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Characteristics of probiotic tapai made by the addition of *Lactobacillus plantarum* 1

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Abstract Tapai is one of the fermented products made from starch sources such as glutinous rice and cassava. The indigenous amylolytic lactic acid bacteria in the production of tapai was utilized and found the chemical, microbiological and sensory characteristics of the probiotic tapai. Tapai by the addition of various strains of *Lactobacillus plantarum* 1 as starter. The results showed that tapai added by various strains of *L. plantarum* 1 had slightly different characteristics with tapai made with the addition of yeast only as starter. Tapai supplemented by *L. plantarum* 1 strains had a lower pH value and alcohol content than tapai added with yeast only. The number of lactic acid bacteria ranged from 10^8 to 10^9 CFU/ml. Overall assay showed that tapai supplemented by *L. plantarum* 1 strains was preferably liked by panelists. Especially tapai supplemented by *L.plantarum* 1 RN2-53 was more preferred by panelists than tapai supplemented by *L. plantarum* 1 RN2- 12112 and *L. plantarum* 1 RN1-23121 in term of colour, aroma, taste, and texture.

Keywords : *Lactobacillus plantarum* 1, glutinous rice, cassava, tapai

Introduction

Awareness of relation between food and the possibility of various diseases has changed people's opinion about food that is not only to fill up the hunger and as a source of nutrients, but also to maintain health. Foods that have the ability to influence physiological processes so that improve health or prevent from diseases are known as functional foods. Fermented products, a type of functional foods have beneficial health effects such as hypocholesterolemic effect, antihypertensive, and so on.

The beneficial health effects for fermented products derive from lactic acid bacteria (LAB) as well as bioactive components produced during the fermentation process. Research on the role of LAB in reducing plasma cholesterol levels has been widely carried out. Cholesterol reduction process by LAB can be performed directly or indirectly.

The direct reduction mechanism is carried out through cholesterol assimilation and the indirect reduction happens through the mechanism of

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bile salts deconjugation (Pereira and Gibson, 2002; Lestari *et al*., 2004; Liong and Shah, 2005). Lactic acid bacteria play an active role in the body if the LAB is able to grow properly inside our digestive system. Therefore LAB is potential probiotic to be used in producing functional food.

The use of probiotic LAB has been widely applied to various food products, both fermented and non-fermented products. However not all LAB can act as probiotic agents. Some LAB strains have the potential to be used as probiotic agents such as *Lactobacillus casei*, *Lactobacillus acidophilus, Bifidobacterium longum*, and *L. plantarum*. These bacteria are widely used in producing fermented food products. Some amylolytic LAB isolates can also be used in production of starch-based food products such as tapai. Different LAB strains will likely produce products with different characteristics. Yusmarini *et al*. (2017) reported that LAB isolated from the sago starch processing industry were dominated by *Lactobacillus plantarum* 1 strains which have amylolytic property. Yusmarini *et al*. (2016) reported that isolates of *Lactobacillus plantarum* 1 strains were resistant to low pH up to 2.5 and were resistant to bile salts (oxgall) as well as inhibited the growth of *Staphylococcus aureus* FNCC 158 and *Escherichia coli* FNCC 195. Utilization of LAB and various raw materials in the making of tapai, have been done by several researchers such as the use of probiotic *Lactobacillus acidophilus* SNP-2 and sticky rice to make tapai and brem (Purwandhani *et al*., 2008) and lotus seeds to make tapai (Khairina *et al*., 2008). Khasanah and Wikandari (2014) used *L. plantarum* B1765 isolated from bekasam to make tapai. Study on the benefits of amylolytic *Lactobacillus plantarum* 1 strains isolated from sago starch processing industry in the making of tapai probiotics is necessary to conduct. The research aimed to study the potential of *Lactobacillus plantarum* 1 isolates combined with yeast in the making of tapai probiotic and to evaluate the characteristics of the tapai produced.

