

# THE POTENTIAL OF GLUTINOUS RICE TAPE ADDED WITH *LACTOBACILLUS PLANTARUM* DAD-13 AND *SACCHAROMYCES BOULARDII* AS A PROBIOTIC FOOD

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## ABSTRACT

Glutinous rice tape has lactic acid bacteria (LAB) as the dominant microorganism apart from mold and yeast. However, adding *Lactobacillus plantarum* Dad-13 and *Saccharomyces boulardii* can increase its potential as a probiotic food. This study is aimed to determine the effect of using glutinous rice varieties and the addition of probiotic cells on the number of BAL and yeast, levels of anthocyanins, antioxidant activity, as well as the preference level of glutinous rice tape produced. A completely randomized design with a factorial pattern was used in this study. The first factor was glutinous rice varieties (white and black), while the second was the probiotic inoculum type (*Lactobacillus plantarum* Dad 13 and *Saccharomyces boulardii*) which were added simultaneously or individually. The result show that adding the probiotic cells simultaneously or individually in the production of white and black glutinous rice tape increased the LAB amount by 2 log cycles and yeast by 1 log cycle. The addition of *Saccharomyces boulardii* together with *Lactobacillus plantarum*, or individually, resulted in higher anthocyanin levels in black glutinous rice tape than white glutinous tape. The antioxidant activity of white glutinous rice tape was improved by the addition of probiotic cells. For the black glutinous rice tape, the antioxidant activity (87.57-88.61 %RSA) was higher than the activity of white glutinous rice tape (15.58-51.22 %RSA). Furthermore, adding *Lactobacillus plantarum* simultaneously with *Saccharomyces boulardii* increased the preference level on aroma, color, taste, texture, and overall, and produced the white glutinous rice tape that the panelists most favored.

**Keywords:** black glutinous rice; *Lactobacillus plantarum* Dad-13; probiotic glutinous rice tape; *Saccharomyces boulardii*; white glutinous rice

## ABSTRAK

Tape ketan yang beredar di masyarakat pada umumnya belum mengandung sel probiotik. Penambahan *Lactobacillus plantarum* Dad-13 dan *Saccharomyces boulardii* dapat meningkatkan potensi tape ketan sebagai pangan probiotik. Tujuan penelitian mengetahui pengaruh penggunaan varietas beras ketan dan penambahan *Lactobacillus plantarum* Dad-13 dan *Saccharomyces boulardii* terhadap jumlah bakteri asam laktat (BAL) dan yeast, kadar antosianin dan aktivitas antioksidan, serta tingkat kesukaan oleh panelis pada tape ketan probiotik yang dihasilkan. Penelitian ini menggunakan rancangan acak lengkap pola faktorial. Faktor pertama ialah varietas beras ketan (putih dan hitam) dan faktor kedua ialah jenis inokulum probiotik (*Lactobacillus plantarum* Dad 13 dan *Saccharomyces boulardii*) yang ditambahkan secara bersamaan maupun individual. Hasilnya menunjukkan bahwa penambahan *Lactobacillus plantarum* Dad-13 secara bersamaan dengan *Saccharomyces boulardii*, atau sendiri pada pembuatan tape ketan putih dan ketan hitam meningkatkan jumlah BAL sebesar 2 log cycles, sedangkan penambahan *Saccharomyces boulardii* secara bersamaan dengan *Lactobacillus plantarum* Dad-13, atau individual meningkatkan jumlah yeast sebesar 1 log cycle. Penambahan *Saccharomyces boulardii* secara bersamaan dengan *Lactobacillus plantarum* Dad-13, atau individual menghasilkan kadar antosianin pada tape ketan hitam. Penambahan sel probiotik meningkatkan aktivitas antioksidan pada tape ketan putih. Aktivitas antioksidan tape ketan hitam (87,57-88,61 %RSA) jauh lebih tinggi daripada aktivitas tape ketan putih (15,58-51,22 %RSA). Penambahan *Lactobacillus plantarum* Dad-13 secara bersamaan dengan *Saccharomyces boulardii* meningkatkan tingkat kesukaan terhadap aroma, warna, rasa, tekstur, dan keseluruhan, dan menghasilkan tape ketan putih maupun ketan yang paling disukai oleh panelis.

