BIODIVERSITAS
Volume 24, Number 11, November 2023
Pages: 6240-6247

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d241146

Variations in the properties and bacterial community of white glutinous rice tapai under different packaging conditions during fermentation

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Manuscript received: 12 August 2023. Revision accepted: 27 November 2023.

Abstract. *Barus T, Cenniati N, Prasasty VD. 2023. Variations in the properties and bacterial community of white glutinous rice tapai under different packaging conditions during fermentation. Biodiversitas 24: 6240-6247.* White Glutinous Rice Tapai (WGRT) is a traditional Indonesian fermented food. Several types of packaging can be used for white glutinous rice tapai (WGRT). However, information about the effects of different types of packaging on characteristics and bacterial communities has not yet been discovered. Therefore, this study aimed to evaluate the effect of packaging on the quality of WGRT. WGRT was fermented using a plastic box (WGRT-PB), banana leaves (WGRT-BL), and rose apple leaves (WGRT-RAL). Our research employed a novel method using next-generation sequencing (NGS), to explore the bacterial communities in the fermentation process of white glutinous rice tapai, offering valuable insights into how the choice of packaging materials affects the organoleptic properties of the final product. The results showed that the type of packaging did not significantly affect pH, total acid, and alcohol content but significantly reduced sugar content. The highest levels of reducing sugar were found in WGRT-PB. The organoleptic profile showed that WGRT-PB was the most preferred by the panelists, significantly different from WGRT-RAL. The results of the bacterial community analysis between WGRT-PB and WGRT-RAL showed a difference. The difference in the bacterial community was presumably due to the different types of packaging used during fermentation. Thus, the different types of packaging used during fermentation affect the quality of the tapai.

Keywords: Indonesian fermented food, next-generation sequencing (NGS), organoleptic properties, plant-base packaging

INTRODUCTION

Various types of traditional fermented foods are found in each country, part of their cultural heritage. Certain types of fermented foods, comprising about one-third of the human diet, have been produced and enjoyed worldwide (Marco et al. 2022). Investigations into fermented products' positive impact on health are increasingly being reported. Fermented food products are essential for maintaining healthy gut microbiota (Stephanie et al. 2017). Gut microbes influence individual health by synthesizing essential amino acids, vitamins, and short-chain fatty acids (Singh et al. 2017). Gut microbes influence the modulation of the immune system, metabolism, and neurobehavioural traits (Valdes et al. 2018). The fermentation process can increase the bioavailability and bioactivity of phytochemicals from food products. Pigmented rice can produce phytochemicals that increase often due to fermentation (Mishra et al. 2022). Phytochemicals derived from fermented foods play an important role in health (Baciu et al. 2023).

Tapai is a traditional fermented food that is quite popular in Indonesia (Yovani 2019). In Indonesia, tapai is often processed with various substrates, and white glutinous rice is one of the substrates used to produce white glutinous rice tapai (WGRT) (Cempaka 2021). WGRT exhibits a slightly sweet-sour, slightly alcoholic taste and aroma with a fresh sensation (Mueedin 2020). According to Halal regulations for Muslim people, the alcohol content in food or beverages must be below 5% (Mueedin 2021). The alcohol content of WGRT is relatively low, ranging from 2.2 to 4.9% (Pauzi et al. 2019), and its pH is around 4 (Yusmarini et al. 2019).

White glutinous rice (*Oryza sativa* L. var *glutinosa*), also known as white sticky rice or *ketan* in Indonesian, is widely used for traditional snacks or desserts in Southeast Asian countries like Indonesia, Malaysia, Myanmar, Thailand, and Vietnam (Chanthao 2020; Perdani et al. 2018). The starch content in white glutinous rice is approximately 80-85%. White glutinous rice has an amylose content of about 1-2% and comprises approximately 98-99% amylopectin (Perdani 2018; Yovani 2019). The high amylopectin content in white glutinous rice gives it a stickier texture than other rice types found in Southeast Asian countries. In contrast, general rice varieties like white rice contain 13-25% amylose (Yovani 2019). White glutinous rice has a high carbohydrate content of 78.9 g per 100 g, contributing to the higher alcohol content in tapai. The fat content of white glutinous rice is around 1.1%, moisture is 10.0%, protein is 8.1%, ash content is 0.8%, and it contains vitamins such as thiamin, riboflavin, and niacin (Shakri et al. 2020).

In Indonesia, tapai is typically wrapped with banana leaves (*Musa balbisiana*), and rose apple leaves (*Syzygium aqueum*), or placed in a plastic box. The packaging of tapai can influence the microorganisms residing within and the

product's characteristics, including taste, aroma, appearance, texture, and more (Zin et al. 2021). According to Zin et al. (2021), packaging tapai with rubber leaves can enhance its aroma and delicacy. The pores of the wrapping materials used for white glutinous rice tapai influence its organoleptic properties and the microorganisms present. A plastic box is chosen for packaging tapai due to its capacity for large-scale fermentation, easy availability and usage, affordability, attractiveness, safety, and durability. However, using plastic containers can compromise the distinct aroma of the food product (Ismail et al. 2021). Barus and Wijaya (2011) reported that the synergy of *Bacillus subtilis* or *Lactobacillus plantarum* with *Saccharomyces cerevisiae* yields high-quality tapai with a balanced sweet taste, soft texture, and an alcohol aroma. Nevertheless, no studies have reported on the bacterial community contained in tapai.