Materials and methods

Materials

The study used *Lactobacillus plantarum* 1 RN2-53, *L. plantarum* 1 RN1-23121, and *L. plantarum* 1 RN2-12112 isolated from the sago starch processing industry. The raw material, medium and chemicals used were glutinous rice, cassava MRS broth and agar (Merck), NaCl (Merck), NaOH (Merck), alcohol, distilled water, and phenolphthalein indicators.

Preparation of glutinous rice tapai

Glutinous rice as much as 0.5 kg were cleaned and then cooked. Cooked sticky rice then cooled in a container that has been coated with banana leaves and sprinkled with yeast as much as 1% (w/w) and added with *L. plantarum* 1 strains as much as 1% and spread evenly on the sticky rice, then closed the container tightly. Glutinous rice that has been inoculated with yeast and *L. plantarum* 1 was incubated for 2 days at room temperature (± 30˚C).

Preparation of cassava tapai

Cassava 0.5 kg were cleaned, cut and washed, then steamed half-cooked. Cassava which had been cooked then cooled in a container that has been coated with banana leaves and sprinkled with yeast as much as 1% (w/w) and added with *L. plantarum* 1 strains as much as 1% and spread evenly on the cassava, then the container was closed tightly. Cassava inoculated with yeast and *L. plantarum* 1 strains was incubated for 2 days at room temperature (± 30˚C).

Parameters of probiotic tapai

Parameters were observed as pH value, total lactic acid, alcohol level, total LAB, total yeast and sensory properties. The pH value was measured using pH meters, protein, ash, total solids, moisture and fat content were analyzed according to Sudarmadji *et al*. (1997), the total lactic acid was carried out by alkalimetric titration using 0.1 N NaOH, and the total BAL was calculated according to Fardiaz (1992).

Data analysis

The data obtained were analyzed by analysis of variance (ANOVA). If the test results show that F count was greater than or equal to F table then further testing is done using Duncan New Multiple Range Test (DNMRT) at the level of 5% to determine the differences in each treatment.

Results

pH value

The pH value is one of the main parameters in the process of making tapai. The average pH value is shown in Figure 1. The pH value ranged from 3.3 to 3.85 for sticky rice tapai and 4.0 to 4.6 for cassava tapai. In general, pH value of cassava tapai was higher than glutinous rice tapai.

Total lactic acid

The total lactic acid was inversed proportional to the pH value. The higher the total lactic acid was the lower the pH value. Total lactic acid in tapai is presented in Figure 2. Figure 2 showed that the lowest total lactic acid in tapai made by the adding yeast only and without the addition of *Lactobacillus plantarum* 1.

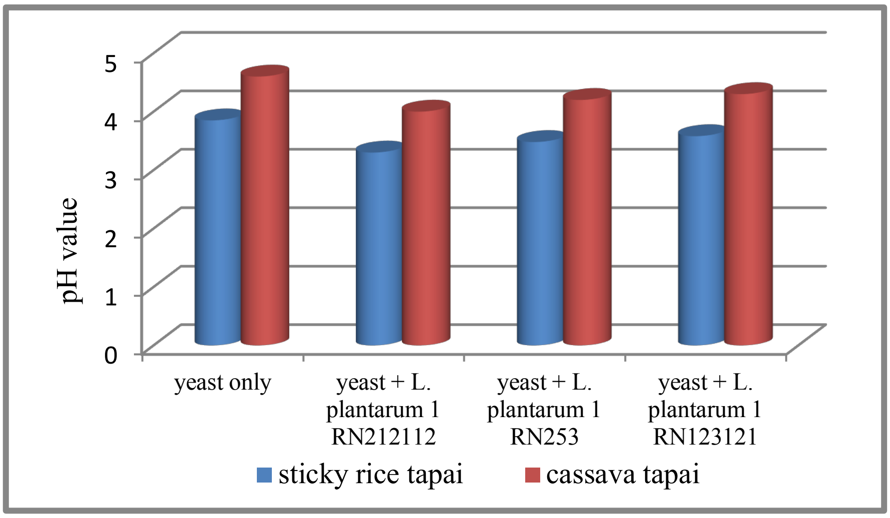

Figure 1. pH value of tapai , The numbers followed by different lowercase letters indicate significant difference at the 5% level

