International Food Research Journal 31(5): 1144 - 1154 (October 2024)
Journal homepage: *http://www.ifrj.upm.edu.my*

Characteristics and bacterial community of black glutinous rice *tapai* with various packaging types during fermentation

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Article history

Received:
14 July 2022
Received in revised form:
30 January 2024
Accepted:
16 July 2024

Keywords

black glutinous rice tapai, bacterial community, next-generation sequencing, sensory evaluation, physicochemical properties

Abstract

Black glutinous rice *tapai* (BGRT) is a traditional Indonesian fermented food. Various packaging materials have been used during the fermentation process. However, there is a lack of information regarding how different packaging types would affect the quality of BGRT. Therefore, the present work aimed to assess the impact of various packaging methods on BGRT quality and bacterial diversity. The packaging types employed included plastic boxes (BGRT-PB), banana leaves (BGRT-BL), and rose apple leaves (BGRT-RA). Results indicated that packaging type had no significant effect on the physicochemical characteristics of BGRT, except for reducing sugar content, where BGRT-BL displayed the highest reducing sugar content. Organoleptic profiles revealed a preference over BGRT-BL products among panellists, whereas BGRT-PB products were comparatively less preferred. Utilising the Illumina next-generation sequencing (NGS) platform, BGRT-PB generated 158,406 reads, and BGRT-BL generated 140,546 reads. NGS outcomes demonstrated differences in bacterial community composition between BGRT-BL and BGRT-PB. In conclusion, packaging type significantly affected sugar content, organoleptic profiles, and the bacterial community composition of BGRT. Among the three packaging types, BGRT-BL outperformed BGRT-PB and BGRT-RA. BGRT-BL exhibited the highest proportion of the genera *Pediococcus* and *Limosilactobacillus*. Therefore, further investigation is necessary to elucidate the roles of these bacteria in determining BGRT quality.

DOI
https://doi.org/10.47836/ifrj.31.5.06

Introduction

Black glutinous rice *tapai* (BGRT) is a popular fermented food from Indonesia. BGRT is produced through the fermentation of black glutinous rice using "*ragi tapai*" as the starter culture. Black glutinous rice is one of Indonesia's significant agricultural products. It contains 13.32% moisture, 24.54% carbohydrate, 3.76% crude protein, 1.20% crude fat, 16.92% dietary fibre, and 5.78% ash (Tamprasit *et al.*, 2019). Starch in black glutinous rice has low amylose content (1 - 2%), and high amylopectin content (98 - 99%). The elevated amylopectin content results in the sticky texture observed when cooking black glutinous rice (Rini *et al.*, 2019). The deep purple-black hue of the rice is attributed to anthocyanin pigments produced in the aleurone and endosperm. The anthocyanin content of BGRT surpasses that of regular black glutinous rice, measuring 122.2 mg/100 g dry weight for BGRT compared to 111.1 mg/100 g dry weight for regular black glutinous rice (Plaitho *et al.*, 2013).

BGRT is a fermented food with substantial potential for further study and research. Anthocyanin pigments are widely recognised for their numerous health benefits, primarily stemming from their antioxidant activity, which shields cells from free radical compounds (Reis *et al.*, 2016). According to Liu *et al.* (2016), anthocyanin has been shown to lower LDL cholesterol levels in dyslipidemic patients. This reduction is attributed to anthocyanin's capacity to inhibit the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG Co-A reductase), a pivotal factor in cholesterol synthesis. The inhibition of this enzyme subsequently curtails cholesterol production, leading to a decrease in LDL cholesterol levels in the bloodstream. Furthermore, anthocyanin offers an array of additional advantages, including anti-cancer, anti-diabetic, anti-obesity, and

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anti-inflammatory properties, as documented by Sivamaruthi *et al.* (2018).

In producing BGRT in Indonesia, various types of packaging have been employed throughout the fermentation stages. Materials such as glass, plastic, and natural elements like leaves (banana, rose apple, and rubber tree leaf) have been utilised for fermentation packaging (Owens, 2015). The influence of packaging type during the fermentation process has been documented on the quality of *tapai* made from white glutinous rice (WGRT). For instance, WGRT packaged in plastic boxes displays a lower pH and a firmer texture than WGRT packaged with rubber tree leaves. The microorganisms inherent in the rubber leaves are believed to contribute significantly to this texture variation (Mohd *et al.*, 2021). However, information concerning the impact of packaging on BGRT remains lacking, thus necessitating further investigation.