Our study introduced a novel approach by utilizing next-generation sequencing (NGS) to investigate the bacterial communities in white glutinous rice tapai during fermentation, providing insights into the impact of packaging materials on its organoleptic properties. NGS has been utilized to determine bacterial communities in Chinese rice wine (Liu et al. 2016) and over-fermented tempeh (Pangastuti et al. 2019). Previous studies reported that NGS is widely used for bacterial community identification in fermented rice-based products, such as analysis during the traditional fermentation of Nepali kiwinep (Shrestha et al. 2020), bacterial diversity and functional potential of fermented gelatinous rice products from Central India (Rai et al. 2023), and bacterial diversity of fermented rice-based foods from Bangladesh (Thapa et al. 2022). Therefore, this study aims to employ the NGS method to identify bacterial communities and specify characteristics using pH, total titratable acid (TAT), reducing sugar, and organoleptic tests for white glutinous rice tapai (WGRT) with various packaging types during fermentation.

MATERIALS AND METHODS

The materials employed in this study included white glutinous rice (sourced from Jakarta, Indonesia), banana leaves (sourced from Jakarta, Indonesia), rose apple leaves (sourced from Jakarta, Indonesia), and tapai inoculum NKL (Na Kok Liong).

Fermentation of white glutinous rice tapai (WGRT)

The white glutinous rice was procured from a traditional market in Jakarta, Indonesia. The fermentation process of WGRT was conducted following the method described by Zin et al. (2021). Approximately 2 kg of white glutinous rice was washed twice with tap water and then soaked overnight. The rice was steamed for around 35 minutes until it achieved a soft and sticky consistency. After cooling to room temperature (25 to 30°C), 1% (w/w) of powdered tapai inoculum was added and thoroughly mixed. The resulting mixture was then packed using plastic boxes (WGRT-PB), banana leaves (WGRT-BL), and rose apple leaves (WGRT-RAL). The WGRT was fermented for 72 h at 27°C in a Memmert IN110 incubator.

pH analysis

The pH levels were measured from day 0 to day 3 of the fermentation process. A total of 10 g of WGRT samples were mashed with 10 mL of distilled water and homogenized. The pH was determined using a pH meter (Mettler Toledo F20, Switzerland). This analysis was conducted in triplicate.

Alcohol analysis

Alcohol analysis was carried out according to the method outlined by Berlian et al. (2016). Initially, 10 g of the sample was mixed with 50 mL of distilled water in a volumetric flask until homogenized. Subsequently, 3 drops of phenolphthalein (Merck) solution were added. The mixture was stirred and titrated using NaOH 0.1 (Merck) solution until the color of the sample changed from white to light pink. The volume of NaOH solution used was recorded and could be utilized for a preliminary calculation of the total alcohol content and total titratable acid (TAT) in the tapai. The alcohol content was calculated using the following formula:

$$\%\text{Alcohol} = \frac{(A \times N \times MW \times DF)}{V \times 100} \times 100\%$$

Where:
A : Average titration result (mL)
N : Molarity of NaOH (0.1N)
MW : Molecular Weight of C_2H_5OH (MW=46)
DF : Dilution factor
V : Volume of sample (mL)

Total acid titrable (TAT) analysis

The total acid content was determined using the titrable method based on the approach described by Lan et al. (2019). Initially, 10 g of the sample was mixed with 50 mL of distilled water in a volumetric flask to ensure homogeneity. Following this, 3 drops of phenolphthalein (Merck) solution were added, and the mixture was stirred. In contrast, titrated using NaOH 0.1 (Merck) solution until the color of the sample shifted from white to light pink. The volume of NaOH solution utilized was recorded and could be used for a preliminary estimation of the total alcohol content and total acid titrable (TAT) in the tapai. The formula below was employed to calculate TAT value.

$$\%\text{TAT} = \frac{(V_{(NaOH)} \times N \times CF)}{m} \times 100\%$$

Where:
V : Volume (mL) of titrated NaOH (mL)
N : Molarity of NaOH (0.1 N)
CF : Conversion factor for citric acid (0.064)
m : mass of the aliquot sample (g)

Reducing sugar content analysis

The DNS method was employed for the analysis of reducing sugar content from day 0 to day 3 of fermentation based on Kapoor et al. (2020). First, a standard glucose

curve was established to correlate glucose concentration with optical density at a wavelength of 540 nm. The standard glucose solution curve was constructed using 0, 200, 400, 600, 800, and 1000 ppm glucose concentrations to derive the linear regression equation (Khatri and Chhetri 2020). The sample was diluted 200 times, and the DNS solution was prepared by dissolving 1 g of DNS powder (Sigma-Aldrich) into 20 mL of NaOH 2M (Merck) and 20 g of potassium sodium tartrate (Merck), combining them in 100 mL of distilled water. One mL of diluted sample was mixed with 1 mL of DNS reagent and 2 mL of aquadest in a reaction tube coated with aluminium foil. The reaction tube was then heated and homogenized in a water bath shaker at 100°C for 5 minutes until the solution turned red-brown. Spectrophotometric measurements were taken at a wavelength of 540 nm using a spectrophotometer (Thermo Scientific Genesys 20, USA). The reducing sugar content was calculated from standard D-glucose calibration curve. The reducing sugar content was calculated using the formula:

$$\%\text{Reducing sugar} = \frac{\text{Mass of reducing sugar in sample}}{\text{Mass of sample}} \times 100\%$$

Organoleptic test

The organoleptic test was conducted using a hedonic approach based on Dwijatmoko et al. (2016), involving 30 untrained panelists. The evaluation parameters included color, aroma, taste, texture, and overall impression. The panelists rated the samples on a scale of 1 (most dislike), 2 (dislike), 3 (neutral), 4 (like), and 5 (most like).

