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# Evaluation of *jamu kunyit asam* (*Curcuma domestica* Val. - *Tamarindus indica* L.) as probiotic carrier of *Lactobacillus plantarum* BP102

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**Abstract**

*Jamu kunyit asam* is a herbal health product with the main ingredients of turmeric (*Curcuma domestica*) and tamarind (*Tamarindus indica*). The addition of probiotics in *jamu kunyit asam* will contribute to better-quality fermented drink. The purposes of the present work were (1) to determine the viability and probiotic potential of *Lactobacillus plantarum* BP102 in *jamu kunyit asam*, (2) to determine the effect of the addition of *L. plantarum* BP102 on the antioxidant activity of *jamu kunyit asam*, and (3) to determine the organoleptic changes of fermented *jamu kunyit asam*. The probiotic strain *L. plantarum* BP102 was able to grow in *jamu kunyit asam* with a density of $10^7$ - $10^8$ CFU/mL, followed by a decrease in pH value after 5-d storage. The *L. plantarum* BP102 in *jamu kunyit asam* also showed its character as a probiotic, namely, survival rates at low pH and in the presence of bile salts of 63 - 70 and 73 - 83%. The antioxidant activity of fermented *jamu kunyit asam* did not show an increase. The addition of *L. plantarum* BP102 was able to improve the organoleptic quality of fermented *jamu kunyit asam*, especially in terms of flavour and colour. Therefore, *jamu kunyit asam* can act as a probiotic carrier of *L. plantarum* BP102 for the development of fermentation-based functional food products.

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## Introduction

Probiotics are the most widely used component in functional foods, and defined by the World Health Organization (WHO) as "living microorganisms which, exert various health benefits on the host, when administrated in adequate amounts". Probiotic microorganisms are generally members of the lactic acid bacteria (LAB) group (Yadav *et al*., 2016). Several species belonging to LAB include *Lactobacillus plantarum*, *Enterococcus faecalis*, *Enterococcus faecium*, *Lactococcus lactis*, *Leuconostoc mesenteroides*, and *Pediococcus acidilactici* (Elizaquivel *et al*., 2011).

Nowadays, with increased awareness of health, probiotic products have received a lot of attention. Fermented products are widely consumed all over the world. One fermented beverage that is well known is yogurt, which is milk fermented by LAB into other compounds to improve the quality of the beverage, thus benefiting human health (Taye *et al*., 2021). Fermentation by LAB is known to increase the characteristics of nutrients, sensory properties, and bioactive contents of various plant extracts (Feng *et al*., 2017). One of the LAB species that is often used for fermented plant extracts is *L. plantarum.*

*Jamu kunyit asam* is a herbal health product with the main ingredients of turmeric (*Curcuma domestica*) rhizome and tamarind (*Tamarindus indica*) pulp. Turmeric rhizome has been proven to be beneficial towards health due to the content of curcumin (60 - 70%) which has several biological activities such as antibacterial, antioxidant, and antihepatotoxic (Santoso, 2008). Meanwhile, tamarind pulp contains an abundance of ascorbic acid (201.7 mg/100 g) (Sadiq *et al*., 2016). The addition of probiotics in *jamu kunyit asam* is expected to provide added value as a fermented beverage with better quality.

*Lactobacillus plantarum* BP102 used in the present work was isolated from garlic bulb tissue, and related to *L. plantarum* JCM $1149^T$ with a similarity value of 99.99% (Wardhani, 2019). Endophytic probiotics have higher survival in fermented plant

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extracts because they come from plant tissues. The content of antioxidant activity by curcumin in *jamu kunyit asam* has the potential to be increased through fermentation. In addition, the presence of probiotics is expected to have a positive impact on human digestive health when adequate amounts are given (Jung *et al*., 2019).

Many studies have been carried out on fermented plant extracts using probiotics. However, studies on fermented *jamu kunyit asam* products using *L. plantarum* have never been done. Therefore, it was deemed important to conduct a study to evaluate *jamu kunyit asam* as a probiotic carrier of *L. plantarum* BP102.

## Materials and methods

*Bacterial strains and growth conditions*

The LAB strain used in this study was *L. plantarum* BP102 which was obtained from the collection of the Microbiology Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Malang, Indonesia. The culture was prepared in sterile de Man Rogosa and Sharpe agar (Merck, German), and incubated at 37°C for 24 - 48 h.

*Growth curve of Lactobacillus plantarum BP102*

Inoculum (10 mL) of *L. plantarum* BP102 was inoculated into 100 mL of sterile MRS broth medium, and incubated at 37°C for 24 h (pre-culture). Then, 10% of the pre-culture was inoculated in 250 mL of MRS broth (Merck, Germany), and incubated at 37°C for 48 h. The culture was taken every 2 h for a period of 12 h; and every 4 h afterwards until a period of 24 h was achieved. The optical density (OD) was measured using a spectrophotometer at 600 nm wavelengths, and the cell density was calculated. Cell density (log cells/mL) can be determined by converting OD values with regression equations that have been generated from the standard curve.