The fermentation container stands as a crucial determinant of fermented food quality. The container type employed during fermentation significantly affects the resultant quality of the fermented food. A previous study noted that utilising varying container types influenced the characteristics of the produced *kimchi*. Likewise, distinct containers impact the yeast diversity in *sethemi* fermented milk, subsequently affecting the product attributes (Kebede *et al.*, 2007). The container type influences flavour compounds and fungal diversity during Sichuan pickle fermentation (Liu *et al.*, 2019). Bacteria also wield vital significance in shaping the quality of cassava (*Manihot esculenta*) *tapai*, as demonstrated by Barus and Wijaya (2011). Hence, delving into bacterial diversity in BGRT across different packaging types becomes equally essential.

One of the latest technologies widely employed for assessing microbial diversity in fermented food products is next-generation sequencing (NGS). This technology has found application in diverse products like *kimchi* (Kang *et al.*, 2018) and Thai shrimp paste (Phewpan *et al.*, 2020). NGS offers various advantages, including the capacity to sequence multiple samples simultaneously through massive parallel processing, faster and more accurate results, reduced sample requirements, and cost-effectiveness (Grada and Weinbrecht, 2013). Therefore, the present work aimed to compare the quality of BGRT across distinct packaging types, and explore the bacterial community within BGRT of varying qualities.

Materials and methods

Black glutinous rice tapai production

BGRT was prepared following the method outlined by Mohd *et al.* (2021) with specific modifications. These modifications included extending the soaking process from 2 to 24 h, the steaming duration from 15 min to 1 h, and the fermentation period from 48 to 72 h. Firstly, 200 g of black glutinous rice (sourced from Cibubur, Indonesia) underwent thorough cleaning, a 24-h soaking period, a 30-min boiling phase, and a subsequent 1-h steaming step. After cooking, the black glutinous rice was cooled to room temperature, inoculated with 1% (w/w) of the inoculum (NKL, Indonesia), and uniformly mixed. The inoculated black glutinous rice was subsequently packed into three different packaging types: plastic boxes (BGRT-PB), banana leaves (sourced from Cibubur, Indonesia) (BGRT-BL), and rose apple leaves (sourced from Cibubur, Indonesia) (BGRT-RA). The fermentation process lasted for 72 h at 27°C in an incubator (Memmert IN110).

Reducing sugar analysis

The reduction sugar content analysis was conducted using the DNS assay, following the method outlined by Maryanty *et al.* (2021) with certain modifications. The modifications included adjusting the concentrations of the glucose standard solution, from the original 0.25, 0.5, 0.75, 1, 1.25, and 1.5 ppm to 200, 400, 600, 800, and 1,000 ppm. To prepare the DNS solution, 1 g of DNS powder (Sigma-Aldrich), 20 mL of 2 M NaOH (Merck), and 30 g of potassium sodium tartrate (Merck) was dissolved in 100 mL of distilled water. Subsequently, 10 g of BGRT was diluted 200 times using distilled water. In a reaction tube lined with aluminium foil, 1 mL of the diluted sample, 1 mL of DNS reagent, and 2 mL of distilled water were combined, homogenised using a vortex (Thermo Scientific, USA), and then subjected to 5 min of heating at 100°C in a water bath shaker (GFL-1086, Italy). Following cooling to room temperature, the absorbance was measured at a wavelength of 540 nm using a spectrophotometer (Thermo Scientific Genesys 20, USA). The reducing sugar concentration within the sample was calculated by comparing the absorbance of the glucose standard solution with that of the sample solution, utilising the regression equation $y = ax + b$. The percentage of

reducing sugar content was determined using Eq. 1:

$$\%\ \text{Reducing sugar} = \frac{\text{M x DF}}{\text{W x 1000}}\ x\ 100\% \qquad \text{(Eq. 1)}$$

where, M = reducing sugar concentration (mg/mL); DF = dilution factor; and W = sample weight (mg).

Alcohol analysis

The alcohol content was determined through titration, following the procedure outlined by Berlian *et al.* (2016). In this process, 10 g of BGRT was diluted with 50 mL of distilled water. The resulting diluted sample was introduced into an Erlenmeyer flask, and combined with three drops of phenolphthalein (Merck) indicator before titration with a 0.1 N NaOH (Merck) solution. The conclusion of titration was signalled by a consistent transition in the solution's colour from pink to purple black. The alcohol content was determined using Eq. 2:

$$\%\ \text{Alcohol} = \frac{\text{V1 x N x Mr } C_2H_5OH \text{ x DF}}{\text{V2 x 100}}\ x\ 100\% \qquad \text{(Eq. 2)}$$

where, V1 = average volume of NaOH (mL); V2 = sample volume (mL); N = normality of NaOH; DF = dilution factor; and Mr = molecular mass (g/mol).