Statistical analysis

Data obtained from the tests on reducing sugar, alcohol, total acid, pH, and organoleptic qualities were analyzed using statistical product and service solutions (SPSS) version 24. The analysis was performed in triplicate using the ANOVA method. Results were deemed statistically significant if ($P>0.05$). Further analysis was conducted using Duncan's Multiple Range Test (DMRT) method.

DNA isolation and next-generation sequencing

DNA isolation from WGRT was carried out following the method of Barus et al. (2010) with some modifications. Briefly, 10 g of WGRT-PB and WGRT-RAL were mashed and combined with 90 mL of sterile 0.85% NaCl solution (Merck). The modification involved using a lower-speed centrifugation at 5500 x g due to the heavy sample mass. The resulting mixture was centrifuged at 110 x g for 5 minutes, and the supernatant was transferred to a new conical tube and centrifuged again at 5500 x g rpm for 10 minutes. The supernatant was discarded, and the pellet was retained. DNA isolation was performed using the DNA Extraction Kit (Geneaid, China), and obtained DNA was quantified using Nanodrops (Singh et al. 2014).

If the DNA obtained met the standard, Next Generation Sequencing (NGS) was performed using an Illumina platform by Macrogen to identify the bacterial community within WGRT. The NGS process comprised four steps: sample preparation, library preparation, sequencing, and raw data analysis. The raw data were subjected to assembly, preprocessing, clustering, and diversity analysis. Assembly utilized the Fast Length Adjustment of Short Reads (FLASH) program. Subsequent preprocessing and clustering steps aimed to remove noise data and cluster operational taxonomic units (OTUs) using CD-HIT-OTU or rDNA Tools program. The final step involved diversity analysis to assign taxonomy in the form of bar charts (Macrogen).

RESULTS AND DISCUSSION

Physicochemical analysis

The measurement of physico-chemical parameters such as pH level, TAT, alcohol, and reducing sugar have been done. During the fermentation of WGRT from day 0 to day 3, the pH level decreased, while alcohol content, TAT, and reducing sugar increased. The measurement results indicate that the packaging used for WGRT samples did not significantly differ in terms of TAT, pH, and alcohol content in WGRT. However, the reduction in sugar content of WGRT was significantly influenced by the type of packaging used. Notably, the reduction sugar content was the highest in WGRT-PB, whereas it was the lowest in WGRT-RAL, and these differences were statistically significant. The fermentation time needed to produce WGRT products is three days. Reducing sugar levels continued to increase along with increasing fermentation time (Figure 1). WGRT-PB had the highest reducing sugar content and WGRT-RAL had the lowest reducing sugar content, which were significantly different from each other. However, the difference in reducing sugar content in the two products was not followed by differences in TAT, pH, and alcohol content (Figure 1), which were not significantly different. It is possible that the duration of WGRT fermentation for three days is not enough time to show this difference in TAT, pH, and alcohol levels. Timing for reproduction, metabolism, and interaction of microorganisms play a significant role in determining TAT, pH, and alcohol levels. In addition, the probability of potential microorganisms is relatively similar. However, it requires further investigation.

White glutinous rice tapai (WGRT) organoleptic evaluation

The analysis of organoleptic attributes indicated that the selection of WGRT sample packaging had no statistically significant effect on characteristics such as color, texture, aroma, and overall perception. Nevertheless, a substantial difference was identified in the taste attribute between WGRT-PB and WGRT-RAL. Notably, WGRT-PB received a pronounced preference from the panelists, while WGRT-RAL was comparatively less favored. The analysis of organoleptic attributes indicated that the selection of WGRT sample packaging had no statistically significant effect on characteristics such as color, texture, aroma, and overall perception. Nevertheless, a substantial difference was identified in the taste attribute between WGRT-PB and WGRT-RAL. Notably, WGRT-PB received a pronounced preference from the panelists, while WGRT-RAL was comparatively less favored (Table 1).

