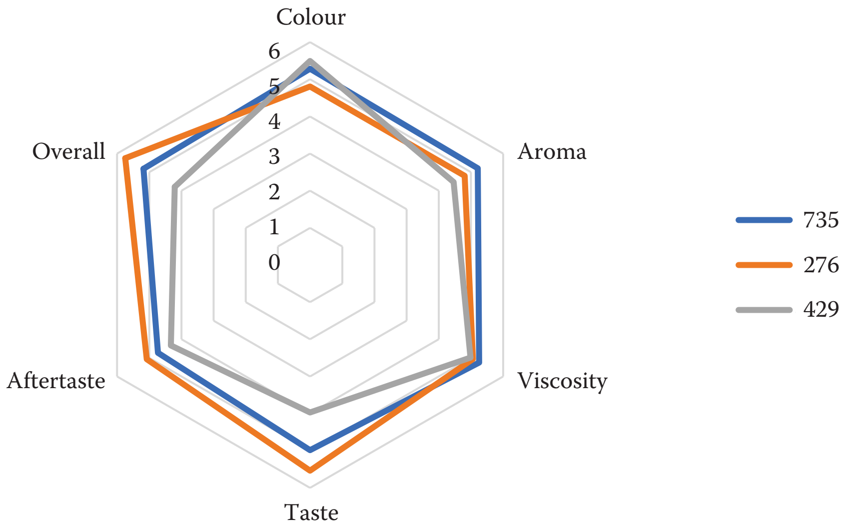

Figure 4. Sensory test of probiotic pineapple juice (n = 67)

No added sucrose 0% (429), sucrose 4% (735), sucrose 8% (276), after 16 h of fermentation, 37 °C. Data are mean values ± standard deviation (SD). Each score was generated by 67 individuals using a 7-point sensory scale ranging from 1 – dislike very much, to 7 – like very much

Figure 5 shows the sensory profile of the tested beverages: From Figure 5, adding 8% sucrose (sample 276) leads to a decrease in the sour taste and an increase in the sweet taste. This is why the panellists in this study chose or preferred the sample with the addition of 8% sucrose. These results are in line with Andradea et al. (2019), who suggested that sweeter products with less sour taste are preferred.

Storage of probiotic pineapple juice. Figure 6 shows that there was still the metabolic activity of LAB during storage, with a significant decrease (P < 0.05) in pH and an increase in titratable acidity. On day 42, the pH of the probiotic pineapple decreased significantly from 3.52 ± 0.02^{a} on day 0 to 3.21 ± 0.01^{g}. And the titratable acidity increased significantly from 0.58% ± 0.01^{a} on day 0 to 0.93% ± 0.01^{e} on day 42. The results are in line with those of Espinoza and Navarro (2010) and Palachum et al. (2021), who also showed that there was a decrease in pH and production of lactic acid, making the medium more acidic, and they are responsible for the viability losses at the end of the storage period. Meanwhile, research (Ding

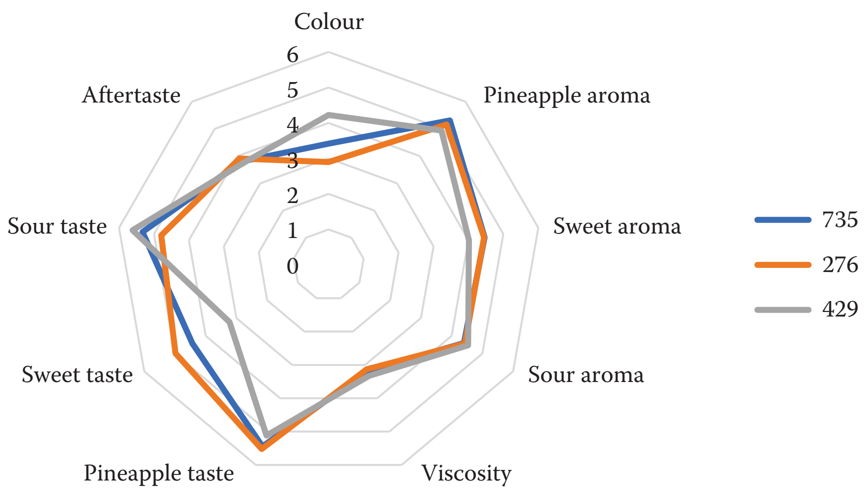

Figure 5. Probiotic pineapple juice sensory profile

No added sucrose 0% (429), sucrose 4% (735), sucrose 8% (276), after 16 h fermentation, 37 °C. Data are mean values ± standard deviation (SD) (n = 67)