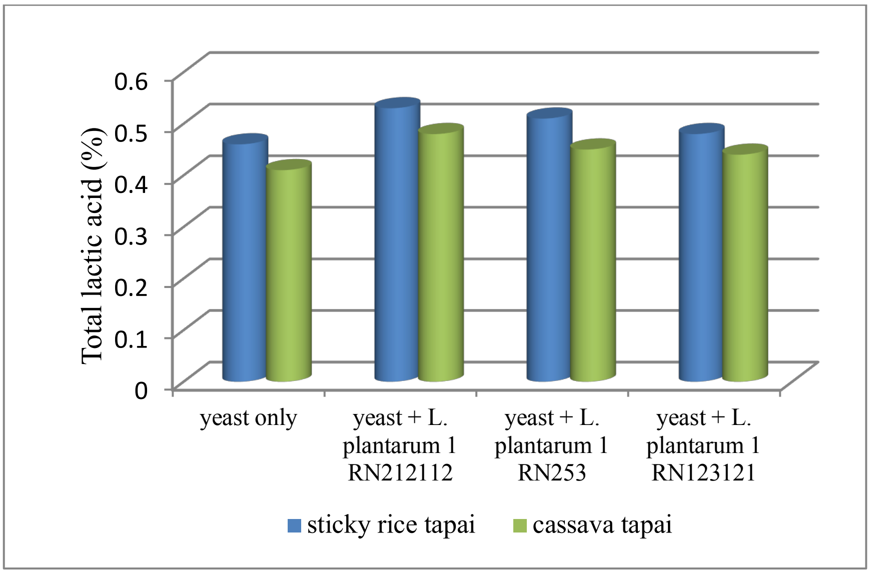

Figure 2. Total lactic acid of tapai, The numbers followed by different lowercase letters indicate significant difference at the 5% level

Total lactic acid bacteria

Total lactic acid bacteria in tapai were calculated after incubation for 48 hours as shown in Table 1. Data in Table 1 showed that the total LAB increased with the addition of *Lactobacillus plantarum* 1.

Table 1. Total lactic acid bacteria in tapai

Treatment	Total LAB (CFU/ml)	
	sticky rice tapai	**cassava tapai**
Yeast only	3,98 x 10^7	2,45 x 10^7
Yeast + *L. plantarum 1* RN212112	2,45 x 10^9	4,50 x 10^8
Yeast + *L. plantarum 1* RN253	5,95 x 10^8	3,63 x 10^8
Yeast + *L. plantarum 1* RN123121	3,75 x 10^8	3,38 x 10^8

Alcohol level

The fermentation process of glutinous rice and cassava into tapai produced acid and alcohol as the result. The average alcohol content in tapai is presented in Figure 3. The data in Figure 3 showed that the tapai made with the addition of yeast only produced more alcohol when compared to the tapai made by the mixture of yeast and *Lactobacillus plantarum* 1.