*Starter culture preparation*

Inoculum (10 mL) of *L. plantarum* BP102 was inoculated into 100 mL of MRS broth, and incubated at 37°C for 24 h (end of log phase). The cell number was determined to be $10^8$ CFU/mL. The 10 mL of starter culture with a density of $10^8$ CFU/mL was added in two sterile propylene tubes, then centrifuged at 5,000 rpm for 7 min to obtain pellets (Elizaquivel *et al*., 2011).

*Preparation of fermented jamu kunyit asam*

*Jamu kunyit asam,* with a composition as shown in Table 1, was pasteurised at 65°C for 30 min. In a sterile container, 100 mL of pasteurised *jamu kunyit asam* was added and cooled to 35 - 40°C. The pellets in two propylene tubes were inoculated in 100 mL of *jamu kunyit asam*. *Jamu kunyit asam* without the *L. plantarum* BP102 inoculum was used as a control. Inoculated *jamu kunyit asam* and negative control were stored in the dark, under aerobic conditions, at room temperature, for 5 d. The potential of *L. plantarum* BP102 as a probiotic was evaluated during the storage periods (days 1, 3, and 5). The parameters observed included pH value, LAB cell density, resistance to low pH, resistance to bile salts, antioxidant activity, and organoleptic properties (scent, colour, flavour, and mouthfeel) (Elizaquivel *et al*., 2011).

**Table 1.** Composition of *jamu kunyit asam* (in 1 L).

| Ingredient | Mass (g) |
|---|---|
| Turmeric | 57 |
| Tamarind | 17 |
| Brown sugar | 51.4 |
| Sugar | 30 |

*Viability of the LAB in jamu kunyit asam during storage*

The LAB cell density was determined by serial dilution on fermented or control *jamu kunyit asam* during storage periods (days 1, 3, and 5) at room temperature. A total of 0.1 mL of *jamu kunyit asam* was suspended in 0.9 mL of sterile saline solution (0.85% NaCl). Appropriate serial dilutions were prepared before pour-plating onto MRS agar (with added 1% $CaCO_3$) and incubated at 37°C for 24 - 72 h (Elizaquivel *et al*., 2011).

Measurements of pH were carried out to observe the level of acidification during storage on days 1, 3, and 5. The percentage of acidification was determined using Eq. 1 (Mulaw *et al*., 2019):

$$\text{Acidification level (\%)} = (pH_{initial} - pH_{final}) / pH_{initial} \times 100 \quad \text{(Eq. 1)}$$

*Resistance of LAB in fermented jamu kunyit asam to acidic conditions*

The LAB contained in *jamu kunyit asam* were tested for their resistance to low pH during storage on days 1, 3, and 5 at room temperature. Then, 2 mL of

fermented *jamu kunyit asam* was added to PBS (phosphate buffer saline) solution with the pH value being adjusted to 2.0 and 3.0 by adding 5 M HCl (Merck, German). The mixture was incubated at 37°C for 2 h. Further, the LAB cell number (CFU/mL) was determined using total plate count (TPC) at 0 and 2 h of incubation, and the survival rate was determined using Eq. 2:

$$\text{Survival rate (\%)} = (\text{Log CFU } N_1 / \text{Log CFU } N_0) \times 100 \quad \text{(Eq. 2)}$$

where, $N_1$ and $N_0$ = total numbers of bacteria, after and before treatment, respectively.

*Resistance of LAB in fermented jamu kunyit asam to bile salts*

The test of the resistance of the LAB in *jamu kunyit asam* to bile salts was carried out during storage on days 1, 3, and 5 at room temperature. Briefly, 2 mL of fermented *jamu kunyit asam* was added to a 20 mL PBS solution containing bile salts (oxgall) with concentrations of 1 and 2%. The mixture was incubated at 37°C for 5 h. Furthermore, the LAB cell number (CFU/mL) of each aliquot was calculated using TPC at 0 and 5 h of incubation, and the survival rate was also determined using Eq. 2.

*Antioxidant activity test with 1,1-diphenyl-2-picrylhydrazyl (DPPH)*

The determination of antioxidant activity was carried out on fermented *jamu kunyit asam*, non-fermented *jamu kunyit asam*, *L. plantarum* BP102 culture, MRS broth medium, and ascorbic acid, which were incubated at room temperature during storage (days 1, 3, and 5). The stock solution of the radical was prepared by dissolving 12 mg of DPPH (Sigma Aldrich) in 50 mL of methanol (99.9%). The working solution of the radical was prepared by diluting the stock solution of DPPH with methanol to obtain an absorbance of about 0.98 (± 0.02) at 517 nm. Insoluble debris from the samples was removed by centrifugation at 10,000 *g* for 5 min at 25°C. In a test tube, 4 mL of supernatant was mixed with 400 µL of DPPH working solution. The absorbance was measured at 517 nm wavelengths for a period of 30 min in the dark. The radical scavenging activity or the percent antioxidant was determined using Eq. 3. The control used was 4 mL of methanol (Ahmed *et al*., 2015).

