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Received: 11.7.2023
Revised: 7.1.2024
Accepted: 9.2.2024
Published: 19.2.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 109-121
https://doi.org/10.5219/1898
ISSN: 1337-0960 online
www.potravinarstvo.com

Characteristics of probiotic glutinous rice tapai with the addition of *Lactobacillus plantarum* 1 RN2-53 and some natural dyes

Yusmarini, Shanti Fitriani, Vonny Setiaries Johan, Netti Herawati, Irhamdi, Emma Riftyan, Usman Pato

ABSTRACT

Tapai is a traditional Indonesian food usually made from cassava and glutinous rice. The study aims to determine natural dyes that produce the highest quality probiotic glutinous rice tapai. The study was conducted experimentally using a complete randomized design consisting of four treatments, and each treatment was repeated four times. The treatment in this study was the soaking of glutinous rice into natural dye extracts, namely red dragon fruit extract, purple sweet potato extract, and suji leaf extract, with different concentrations, namely 10%, 15%, 20%, and 25%. The data obtained were statistically analyzed using variance analysis and continued with Duncan's new multiple-range test (DNMRT) at a level of 5%. The results showed that natural dye extracts with different concentrations have a noticeable influence on total lactic acid, alcohol content, total lactic acid bacteria, antioxidant activity, and sensory characteristics of color, aroma, taste, and hardness. The soaking treatment in red dragon fruit extract and purple sweet potato extract with a concentration of 20% resulted in glutinous rice tapai, preferred by panelists. Furthermore, the characteristic pH value of 3.35-3.41, total lactic acid 0.61-0.70%, alcohol content 0.33-0.42%, total lactic acid bacteria 9.11-9.40 CFU/mL, and antioxidant activity 167.35-102.51 ppm.

Keywords: Glutinous rice tapai, Probiotics, *Lactobacillus plantarum* 1 RN2-53, natural dyes

INTRODUCTION

Tapai is a traditional Indonesian food made from high-carbohydrate ingredients such as glutinous rice and cassava, with a fermentation process involving tapai yeast. Tapai can also be manufactured using a mixture of yeast and lactic acid bacteria (LAB). Khasanah and Prima **[1]** added *Lactobacillus plantarum* B1765 as much as 10^5 CFU/g with a fermentation time of 48 hours in making cassava tapai. Other studies were conducted by Yusmarini et al. **[2]** with a mixture of yeast and some types of BAL strain *Lactobacillus plantarum* 1 RN2-53 to manufacture white glutinous rice tapai. The results showed that tapai, with the addition of *Lactobacillus plantarum* 1 RN2-53, was preferred by panelists because of its physical properties and better quality. The resulting white glutinous rice tapai has a less attractive color, yellowish-white, so improving sensory quality and increasing consumer preferences can be done by adding dyes.

The use of natural color is prevalent in the food processing process in Indonesia. Natural dyes are pigments obtained from plants, animals, or mineral sources. Natural dyes have long been used for food coloring, and until recently, their use was considered safer than synthetic dyes. Some agricultural products are often used as natural dyes in food, including red dragon fruit, purple sweet potato, and suji leaves. These commodities have a striking color, so if used in food products, they will improve sensory quality, especially appearance. Besides being easy to get, red dragon fruit, purple sweet potato, and suji leaves contain antioxidant compounds. According to

Nataliani et al. **[3]**, Red Dragon Fruit Extract has an antioxidant activity of 87.11% heating process, and extended storage room temperature and cold temperature will cause a decrease in antioxidant activity.

Another plant that can also be used as a natural dye is the purple sweet potato, which has anthocyanin compounds that give it an intense color, and the compound is also an antioxidant. Based on the research of Husna et al. **[4]**, concentrated purple sweet potatoes have an anthocyanin content of 61.85 mg/100g, while light purple sweet potatoes have 3.51 mg/100g. Another natural dye often used is suji leaves, which contain the natural green chlorophyll pigment. It is one of the compounds that are antioxidants and have a total chlorophyll content of 3,773.9 ppm consisting of chlorophyll a of 2,523.6 ppm and chlorophyll b of 1,250.3 ppm **[5]**. Several studies have used natural dyes to manufacture food products, including those reported by Putri et al. **[6]**, which state that red dragon juice by as much as 15% can increase panelists' liking for yogurt. According to Yusmarini et al. **[7]**, natural dyes from purple sweet potatoes, red dragon fruit, and suji leaves with a 10-25% concentration to manufacture cassava tapai. The study's results showed that adding natural dyes gave a more attractive appearance to the cassava tapai color. The study aims to determine natural dyes that produce the highest quality probiotic glutinous rice tapai.

Scientific Hypothesis

The addition of natural coloring extracts (dragon fruit, purple sweet potato, and suji leaves) significantly affected the quality and antioxidant content of probiotic glutinous rice tapai.

MATERIAL AND METHODOLOGY

Samples

This study was conducted at Universitas Riau, Processing of Agricultural Products Laboratory and Agricultural Products Analysis Laboratory in Pekanbaru. Natural dye materials include red dragon fruit, purple sweet potato, and suji leaves. The researchers developed tapai from white glutinous rice.

Chemicals

The chemicals used consist of deMan rugosa sharp (MRS) broth and agar (Merck), NaCl (Merck), NaOH (Merck), alcohol, distilled water, phenolphthalein indicator, and 1,1-diphenyl-2-picryl hydroxyl solution (Sigma).

Animals, Plants, and Biological Materials

Lactobacillus plantarum 1 RN2-53 (personal collection of Dr. Yusmarini) and instant yeast (*Saccharomyces cerevisiae*) used a commercial brand Fermipan.