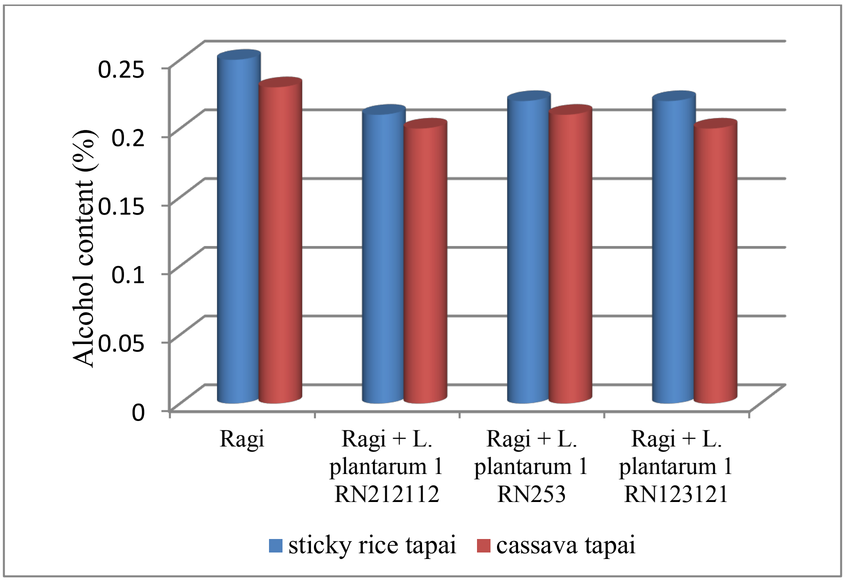

Figure 3. Alcohol content of tapai, The numbers followed by different lowercase letters indicate significant difference at the 5% level

Total yeast

Total yeast in tapai were calculated after incubation for 48 hours as shown in Table 2. The data in Table 2 showed that the highest total yeast was found in tapai that was added yeast only as starter.

Table 2. Total yeast of tapai

Treatment	Total yeast (CFU/g)	
	sticky rice tapai	**Cassava tapai**
Yeast only	9,43 x 10^{8}	1,16 x 10^{9}
Yeast + *L. plantarum 1* RN212112	2,98 x 10^{7}	5,68 x 10^{7}
Yeast + *L. plantarum 1* RN253	3,80 x 10^{7}	6,23 x 10^{7}
Yeast + *L. plantarum 1* RN123121	4,70 x 10^{7}	6,83 x 10^{7}

Sensory properties

Sensory testing is needed to determine the tapai characteristics that produced and determined the level of panelist's preference for the product. The results of the hedonic assessment (colour, aroma, texture, and taste) of tapai are presented in Figure 4.

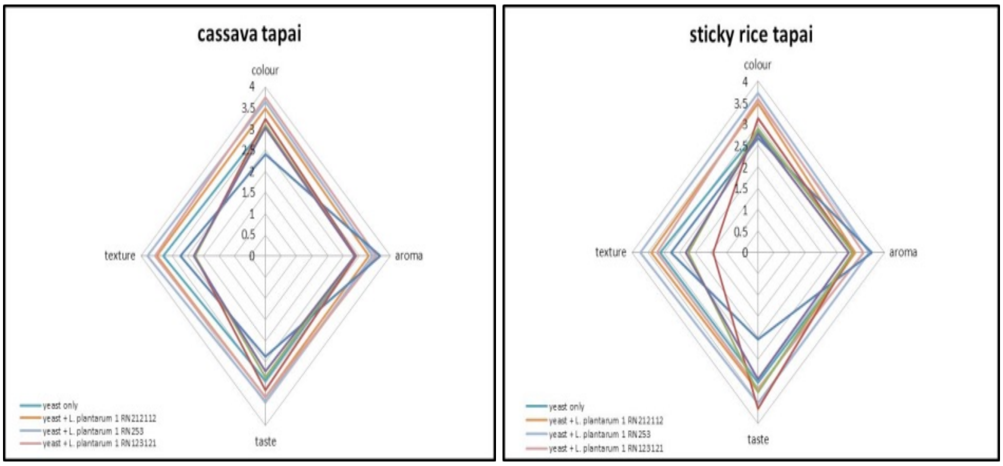

Figure 4. Hedonic assessment of tapai, Information : hedonic score 1 (very dislike); 2 (dislike); 3 (rather like); 4 (likes); 5 (really like)

Based on the data in Figure 4, the panelists were preferred the tapai of cassava and glutinous rice added with *L. plantarum* 1 strains compared to tapai made by the addition of yeast only, both in terms of color, aroma, texture, and taste. The descriptive assessment of tapai is presented in Figure 5. Tapai made with the addition of *L. plantarum* 1 isolates had a yellowish white color while tapai made with the addition of yeast alone was white.