Titratable acidity analysis

The titratable acidity content was assessed *via* titration, following the methodology outlined by Tyl and Sadler (2017). For this procedure, 10 g of BGRT was diluted with 50 mL of distilled water. The resulting diluted sample was transferred into an Erlenmeyer flask, and mixed with three drops of phenolphthalein (Merck) indicator before titration using a 0.1 N NaOH (Merck) solution. The point of titration completion was indicated by a consistent colour shift in the solution, transitioning from pink to purple black. The titratable acidity content was determined using Eq. 3:

$$\%\ \text{Titratable acidity} = \frac{\text{V1 x N}}{\text{V2}}\ x\ 100\% \qquad \text{(Eq. 3)}$$

where, V1 = average volume of NaOH (mL); V2 = sample volume (mL); and N = normality of NaOH.

pH analysis

The pH was determined by adding 10 g of BGRT with 10 mL of distilled water, pulverisation using a mortar and pestle, and pH determination using a pH meter (Mettler Toledo F20, Switzerland).

Organoleptic test

The organoleptic test was performed according to Dwijatmoko *et al.* (2016). This hedonic test engaged 30 untrained panellists, encompassing colour, flavour, texture, aroma, and overall impression. Each parameter was assessed on a scale of 1 to 5, representing 1 (dislike very much), 2 (dislike), 3 (neutral), 4 (like), and 5 (like very much).

DNA Isolation

The preparation of BGRT samples was adapted from Barus *et al.* (2013) before the isolation of total bacterial DNA. Random BGRT samples were taken, amounting to 50 g each, and homogenised in 100 mL of sterile 0.85% NaCl (Merck) utilising a Philips HR2061 blender (Koninklijke Philips, Amsterdam, Netherlands) for 30 sec. Subsequently, each homogenised sample was centrifuged at 1,000 *g* for 5 min, and the resulting supernatants were collected. Each pellet was then subjected to total microbial DNA extraction following the protocols of the DNA extraction kit (Geneaid, Taiwan). The concentration and purity of the extracted DNA were assessed using a nanodrop (ThermoFisher Scientific, USA).

The V4 region of the 16S rDNA from different regions was amplified using the primer 515F-806R, with a primer concentration of 0.2 µM employed for each reaction. PCR products were visualised on a 2% agarose gel. Samples displaying a prominent band between 400 - 450 bp were selected, and subsequently purified using the Qiagen Gel Extraction Kit (Qiagen, Germany) for subsequent analysis. Sequencing libraries were generated following the protocols of the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA). The library quality was assessed using the Qubit@ 2.0 Fluorometer (Thermo Scientific) and the Agilent Bioanalyzer 2100 system. Ultimately, the library was sequenced on an Illumina HiSeq 2500 platform. This entire process constituted the metagenome library preparation.

Statistical analysis

All analyses were conducted in triplicate or more. The data were analysed using IBM SPSS 24.0 and Microsoft Excel. The SPSS 24.0 analysis included One-way ANOVA followed by Duncan's *post hoc* test, with a significance level set at 5%.

Results

Physico-chemical analysis

Figure 1 illustrates the measurement outcomes for reducing sugar, alcohol, titratable acidity, and pH levels across all BGRT products. The reducing sugar content displayed a consistent increase as fermentation time increased. The various packaging types employed during BGRT fermentation exhibited no significant impact on reducing sugar content. BGRT-BL showcased the highest reducing sugar content, with no significant difference observed compared to BGRT-RA. Conversely, BGRT-PB recorded the lowest reducing sugar content.

The various packaging types utilised during BGRT fermentation exhibited no significant impact on alcohol, titratable acidity, and pH levels. Both alcohol and titratable acidity contents displayed a progressive increase as fermentation time progressed. Conversely, the pH level appeared to decrease as fermentation time increased.