Instruments

The equipment comprises glassware, test tubes, Petri dishes, erlenmeyer, drip pipettes, stirrers, measuring cups, and cups. Other tools consist of digital scales (Shimadzu), pH meters (Loviband), autoclaves (All American), laminar airflow (Elisa), incubators (Memmert), vortex-mixers (Taiyo s-100), micropipettes (Soccorex), hot plates, bunsen lamps, and tips.

Laboratory Methods

Parameters were observed as pH value, total lactic acid, alcohol level, total LAB, total yeast, antioxidant activity, and sensory properties. Sensory testing includes a descriptive test to describe the tapai produced and a hedonic test to see the panelist's favorite response to tapai. The panelists for the descriptive test were 30 people; for the hedonic test, 80 were students of the Faculty of Agriculture, University of Riau, aged 20-23 years. The panelists tested each booth to avoid bias. At each sample change, the panelists neutralized their taste by drinking mineral water and their sense of smell by resting for ±10 seconds.

pH value: Determination of the degree of acidity (pH) refers to **[8]**. The pH value was measured using a pH meter. The electrodes are rinsed with distilled water and dried with a tissue. The pH meter tool is calibrated first using a buffer solution of 7.0 and 4.0 left to stabilize. The electrode on the pH meter is rinsed with distilled water. A Tapai sample of as much as 10 g was then crushed, and 100 ml of distilled water was stirred until homogeneous. Measurement of the sample by dipping the electrode into the sample and leaving it until a stable reading is obtained.

Total Lactic Acid: Determination of total acid content was carried out by titration, referring to **[9]**. As much as 10 mL of glutinous rice solution was put into the Erlenmeyer. Then, three drops of 1% phenolphthalein indicator were added to the sample and titrated with 0.1 N NaOH solution until the end point of the titration was reached, i.e., the formation of a pink color. Total acid (w/v) is calculated as percent acid lactate with the following formula:

$$Lactic\ Acid\ (\%) = \frac{V_{NaOH} \times N_{NaOH} \times {}^{90}/_{1000}}{V\ sample} \times 100$$

Alcohol Level: Measurement of alcohol content refers to **[10]**. Tapai 10 g was weighed, put into an Erlenmeyer, added with 50 mL distilled water, and added 1% phenolphthalein indicator as much as three drops. The sample is stirred and titrated with NaOH until the tapai solution turns pink. After When the titration changes color, stop and then look at the volume of the NaOH solution. Then the amount is used to calculate the alcohol content contained in tapai. Alcohol level (w/v) is calculated as percent alcohol with the following formula:

$$\textit{Alcohol Level}\ (\%) = \frac{a \times M \times Mr\ C_2H_5OH}{\textit{Fermentation Time}} \times 100$$

Where:
a = V NaOH; M = Concentration of NaOH (0,1 M); Mr = Mass Relative C_2H_5OH.

Antioxidant Activity: Analysis of antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) refers to **[11]**. The sample is 0.5 g extracted in 5 mL of 95% ethanol and allowed to stand for 24 hours in a dark room. The extract was taken as much as 1.3 mL and reacted with 5 ml of ethanol 95% and 5 mL of DPPH solution prepared by dissolving 0.0001 g of DPPH in 100 mL of 95% ethanol. The samples were then incubated in a dark place for 30 minutes, and then the absorbance was measured at a wavelength of 517 nm. Antioxidant activity is expressed as % inhibitor, which is formulated as follows:

$$\%\ \textit{Inhibitor} = \left[{}^{(A_B - A_B)}/_{AB}\right] \times 100$$

Where:
A_A = Sample Absorbance; A_B = Control Absorbance.

Total Lactic Acid Bacteria: The procedure for calculating the total number of LAB refers to **[12]** – a microbiological test using the spread method (spread surface plate). Calculating the amount of LAB was carried out by taking 1 mL of tapai liquid sample using a micropipette, then put into 9 mL of 0.85% physiological saline for a 10^{-1} dilution and continued until the 10^{-8} dilution. Taken 0.1 mL from the 10^{-6} dilution to 10^{-8} for inoculating on MRS Agar medium by dropping the sample in a petri dish containing MRS Agar, and the sample is leveled over the entire surface with a hockey stick that has been sterilized by burning over a Bunsen burner. Inoculation was carried out in laminar flow. The inoculated Petri dishes were then incubated in the incubator for 24 hours at a temperature of 37 ºC in reverse to avoid water droplets that may adhere to the inner wall of the cup lid. Colony The growing LAB was counted directly. Total LAB is expressed in logs CFU/mL.

Description of the Experiment

Sample preparation: The sample preparation for probiotic glutinous rice tapai with natural dyes can be seen in Figure 1.