$$\text{Antioxidant activity (\%)} = (A_{control} - A_{sample}) / A_{control} \times 100 \quad \text{(Eq. 3)}$$

*Organoleptic test of jamu kunyit asam during storage*

The organoleptic test used a hedonic test to evaluate differences among the six types of samples. The samples to be tested were fermented and non-fermented *jamu kunyit asam* which were stored for 1, 3, and 5 d. Ten panellists were selected within an age range of 18 - 22 years old (Mehran, 2015). Each 10 mL sample was distributed into disposable cups that had been coded. Six cups were given to each panellist to be tested. The indicators evaluated through the hedonic test included the scent, colour, flavour, and mouthfeel with a scale value of 1 - 5 (1: dislike very much; 2: dislike; 3: moderate; 4: like; and 5: like very much). The panellists were given water before moving on to the next sample to reduce the effect of one sample on another (Figueiredo *et al*., 2019).

*Statistical analysis*

All the experiments were conducted in triplicates. All data tests were analysed using two-way analysis of variance (ANOVA) with IBM SPSS statistics software version 20 (IBM Corp, USA). $p < 0.05$ was considered significant.

## Results and discussion

*Viability of LAB and total bacteria in jamu kunyit asam during storage*

*Jamu kunyit asam* was inoculated with the probiotic *L. plantarum* BP102 with an initial cell density of $10^8$ CFU/mL. *Lactobacillus plantarum* BP102 showed the ability to utilise *jamu kunyit asam* for cell synthesis although it required adaptation to a new substrate. Total LAB on MRS agar showed an increase in cell density after 3-d storage, from 7.9 to 8.21 log CFU/mL (Figure 1), which was in accordance with the minimum of probiotic cell number in food that is $10^6$ - $10^9$ CFU/mL (Speranza *et al*., 2018). A decrease in LAB cell density occurred after 5-d storage to 8.07 log CFU/mL (Figure 1). However, this cell density was still within the standard for probiotics in food, which means that a storage duration of up to 5 d is still acceptable (time to maintain the viability of the probiotics to an acceptable concentration) (Speranza *et al*., 2018). The non-fermented *jamu kunyit asam* did not show LAB colony growth during storage. This was because the

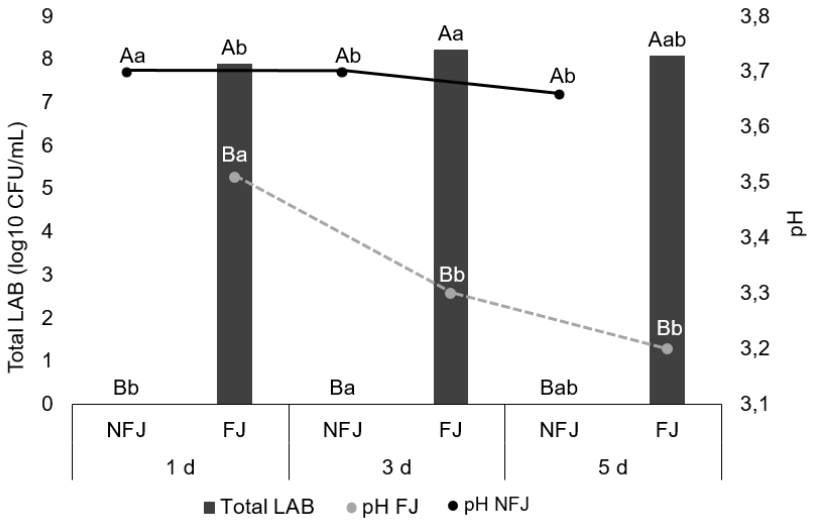


**Figure 1.** pH values of total BAL in *jamu kunyit asam* during storage. The initial LAB density as starter was $10^8$ CFU/mL. NFJ: non-fermented *jamu*; and FJ: fermented *jamu*.

non-fermented *jamu kunyit asam* was not inoculated with starter *L. plantarum* BP102. In addition, the *jamu kunyit asam* was sterile because it went through a pasteurisation stage.

*Lactobacillus plantarum* BP102 was able to grow well until day 3 of storage which indicated that *jamu kunyit asam* can be a substrate for the growth of probiotic bacteria. Since the strain originated from plant tissues (endophytes), *L. plantarum* BP102 was able to grow and adapt well to the plant extract beverage. Therefore, *jamu kunyit asam* could be a suitable carrier of the probiotic *L. plantarum* BP102. *Jamu kunyit asam* contains several types of sugars, such as glucose and sucrose, which can be carbon sources for *L. plantarum* BP102 (Nguyen *et al*., 2019).