**Kata kunci:** ketan hitam; ketan putih; *Lactobacillus plantarum* Dad-13; *Saccharomyces boulardii*; tape ketan probiotik

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## INTRODUCTION

Probiotic foods are known to have beneficial effects on consumers. WHO/FAO (2006) defined probiotics as live microorganisms that will offer health benefits to users, provided they are consumed in sufficient quantities ( $> 6-7 \log$  CFU/g). To ensure the beneficial effects on consumers, the current criteria for probiotics must have the following characteristics: resistance to gastric acidity, bile acid resistance, adhesion to mucus and epithelial cells, antimicrobial activity against pathogenic microorganisms, co-aggregates with pathogens, and bile salt hydrolase activity to fight (resistant) digestive tract complications (Kassaa, 2017).

Based on various strains of lactic acid bacteria (LAB), several *Lactobacillus*, *Bifidobacterium*, and only one yeast species, *Saccharomyces boulardii* are recognized as the main probiotic microorganisms (Kitazawa et al., 2014). Furthermore, bacterial groups, especially *Lactobacillus plantarum* Dad-13 isolated from curd, have shown their potential as probiotics. This includes providing preventive effects such as antidiarrheal and immunomodulatory agents in male rats treated with enteropathogenic treatment (Tari et al., 2016) and reducing the population of *Escherichia coli* and non-*E. coli* coliform bacteria in school-aged children in Indonesia (Rahayu et al., 2021), as well as improving the metabolic syndrome in diabetic rats (Wulandari, 2021). Furthermore, the LAB can produce high folic acid, an essential element in preventing infant defects (Purwandhani et al., 2017). Meanwhile, the interaction of *Saccharomyces boulardii* with the innate immune system has recently opened up new therapeutic potential in cases of intestinal infections and other pathologies associated with dysbiosis, such as inflammatory diseases (Czerucka and Rampal, 2019). In fact, during the Covid-19 pandemic, *Saccharomyces boulardii* has been reported to have a positive (healthy) effect on the host (Pourhossein and Moravejolahkami, 2020).

However, application efforts, especially probiotic yeasts in Indonesia, are still limited. This country is known for having fermented food products that are used as a vehicle for the growth and development

of probiotic cells, including glutinous rice tape, both white and black. The glutinous rice tape was selected as a probiotic cell carrier agent because it can be easily made, favored by the public, and consumed daily. Black glutinous rice tape is known to have functional food potential because it is rich in phytochemical compounds in the form of anthocyanins as antioxidant agents. Consumption of black glutinous rice tape up to a certain amount can prevent metabolic syndrome. Respondents who consumed  $\leq 11.5$  grams of black glutinous rice tape daily had a higher proportion of metabolic syndrome (82.1%). The proportion of the non-metabolic syndrome was greater in respondents who consumed black glutinous rice tape  $>11.5$  grams per day (77.2%) (Fauziyah et al., 2018). Therefore, the probiotic cells of lactic acid bacteria and *Saccharomyces boulardii* are a required ingredient during the manufacture of tape. This study aimed to determine the effect of adding *Lactobacillus plantarum* Dad-13 and *Saccharomyces boulardii* on the amount of LAB and yeast, anthocyanin levels, antioxidant activity of glutinous rice tape, and their preference level by the panelists.

## MATERIALS AND METHOD

### Materials and tools.

Black and white glutinous rice obtained from Mirota Supermarket in Sleman, Yogyakarta, was used in this study. The lactic acid bacteria used as a source of probiotics were *Lactobacillus plantarum* Dad-13 obtained from the Center for Food and Nutrition Studies (CNFS), Gadjah Mada University, Yogyakarta, Indonesia. Meanwhile, *Saccharomyces boulardii* with the brand Now foods ORI USA were purchased through the online shop Tokopedia. MRS Agar was used as a medium for calculating LAB and PDA as a medium for counting the number of yeast cells. This study also used KCl buffer 0.025 pH 1, sodium acetate buffer 0.4 M pH 4.5, pure methanol, HCL for anthocyanin analysis, pure methanol liquid, and DPPH methanol (0.2M) for antioxidant activity analysis. The main tools used were UV-Vis spectrophotometry (Shimadzu UV mini1240), laminar airflow, Petri dishes, vortex (Maxi Mix II type 37600), and glassware.