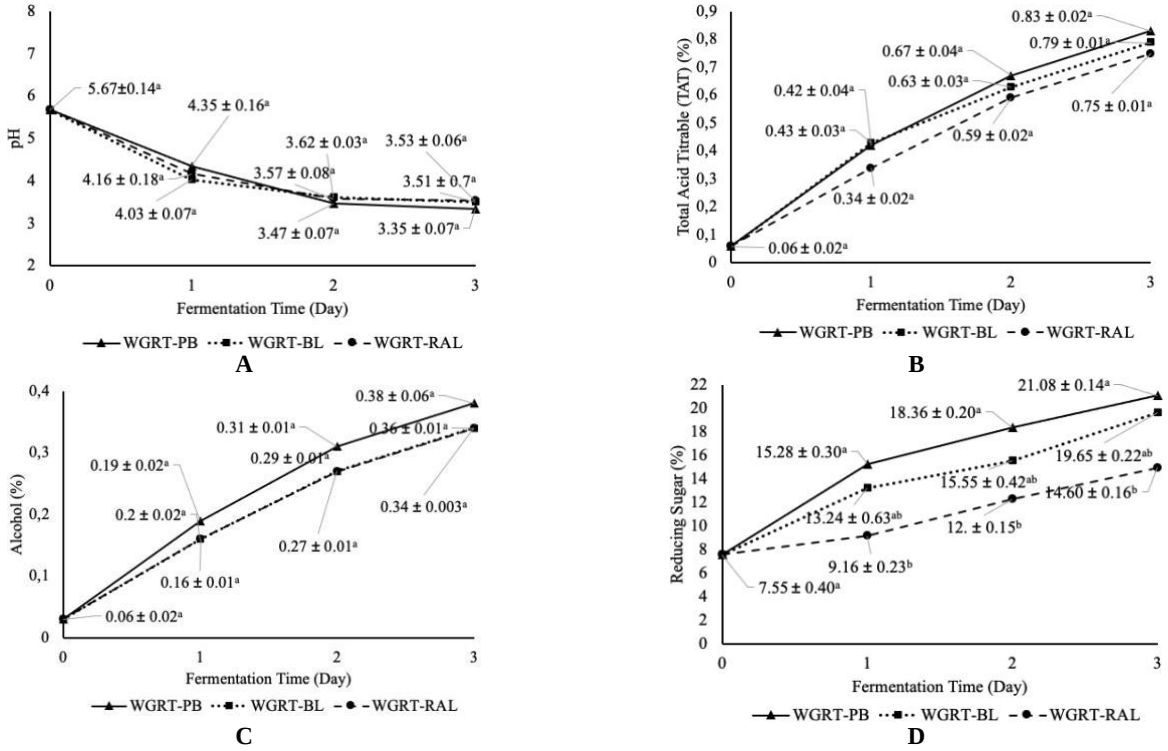

Figure 1. Dynamics of pH level (A), TAT (B), alcohol (C), and reducing sugar (D) content in white glutinous rice tapai (WGRT) with various types of packaging during fermentation

Table 1. Organoleptic property of white glutinous rice tapai (WGRT) with different types of packaging

Sample	Color	Taste	Texture	Aroma	Overall
WGRT-PB	3.66±0.85[a]	3.78±0.93[a]	3.1±0.94[a]	3.33±0.85[a]	3.45±0.78[a]
WGRT-BL	3.66±0.69[a]	3.51±0.97[ab]	3.2±0.90[a]	3.26±0.84[a]	3.44±0.81[a]
WGRT-RAL	3.61±0.76[a]	3.21±1.05[b]	3.1±1.08[a]	3.29±0.85[a]	3.36±0.85[a]

Note: Samples with the same letter in the same row indicate no significant difference

Bacterial community in white glutinous tapai with plastic box and rose apple leaves

The preference results demonstrated that WGRT-PB was the most favored among the panelists, whereas WGRT-RAL received the least preference. The findings indicated that the dominant phyla were *Firmicutes* and *Proteobacteria*, along with smaller proportions of Cyanobacteria and other phyla. Notably, there were distinct differences in the composition of *Firmicutes* and *Proteobacteria* between WGRT-PB and WGRT-RAL. Specifically, WGRT-PB exhibited a phylum composition of 9.2% *Cyanobacteri*a, 38% *Firmicutes*, 47.5% *Proteobacteria*, and other components. In contrast, WGRT-RAL showed a composition of 8.5% *Cyanobacteria*, 55% *Firmicutes*, 28.4% *Proteobacteria*, and other components (Figure 2).

Discussion

The acidity level of WGRT may increase due to the fermentation process (Figures 1.A and 1.B). The increase in acidity level observed in this study was accompanied by a direct proportional decrease in pH value as the %TAT value increased (ul-Haque et al. 2021). White glutinous rice has a pH value of 5.67 with a TAT of 0.06%. The fermentation process lowers the pH level and increases the TAT level. After fermentation, the pH values were as follows: WGRT-PB (3.35), WGRT-BL (3.51), and WGRT-RAL (3.53). Meanwhile, %TAT levels were WGRT-PB (0.84%), WGRT-BL (0.79%), and WGRT-RAL (0.75%). This study indicates that the packaging differences for WGRT products have no significant effect. Longer fermentation times result in more acid formation, leading to lower pH values and increased %TAT due to the fermentation metabolism of *Rhizopus oryzae*. The sugar formed in WGRT is converted to ethanol by zymase and catalyzed by lactate dehydrogenase to produce lactic acid (Xu et al. 2021). The production of glutinous rice tapai was examined using plastic box packaging that was coated with banana leaves. A control group was established using 1% yeast, while various strains of *Lactobacillus plantarum* 1% were used as treatment. The results showed that the tapai treated with *Lactobacillus plantarum* had pH values ranging from 3.3 to 3.85 and total lactic acid values ranging from 0.43 to 0.50% (Yusmarini et al. 2019). The analysis results in this study align with previous research. However, no studies report on the total acid content of WGRT products.

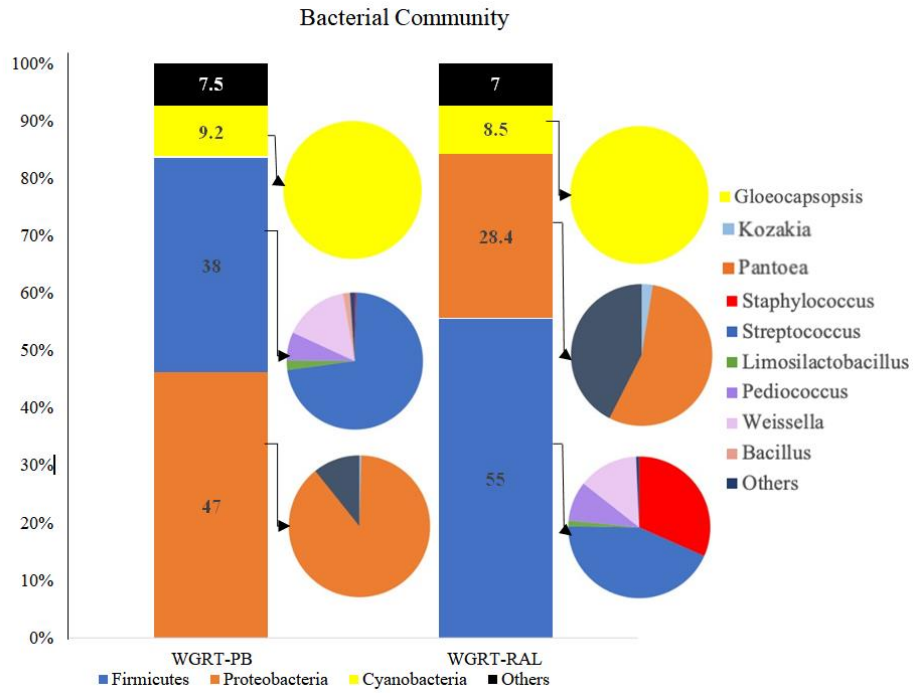

Figure 2. Bacterial community composition comparison of white glutinous rice tapai with plastic box (WGRT-PB) and with rose apple leaves (WGRT-RAL)