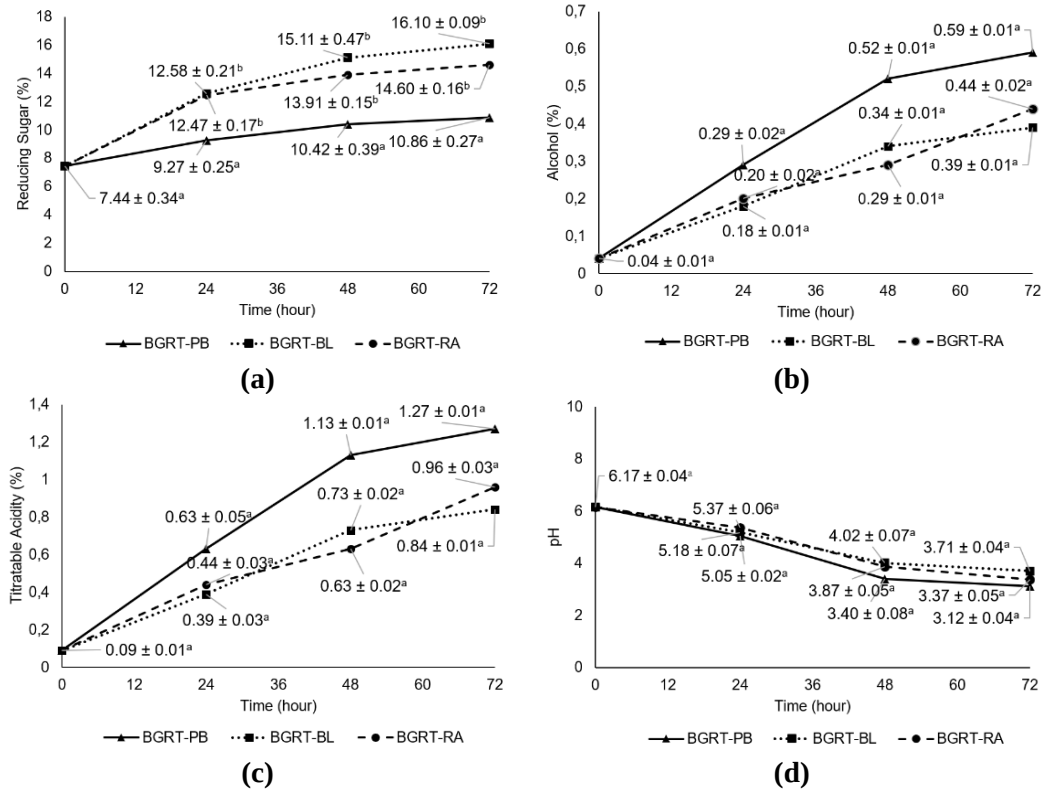

Figure 1. Results on reducing sugar **(a)**, alcohol **(b)**, titratable acidity **(c)**, and pH level **(d)** of BGRT with plastic box (BGRT-PB), banana leaf (BGRT-BL), and rose apple leaf (BGRT-RA) packaging during fermentation.

Organoleptic profile

Table 1 and Figure 2 reveal that the various types of packaging significantly impact the organoleptic profile of BGRT, excluding the colour parameter. Overall, panellists preferred BGRT-BL concerning flavour, texture, aroma, and overall impression.

Bacterial community profile

Figure 3 illustrates the disparities in bacterial communities between BGRT-PB and BGRT-BL, as revealed by NGS. The bacterial composition in both product types encompassed *Firmicutes* and *Proteobacteria*. Specifically, the BGRT-PB product comprised 91.7% *Firmicutes* and 8.3% *Proteobacteria*, while the BGRT-BL product contained 93.7% *Firmicutes* and 6.3% *Proteobacteria*. In the BGRT-PB product, *Firmicutes* was constituted by *Limosilactobacillus* at 54.3%, *Weissella* at 24%, *Pediococcus* at 12%, *Gluconobacter* at 7.5%, *Lactiplantibacillus* at 0.6%, and *Pantoea* at 0.4%. Conversely, the *Firmicutes* composition in the BGRT-BL product was distributed as follows: *Limosilactobacillus* at 45.1%, *Weissella* at 18.6%, *Pediococcus* at 23.9%, *Gluconobacter* at 5.5%, *Lactiplantibacillus* at 4.1%, and *Pantoea* at 0.6%.

Table 1. Organoleptic profile of BGRT with plastic box (BGRT-PB), banana leaf (BGRT-BL), and rose apple leaf (BGRT-RA) packaging during fermentation.