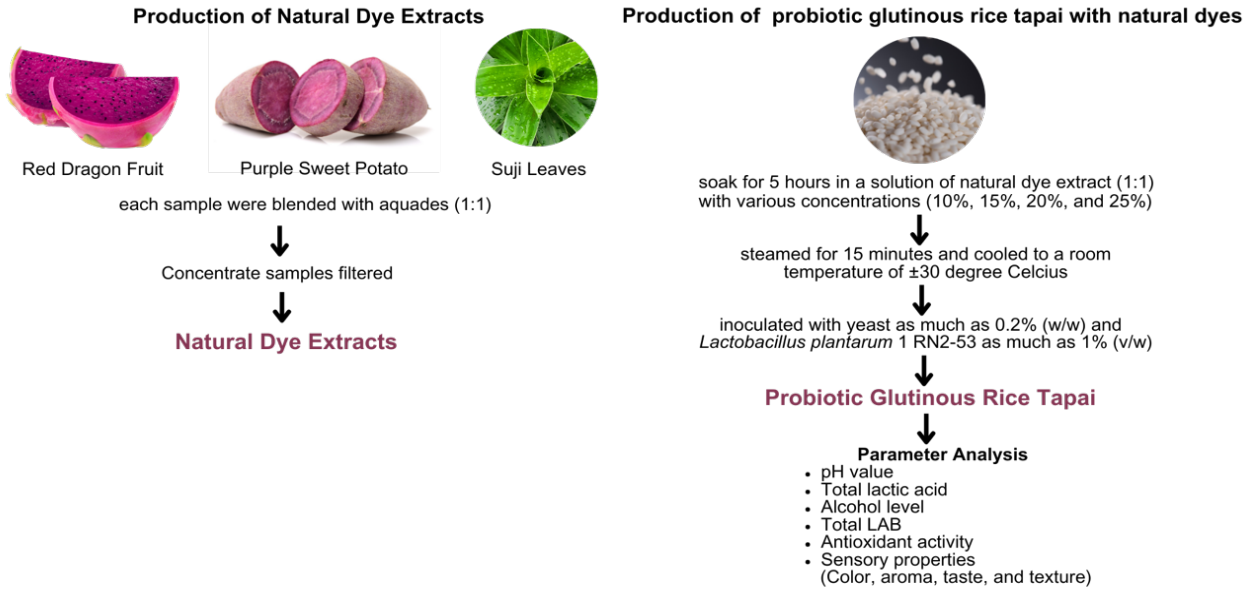


Figure 1 Production of natural dye extract and Probiotic glutinous rice tapai.

Production of natural dye extracts: The red dragon fruit, purple sweet potato, and suji leaves were washed clean. The flesh of samples was weighed as much as 500 g, cut into pieces to reduce the size, and then mashed using a blender by adding 500 mL of water (the ratio of ingredients and water is 1:1). Samples were filtered with a cloth for extraction.

Production of probiotic glutinous rice tapai with natural dyes: Probiotic glutinous rice tapai manufacture refers to **[2]** with minor modifications. The first step in making glutinous rice tapai is to weigh 500 g of glutinous rice, then wash and soak it for five hours in a solution of natural dye extract with various concentrations, namely 10%, 15%, 20%, and 25%. Comparison of glutinous rice and natural dye extract 1:2. Furthermore, glutinous rice is steamed for 15 minutes, and the glutinous rice that is still warm is soaked into a natural dye extract solution with a ratio of glutinous rice and a 1:1 dye extract solution. Soaking is carried out for 15 minutes. The soaked glutinous rice is then steamed for 15 minutes and cooled to a room temperature of ±30 °C. Glutinous rice is then inoculated with yeast as much as 0.2% (w/w) and *Lactobacillus plantarum* 1 RN2-53 as much as 1% (v/w). Glutinous rice inoculated starter is stirred thoroughly and incubated for 48 hours at ±30 °C.

The number of samples analyzed: We analyzed 48 samples, where three natural dyes were red dragon fruit extract, purple sweet potato extract, and suji leaf extract, and the four concentrations used were 10%,15%, 20%, and 25% (v/v). Each treatment was repeated for four replications.

Number of repeated analyses: All measurements of instrument readings were performed two times (Duplo).

Number of experiment replications: Each treatment was repeated four replications.

Design of the experiment: The study was conducted experimentally using a complete randomized design (CRD) with a two-factor experiment where Factor I: three natural dyes (red dragon fruit extract, purple sweet potato extract, and suji leaf extract) are applied with Factor II: four concentration (10%, 15%, 20% and 25%). This is a complete two-factor experiment with 4 x 3 = 12 combinations of the two factors (type of natural dyes and concentration). Analysis parameters are conducted with a CRD two-factor experiment, including pH, total LAB, and total alcohol. Hence, antioxidant, activity antioxidant, and sensory parameters were analyzed as descriptive.

Statistical Analysis

The results of chemical parameters, including pH and total alcohol, were analyzed by SPSS software (version 23) through one-way analysis of variance (ANOVA). Further testing was conducted using the Duncan New Multiple Range Test (DNMRT) at the confidence level of 5% (p <0.05), level to determine differences in each treatment if $Fcount \geq Ftable$.

RESULTS AND DISCUSSION

pH Value of Probiotic Glutinous Rice Tapai

A decreased pH value or degree of acidity in glutinous rice tapai indicates that yeast and lactic acid bacteria have been added to the fermentation process. The addition of natural dye extracts with different concentrations undeniably influences the pH value of glutinous rice tapai, as shown in Figure 1.

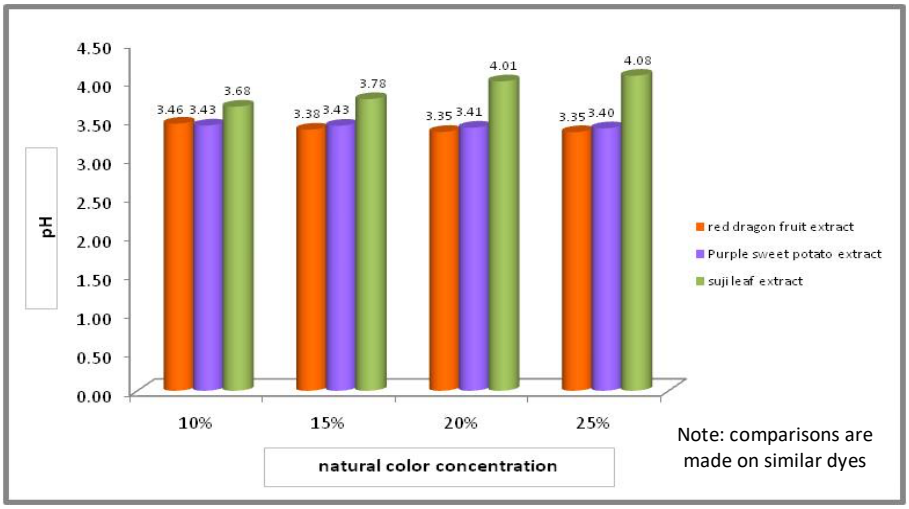


Figure 2 pH value of probiotic glutinous rice tapai.