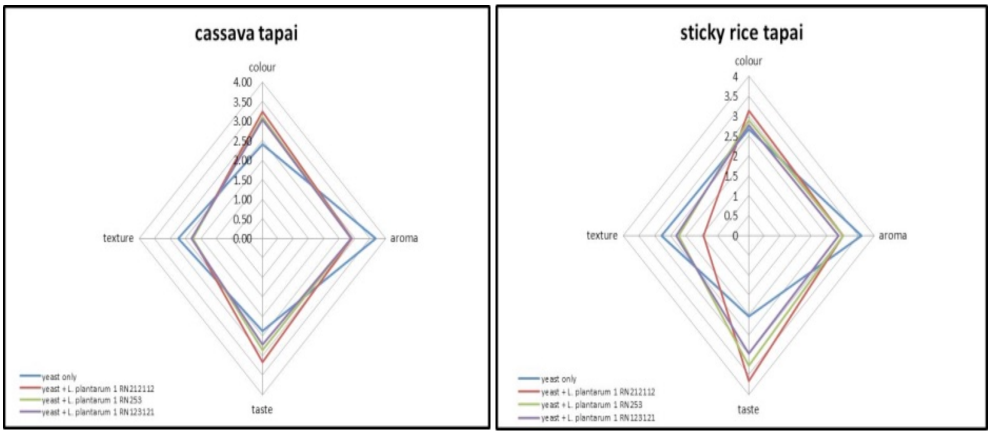

Figure 5. Descriptive assessment of tapai, Colour score: 1 (very white); 2 (white); 3 (yellowish white); 4 (yellow); 5 (very yellow); Aroma score: 1 (very flavorful); 2 (sour smell); 3 (smells of acid and alcohol); 4 (flavored alcohol); 5 (very alcoholic); Taste score: 1 (very sweet taste); 2 (sweet taste); 3 (sweet and sour taste); 4 (sour taste); (very sour); Texture score: 1 (very soft); 2 (soft); 3 (soft little hard); 4 (hard); 5 (very hard)