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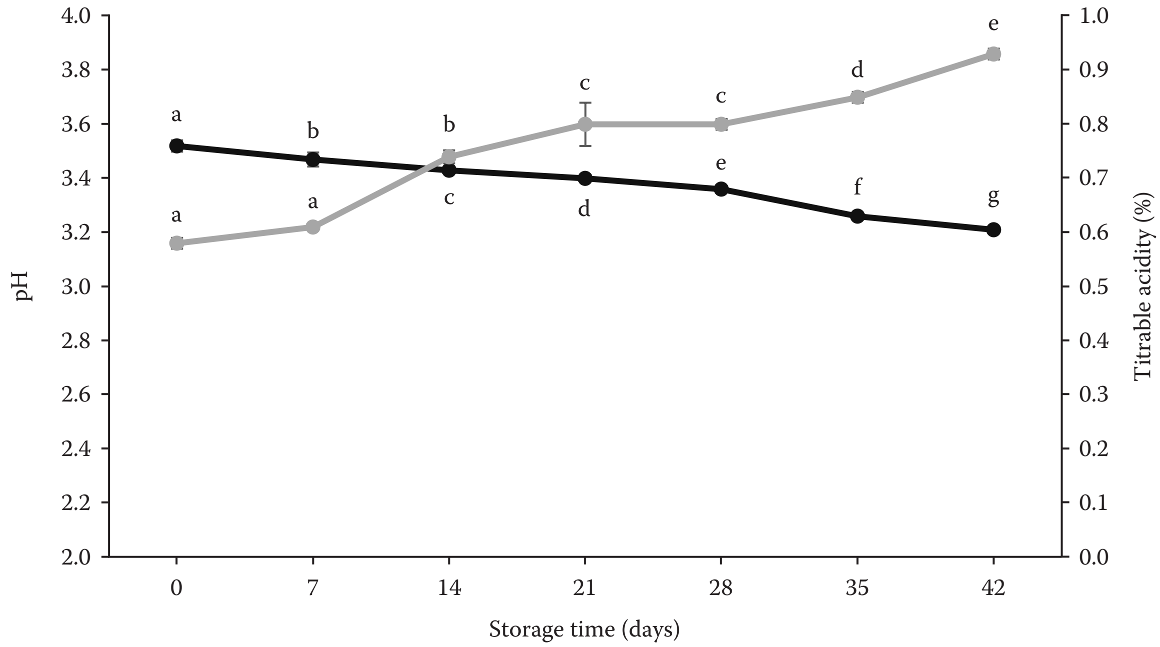

Figure 6. pH and titratable acidity (%) during storage at 4 °C, 42 days

a–g – significant differences ($P < 0.05$)

and Shah 2008) revealed that dead cells would release enzymes to hydrolyse sugar, thus decreasing the pH. This indicates that the longer the storage time, the more increased the acidity level.

Figure 7 shows the viable cells during storage. After 42 days, the probiotic pineapple juice was kept in storage. There was no significant decrease in the number of cells from 8.85 ± 0.06[a] log CFU·mL^{-1} at the initial time of storage to 8.81 ± 0.04[a] log CFU·mL^{-1} on day 42.

However, the viable cell counts were higher than 8 log CFU·mL^{-1}, which can be a good source for fermented goods containing probiotics. Our research explains that *L. plantarum* Dad-13 can survive in the acidic environment of pineapple juice. In addition, *L. plantarum* Dad-13 can maintain a minimum cell count > 10^6 CFU·mL^{-1} (Zandi et al. 2016) when consumed on the 42nd day to provide health benefits. Sensory testing during the storage of pineapple juice fermented products with the addition of 8% sucrose

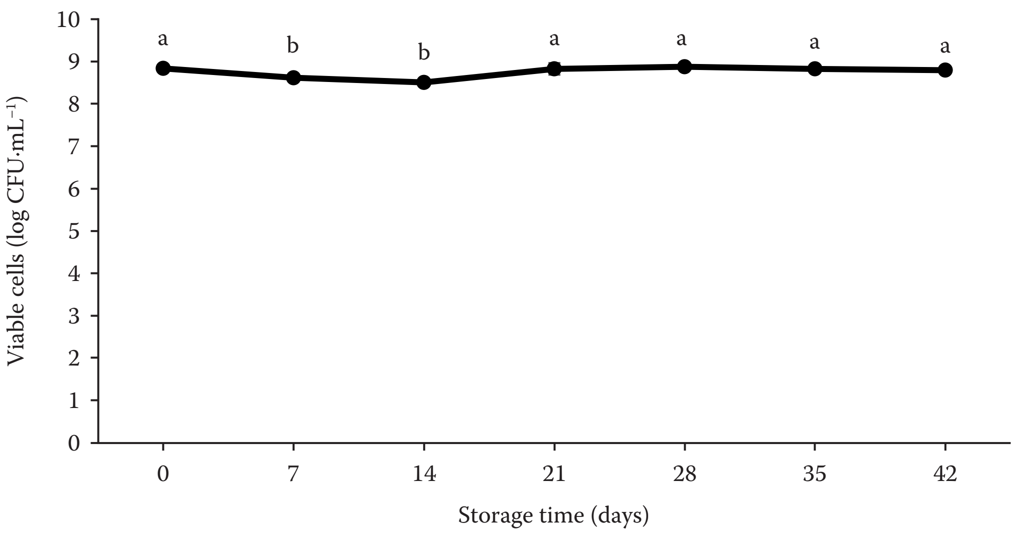

Figure 7. Viable cells during storage at 4 °C, 42 days

a, b – significant differences ($P < 0.05$), but the number of cells remained in the same log until the 42nd day of observation

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was carried out by the researchers themselves, showing an increase in sour taste with increasing storage time but still suitable for consumption in sensory terms until the 42nd day [see Electronic Supplementary Material (ESM), Table S1]. Our research proves that fermented honey pineapple juice is a suitable carrier for *L. plantarum* Dad-13, so it has the potential as an alternative functional food product.

CONCLUSION

This study shows the use of pineapple juice as a potential probiotic substrate because *L. plantarum* Dad-13 cells can grow up to 8.86 log CFU·mL^{-1} after 16 h of fermentation in environmental conditions of acidic pineapple juice. In the sensory test, adding 8% sucrose was the most preferred, with an overall acceptance of 5.74 (slightly like). The sensory attribute that plays a significant role was the taste attribute. Adding 8% sucrose can reduce the sour taste and improve the sweet taste. Subsequently, the probiotic juice was stable for up to 42 days of refrigerated storage period without significant loss of viability (8.81 log CFU·mL^{-1}), even though there were a decrease in pH and an increase in titratable acidity. Therefore, pineapple juice fermented with *L. plantarum* Dad-13 is proven as a suitable carrier for probiotic products. Further studies are necessary also to investigate sucrose concentration, sensorial analysis after storage, and changes in the aroma compounds of fermented honey pineapple.

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