Type	Colour	Flavour	Texture	Aroma	Overall
BGRT-PB	3.68 ± 0.93[a]	2.42 ± 0.91[a]	2.92 ± 0.91[a]	3.14 ± 0.97[a]	2.83 ± 0.80[a]
BGRT-BL	3.84 ± 0.82[a]	3.37 ± 1.09[b]	3.38 ± 0.81[b]	3.69 ± 0.86[b]	3.48 ± 0.85[b]
BGRT-RA	3.78 ± 0.80[a]	3.00 ± 0.96[c]	3.16 ± 0.89[ab]	3.39 ± 0.98[a]	3.22 ± 0.82[c]

Means with different lowercase superscripts in the same column are significantly different from each other ($p < 0.05$). Score 1: dislike very much; Score 5: like very much.

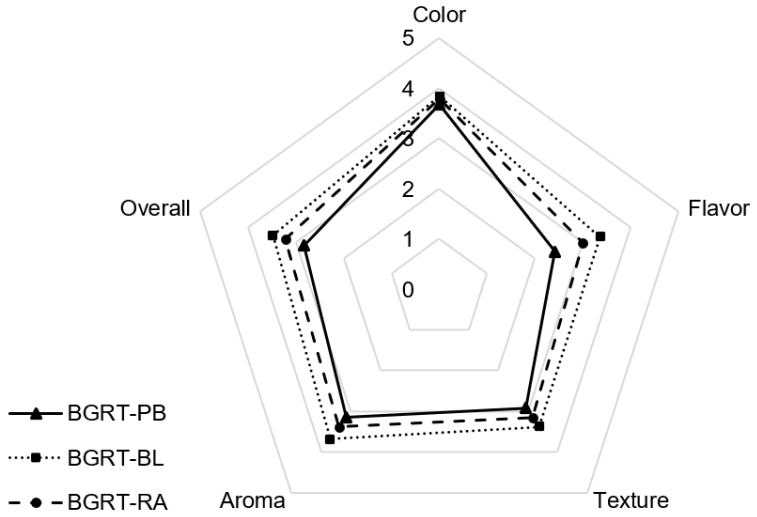

Figure 2. Organoleptic profile of BGRT with plastic box (BGRT-PB), banana leaf (BGRT-BL), and rose apple leaf (BGRT-RA) packaging during fermentation.

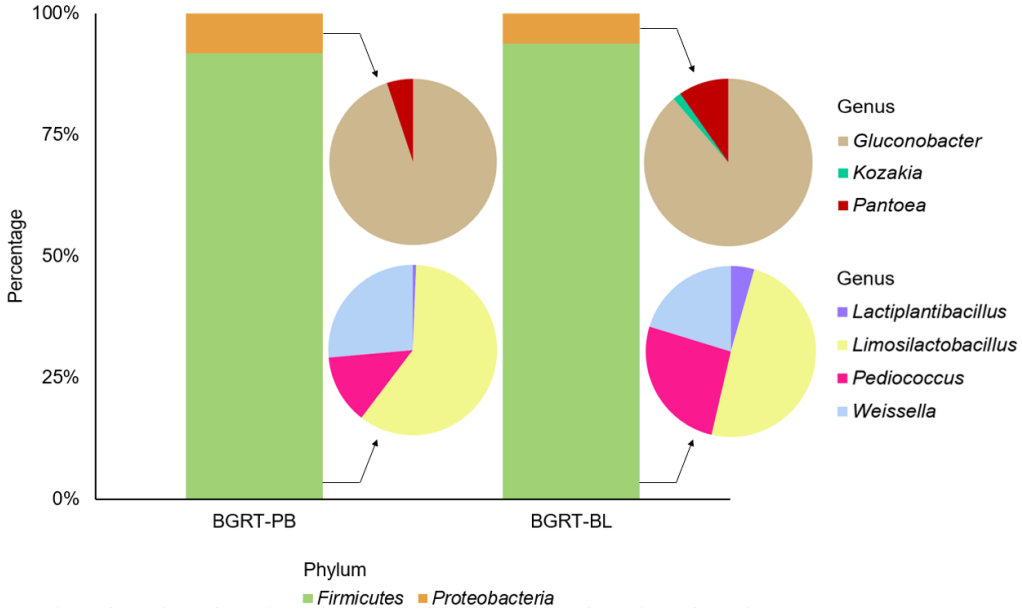

Figure 3. Bacterial community profile on BGRT with banana leaf (BGRT-BL) and plastic box (BGRT-PB) packaging.