Discussion

Tapai is usually made by using starter containing yeast only. In this study, tapai was made by using yeast with or without addition of *L. plantarum* 1. In general, pH value of cassava tapai was higher than glutinous rice tapai. This is in line with the finding of Gultom (2017) who stated that the pH value of cassava tapai was 4.93 - 5.12 while glutinous rice tapai was 4.01 - 4.04. The data in Figure 1 show that the pH value of tapai was lower for treatment of the addition of *L. plantarum* 1 isolates for both glutinous rice tapai and cassava tapai. It showed that *L. plantarum* 1 could grow well on the substrate in the form of glutinous rice and cassava. *L. plantarum* 1 strains were amylolytic LAB isolated from the sago starch processing industry and during the fermentation process the LAB isolate together with yeast fermented the carbohydrates found in glutinous rice and cassava. The fermentation process produced organic acids which cause a decrease in pH value. The addition of *L. plantarum* 1 significantly decreased the pH compared to treatment that only used yeast. Furthermore, tapai in addition to *L. plantarum* 1 RN2- 12112 had the lowest pH value. It is suspected that *L. plantarum* 1 RN2 12112 was more effective in breaking down carbohydrates into organic acids compared to other *L. plantarum* 1 isolates. Yusmarini *et al.* (2017) stated that *L. plantarum* 1 RN2-12112 had greater amylolytic activity compared to isolates RN2-53 and RN1-23121. Amylolytic activity is closely related to the fermentation process of starch-rich food products such as cassava and glutinous rice. The results of research by Khasanah and Wikandari (2014) stated that the pH value of tapai cassava made with the addition of *L. plantarum* B1765 was lower than tapai which was only made with the addition of yeast. The range of pH values of glutinous rice and cassava of 3.3-4.6 were not much different from the tapai of lotus seeds reported by Khairina *et al.* (2008) of 3.88-4.39, but much lower compared to cassava tapai reported by Asnawi *et al.* (2013) of 5.69. Khairina *et al.* (2008) also added *L. acidophilus* SNP-2 in making tapai while Asnawi *et al.* (2013) only used yeast. This proves that the addition of LAB isolates has an impact on the low pH value of tapai. The lowest total lactic acid in tapai made by the adding yeast only and without the addition of *L. plantarum* 1. The addition of LAB isolates significantly increased the amount of organic acids produced and along with this increased the total lactic acid. In line with the measurement of the pH value, the tapai added with Lactobacillus plantarum 1 RN2-12112 produced the highest total lactic acid and the lowest pH. This proved that LAB isolates which have high amylolytic activity were able to ferment more starch and produce more organic acids, especially lactic acid. Reddy *et al.* (2008) stated that several strains of Lactobacillus spp. that produce extracellular amylase will ferment the starch directly into lactic acid. During fermentation, amylolytic LAB would combine two processes, namely enzymatic hydrolysis of carbohydrate substrate (starch) as well as fermentation which utilizes sugar to produce lactic acid. The pH value and the amount of lactic acid were very correlated to the number of total LAB in tapai. Total LAB increased with the addition of *L. plantarum* 1. *L. plantarum* 1 strains were amylolytic isolates and able to utilize carbohydrate sources, especially starch found in glutinous rice and cassava to grow and multiply. This fact is evidenced by the large number of LAB in tapai added with *L. plantarum* 1. The total LAB is directly proportional to the total lactic acid. The more number of LAB, the higher amount of titrated acid was recorded. The amount of LAB in tapai was still within the limits of probiotic food requirements, which was a minimum of 10^7 CFU/g. The research results of Khairina *et al*. (2008) showed that the number of LAB on the second day of fermentation was 10^6 CFU/g using *L. acidophilus* SNP-2 in the manufacture of tapai from lotus seeds. Similar result study was also reported by Khasanah and Wikandari (2104) who stated that the total LAB on the second day of incubation was about 10^6 CFU/g. The results of the present study using *L. plantarum* 1 isolates contained a higher number of LAB on the same incubation day which was 10^8 CFU/g. This probably due to the different characteristics of LAB isolates and *L. plantarum* 1 strains used in this study were amylolytic so they were able to utilize starch to grow and multiply.