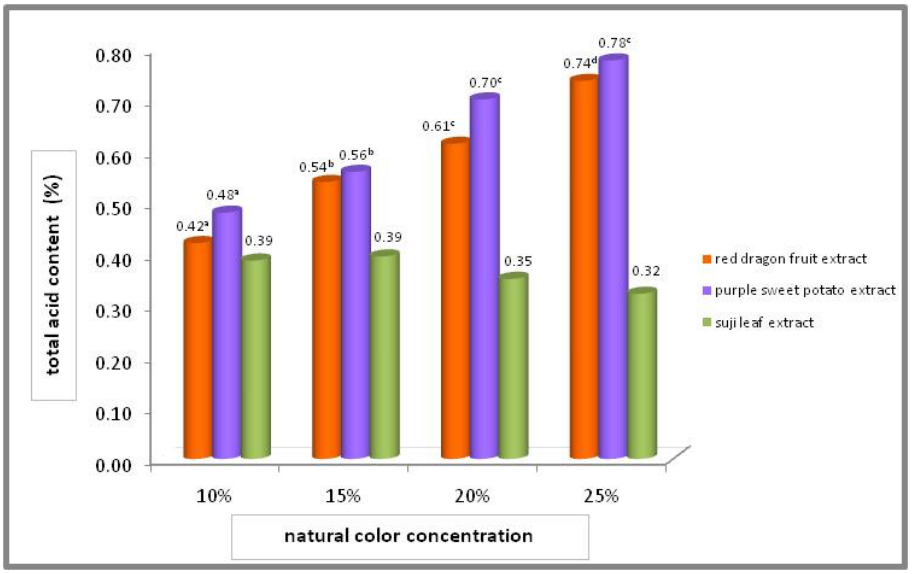
Figure 2 shows that the pH value of probiotic glutinous rice tapai tends to be the same for all natural dye concentration treatments added. Based on the ANOVA test results, it is known that the pH analysis between

factors does show no significance (p >0.05), as well as within factor I (type of natural dyes) and factor II (concentration) shows no significant results (p >0.05). The average pH value of glutinous rice tapai for natural dyes from red dragon fruit extract, purple sweet potato extract, and suji leaf extract, respectively, is between 3.35-3.46, 3.40-3.43, and 3.67-4.08. The resulting glutinous rice tapai has a low pH and an acidic taste. During the fermentation process, there is an overhaul of starch compounds by yeast and *L. plantarum* 1 RN-53 into organic acids, which causes a decrease in pH values. The *L. plantarum* 1 RN-53 used is amylolytic and can convert starch into simple sugars, which are then converted into organic acids. Adding *L. plantarum* 1 RN-53 will accelerate the decomposition of starch into organic acids. Yusmarini et al. **[2]** added *L. plantarum* 1 RN-53 resulted in a lower pH value than the treatment only added with yeast. The study indicates that adding *L. plantarum* 1 RN-53 will speed up fermentation. According to Yusmarini et al. **[13]**, *L. plantarum* 1 RN-53 is an amylolytic lactic acid bacterium. The decrease in pH value is also triggered by natural dye extracts that can be used as an energy source for yeast and *L. plantarum* 1 RN-53.

The pH value of the tapai sticky rice produced in this study was not too much different from the tapai white sticky rice added with dragon fruit extract as a colorant as a result of research by Isnaini et al. **[14]**, which ranged from 3.27 to 3.57, as well as the pH value of the tapai probiotics reported by Dede et al. **[15]** of 3.62.

Total Lactic Acid Tapai Glutinous Rice Probiotics

Total lactic acid is inversely proportional to the pH value. If high in total lactic acid, the pH value of the glutinous rice tapai produced will be lower. The total lactic acid of glutinous rice tapai is presented in Figure 2.



The different letters listed with the mean values in the graph represent statistically significant differences between the observed varieties (p <0.05).

Figure 3 Total lactic acid bacteria (LAB) tapai glutinous rice probiotics.

According to ANOVA results, it is known that the total LAB analysis between factors does show no significance (p >0.05), however, factor II (concentration of natural dyes) shows significant results (p <0.05). The data in Figure 2 shows that the more natural dye extracts derived from the red dragon fruit and purple sweet potatoes, the higher the total lactic acid produced, but this is not the case with suji leaf extract. Red dragon fruit extract and purple sweet potato extract are better able to provide substrates containing nutrients essential for yeast and LAB growth. These results align with the studies reported by Yusmarini et al. **[7]**, which state that cassava tapai with red dragon fruit extract and purple sweet potato extract has a higher total lactic acid than the addition of suji leaf extract. According to Nurul and Asmah **[16]**, red dragon fruit contains 12.97% of these carbohydrates containing glucose and fructose, which can be used by yeast and *L. plantarum* 1 to grow, multiply, and produce acid. Kakade et al. **[17]** state that red dragon fruit contains glucose of 5.70% and fructose of 3.20%. Kurnianingsig et al. **[18]** stated that purple sweet potatoes originating from the island of Java contain carbohydrates about 31.36-39.39% of 100 g. In addition, purple sweet potatoes also contain fairly complete amino acids needed by microbes to grow and multiply. Suji leaves contain more carbohydrates in the form of dietary fiber. According to Murtini et al. **[19]**, powdered suji leaves contain 42% dietary fiber, both soluble and insoluble, and generally cannot be utilized by LAB.