The occurrence of fermentation was also confirmed by the decrease in pH, which allowed for a hypothesised fermentation action in *jamu kunyit asam*. The pH of the fermented *jamu kunyit asam* decreased gradually during storage, while the pH of the non-fermented *jamu kunyit asam* remained constant (Figure 1). The non-fermented *jamu kunyit asam* did not show a significant change in pH during storage, except on day 5, which was 0.04. This decrease might also be due to the breakdown of citric and ascorbic acids in the sugar components (Hidayat *et al*., 2021). The initial pH value of *jamu kunyit asam* in the present work (pH 3.7) was not much different from the pH value of commercial *jamu kunyit asam* sold by traders, which is around pH 3.6. The most visible change in acidification rate was found in the fermented *jamu kunyit asam* ($p < 0.05$). The fermented *jamu kunyit asam* had a higher acidification rate than the non-fermented *jamu kunyit asam*. The storage time affected the increase in acidification of the fermented *jamu kunyit asam* because the acidification rate increased along with the storage period (Figure 2).

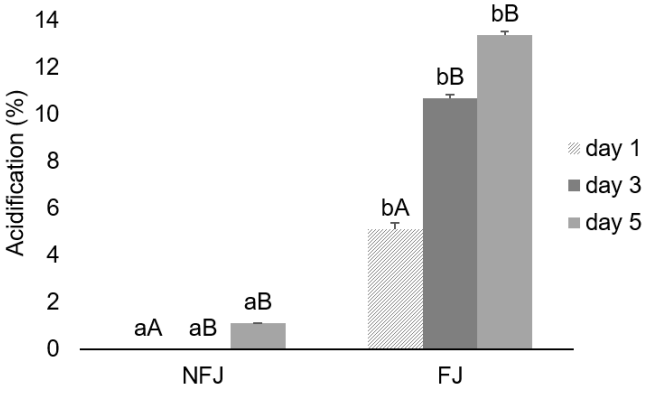


**Figure 2.** Acidification activity of beverages during storage. NFJ: non-fermented *jamu*; and FJ: fermented *jamu*. Different lowercase letters indicate significant difference between samples, and different uppercase letters indicate significant difference between storage durations.

The increase in acidity of the fermented *jamu kunyit asam* was due to the accumulation of lactic acid produced by *L. plantarum* during fermentation. Lactic acid bacteria metabolise monosaccharides into glucose-6-phosphate or fructose-6-phosphate, which then goes through the Embden Meyerhoff Parnas (EMP) pathway, and produces the final product of lactic acid (Zubaidah *et al*., 2012; Ayed *et al*., 2020).

*Tolerance of lactic acid bacteria in jamu kunyit asam to acidic conditions*

Besides the carrier food during storage, probiotics must also survive in acid gastric conditions. Therefore, *L. plantarum* BP102 as a probiotic in *jamu kunyit asam* was subjected to stress conditions simulating the human gut. The *L. plantarum* BP102 in fermented *jamu kunyit asam* showed a survival rate ranging from 63 to 70% at pH 3, with the highest percentage being on days 3 and 5, which was 70%. *L. plantarum* BP102 was not able to grow at pH 2 (Figure 3). Therefore, the *L. plantarum* BP102 in the fermented *jamu kunyit asam* showed better survival at pH 3 than 2. Non-fermented *jamu kunyit asam* did not show cell growth during storage. The acidity level of the medium affected the survival rate of *L. plantarum* BP102 ($p < 0.05$). The duration of beverage storage did not affect the survival rate of *L. plantarum* BP102 (Figure 3). According to Mulaw *et al*. (2019), *L. plantarum* OL12, OL9, and OL33 isolated from fermented olives showed survival rates of 55, 49, and 57%, respectively, after incubation in pH 2 medium for 2 h. A study by Nigatu *et al*. (2015) showed low to high survival rates (1.03 - 100%) of six *Lactobacillus* species at pH 2 and 3 for 3 h of incubation.

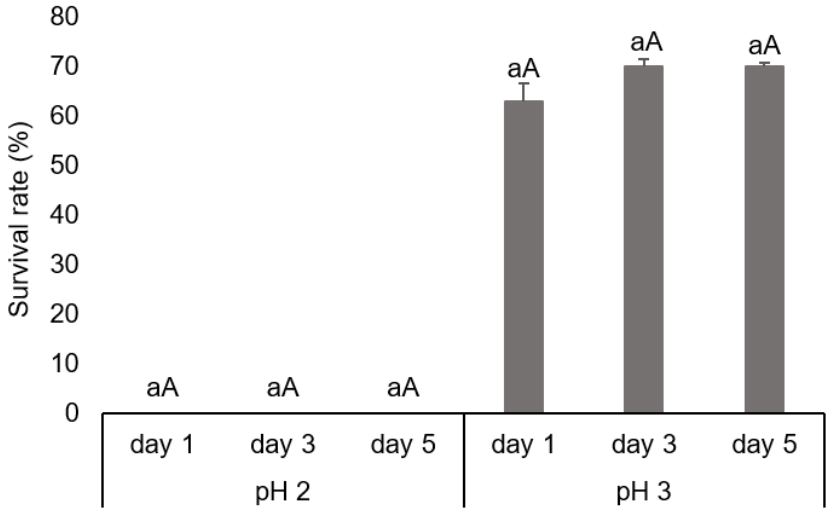


**Figure 3.** Survival rate of LAB in fermented *jamu kunyit asam* under low pH conditions. Different lowercase letters indicate significant difference between storage durations, and different uppercase letters indicate significant difference between medium acidity.