### Inoculum preparation.

Yeast inoculum was prepared by crushing 4 grains of NKL yeast (10 g) and filtering. Fine yeast was added to 15 g of finely roasted rice flour, and 1.6 g of yeast was used for every 100 g of cooked glutinous rice. The preparation of *Lactobacillus plantarum* Dad-13 was performed using 1 g of its freeze-drying granules ( $2.10^9$  cells) and 19 g of roasted rice flour. Also, 1 g of its starter was used for 100 g of cooked glutinous rice. The yeast was prepared by mixing 1 capsule of (0.28 g containing  $5.10^9$  cells) *S. boulardii* with 27.72 g of roasted rice flour. Furthermore, 1 g of yeast starter was used for every 100 g of cooked glutinous rice.

### The process of making probiotic glutinous rice tape.

The method begins with sorting glutinous rice (1 kg), washing it seven times, and soaking it in well water (1.3 L) for 7 hours. It was rewashed, cooked for 25 minutes using a rice cooker, and produced about 1,400 g of glutinous rice. After cooling of 1-2 hours, 100 g of cooked glutinous rice were added to 6 g of sugar and inoculated with 1 g of NKL yeast, 1 g of *Lactobacillus plantarum* Dad-13, and 1 g of *Saccharomyces boulardii*. Starter inoculation or inoculum according to the experimental design, namely control (only NKL yeast), NKL + *Lactobacillus plantarum* Dad-13, NKL + *Saccharomyces boulardii*, and NKL + a combination of *Lactobacillus plantarum* Dad-13 with *Saccharomyces boulardii*, were used both for cooked white and black glutinous rice. Fermentation was performed for 2 days for white glutinous rice tape and 3 days for black glutinous rice tape (from results of the preliminary study).

### Analysis.

Analysis of the research conducted on this probiotic glutinous rice tape includes the number of lactic acid bacteria and yeast (Niamah, 2017), anthocyanin levels (Lee et al., 2005), antioxidant activity determined by the DPPH method (Li et al., 2007), and preference level (Kartika, et al., 1987). The test of preference level includes aroma, color, taste, texture, and overall. This preference level test was conducted with the participation of 30

untrained panelists (laboratory technicians and students of the Agricultural Product Technology Study Program, Mercu Buana University Yogyakarta). The organoleptic test of this glutinous rice tape uses 5 levels of preference scale, namely 1 = Very dislike, 2 = dislike, 3 = slightly like, 4 = like, and 5 = very like.

### Experimental design and data analysis.

This study employed a factorial, completely randomized design (CRD) with two treatment factors and two replications. The first factor is the type of glutinous rice (white and black), and the second factor is the addition of inoculum types (NKL yeast, NKL yeast + *Lactobacillus plantarum* Dad-13, NKL yeast + *Saccharomyces boulardii*, NKL yeast + *L. plantarum* Dad-13 + *S. boulardii*). The data obtained were evaluated using the ANOVA statistical method with SPSS software version 22.0, and if there was a significant difference between treatments, Duncan's Multiple Range Test ( $p < 0.05$ ) was used.

## RESULTS AND DISCUSSION

### The number of lactic acid bacteria and yeast.

Table 1 shows the results of calculating the number of lactic acid bacteria and yeast on white glutinous rice tape after two days of fermentation and on black glutinous rice tape after three days. Tape quality testing is carried out when the two products reach their optimal maturity. According to the data, adding LAB (*Lactobacillus plantarum* Dad-13) and yeast (*S. boulardii*) can increase the total amount of LAB and yeast at the end of the fermentation of white and black glutinous rice tape.

The use of NKL yeast inoculum resulted in the amount of LAB and yeast on white and black glutinous rice tape,  $4.4 - 4.5 \times 10^6$  CFU/g and  $3.7 - 3.9 \times 10^5$  CFU/g, respectively. After adding the LAB and yeast probiotic cells, the amount of LAB and yeast either separately or simultaneously on white and black glutinous rice tapes ranged from  $4.7 - 8.04 \times 10^8$  CFU/g for LAB and  $4.9 - 7.4 \times 10^7$  for yeast. Comparing NKL alone, the inclusion of LAB and yeast could increase 100 times or 2 log cycles from  $10^6$  to  $10^8$  CFU/g for LAB and from

10<sup>5</sup> to 10<sup>7</sup> CFU/g for yeast. The two types of raw materials, white and black glutinous rice used, show almost the same population. Therefore, these materials can be used to make probiotic tape once combined with LAB (*Lactobacillus plantarum* Dad-13) and yeast (*S. boulardii*). The table also reveals that the maximal amounts of

LAB and yeast were produced by this combination. These findings also point to synergistic growth between the two added probiotic cells. In general, the amount of LAB at the end of fermentation qualifies as a probiotic food with a minimum LAB content of 10<sup>6</sup>-10<sup>7</sup> CFU/g (WHO/FAO, 2006).