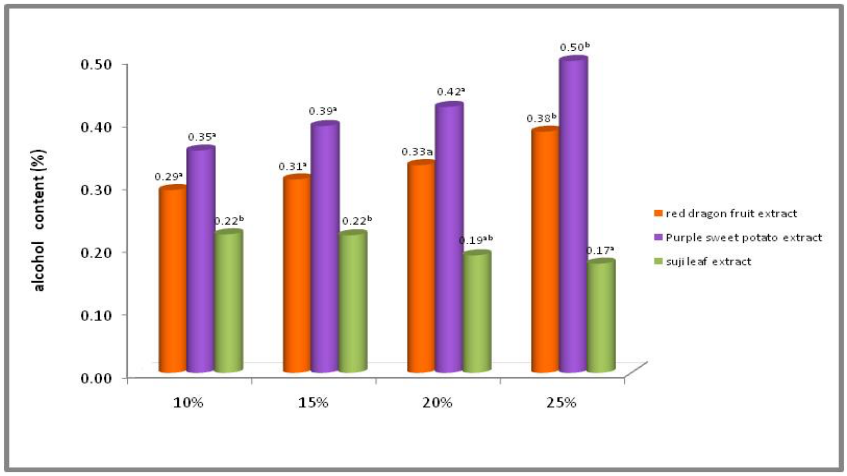
The total lactic acid of glutinous rice tapai soaked with red dragon fruit extract and purple sweet potato at a concentration of 20 to 25% ranged from 0.61-0.78% higher than the glutinous rice tapai reported by Kurniawan

et al. **[20]** and Yusmarini et al. **[2]** which was around 0.5%. Dede et al. **[15]** stated that sticky rice made by adding *Lactobacillus rhamnosus* SKG 34 had a total acidity of 0.57%. Based on the data presented, it can be seen that the addition of natural coloring extracts in the form of extracts of red dragon fruit and purple sweet potato can increase the total acid in tapai.

Total Alcohol Tapai Glutinous Rice Probiotics

Alcohol is one of the compounds produced during the process of making tapai. The more the use of natural dye extracts, there is tendency to increase alcohol levels in glutinous rice tapai, especially in the treatment with which red dragon fruit extract and purple sweet potato extract are added. Alcohol levels of glutinous rice tapai are presented in Figure 3.

The alcohol content in probiotic glutinous rice tapai ranges from 0.17-0.50%. The alcohol content in the treatment soaking with red dragon fruit extract and purple sweet potato was higher than in the treatment with suji leaf extract. The higher the concentration of natural dye extract used, the higher the alcohol content increases. It is closely related to the content of nutrients available to microorganisms used to manufacture tapai, especially yeast. Khamir plays a role in producing alcohol. Walker and Stewart **[21]** state that glucose, sucrose, fructose, maltose, and maltotriose are sugars that can be fermented into ethanol and carbon dioxide by Saccharomyces cerevisiae. According to Kurniawan et al. **[20]**, yeast will produce the zymase enzyme, which can break down sucrose into glucose and fructose, and the invertase enzyme, which plays a role in converting glucose into bioethanol. The lactic acid bacteria *L. plantarum* RN2-53 added to making tapai can help yeast decompose starch into glucose to produce alcohol. This result was approved by Yusmarini et al. **[2]**, who stated that yeast and lactic acid bacteria during fermentation are symbiotic mutualisms in utilizing the carbon source contained in the substrate. Amylolytic LAB hydrolyzes starch into simple sugars to produce lactic acid, while yeast will convert it into alcohol.



The different letters listed with the mean values in the graph represent statistically significant differences between the observed varieties (p <0.05).

Figure 4 Total alcohol tapai glutinous rice probiotics.

Based on the results of the ANOVA test, it is known that the overall LAB analysis between factors shows no significance (p >0.05). Still, factor I (type of natural dyes) shows significant results (p <0.05). The alcohol content of probiotic glutinous rice tapai with natural dye extract of red dragon fruit and purple sweet potato is higher than that of glutinous rice tapai. In the previous study Yusmarini et al. **[2]**, it is 0.22% but lower than the results of the study reported by Berlian et al. **[22]**, which is 0.58%, and the alcohol content reported by Marniza et al. **[23]** is 1.59%. Alcohol is produced by yeast, which is found in tapai yeast. The more yeast added and the longer the fermentation time, the higher the alcohol content of the tapai. Anisa et al. **[24]** stated that tapai made with 1% yeast produced much higher alcohol than tapai made with 0.25% yeast. Furthermore, it was stated by Marniza et al. **[23]** that the alcohol (ethanol) content of black sticky rice increased from 0.8% at 24 hours of incubation to 3.19% at 72 hours of incubation. This alcohol content is still classified as safe for consumption based on the Fatwa of the Indonesian Ulema Council **[25]**, which states that food and beverage products included in the khamr category or haram consumed are those that contain more than 0.5% alcohol.

Total Lactic Acid Bacteria

The calculation of total lactic acid bacteria (LAB) aims to determine the ability of *L. plantarum* 1 RN2-53 to grow and multiply together with yeast in making glutinous rice tapai soaked with natural dyes. The total LAB is presented in Table 1. The total LAB in the treatment soaked with red dragon fruit extract was 8.83-9.21 CFU/g, soaking in purple sweet potato extract was 8.99-9.53 CFU/g, and soaking in suji leaf extract was 8.17-8.84 CFU/g. Using natural dye extract from red dragon fruit and purple sweet potato does not inhibit the growth of *L. plantarum* 1 RN2-53. The result can be seen from the relatively high amount of LAB after glutinous rice is fermented for 48 hours at room temperature. The study results show that total LAB in the soaking treatment with red dragon fruit extract and purple sweet potato is higher than that with suji leaf extract, especially at 15%, 20%, and 25%. The extracts of red dragon fruit and purple sweet potatoes provide nutrients for the growth of LAB. The higher the concentration of natural dye extracts, the more LAB increases. It is proven that the natural dye extracts, in addition to improving the color of glutinous rice tapai, can also increase the amount of LAB.

Table 1 Total lactic acid bacteria of tapai glutinous rice probiotics.