Probiotic bacteria are tolerant to acid stress conditions, which enables them to survive in the gastric area with acidic conditions of pH 1.5 - 3. According to Harnentis *et al*. (2020), good probiotic candidates are isolates that have survival rates of more than 50% under low pH conditions. In the present work, at 2 h of incubation, *L. plantarum* BP102 could survive at pH 3 with a resistance rate of at least 60%, which indicated that *jamu kunyit asam* could have the potential to be a probiotic carrier of *L. plantarum* BP102. The LAB did not show survival at pH 2. The optimum acidity level for LAB growth is 5.5 - 5.8. In the present work, *L. plantarum* BP102 was not able to survive at pH 2, which might be due to the pH of the previous growth medium. The *jamu kunyit asam* had a low pH value for optimum LAB growth, which was 3.51. Therefore, after being incubated at pH 2, the LAB survival was lower than it was at pH 3 (Ding *et al*., 2017).

The tolerance of probiotic bacteria to acids is influenced by the ability of bacteria to maintain cytoplasmic pH that is more alkaline than the extracellular pH. However, the decrease in intracellular pH continues as the extracellular pH decreases (Apridiani *et al*., 2014).

*Tolerance of lactic acid bacteria in jamu kunyit asam to bile salts*

The survival of LAB in the presence of bile salts is an important criterion for LAB as candidate for probiotics because it affects the functional activity in the digestive tract. Therefore, *L. plantarum* BP102 as a probiotic in *jamu kunyit asam* was subjected to stress conditions simulating the human gut. *Lactobacillus plantarum* BP102 in fermented *jamu kunyit asam* showed survival against 1 and 2% bile salts for 5 h with resistance rates of 74 - 83 and 73 - 83%, respectively. The highest survival rate in 1 and

2% bile salts demonstrated by *L. plantarum* BP102 was 83% as observed on day 1 (Figure 4). Although bile salts in the medium inhibited the growth of *L. plantarum* BP102, the number of surviving cells in this condition was higher than the number of surviving cells under low pH conditions. Non-fermented *jamu kunyit asam* did not show cell growth during storage. The acidity of the medium and the storage periods did not affect the survival rate of *L. plantarum* BP102 in the presence of bile salts (Figure 4).

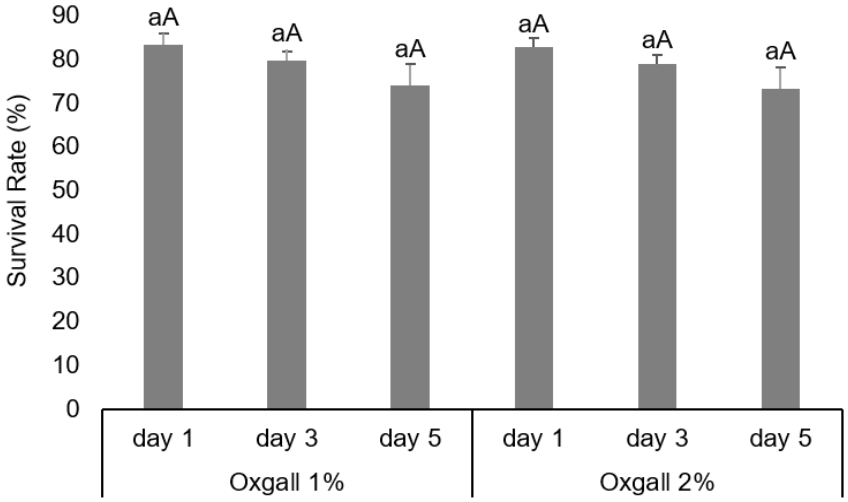


**Figure 4.** Survival rate of LAB in fermented *jamu kunyit asam* against bile salt conditions. Different lowercase letters indicate significant difference between storage durations, and different uppercase letters indicate significant difference between oxgall concentrations.

According to Lapsiri *et al*. (2011), *L. plantarum* OL9 and OL36 isolated from fermented olives showed high tolerance rates (65 and 59%, respectively) to 0.45% bile salts. In addition, Unban *et al*. (2021) also showed that *L. plantarum* A9-2 isolated from fermented tea leaves had a high tolerance rate to 0.3% bile salts, which was 91.8%.

Probiotic bacteria have tolerance to bile salts, which supports them to survive for 1 - 2 h in the upper intestinal tract with bile salts percentage ranging from 0.3 - 2%. According to Harnentis *et al*. (2020), good probiotic candidates are isolates that have survival rates of more than 50% against bile salts. In the present work, at 5 h of incubation, *L. plantarum* BP102 could survive against 1 - 2% bile salts with a resistance rate of at least 70%, which indicated that *jamu kunyit asam* could have the potential to be a probiotic carrier of *L. plantarum* BP102.