**Table 1.** The Number of LAB and Yeast of Glutinous Rice Tape Type with the Addition of Probiotic Cells

Type of probiotic addition	Amount of BAL (CFU/g)		Amount of yeast (CFU/g)	
	Type of glutinous rice			
	White	Black	White	Black
NKL	4.4×10 <sup>6</sup>	4.5×10 <sup>6</sup>	3.9×10 <sup>5</sup>	3.7×10 <sup>5</sup>
NKL + <i>Saccharomyces boulardii</i>	5.5×10 <sup>6</sup>	6.8×10 <sup>6</sup>	4.9×10 <sup>7</sup>	5.3×10 <sup>7</sup>
NKL + <i>Lactobacillus plantarum</i> Dad-13	4.7×10 <sup>8</sup>	7.2×10 <sup>8</sup>	4.8×10 <sup>7</sup>	4.5×10 <sup>7</sup>
NKL + <i>Saccharomyces boulardii</i> + <i>Lactobacillus plantarum</i> Dad-13	6.24×10 <sup>8</sup>	8.0×10 <sup>8</sup>	7.1×10 <sup>7</sup>	7.4×10 <sup>7</sup>

The values in the table are the mean of two replicates and two batches

**Anthocyanin levels and antioxidant activity.**

Table 2 presents the average anthocyanin levels and antioxidant activity of probiotic tape from glutinous rice varieties with the addition of inoculum types. In general, the average anthocyanin content in black glutinous rice tape (2.35 mg/100 g) was relatively higher than that of white glutinous rice tape (0.63 mg/100 g). The addition of *Saccharomyces boulardii* simultaneously with *Lactobacillus plantarum* Dad-13 or separately resulted in higher anthocyanin levels in black glutinous rice tape than in white glutinous rice tape. The anthocyanin levels of black glutinous rice are not much different from the results of the research by Fauziyah et al (2018). The results showed the average anthocyanin content in black glutinous rice tape on the 3rd to 5th day of fermentation was 3.02 mg/100 g. Furthermore, this study's average anthocyanin content was 3.73 times that of white glutinous rice tape. This is owing to the higher anthocyanin concentration of black glutinous rice raw material than white glutinous rice. According to Indrasari et al. (2010), the anthocyanin content in Cihorang rice (white rice) with a milling degree of 100% was 0.26 mg/100 g, while the brown rice variety BH390-MR-11-1-1-6 with a milling degree of 80% and 100% were 2.02 mg/100 g and 2.01 mg/100 g,

respectively. The average reduction in anthocyanin content during the cooking process from milled rice with 80% and 100% milling degrees to cooked rice was about 81% and 83%, respectively. Sompong et al. (2011) reported anthocyanin levels in black rice (glutinous) ranged from 109.52 - 256.61 mg/100 g, while in brown rice, they ranged from 0.33 to 1.39 mg/100 g. Furthermore, cyanidin 3-glucoside and peonidin 3-glucoside were confirmed as dominant anthocyanins in black rice varieties, with contents ranging from 19.4 to 140.8 mg/100 g dry weight and 11.1–12.8 mg/100 g dry weight, respectively. Anthocyanins present in plants are glycosides linked to sugar components (Ávila et al., 2009). The first step in the breakdown and absorption of anthocyanins in the body is the hydrolysis of anthocyanin glycosides (Keppler and Humpf, 2005). Several species of lactic acid bacteria have glucosidase enzyme activity and contribute to the hydrolysis of dietary glycosides (Ávila et al., 2009). The β-glucosidase enzyme is an enzyme that is trapped in the cell wall and has extracellular activity. It is produced by the bacteria *L. plantarum* Mut 7 (Suhartatik et al., 2013), *Pediococcus pentosaceus* N11.16 (Suhartatik et al., 2014), a subspecies of *Lactobacillus plantarum*, *Lactobacillus casei* LC-01 and *L. casei* IFPL7190 (Ávila et al., 2009).