Natural color concentration	Red dragon fruit extract (CFU/g)	Purple sweet potato Extract (CFU/g)	Suji leaf Extract (CFU/g)
10%	8.83	8.99	8.84
15%	9.10	9.10	8.68
20%	9.11	9.40	8.57
25%	9.21	9.53	8.17

Total LAB is closely related to the acid content and pH value. The more LAB in glutinous rice tapai, the more carbohydrates will be overhauled into organic acids. The lactic acid content will be higher and the pH value lower. Lactic acid bacteria will grow well if the need for nutrients is more available so that they can be utilized to grow and multiply.

Hasanah et al. **[26]** stated that sticky rice tapai produced by home industries in Bogor contains LAB of 7.9-8.5 CFU/g. The results of the research by Dede et al. **[15]** showed that the addition of *Lactobacillus rhamnosus* SKG 34 before it was fermented produced tapai sticky rice with a total LAB of 3.5 x 10^8 CFU/g or the equivalent of 8.53 CFU/g. Research conducted by Yusmarini et al. **[2]** regarding the production of glutinous rice tapai with the addition of *Lactobacillus plantarum* 1 produced tapai with a LAB content of 8.77 log CFU/ml. Several researchers reported results were slightly lower than that of glutinous rice made using natural dyes from red dragon fruit extract and purple sweet potato, but not much different from the suji leaf extract treatment. Total LAB is related to the additional nutritional content of red dragon fruit and purple sweet potato extracts. Furthermore, it is indicated that the added natural dyes do not inhibit LAB activity but can be additional nutrients for LAB. Moreover, *Lactobacillus plantarum* 1 is a type of lactic acid bacteria (LAB) that can be used as a probiotic culture or as a food additive. It can tolerate acidic and bile environments **[27]**, allowing it to survive and thrive in the digestive system. This strain has been found to have various therapeutic benefits, such as preventing mutations and inhibiting the growth of cancer cells **[28]**. It also possesses properties that help break down bile salts and bind cholesterol **[29]**. Additionally, it has been shown to modulate the immune system in the intestinal tract **[30]**.

However, the results of this study were encouraging as they showed that glutinous rice tapai with natural dyes still had a higher nutritional content than tapai made with suji leaf extract. According to this study, it can be seen that the addition of natural dye extracts, as much as 20%, has given good results; therefore, for testing antioxidant activity and sensory assessment is only carried out on the treatment of immersion in natural dye extracts as much as 20%.

Antioxidant Activity

The antioxidant activity found in glutinous rice tapai was made by adding *Lactobacillus plantarum* 1 RN2-53 and soaking in natural dye extract by 20%, ranging from 102.51-354.25 ppm. Data on antioxidant activity are presented in Table 2.

Table 2 Antioxidant activity of tapai glutinous rice probiotics.

Treatments	Antioxidant activity IC_{50} (ppm)
Addition of 20% red dragon fruit extract	167.345
Addition of 20% purple sweet potato extract	102.510
Addition of 20% suji leaf extract	354.248

Table 2 shows that soaking with purple sweet potato extract has a higher antioxidant activity than red dragon fruit extract and suji leaves. According to Molyneux **[31]** a compound is said to have robust group antioxidant activity if the IC_{50} value is less than 50 ppm, the strong group IC_{50} 50-100 ppm, the medium group IC_{50} 101-150 ppm, the vulnerable group IC_{50} 151-200 ppm, and more than 200 ppm are said to be weak. It can be stated that the treatment with purple sweet potato extract produces probiotic tapai with moderate antioxidant activity. Soaking with red dragon fruit extract and suji leaves produces tapai with fragile antioxidant activity. According to Kurnianingsih et al. **[18]**, purple sweet potatoes contain anthocyanins as a natural pigment of 283.1 mg/100 g, with antioxidant activity of 90.47% or equivalent to 9.047 ppm. During the processing process, antioxidant activity will decrease. The study results show that the antioxidant activity in glutinous rice powder is 102.514 ppm. The higher the IC_{50}, the smaller the antioxidant activity. Therefore, these results suggest that the antioxidant activity of purple sweet potatoes decreases significantly during the processing process. The research results of Salim et al. **[32]** showed that the steaming process in sweet potatoes would cause a reduction in antioxidant activity, which was initially 5 mg/L to 47.82 mg/L. Tapai making process involves a high temperature when glutinous rice is steamed for 30 minutes, which causes a decrease in antioxidant activity.

Red dragon fruit contains betacyanin, a natural dye and antioxidant. The super red-meat dragon fruit extract has antioxidant activity with an IC_{50} of 89.1 ppm **[33]**. The heating process will reduce the antioxidant activity of the red dragon fruit. Nataliani et al. **[3]** show that the longer and higher the heating temperature, the value of the antioxidant activity of the dragon fruit flesh natural dye solution decreases. Due to betacyanin found in the red dragon fruit, it cannot stand the high temperature. Furthermore, Reshmi et al. **[34]** show that betacyanin pigmentation occurs if heated at temperatures of 40, 50, and 60 °C.

Probiotic glutinous rice tapai on soaking treatment with suji leaf extract has lower antioxidant activity than soaking treatment in red dragon fruit and purple sweet potato extracts. Suji leaves contain chlorophyll, which acts as a dye and has antioxidant properties. The stability of the pigment chlorophyll is influenced by pH, temperature, and light **[35]**. The decrease in pH due to the fermentation process in the manufacture of glutinous rice tapai causes chlorophyll instability, which decreases its antioxidant activity.

Sensory Characteristics of Probiotics Glutinous Rice Tapai with the Addition of Natural Dyes

Glutinous rice tapai made with the addition of *Lactobacillus plantarum* 1 RN2-53 and immersion treatment into natural dye extracts have different sensory properties. Descriptive and hedonic assessments of glutinous rice tapai are presented in Figure 4.