Bile salts are fluids in the digestive system that can dissolve lipids, and affect the structure of cell membranes. Therefore, bile salts tolerance is an important ability for microbial survival and colonisation in the digestive tract. LAB can survive in bile salt conditions because of the activity of the enzyme bile salt hydrolase (BSH) which is able to decompose conjugated bile acids into free bile acids, and release the amino acids glycine or taurine. Free bile acids can play a role in various metabolic processes, such as cholesterol metabolism and regulation of fat absorption, thus creating homeostatic conditions in bacterial membranes (Harnentis *et al*., 2020). *Lactobacillus plantarum* WCFS1 was thought to contain four genes for BSH, including *bsh1* to *bsh4*, which are distributed throughout the genome. The *bsh1* gene is fully responsible for the bile acid metabolism of *L. plantarum* strains (Prete *et al*., 2020).

*Antioxidant activity in jamu kunyit asam during storage*

The antioxidant or free radical scavenging activities of fermented and non-fermented *jamu kunyit asam* were determined using DPPH radical scavenging assay. Results showed that each sample had different DPPH radical scavenging activities ($p < 0.05$). However, the duration of storage did not significantly affect the antioxidant activity. The non-fermented *jamu kunyit asam* showed higher antioxidant activity (53.9 - 61.2%) as compared to the fermented *jamu kunyit asam* (43.6 - 48.4%) during 5-d storage (Figure 5). Different from most recent reports related to the study of fermentation of plant

extracts by LAB which resulted in an increase in antioxidant activity, fermentation with *L. plantarum* BP102 caused a decrease in antioxidant activity of *jamu kunyit asam*. The non-fermented *jamu kunyit asam* on day 3 of storage had the highest antioxidant activity, which was 61.2%. This differed from those presented by Li *et al*. (2011) who showed that fermentation by *L. plantarum* ATCC14917 in apple juice for 24 h significantly increased DPPH radical scavenging activity by 23%. However, according to Nazzaro *et al*. (2008), decreased DPPH radical scavenging also occurred in carrot juice fermentation by *L. rhamnosus.* Fermentation of carrot juice by probiotics showed increased DPPH radical scavenging activity when the juice was enriched with prebiotic components such as fructooligosaccharides (FOS). The same thing was observed by Hunaefi *et al*. (2012) who reported a drastic decrease in antioxidant activity during the 24, 48, and 72 h of fermentation of *Orthosiphon aristatus* extract. Fermentation by *L. plantarum* caused a greater loss of antioxidant activity than *L. acidophilus* (Hunaefi *et al*., 2012).

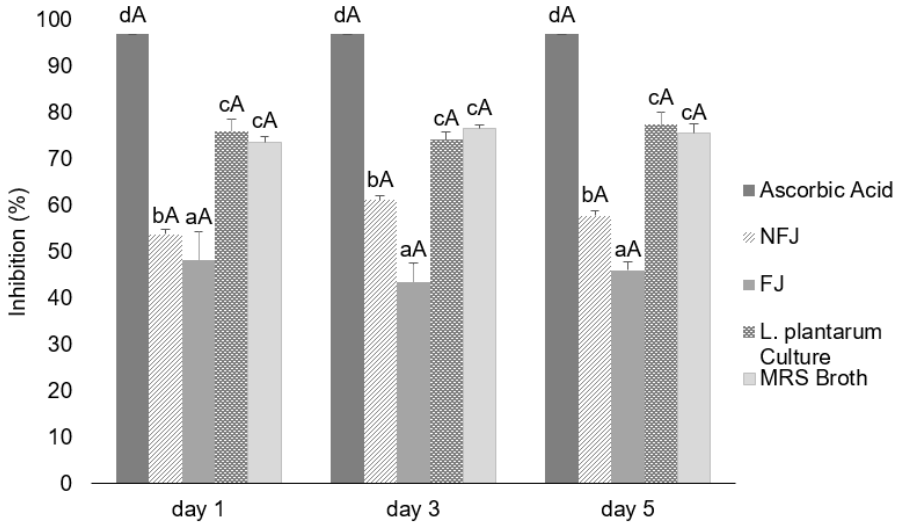


**Figure 5.** Percentage of inhibition of *jamu kunyit asam* as per DPPH assay after an incubation of 30 min. NFJ: non-fermented *jamu*; and FJ: fermented *jamu*. Different lowercase letters indicate significant difference between samples, and different uppercase letters indicate significant difference between storage durations.

The gradually weakening antioxidant activity might be related to the decrease in antioxidant components, such as total phenolics, which were degraded and utilised by fermenting microorganisms. It has been generally known that the growth of *L. plantarum* leads to the depolymerisation of phenolic compounds. This strain has the ability to degrade many types of phenolic acids. From the hydroxycinnamic group, *L. plantarum* is able to metabolise *p*-coumaric, caffeic, ferulic, and *m*-coumaric acids. It is also able to metabolise hydroxybenzoic acids such as gallic and protocatechuic acids. The decarboxylation and reduction reaction of phenolic acids are involved in their metabolism. The formation of ethyl derivatives from the degradation of the hydroxycinnamic group and some compounds such as pyrogallol and catechol might be beneficial for the growth and metabolism of *L. plantarum* (Hunaefi *et al*., 2012).