The duration of fermentation is a variable that enhances the alcohol content in WGRT (Figure 1.C). The initial alcohol content in white glutinous rice was 0.03%. Subsequently, following fermentation, WGRT-PB, WGRT-BL, and WGRT-RAL emerged as the products with the highest alcohol content at 0.38, 0.34, and 0.34%, respectively. The alcohol content of the three WGRT products treated with different packaging showed no significant effect. The conversion of simple sugars into alcohol by the microbial zymase enzyme (Yovani 2019) increases alcohol content. Rofiqoh et al. (2019) reported alcohol content in WGRT products with plastic without holes (0.65%), WGRT with banana leaves (0.53%), and WGRT with rose apple leaves (0.5%). Alcohol content composition can enhance the taste and aroma of WGRT products. Previous research indicates that fermentation using microorganisms can improve fermented products' taste, aroma, and nutritional value (Zin et al. 2021; Shiferaw Terefe and Augustin 2020).

Reducing sugar level was influenced by fermentation time (Figure 1.D). The reducing sugar content in white glutinous rice was 7.55%. Fermentation over three days increased, reducing sugar content compared to before fermentation. The highest reducing sugar levels were in WGRT-PB (21.08%), followed by WGRT-BL (19.65%), and WGRT-RAL (14.96%). The test results on reducing sugar content indicated a significant difference in packaging for tapai, specifically between WGRT-PB and WGRT-RAL. Therefore, significantly different WGRT products will continue to be tested using the NGS method to identify differences in the bacterial community present in tapai. The results of the analysis cannot be directly compared due to limited information about reducing sugar in fermented white glutinous rice. Sugar production is induced by bacteria with amylolytic characteristics capable of hydrolyzing starch into sugars (ul-Haque et al. 2020). Amylolytic microorganisms in tapai yeast include *Amylomyces rouxii*, *S. cerevisiae*, *Candida utilis*, *R. oryzae*, *Mucor* spp., and various bacterial types. Amylolytic bacterial species, such as *B. subtilis*, have also been found in tapai (Barus et al. 2013).

Organoleptic tests were conducted on WGRT products with different packaging types, assessing color, taste, texture, aroma, and overall attributes. Test results showed that, according to 30 panelists, the packaging had no significant impact on color, texture, aroma, and overall attributes. However, panelists significantly preferred the taste of WGRT-PB compared to WGRT-RAL. Each analysis carried out influences the organoleptic profile of WGRT products. Reducing sugar plays a role in imparting sweetness to WGRT (ul-Haque et al. 2021). This aligns with previous analyses where reducing sugar composition was higher in WGRT-PB (21.08%) and lowest in WGRT-RAL (14.96%). This analysis suggests that panelists tend to prefer the sweeter taste of tapai. Acidity levels, determined through pH and TAT tests, contribute to the sour taste and distinctive aroma of WGRT products (Yusmarini et al. 2019).

Additionally, tapai alcohol content impacts both the alcoholic taste and aroma of WGRT products (ul-Haque et al. 2021). Alcoholic organic acids can form aromatic ester bonds, contributing to the distinctive aroma of WGRT products (Putra et al. 2019). However, the analysis indicated that acidity levels (pH and TAT) and alcohol

content of WGRT products with different packaging types did not yield significant results. According to Yusmarini et al. (2019), the texture of WGRT products is influenced by fermentation time; longer fermentation leads to softer tapai. The synergistic interactions between the diverse bacterial communities during the fermentation process of WGRT products can lead to enhanced sensory characteristics compared to pre-fermentation stages. This modulation of the sensory profile is attributed to the production of aroma compounds, volatile organic acids, and flavor precursors by the fermenting bacteria (Zin et al. 2020; Suffys et al. 2023).

Bacterial community identification utilized the NGS method for two distinct products: WGRT-PB and WGRT-RAL. The results exhibited significant variations in reducing sugar analysis and organoleptic taste attributes between WGRT-PB and WGRT-RAL. This approach identified the discrepancies in the bacterial community within WGRT. In terms of bacterial phyla, WGRT-PB displayed *Cyanobacteria* (9.2%), *Firmicutes* (38%), Proteobacteria (47%), and other components (depicted in Figure 2). Conversely, WGRT-RAL demonstrated *Cyanobacteria* (8.5%), *Firmicutes* (55%), *Proteobacteria* (28.4%), and other phyla. *Firmicutes* and *Proteobacteria* emerged as the dominant phyla in WGRT products, comprising *Bacillus*, *Staphylococcus*, *Weissella*, *Streptococcus*, *Limosilactobacillus*, *Pediococcus*, *Kozakia*, *Pantoea*, among others. Prior research on fermented paocai highlighted the prevalence of *Firmicutes* and *Proteobacteria* (Liang et al. 2018). The presence of *Cyanobacteria* underscores the distinctive microbial composition observed in WGRT.