Tapai made with the addition of yeast only produced more alcohol compared to the tapai made by the mixture of yeast and *Lactobacillus plantarum* 1. During the fermentation process yeast converted sugar to alcohol, while lactic acid bacteria predominantly fermented sugar into organic acids, especially lactic acid. This would have an impact on the final fermentation product, tapai that was only given yeast produced more alcohol and the tapai with addition of *L. plantarum* 1 isolates produced more acid. The research finding by Khasanah and Wikandari (2014) showed that alcohol content in tapai inoculated with yeast and *L. plantarum* B1765 was lower than tapai fermented with yeast only. Under anaerobes conditions, yeast hydrolyzed sugar to pyruvic acid and then pyruvic acid be converted to acid, and, acetaldehyde be converted to ethanol by dehydrogenase produced by yeast. The amount of ethanol produced also depend on the incubation period. The length of the incubation period increased the amount of alcohol. The research finding of Hasanah *et al.* (2012) stated that the alcohol content of cassava tapai increased with the incubation period. Alcohol content was directly proportional to the total yeast in tapai. The more the amount of yeast, the alcohol content will increases. Saono (1982) stated that enzymes that can convert glucose to alcohol and carbon dioxide are complex enzymes called zimase which is produced by *Sacharomyces cerevisiae*. The alcohol content in the white glutinous rice tapai was higher than cassava tapai. This proved that microbes used in fermentation are more effective in utilizing carbon contained on glutinous rice. These findings were similar to the result study reported by Berlian *et al.* (2016) that sticky rice tapai contained higher alcohol than cassava tapai with values of 0.58 and 0.41% respectively. The level of alcohol was closely related to the number of yeast in tapai. The highest total yeast was found in tapai that was added yeast only as starter. The microbes were also found in leavening agent besides *Sacccharomyces cerevisiae* and this leavening agent play a major role in converting carbohydrates into alcohol. Leavening agent containing yeast and other microbes as well as LAB played a mutualism symbiosis in utilizing carbon sources contained in the substrate. In the first stage, the amylolytic LAB will break down the starch into simple sugars and then converted lactic acid, while *S. cerevisiae* converted to alcohol. The total yeast in this study ranged from 10^7 to 10^8 CFU/g; the count were higher than that reported by Khairina *et al.* (2008) which were 10^5 CFU/g. The difference in total yeast is likely caused by different substrates. Khairina *et al.* (2008) used lotus seeds as a substrate while in this study using glutinous rice and cassava which contained more starch than lotus seeds. This starch is contained in glutinous rice and cassava which is the source of carbon for yeast to grow and multiply. Sensory evaluation showed that panelists preferred tapai of cassava and glutinous rice added with *L. plantarum* 1 strains compared to tapai made by the addition of yeast only, both in terms of color, aroma, texture, and taste. Tapai made with the addition of *L. plantarum* 1 isolates had a yellowish white color while tapai made with the addition of yeast alone was white. However panelists preferred yellowish white colour for cassava tapai. Whereas glutinous tapai made with the addition of *L. plantarum* 1 isolates had almost the same colour with cassava tapai which was yellowish white and was favored by panelists. Tapai made with the addition of yeast alone had a stronger alcoholic aroma when compared to tapai that was added with *L. plantarum* 1 isolates. Stronger aroma of alcohol caused panelists to dislike this tapai. Tapai made with the addition of *L. plantarum* 1 strains had an acidic aroma mixed with alcohol. Acidic aroma was more dominantly produced by LAB, while the aroma of alcohol was produced by yeast. The panelists preferred tapai with sour aroma and a little aroma of alcohol. Tapai made with the addition of *L. plantarum* 1 strains had a slightly different taste from tapai which was only made with the addition of yeast. Tapai made with addition of LAB tasted sweet and sour, while tapai made from the addition of yeast only had a more dominant sweet taste. The tapai added with *L. plantarum* 1 RN2-12112 had a stronger acidic taste than the tapai added with *L. plantarum* 1 RN2-53 and *L. plantarum* 1 RN1-23121. This was related to the amylolytic ability of the LAB isolates. Yusmarini *et al*. (2017) research result showed that *L. plantarum* 1 RN2-12112 had greater amylolytic activity than *L. plantarum* 1 RN2-53 and *L. plantarum* 1 RN1-23121. Isolate *L. plantarum* 1 RN2-53 had an amylolytic activity greater than and *L. plantarum* 1 RN1-23121. Amylolytic activity is important in the fermentation process of starch into organic acids. The stronger the amylolytic activity, the more starch would be changed to organic acids so that it produced tapai which was more acidic. Tapai made with the addition of yeast alone has a harsher texture compared to tapai added with *L. plantarum* 1 isolates. This is related to the ability of *L. plantarum* 1 in metabolizing starch so that the more metabolized starch, the softer the tapai was produced. Asnawi *et al*. (2013) stated that the longer the fermentation time, the softer the tapai produced. The difference in cassava tapai hardness was significant after fermentation for 24 hours.

It is concluded that Tapai made from glutinous rice and cassava supplemented with yeast and *L. plantarum* 1 isolates were slightly different from glutinous rice tapai and cassava which added with yeast only. The result shows that tapai had a lower pH and alcohol content with sweet and sour taste, scent of alcohol, with a softer texture and preferred by panelists. Overall tapai supplemented by *L. plantarum* 1 were favored by panelists but tapai supplemented with *L. plantarum* 1 RN2-53 was more preferred than tapai supplemented by *L. plantarum* 1 RN2- 12112 and *L. plantarum* 1 RN1-23121 in terms of colour, aroma, taste and texture. The amount of lactic acid bacteria found in tapai added with *L. plantarum* 1 had met the criteria for functional food which must contain at least 10^8 CFU/g of probiotic.

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