The cultures of *L. plantarum* BP102 and MRS broth (MRSB) medium showed higher antioxidant activity (74.3 - 77.5 and 73.79 - 76.6%, respectively) than fermented and non-fermented *jamu kunyit asam* (Figure 5). Since the implementation was carried out at different times, differences in the test materials used could affect the results of antioxidant activity. However, Suhartatik *et al*. (2014) showed that the culture of *L. plantarum* BP102 in MRS broth medium had an antioxidant activity of about 65%. This antioxidant activity occurred due to the presence of phenol compounds in the culture, which was possible from the components of MRS broth which had a phenolic structure, such as amino acids in the beef extract.

The antioxidant activity was also determined as a function of time to determine the optimum incubation time for antioxidants in DPPH radical scavenging (Figure 6). The results showed that there

was a gradual increase in antioxidant activity with an incubation time of 30 min, in both fermented and non-fermented *jamu kunyit asam.* This indicated that the optimum incubation time of antioxidant compounds in DPPH radical scavenging was 30 min. DPPH radicals are capable of accepting an electron as well as hydrogen from antioxidant compounds, and binding to a free electron in radical compounds so that they neutralise them into non-radical compounds. *Jamu kunyit asam* could perform free radical scavenging due to the presence of polyphenolic compounds as antioxidants (Ahmed *et al*., 2015).

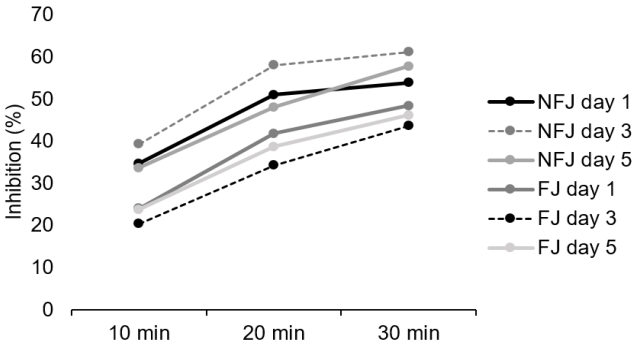


**Figure 6.** Percentage of inhibition of *jamu kunyit asam* as per DPPH assay as a function of time for 30 min. NFJ: non-fermented *jamu*; and FJ: fermented *jamu*.

*Organoleptic evaluation of jamu kunyit asam during storage*

Panellists were given six samples to evaluate, including fermented and non-fermented *jamu kunyit asam* samples which were stored for 1, 3, and 5 d. Results showed that fermented *jamu kunyit asam* after 3-d storage had the highest values for flavour and colour properties. Meanwhile, the highest values for scent and mouthfeel were obtained by non-fermented *jamu kunyit asam* after 1-d storage, and fermented *jamu kunyit asam* after 5-d storage (Figures 7 and 8). The sensory properties of fermented *jamu kunyit asam* are derived from the molecules and metabolites produced during fermentation (exopolysaccharides, aromatic compounds, and organic acids) (Ayed *et al*., 2020).

After 3-d storage, fermented *jamu kunyit asam* had the highest value for colour, which was 4.1, with brownish-yellow colour perception. The yellow colour of *jamu kunyit asam* is influenced by curcumin derived from turmeric. Curcumin is yellow at pH 1 – 7, and turns red at pH > 7.5. The brown colour of *jamu kunyit asam* is influenced by the addition of tamarind and brown sugar. After 5-d storage, *jamu kunyit asam* had a darker brown colour than the colour after 3-d storage. This condition was thought to be due to fat oxidation in beverages caused by exposure to more oxygen and light. Similar occurrence was noted in the storage of turmeric slices in an open room (Septiana *et al*., 2018). Curcumin is the component of turmeric that affects the colour of *jamu kunyit asam.* The effect of the storage lighting on beverage colour based on curcumin damage was still unclear. According to Kumavat *et al*. (2013), light can cause curcumin to decompose, thus resulting in a colour change from yellow to brownish-yellow. However, Kadam *et al*. (2013) showed that visible light did not affect the colour of turmeric powder, except for ultraviolet (UV) light. The lighting during storage was derived from an LED light, which emits visible light. Therefore, the research showed that the light in the beverage storage area did not affect the colour.