Various bacteria, including *Bacillus*, *Lactobacillus*, *Pseudomonas*, and *Proteus*, can produce amylase to break down starch into simple sugars. According to Izyan et al. (2020), reducing sugar content is directly proportional to the presence of amylolytic bacteria. *Bacillus* is present in fermented food and tapai cassava (Barus et al. 2013). *Bacillus* is recognized for producing alpha-amylase that breaks down starch into sugar in white glutinous rice (Simair et al. 2017). Additionally, *Bacillus* plays a role in improving the quality of cassava tapai, including taste (Barus et al. 2013). WGRT-PB contains 0.6% *Bacillus*, which is absent in WGRT-RAL. WGRT products also contain amylolytic lactic acid bacteria (ALAB) such as *Limosilactobacillus* and *Pediococcus*, which perform both amylase production and lactic acid fermentation (Haydersah et al. 2012; Bolaños-Núñez et al. 2021). WGRT-PB contains 0.8% *Limosilactobacillus*, and WGRT-RAL contains 0.7%. *Limosilactobacillus* can contribute to distinct fermented food aromas (Wang et al. 2020). Additionally, *Pediococcus* is present in WGRT-PB (2.4%) and WGRT-RAL (5%). *Pantoea*, found in WGRT-PB (41.3%) and WGRT-RAL (15%), can produce amylase (Suman et al. 2020) and is present in traditional Mexican fermented beverages and Chinese rice wine. WGRT-PB contains 0.2% *Kozakia*, and WGRT-RAL contains 0.7%. These amylolytic bacteria are higher in WGRT-PB than in WGRT-RAL, aligning with the higher reducing sugar content observed in WGRT-PB. Moreover, the organoleptic test results indicated that panelists preferred the sweeter taste of WGRT-PB.

Bacteria affect the sensory profile of fermented products which lactic acid bacteria (LAB) can produce lactic acid and lowering the pH value (Sari et al. 2020). Sari et al. (2020) found LAB such as *Weissella cibaria* and *Pediococcus acidilactis* in cassava tapai beverage. It indicates that the bacterial community present can influence the acidity of WGRT products. *Staphylococcus* is heterofermentative, producing various organic acids, such as lactic and acetic acids, affecting taste, aroma, and color. *Weissella*, a heterofermentative LAB and non-amylolytic, is also present (Pereira et al. 2012; Bolaños-Núñez et al. 2021). WGRT-PB contains 5.5% *Weissella*, while WGRT-RAL contains 7.5%. *Streptococcus*, a LAB found in stinky tofu and fermented soy curds (Gu et al. 2018), is present in both WGRT-PB (26%) and WGRT-RAL (24%). *Limosilactobacillus* and *Pediococcus* can also impact WGRT acidity as LABs (Porto et al. 2017). Lastly, Acetic Acid Bacteria (AAB) like *Kozakia*, found in tapai yeast or ragi (Rahayu 2016; Brandt et al. 2016), were present in WGRT-PB (0.2%) and WGRT-RAL (0.7%). While the percentage of LAB and AAB are lower in WGRT-PB than in WGRT-RAL, acidity levels between WGRT-PB and WGRT-RAL did not differ significantly. NGS data demonstrated that bacterial community differences contribute to tapai quality.

In conclusion, fermentation significantly impacts the acidity, alcohol content and reducing sugar levels of White Glutinous Rice Tapai (WGRT). The study reveals a direct correlation between fermentation duration and increased acidity, driven by the microbial activity of *R. oryzae*. Packaging variations, however, show no significant effects on the analyzed parameters. The organoleptic tests indicate that taste preference aligns with the higher reducing sugar content, suggesting a preference for sweetness in tapai. Bacterial community analysis using Next-generation sequencing (NGS) identifies significant differences between WGRT-PB and WGRT-RAL, emphasizing the role of specific bacteria, such as *Bacillus*, *Limosilactobacillus*, and *Pediococcus*, in influencing tapai quality. These findings contribute valuable insights into the intricate interplay between fermentation conditions, bacterial communities, and sensory attributes, providing a foundation for further research in optimizing the production of high-quality WGRT.

ACKNOWLEDGEMENTS

The authors thank Unika Atma Jaya, Tangerang, Indonesia for financially supporting our research through a competitive grant.

REFERENCES

Baciu AM, Opris RV, Filip GA, Florea A. 2023. Effects of phytochemicals from fermented food sources in alzheimer's disease in vivo experimental models: a systematic review. Foods 1 (11): 2102. DOI: 10.3390/foods12112102.

Barus T, Griselda HN, Suwanto A, Tan AW. 2020. Metagenomic analysis of bacterial diversity in tempe using terminal restriction fragmen length polymorphism (T-RFLP) technique. Biota 15: 273-280.

Bolaños-Núñez S, Santiago-Urbina JA, Guyot JP, Díaz-Ruiz G, Wacher C. 2021. Microbial interactions between amylolytic and non-amylolytic lactic acid bacteria strains isolated during the fermentation of pozol. Foods 10 (11): 2607. DOI: 10.3390/foods10112607.

Barus T, Wijaya LN. 2011. Dominant microbiota and their role in taste of cassava tapai. J Biota 16 (1): 354-361. DOI: 10.24002/biota.v16i2.119.

Barus T, Kristiani A, Yulandi A. 2013. Diversity of amylase-producing Bacillus spp. from "tapai" (fermented cassava). J Hayati 20 (2): 94-98. DOI: 10.4308/hjb.20.2.94.