**Table 2.** Anthocyanin Content and Antioxidant Activity of Glutinous Rice Tape Type with the Addition of Probiotic Cells

Type of probiotic addition	Anthocyanin content (mg/100 g)		Antioxidant activity (%RSA)	
	Type of glutinous rice			
	White	Black	White	Black
NKL	0,85 ± 0,00 <sup>a</sup>	1,90 ± 0,11 <sup>abc</sup>	15,58 ± 0,36 <sup>a</sup>	87,57 ± 3.20 <sup>e</sup>
NKL + <i>Saccharomyces boulardii</i>	0,41 ± 0,08 <sup>a</sup>	3,13 ± 0,13 <sup>c</sup>	20,95 ± 0,24 <sup>b</sup>	87,99 ± 4.17 <sup>e</sup>
NKL + <i>Lactobacillus plantarum</i> Dad-13	0,62 ± 0,08 <sup>a</sup>	1,46 ± 0,19 <sup>ab</sup>	32,93 ± 0,33 <sup>c</sup>	88,02 ± 8.12 <sup>e</sup>
NKL + <i>Saccharomyces boulardii</i> + <i>Lactobacillus plantarum</i> Dad-13	0,62 ± 0,08 <sup>a</sup>	2,92 ± 0,48 <sup>bc</sup>	51,22 ± 1,09 <sup>d</sup>	88,61 ± 3.24 <sup>e</sup>

Numbers followed by different letter notations in the same column are significantly different (p<0.005)

Table 2 shows that the addition of the inoculum did not significantly affect the anthocyanin levels in white glutinous rice tape. Meanwhile, the content of black glutinous rice was significantly affected (p<0.05) and adding *S. boulardii* increased the anthocyanin levels compared to adding *L. plantarum* Dad-13. The increase in anthocyanin levels in the black glutinous rice tape could be due to the higher β-glucosidase activity produced by *S. boulardii* than that of *L. plantarum* Dad-13. This enzyme helps the process of hydrolysis of glycosides into sugars and their aglycones.

The antioxidant activity of white glutinous rice tape is not affected by anthocyanin levels. Although, it can be induced by enzyme activity, the number of probiotic cells, and microorganisms from NKL. Table 2 also shows that adding inoculum did not significantly affect anthocyanin levels (0.41-0.85 mg/100 g) in white glutinous rice tape but greatly increased its antioxidant activity. Adding *L. plantarum* Dad-13 with *S. boulardii* on white glutinous rice tape either simultaneously or separately increased antioxidant activity compared to control glutinous rice tape. The increase in antioxidant activity can be due to the action of *L. plantarum* Dad-13 and *S. boulardii*, which can potentially help the degrading of anthocyanin content and total phenol to increase antioxidant activity. According to Fauziyah et al. (2018), the highest activity of black glutinous rice tape at 70.2% with a total phenol of 73.38 mg/100 g and anthocyanins (flavonoids) of 2.57 mg/100 g was achieved on the 3rd day of fermentation. The combination of *L. plantarum* Dad-13 and *S.*

*boulardii* provided the maximum antioxidant activity in white glutinous rice tape (51.22%), followed by *L. plantarum* Dad-13 (32.93%), *L. plantarum* Dad-13 (20.95) %, and NKL (15.58%). Table 1 shows that the magnitude of this antioxidant activity is in line with the size of the total cells of lactic acid bacteria and yeast in white glutinous rice tape.

Meanwhile, the antioxidant activity of black glutinous rice tape ranged from 87.57-88.61 (%RSA). This was higher than that of white glutinous rice tape (15.58-51.22%RSA) and was not affected by the addition of inoculum type. The high antioxidant activity of black glutinous rice tape may be due to a combination of anthocyanin levels and compounds fermented by black glutinous rice tape. According to Datta et al. (2017), the extracellular fraction of the *S. cerevisiae* var. *boulardii* cultures was rich in polyphenolic metabolites such as vanillic acid, cinnamic acid, phenyl ethyl alcohol (rose oil), erythromycin, amphetamine and vitamin B6. This resulted in the strain having a 6-10-fold more significant antioxidant potential judged by the DPPH assay. Moreover, Avila et al. (2009) reported that with malvidin-3-glucoside as a substrate, aglycones would be fermented into gallic acid, homogentisic, syringic, p-coumaric acid, sinapic, and DMB propionic acid. This also included other compounds not identified by cell-free extracts of *Bifidobacterium lactis* BB-12, *Lactobacillus plantarum* IFPL722, *L. casei* LC-01, *L. acidophilus* LA-5. These bacteria also showed β-glucosidase enzyme activity. According to Parrella