Aroma: The results showed that soaking with natural dye extracts will produce tapai that smells of acid and alcohol. Hedonic panelists preferred treatment with immersion in red dragon fruit extract. Sour aromas of tapai with a slightly alcoholic aroma dominate the resulting aroma. While in other treatments, it has a more pungent smell of alcohol. Cassava tapai was added that the panelists liked the most, namely that the alcohol aroma was not too sharp **[36]**. Marniza et al. **[23]** stated that fermented glutinous rice for 2 days is preferable to fermented glutinous rice made with a 3-day fermentation period. Fermentation time is related to the high alcohol content in tapai. The longer the fermentation, the more sugar will be broken into alcohol. In addition, the purple sweet potato extract treatment produces another aroma, which is slightly off-flavor scented, while suji leaf extract still leaves the aroma of the leaves. The panelists liked the side scent.

Color: Color is one of the sensory characteristics that are very important because it can determine the level of consumer acceptance of a product. The research results by Widiyanti and Sukarta **[37]** state that tapai made from white glutinous rice has a white color and is not liked by panelists. One of the purposes of using natural dyes in making white sticky rice is to improve the color of the white sticky rice, which was initially yellowish-white. The color of probiotic glutinous rice tapai soaked with natural dye extract of as much as 20% can be seen in Figure 6.

Using 20% natural dye extract in soaking water produces glutinous rice tapai with an attractive color according to the type of natural dye used. The use of red dragon fruit extract produces tapai with a slightly orange-red color. In contrast, tapai in the soaking treatment in purple sweet potato extract produces purple tapai, and soaking in suji leaf extract produces green tapai. The cooking and fermentation process can cause a decrease in color density. Dragon fruit extract, which was originally a deep red color after undergoing a process with high temperature and an acidic atmosphere, has decreased its red concentration.

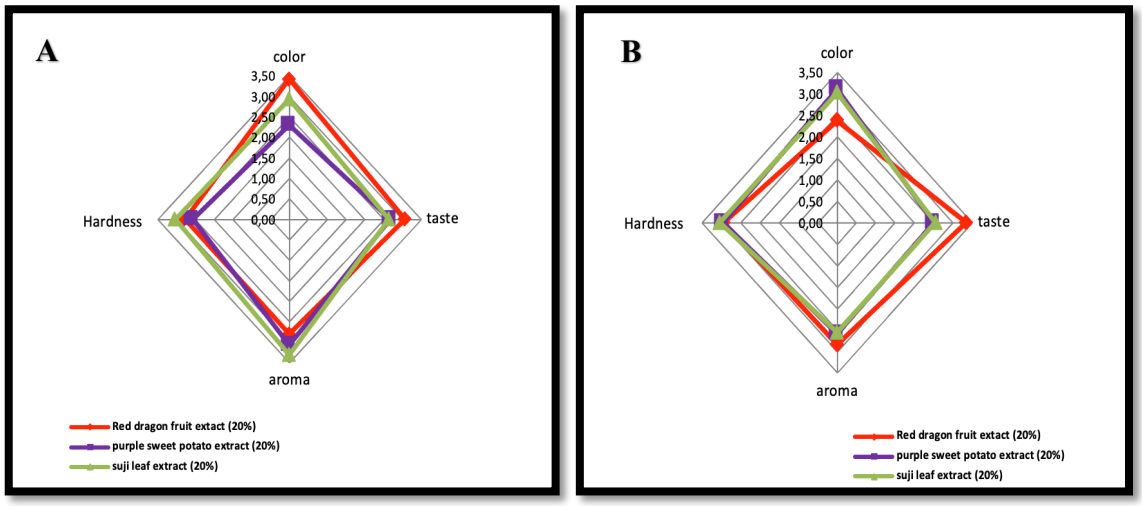


Figure 5 Sensory characteristics of probiotics glutinous rice tapai with the addition of natural dyes (A. Descriptive Analysis; B. Hedonic Analysis).

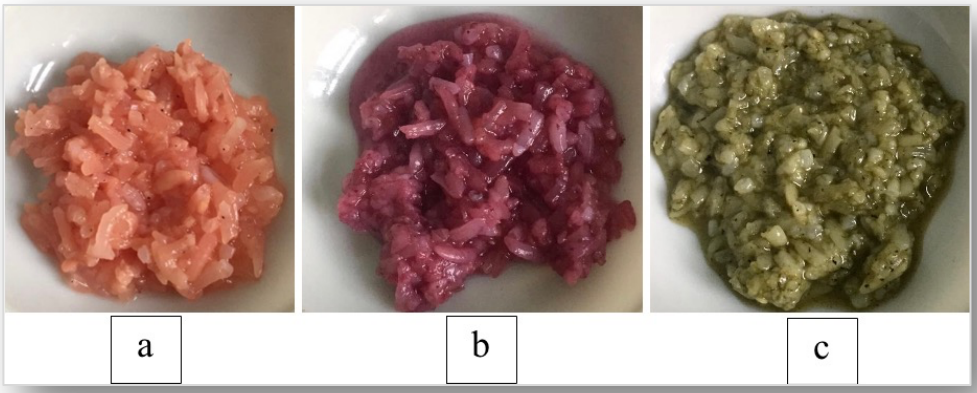


Figure 5 Color of probiotic glutinous rice (a) red dragon fruit extract (b) purple sweet.

According to Agne et al. **[38]**, temperatures above 40 °C will cause a change in betacyanin compounds from red to orange and then yellow. The research results of Husna et al. **[4]** state that the heating process by steaming purple sweet potatoes will result in a 34.14% decrease in anthocyanin pigment. Furthermore, Nugraheni **[5]** stated that heat treatment with a temperature of 100 °C degrades the pigment chlorophyll by 82.68%, and heating will convert chlorophyll into pheophytin by magnesium substitution by hydrogen so that the chlorophyll content becomes low. Based on the results of the hedonic sensory assessment, it is known that panelists prefer glutinous rice tapai color by soaking in purple sweet potato extract and suji leaf extract.