Berlian Z, Aini F, Ulandari R. 2016. Uji kadar alkohol pada tapai ketan putih dan singkong melalui fermentasi dengan dosis ragi yang berbeda. J Biota 2 (1): 106-110. [Indonesian]

Brandt JU, Jakob F, Behr J, Geissler AJ, Vogel RF. 2016. Dissection of exopolysaccharide biosynthesis in Kozakia baliensis. J Micro Cell Factories 15 (1): 1-3. DOI: 10.1186/s12934-016-0572-x.

Cempaka L. 2021. Peuyeum: Fermented cassava from Bandung, West Java, Indonesia. J Ethn Foods 8 (3): 1-7. DOI: 10.1186/s42779-021-00079-3.

Chanthao R. 2020. Bamboo rice: food cultural heritage in southeast asia. Intl J Educ Soc Sci Res 3 (6): 111-115. DOI: 10.37500/IJESSR.2020.3608.

Dwijatmoko MI, Praseptiangga D, Muhammad DRA. 2016. Effects of cinnamon essential oils addition in the sensory attributes of dark chocolate. Nusantara Biosci 8 (2): 301-305. DOI: 10.13057/nusbiosci/n080227.

Gu JS, Liu TJ, Sadiq FA, Yang HY, Yuan L, Zhang GH, He GQ. 2018. Biogenic amines content and assessment of bacterial and fungal diversity in stinky tofu – A traditional fermented soy curd. LWT 88: 26-34. DOI: 10.1016/j.lwt.2017.08.085.

Haydersah J, Chevallier I, Rochette I, Mouquet-Rivier C, Picq C, Marianne- Pépin T, Icard-Vernière C, Guyot JP. 2012. Fermentation by amylolytic lactic acid bacteria and consequences for starch digestibility of plantain, breadfruit, and sweet potato flours. J Food Sci 77 (8): M466-M472. DOI: 10.1111/j.1750-3841.2012.02811.x.

Ismail N, Karim MSA, Ishak FAC, Arsyad MM, Karnjamapratum S, Sirison J. 2021. The Malay's traditional sweet, dodol: a review of the Malaysia's heritage delicacy alongside with the rendition of neighbouring countries. J Ethn Foods 8 (19): 1-13. DOI: 10.1186/s42779-021-00095-3.

Izyan NS, Azman DN, Mohd Saad NA, Sauid SM, Hamzah F. 2020. Effect of tacca starch loading on production of amylolytic enzymes from ragi tapai. Mater Sci Forum 987: 118-123. DOI: 10.4028/www.scientific.net/MSF.987.118.

Kapoor K, Tyagi AK, Diwan RK. 2020. Effect of gamma irradiation on recovery of total reducing sugar from delignified sugarcane bagasse. Radiat Phys Chem 170: 108643. DOI: 10.1016/j.radphyschem.2019.108643.

Khatri D, Chhetri SBB. 2020. Reducing sugar, total phenolic content, and antioxidant potential of Nepalese plants. J BioMed Res Intl 2020: 1-7. DOI: 10.1155/2020/7296859.

Lan W, Zhang R, Ahmed S, Qin W, Liu Y. 2019. Effects of various antimicrobial polyvynil alcohol/tea polyphenol composite films on the shelf life of packaged strawberries. Lwt 113: 108297. DOI: 10.1016/j.lwt.2019.108297.

Liang H, Yin L, Zhang Y, Chang C, Zhang W. 2018. Dynamics and diversity of a microbial community during the fermentation of industrialized Qingcai paocai, a traditional Chinese fermented vegetable food, as assessed by Illumina MiSeq sequencing, DGGE and qPCR assay. J Ann Microbiol 68 (2): 111-122. DOI: 10.1007/s13213-017-1321-z.

Liu SP, Yu JX, Wei XL, Ji ZW, Zhou ZL, Meng XY, Mao J. 2016. Sequencing- based screening of functional microorganism to decrease the formation of biogenic amines in Chinese rice wine. J Food Control 1 (64): 98-104. DOI: 10.1155/2012/251364.

Marco ML, Sanders ME, Gänzle M, Arrieta MC, Cotter PD, De Vuyst L, Hill C, Holzapfel W, Lebeer S, Merenstein D, Reid G. 2021. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on fermented foods. Nat Rev Gastroenterol Hepatol 18 (3): 196-208. DOI: 1038/s41575-020-00390-5.

Mishra S, Aravind SM, Charpe P, Ajlouni S, Ranadheera CS, Chakkaravarthi S. 2022. Traditional rice-based fermented products: Insight into their probiotic diversity and probable health benefits. Food Biosci 2022: 102082. DOI: 10.1016/j.fbio.2022.102082.

Mueedin NS. 2020. Fermentation of tapai and alcohol content release from tapai. IOP Conf Ser: Chem Mater Eng 1053 (1): 012050. DOI: 10.1088/1757-899X/1053/1/012050.

Pangastuti A, Alfisah RK, istiana NI, Sari SL, Setyaningsih R, Susilowati A, Purwoko T. 2019. Metagenomic analysis of microbial community in over-fermented tempeh. Biodiversitas 20 (4): 1106-1114. DOI: 10.13057/biodiv/d200423.

Pauzi N, Man S, Nawawi MSA, Abu-Hussin MF. 2019. Ethanol standard in halal dietary product among southeast asian halal governing bodies. Trends Food Sci Technol 86: 375-380. DOI: 10.1016/j.tifs.2019.02.042.

Perdani AY, Mulyaningsih ES, Paradisa YB. 2018. Diversity of some Indonesia local glutinous rice (*Oryza sativa* L. Var. Glutinous) based on agromorphological and RAPD markers. SABRAO J Breed Genet 50 (2): 85-100.