Discussion

Indonesia boasts a diverse array of fermented food products, and among the most renowned is *tapai*. This traditional delight can be produced from various raw materials, including cassava and white sticky rice. The present work was focussed on BGRT due to its utilisation of black glutinous rice, a variety recognised for its high content of phenols, flavonoids, and notably, anthocyanins (Shen *et al.*, 2009). Anthocyanins, a subgroup of flavonoids, play a pivotal role in imparting colours to plants.

Numerous studies have highlighted the significant impact of dietary anthocyanins on health. Consumption of dietary anthocyanins has been linked to promoting a favourable gut-microbiota composition ratio, attributed to the generation of beneficial health-associated metabolites such as short-chain fatty acids (SCFA), bile acids, and phenolic acids, which in turn regulate diverse biological activities (Kapoor *et al.*, 2023; Liang *et al.*, 2023). Anthocyanins also exhibit antioxidant properties that counteract free radical activity, thereby mitigating cell damage (Hou, 2003; Fimognari *et al.*, 2008; Junka *et al.*, 2017). Additionally, a diet enriched with anthocyanin has been shown to support cognitive and neural functions in older adults, thereby contributing to an improved quality of daily life (Feng *et al.*, 2023). The present work marks the pioneering effort to comprehensively investigate the impact of various packaging types on the alteration of metabolites and bacterial community composition in BGRT products, a crucial endeavour to enhance the quality of BGRT products.

The fermentation of black glutinous rice into BGRT products resulted in an elevation of reducing sugar levels, as depicted in Figure 1, progressing from 7.44 - 10.86% to 13 - 16.1%. The findings of the present work underscore the substantial influence of packaging type on the significant increase in reducing sugar levels during fermentation. Notably, the BGRT-BL product displayed the highest reducing sugar content at 16.1%, followed by BGRT-RA at 14.6%, with a statistically significant difference from BGRT-PB at 10.86%. The variation in reducing sugar levels could have been due to the differing microorganisms present during fermentation. This observation was further supported by the contrasting abundance of microorganisms between BGRT-BL and BGRT-PB products, as assessed through NGS (Figure 3). Specifically, *Limosilactobacillus* and *Weissella* were found to be dominant in BGRT-BL at 54.3 and 24%, respectively, while *Pediococcus* at 23.9% prevailed in the BGRT-PB product. Alongside numerical variations, variations in the metabolic attributes of microorganisms also exist. Barus and Wijaya (2011) noted that even identical *Bacillus* strains could exhibit different amylase activities in *tapai*. Microorganisms generating amylase enzymes are pivotal in determining *tapai*'s reducing sugar levels (Owens, 2015; Haque *et al.*, 2020). *Bacillus* spp. with amylase-producing enzymes are abundant in *tapai* (Barus *et al.*, 2013).

Variations in the levels of reducing sugars within food products influence the sensory attributes directly. In harmony with this principle, an increase in reducing sugar content corresponds to an elevated perception of sweetness in the product (Dashdorj *et al.*, 2015). Based on the outcomes of the organoleptic assessment in the present work, the BGRT-BL product emerged as the most favoured, while the BGRT-PB product was the least favoured. Based on the panellists' evaluations, the BGRT-BL product was identified as possessing a sweeter taste and a more tender texture. This enhanced texture could have been due to the heightened activity of starch hydrolysis in BGRT-BL (Bornhorst *et al.*, 2014). This sweetness aligned with the elevated reducing sugar content in BGRT-BL. The taste of *tapai* is intrinsically intertwined with sweetness. It has been documented that a *tapai* variety having a sweet taste complemented by a subtle tang and a mild alcohol undertone obtained the highest favour among panellists (Barus and Wijaya, 2011). Furthermore, BGRT-BL products exhibited the lowest pH (0.4%) compared to BGRT-PB and BGRT-RA products.

The fermentation duration constitutes a pivotal factor contributing to the increase in alcohol content in BGRT (as illustrated in Figure 1). The alcohol content in black glutinous rice was originally 0.04%, subsequently increasing upon fermentation. Among the BGRT products, BGRT-PB displayed the highest alcohol content at 0.59%, followed by BGRT-RA at 0.44% and BGRT-BL at 0.39%. This could have been due to the conversion of simple sugars through microbial zymase enzymes during the fermentation process of black glutinous rice, resulting in the formation of alcohol (Yovani, 2019). The packaging's density influences oxygen content and the rate of alcohol evaporation during fermentation (Barus *et al.*, 2023). Oxygen can significantly impact metabolism and microbial growth. Under aerobic

conditions, *Saccharomyces* spp. convert sugar into carbon dioxide and water, whereas glucose transforms into alcohol and carbon dioxide under anaerobic conditions (Ria *et al.*, 2019).