Taste: Glutinous rice tapai, soaking in natural dye extracts, generally has a mixture of sweet and sour tastes. However, the soaking treatment into purple sweet potato extract has a more robust sweetness than other treatments. The sweet and sour taste is produced during fermentation, remodeling complex carbohydrates into simple, sweet-tasting confectionery. Some simple sugars will be overhauled into organic acids that give tapai a sour taste. Furthermore, Barus and Wijaya **[36]** stated that the yeast's metabolic activities produce carbon dioxide gas that causes the dough to rise and produces alcohol and other flavor compounds. The fermentation process of tapai also produces ethanol and carbon dioxide gas, giving it an alcoholic aroma and a bubbly texture. Panelists preferred tapai glutinous rice probiotics with red dragon fruit extract to other treatments. The flavor characteristics were a balance of sweet and sour tastes that fit on the consumer's tongue and the absence of aftertaste after eating. Tapai with purple sweet potato extract dye still leaves the taste of yam, while suji leaf extract also leaves the particular flavor of suji leaves. Widiyanti and Sukarta **[37]** state that white sticky rice has a sweet taste and is not liked by panelists. Based on this, it can be seen that the panelists liked the balance of sweet, sour, and alcoholic flavors in tapai. Sweet, sour, and alcoholic tastes are influenced by the type of microbes used in making tapai.

Texture: Probiotic glutinous rice tapai generally has almost the same soft texture (hardness) in all treatments. However, in the treatment with suji leaf extract immersion, the tapai texture is slightly more complicated. The treatment concerns the more limited availability of nutrients in suji leaves. Nutrients are required by yeast and lactic acid bacteria during their growth. In the process of metabolism, the overhaul of complex compounds into simple compounds and the release of water causes the texture of tapai to become relatively soft. The texture is also affected by fermentation time. The longer the fermentation time, the softer the tapai texture becomes. Asnawi et al. **[39]** stated that fermented cassava tapai for 2 days was softer than 1 day of fermentation. The panelists' score on hardness was hedonic and ranged from 2.93-3.03, which is somewhat similar. Panelists prefer softer glutinous rice tapai over tapai with a soft or slightly soft texture. The results align with the research of Abdillah et al. **[40]**, which states that the panelists did not like wheat tapai with a very soft texture.

CONCLUSION

Based on the results of research that has been carried out by immersion with three different natural coloring extracts at a various concentration, tapai glutinous rice was selected from the tested parameters and fulfilled the food standards. standards that produced the best tapai quality based on the overall assessment. Tapai glutinous rice made with natural dyes has different physicochemical, microbiological, and sensory characteristics. The treatment in red dragon fruit extract (20%) and purple sweet potato extract (20%) resulted in the best treatment in glutinous rice tapai, which panelists preferred. Resulting in the characteristic pH value of 3.35-3.41, total lactic acid 0.61-0.70%, alcohol content 0.33-0.42%, total lactic acid bacteria 9.11-9.40 CFU/mL, and antioxidant activity 167.345-102.51 ppm.

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Funds:

Universitas Riau funded this research, Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia, with Contract Number 852/UN.19.5.1.3/PT.01.03/2020.

Acknowledgments:

We would like to thank to Research institutions and Community Service Universitas Riau.

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

***Yusmarini**, Universitas Riau, Faculty of Agriculture, Department of Agricultural Technology, HR. Soebrantas KM. 12,5, 28293, Pekanbaru, Indonesia,
Tel.: (+62)761-63272
E-mail: yusmarini@lecturer.unri.ac.id
ORCID: https://orcid.org/0000-0002-8879-4030

Shanti Fitriani, Universitas Riau, Faculty of Agriculture, Department of Agricultural Technology, HR. Soebrantas KM. 12,5, 28293, Pekanbaru, Indonesia,
Tel.: (+62)761-63272
E-mail: shanti.fitriani@lecturer.unri.ac.id
ORCID: https://orcid.org/0000-0002-9693-7679

Vonny Setiaries Johan, Universitas Riau, Faculty of Agriculture, Department of Agricultural Technology, HR. Soebrantas KM. 12,5, 28293, Pekanbaru, Indonesia,
Tel.: (+62)761-63272
E-mail: vonny.setiaries@lecturer.unri.ac.id
ORCID: https://orcid.org/0000-0002-5140-5222

Netti Herawati, Universitas Riau, Faculty of Agriculture, Department of Agricultural Technology, HR. Soebrantas KM. 12,5, 28293, Pekanbaru, Indonesia,
Tel.: (+62)761-63272
E-mail: netti.herawati@lecturer.unri.ac.id
ORCID: https://orcid.org/0000-0002-8579-4748

Irhamdi, Universitas Riau, Faculty of Agriculture, Department of Agricultural Technology, HR. Soebrantas KM. 12,5, 28293, Pekanbaru, Indonesia,
Tel.: (+62)761-63272
E-mail: irhamdibendang@gmail.com
ORCID: https://orcid.org/0000-0003-1198-8467

Emma Riftyan, Universitas Riau, Faculty of Agriculture, Department of Agricultural Technology, HR. Soebrantas KM. 12,5, 28293, Pekanbaru, Indonesia,
Tel.: (+62)761-63272
E-mail: emma.riftyan@lecturer.unri.ac.id
ORCID: https://orcid.org/0000-0001-7919-4319

Usman Pato, Universitas Riau, Faculty of Agriculture, Department of Agricultural Technology, HR. Soebrantas KM. 12,5, 28293, Pekanbaru, Indonesia,
Tel.: (+62)761-63272
E-mail: usmanpato@yahoo.com
ORCID: https://orcid.org/0000-0001-7716-5282

Corresponding author: *