Porto MC, Kuniyoshi TM, Azevedo PO, Vitolo M, Oliveira RS. 2017. *Pediococcus* spp.: An important genus of lactic acid bacteria and pediocin producers. Biotechnol Adv 35 (3): 361-374. DOI: 10.1016/j.biotechadv.2017.03.004.

Rahayu ES. 2016. Lactic acid bacteria in fermented foods of Indonesian origin. Agritech 23 (2): 75-84. DOI: 10.22146/agritech.13515.

Rai A, Pandey A, Tomar SK, Singh VK. 2023. Bacterial diversity and functional potential of fermented rice-based foods of Central India. J Food Sci Technol 60 (1): 169-182. DOI: 10.1007/s13197-022-05552-z.

Rofiqoh I, Suaidah HI, Kinanti AR, Mawaddah A. 2017. Analisis kualitas tape ketan dengan berbagai alternatif bahan pembungkus. [Thesis]. Universitas Negeri Surabaya, Surabaya. [Indonesian]

Sari N, Tahir MM. 2020. Isolation and identification lactic acid bacteria of honey-enriched functional beverage from cassava (*Manihot esculenta*) tapai from Sinjai regency. In IOP Conf Ser: Earth Environ Sci 575 (1): 012016. DOI: 10.1088/1755-1315/575/1/012016.

Stephanie S, Ratih NK, Soka S, Suwanto A. 2017. Effect of tempeh supplementation on the profiles of human intestinal immune system and gut microbiota. Microbiol Indones 11 (1): 2. DOI: 10.5454/mi.11.1.2.

Shakri ANA, Kasim KF, Rukunudin IB. 2021. Chemical compositions and physical properties of selected malaysian rice: A review. IOP Conf Ser: Earth Environ Sci 765: 1-5. DOI: 10.1088/1755-1315/765/1/012024.

Shiferaw Terefe N, Augustin MA. 2020. Fermentation for tailoring the technological and health related functionality of food products. Crit Rev Food Sci Nutr 60 (17): 2887-2913. DOI: 10.1080/10408398.2019.1666250.

Shrestha N, Tamang JP, Watanabe K, Sakakibara H, Xiao H. 2020. Bacterial community dynamics and metagenomic analysis during the fermentation of kiwinep, a traditional fermented rice product of Nepal. Front Microbiol 11: 1273. DOI: 10.3389/fmicb.2020.01273.

Simair AA, Qureshi AS, Khushk I, Ali CH, Lashari S, Bhutto MA, Mangrio GS, Lu C. 2017. Production and partial characterization of α-amylase enzyme from *Bacillus* sp. BCC 01-50 and potential applications. BioMed Res Intl 2017: 1-9. DOI: 10.1155/2017/9173040.

Singh H, Khattar JS, Ahluwalia AS. 2014. Cyanobacteria and agricultural corps. Vegetos 27 (1): 37-44. DOI: 10.5958/j.2229-4473.27.1.008.

Singh RK, Chang HW, Yan DI, Lee KM, Ucmak D, Wong K, Abrouk M, Farahnik B, Nakamura M, Zhu TH, Bhutani T. 2017. Influence of diet on the gut microbiome and implications for human health. J Translational Med 15 (1): 1-17. DOI: 10.1186/s12967-017-1175-y.

Suffys S, Richard G, Burgeon C, Werrie PY, Haubruge E, Fauconnier ML, Goffin D. 2023. Characterization of aroma active compound production during kombucha fermentation: Towards the control of sensory profiles. Foods 12 (8): 1657. DOI: 10.3390/foods12081657.

Suman A, Shukla L, Marag PS, Verma P, Gond S, Prasad JS. 2020. Potential use of plant colonizing Pantoea as generic plant growth promoting bacteria for cereal crops. J Environ Biol 41 (5): 987-994. DOI: 10.22438/jeb/41/5/MRN-1250.

Thapa N, Tamang JP, Watanabe K, Sakakibara H, Shrestha N. 2022. Bacterial diversity and probiotic potential of fermented rice-based foods from Bangladesh. Food Microbiol 108: 103744. DOI: 10.1016/j.fm.2022.103744.

Valdes AM, Walter J, Segal E, Spector TD. 2018. Role of the gut microbiota in nutrition and health. Br Med J 361: 36-44. DOI: 10.1136/bmj.j2179.

Wang Z, Xu Z, Sun L, Dong L, Wang Z, Du M. 2020. Dynamics of microbial communities, texture and taste in Suan zuo yu during fermentation. J Food Chem 332: 127364. DOI: 10.1016/j.foodchem.2020.127364.

Xu Y, Jin Y, Su J, Yang N, Xu X, Jin Z, Wu F. 2021. Changes in the nutritional value, taste, and antioxidant activity of brown glutinous rice during fermentation. Food Biosci 43: 101273. DOI: 10.1016/j.fbio.2021.101273.

Yovani T. 2019. Lamang tapai: the ancient Malay food in Minangkabau tradition. J Ethn Foods 6 (22): 1-9. DOI: 10.1186/s42779-019-0029-z.

Yusmarini, Johan VS, Fitriani S, Rahmayuni, Artanti VF, Pato U. 2019. Characteristics of probiotic tapai made by the addition of *Lactobacillus plantarum* 1. Intl J Agric Technol 15 (1): 195-206.

Zin M, Azman NI, Razak SB, Ibrahim K, Rusli ND, Zainol MK. 2021. Identification of yeast in different rubber leaf (*Hevea brasilensis*) clones and their effects on the physical properties of fermented glutinous rice tapai. J Food Res 5 94: 29-37. DOI: 10.26656/fr.2017.5(4).735.