Nevertheless, the variance in alcohol content among different packaging types during fermentation did not yield significant differences. Disparities in alcohol content within food products yield discernible effects on sensory attributes. Alcohol exhibits a fruity aroma at low concentrations yet turns pungently flavoured and aromatic at higher concentrations (Zhao *et al.*, 2020). Based on organoleptic test results, panellists favoured BGRT-BL over BGRT-PB products. Consequently, the alcohol content is a determining factor in shaping the taste of *tapai*.

The fermentation of black glutinous rice into BGRT products led to increased titratable acidity content and decreased pH levels, as depicted in Figure 1. The titratable acidity content of black glutinous rice increased from 0.09 to 0.84%, reaching 1.27% in BGRT. Meanwhile, the pH level of black glutinous rice decreased from 6.17 to a range of 3.12 to 3.71 in BGRT. Among the BGRT products, BGRT-PB exhibited the highest titratable acidity content at 1.27%, followed by BGRT-RA at 0.96% and BGRT-BL at 0.84%. Regarding pH levels, BGRT-PB also displayed the lowest pH at 3.12, followed by BGRT-RA at 3.37 and BGRT-BL at 3.71. Novelina *et al.* (2019) documented a titratable acidity content of 2.56% and a pH level of 4.53 in BGRT-PB after a 72-h fermentation period. In contrast, the present work demonstrated that BGRT-PB yielded lower titratable acidity content (1.27%) and pH level (3.12). This could have been due to variations in the types of black glutinous rice used and the type of inoculum employed.

The elevation in BGRT's titratable acidity content was likely attributed to the production of organic acids from microbial metabolism during fermentation. As Owens (2015) noted, significant generation of organic acids leads to increased titratable acidity content, concomitant with decreased pH levels. The production of organic acids can occur both pre- and post-alcoholic fermentation. Various processes contribute to the formation of organic acids, including the phosphoenolpyruvate carboxylation process yielding malic acid, the glucose oxidation process yielding gluconic acid, the alcohol oxidation process yielding acetic acid, and more (Ferreira and Mendes-Faia, 2020). Organic acids in food products are advantageous, as they safeguard against contamination by spoilage bacteria and pathogenic microorganisms (Chiang *et al.*, 2006).

Discrepancies in titratable acidity content and pH levels within food products impact the resultant sensory attributes. Organic acids, synthesised during fermentation, tend to interact with alcohol, leading to the formation of ester compounds. These ester compounds contribute distinctive aromas and flavours to *tapai* products. Among the ester compounds detected in *tapai* products, ethyl acetate stands out (Owens, 2015). Esters are also detected within the packaging utilised during fermentation. Banana leaf packaging encompasses ester compounds such as isobutyl acetate and isoamyl acetate, which impart the fragrance of bananas (Nagarajan and Chandiramouli, 2018). Ester compounds are also present in the packaging made from rose apple leaves, like phenyl ethyl benzoate and 2-phenyl ethyl 3-methyl butanoate, imparting the aroma of roses and honey (Wong and Lai, 1996). Based on the results of the organoleptic test, the BGRT-BL product emerged as the favoured choice among panellists due to its mildly tangy taste coupled with the distinctive aroma of banana leaves.

Bacterial communities within BGRT products were only identified in BGRT-PB and BGRT-BL variants. The bacterial communities were analysed based on the outcomes of organoleptic tests applied to the most and least preferred BGRT products. Within BGRT-PB and BGRT-BL products, the dominant phyla were *Firmicutes* (ranging from 91.7 to 93.7%), followed by *Proteobacteria*. Specifically, the BGRT-PB product comprised 91.7% *Firmicutes* and 8.3% *Proteobacteria*, while the BGRT-BL product comprised 93.7% *Firmicutes* and 6.3% *Proteobacteria*. Regarding the general distribution, BGRT-PB products encompassed 0.6% *Lactiplantibacillus*, 12% *Pediococcus*, 24% *Weissella*, 54.3% *Limosilactobacillus*, 7.5% *Gluconobacter*, and 0.4% *Pantoea*. Conversely, BGRT-BL products comprised 4.1% *Lactiplantibacillus*, 18.6% *Weissella*, 23.9% *Pediococcus*, 45.1% *Limosilactobacillus*, 5.5% *Gluconobacter*, 0.1% *Kozakia*, and 0.6% *Pantoea*.