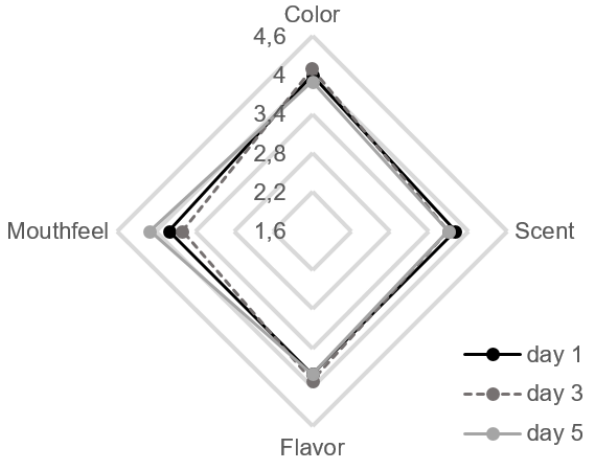


**Figure 7.** Results of sensory analysis of fermented *jamu kunyit asam* during storage.

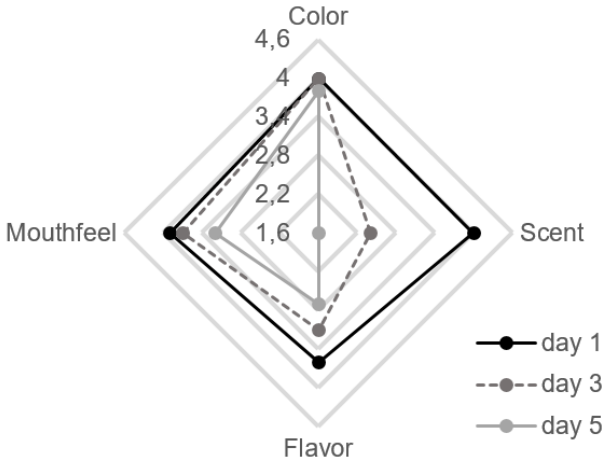


**Figure 8.** Results of sensory analysis of non-fermented *jamu kunyit asam* during storage.

Non-fermented *jamu kunyit asam* after 1-d storage had the highest value for scent, which was 4, with the perception of the herbal turmeric scent being not too strong. Each sample was significantly different for scent properties. Some of the essential oil constituent compounds in turmeric include ketones, sesquiterpenes, turmerone, zingiberene, phellandrene, sabinene, borneol, and cineol (Mulyani *et al*., 2014). In addition, the aromatic compounds formed during fermentation may include alcohols, aldehydes, ketones, esters, or fatty acids, which are derived from the catabolism of carbohydrates, proteins, and fats in *jamu kunyit asam.* The lactic fermentation of *jamu kunyit asam* has led to the formation of aromatic compounds, such as alcohols, ketones, alkanes, and terpenes (Ayed *et al*., 2020).

Fermented *jamu kunyit asam* after 3-d storage had the highest value for flavour, which was 3.9, with the perception of a balanced taste of sweetness, sourness, and herbal spiciness. Each sample was significantly different for flavour properties. The perception of sweetness comes from granulated and brown sugar. Lactic acid bacteria use glucose and sucrose as energy sources, so a shorter fermentation time implies higher sugar content. The perception of sour flavour is considered a refreshing quality in functional beverages. The sour flavour comes from tamarind and the accumulation of hydrogen ions. The main source of these ions is organic acids produced during fermentation, so a longer fermentation time implies that the lactic acid content is higher (Vazquez-Cabral *et al*., 2014). The dominant herbal taste perception comes from turmeric. Turmeric rhizome has a distinctive taste perception, such as hot, bitter, spicy, and bitter tastes (Mulyani *et al*., 2014).

Fermented *jamu kunyit asam* after 5-d storage had the highest value for mouthfeel, which was 4.1, with the perception of being easy to swallow, otherwise known as watery mouthfeel. However, there was no significant difference between samples in mouthfeel properties. Mouthfeel properties refer to the sensation of the beverage in the mouth.

Based on the panellist evaluation, fermented *jamu kunyit asam* after 3-d storage scored the highest value for colour and flavour properties. The mouthfeel properties showed that there was no significant difference between samples, which indicated that fermented *jamu kunyit asam* after 3-d storage was still within the panellists' acceptance limit. This was also supported by the total cell number of LAB, acidification activity, and probiotic properties of the samples. The total LAB of fermented *jamu kunyit asam* after 3-d storage showed the highest yield of $1.64 \times 10^8$ CFU/mL with an acidity level that was still acceptable to the panellists. Probiotic properties showed that fermented *jamu kunyit asam* after 3-d storage had a relatively high *L. plantarum* BP102 survival rate (70 - 80%). Therefore, fermented *jamu kunyit asam* after 3-d storage is a recommended product for consumption.

## Conclusion

The probiotic *L. plantarum* BP102 was able to grow in *jamu kunyit asam*, as indicated by an increase in total LAB during storage (98.2 - 103%). *Jamu kunyit asam* had the potential to be a carrier of the probiotic *L. plantarum* BP102 with higher survival rates of 63 - 70 and 73 - 83% under low pH conditions, and in the presence of 1 and 2 % bile salts, respectively. The addition of the probiotic *L. plantarum* BP102 did not increase the antioxidant activity in *jamu kunyit asam*. Non-fermented *jamu kunyit asam* had higher antioxidant activity (53.9 - 61.2%) than fermented *jamu kunyit asam* (43.6 - 48.4%). The organoleptic quality of fermented *jamu kunyit asam* increased in terms of flavour and colour. Based on the total LAB, acidification activity, probiotic properties, and organoleptic evaluation, fermented *jamu kunyit asam* after three days of storage is a recommendation for herbal-based functional products that can be consumed.

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