The genera *Limosilactobacillus* and *Lactiplantibacillus* are recognised as a subset of amylolytic lactic acid bacteria (ALAB), capable of not only lactic acid production, but also amylase enzyme synthesis. These genera have found application in rice fermentation for producing functional foods and probiotic beverages (Jo *et al.*,

2021; Tran *et al.*, 2021). In contrast, other lactic acid bacteria (LAB) groups, like *Pediococcus*, demonstrate limited amylase enzyme production, whereas *Weissella* lacks this capability altogether (Keerthana and Narayanan, 2020; Nath *et al.*, 2021). *Pantoea*, an endophytic bacterium thriving on cereal plants, is commonly isolated from cereal food fermentation, and known for amylase enzyme production (Suman *et al.*, 2020). Acetic acid bacteria (AAB), such as *Gluconobacter* and *Kozakia*, do not possess the ability to generate amylase enzymes (Srivastava and Rani, 2019). Higher abundance of amylase-producing bacteria present in the BGRT-BL product, together with its elevated reducing sugar content and sweeter taste could have led to this composition disparity compared to BGRT-PB.

Both bacteria and yeasts play pivotal roles in alcohol production during the BGRT fermentation process. Heterofermentative LAB groups, including *Limosilactobacillus* and *Weissella*, exhibit the capacity to employ acetate kinase and phosphate acetyltransferase enzymes for the conversion of acetyl phosphate to either acetate or acetyl Co-A. Consequently, the bifunctional acetaldehyde-alcohol dehydrogenase enzyme facilitates the transformation of acetyl Co-A into ethanol (Manberger *et al.*, 2020; Verce *et al.*, 2021). In contrast, homofermentative LAB groups like *Lactiplantibacillus* and *Pediococcus* lack this ability (De Vuyst *et al.*, 2014). With the BGRT-PB product having a higher abundance of *Limosilactobacillus* and *Weissella* than the BGRT-BL product, the former exhibited high alcohol content due to this microbial composition disparity.

The BGRT-PB product demonstrated the highest titratable acidity content and the lowest pH level due to its elevated levels of LAB and AAB compared to the BGRT-BL product. BGRT-PB products had greater packaging density than BGRT-BL products, leading to more anaerobic fermentation conditions. This environment is optimal for producing organic acids, as LAB falls within the category of aerotolerant anaerobic bacteria. High oxygen content prompts the formation of reactive oxygen species (ROS), which can hinder LAB growth (Feng and Wang, 2020). Moreover, LAB are capable of generating compounds contributing to food flavour. Among these is the volatile compound 2-methyl-propanal, which imparts a malty flavour to Sabah's *tapai*.

In contrast to LAB, AAB represents aerobic bacteria capable of performing a two-step alcohol oxidation process to yield acetic acid. AAB initiates alcohol oxidation by converting alcohol into acetaldehyde through the pyrroloquinoline quinone-dependent alcohol dehydrogenase (PQQ-ADH) enzyme. Subsequently, the acetaldehyde is further oxidised into acetic acid *via* the aldehyde dehydrogenase (ALDH) enzyme. Specific AAB genera, such as *Kozakia*, even possess the ability to engage in acetic acid oxidation, resulting in the production of carbon dioxide and water. It contrasts with *Gluconobacter*, which lacks this capability (Gomes *et al.*, 2018).

BGRT products offer added value due to their having anthocyanin pigments, which confer significant health benefits to humans. Currently, research concerning the impact of anthocyanin content in BGRT products with varying types of packaging during fermentation remains scarce. Plaitho *et al.* (2013) noted that BGRT products exhibited higher anthocyanin levels post-fermentation than their initial state.

Conclusion

The type of packaging significantly influenced sugar content, organoleptic profile, and composition of BGRT bacterial community. Among the three packaging types assessed, BGRT-BL (banana leaf) was the best in comparison to BGRT-PB (plastic box) and BGRT-RA (rose apple leaf). BGRT-BL products displayed the highest proportions of *Pediococcus* and *Limosilactobacillus* genera. Therefore, further exploration on the roles played by these bacteria in shaping BGRT quality is warranted.

Acknowledgement

The present work was financially supported by the Competitive Grant from Atma Jaya Catholic University of Indonesia.

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