et al. (2012), the probiotic yeast *S. boulardii* could use fermented goods to develop, preserve the stability of lactic acid bacterial strains while stored and boost the antioxidant characteristics of the final fermented product. The development of probiotic yeast in combination with LAB seems to increase the stability of these microorganisms. Furthermore, *L. plantarum* was previously found capable of degrading hydroxycinnamic and hydroxybenzoic acids (gallic and protocatechuic acid) through phenolic acid decarboxylation and reduction processes (Rodríguez et al., 2008). Gallic acid, for

example, is decarboxylated to form pyrogallol, a potent antioxidant.

**Preference level.**

The panelists' preference for probiotic glutinous rice tape was determined using an organoleptic test. Aroma, color, taste, texture, and the overall probiotic glutinous rice tape produced were the quality attributes evaluated. Table 3 shows the preference test results on white and black glutinous rice tape with the addition of the inoculum type.

**Table 3.** Preference Level of Glutinous Rice Tape Type with the Addition of Probiotic Cells

Type of glutinous rice tape	Type of probiotic addition	Test parameters				
		Aroma	Color	Taste	Texture	Overall
White	NKL	3,43±0,73 <sup>a</sup>	3,67±0,70 <sup>b</sup>	3,90±0,89 <sup>b</sup>	2,87±1,14 <sup>ab</sup>	3,63±0,85 <sup>b</sup>
	NKL + S.b	3,87±0,73 <sup>ab</sup>	3,73±0,74 <sup>b</sup>	3,77±0,77 <sup>b</sup>	3,43±1,04 <sup>c</sup>	3,77±0,82 <sup>b</sup>
	NKL + L.p	3,53±0,68 <sup>b</sup>	3,50±0,68 <sup>b</sup>	3,60±0,93 <sup>b</sup>	3,23±0,77 <sup>bc</sup>	3,50±0,73 <sup>a</sup>
	NKL + S.b + L.p	4,47±0,63 <sup>c</sup>	4,20±0,81 <sup>c</sup>	4,40±0,72 <sup>c</sup>	4,13±0,073 <sup>d</sup>	4,60±0,56 <sup>c</sup>
Black	NKL	3,60±0,81 <sup>ab</sup>	3,03±0,85 <sup>a</sup>	2,90±0,76 <sup>a</sup>	2,70±1,02 <sup>a</sup>	3,17±0,59 <sup>a</sup>
	NKL + S.b	3,80±0,61 <sup>ab</sup>	3,53±0,63 <sup>b</sup>	2,80±1,13 <sup>a</sup>	3,10±0,85 <sup>abc</sup>	3,17±0,91 <sup>a</sup>
	NKL + L.p	3,90±07 <sup>b</sup>	3,57±0,63 <sup>b</sup>	2,50±1,04 <sup>a</sup>	2,90±1,03 <sup>ab</sup>	3,17±0,87 <sup>a</sup>
	NKL + S.b + L.p	4,47±0,63 <sup>c</sup>	4,43±0,73 <sup>c</sup>	3,77±0,50 <sup>b</sup>	4,30±0,75 <sup>d</sup>	4,27±0,69 <sup>c</sup>

Numbers followed by different letter notations in the same column are significantly different (p<0.005); S.b= *Saccharomyces boulardii*, L.p= *Lactobacillus plantarum* Dad-13; 1=dislike very much, 2=dislike, 3=a little like, 4=like, 5= like very much

**Aroma.** Glutinous rice tape with the addition of *Lactobacillus plantarum* Dad-13 and *Saccharomyces boulardii* simultaneously provided the highest aroma value (like to very like) with a rating of 4.47 for white glutinous rice tape and 4.47 for black glutinous rice tape. The addition of *Lactobacillus plantarum* Dad-13 can also significantly increase the preference level for the aroma of both white (3.53) and black glutinous rice tape (3.90) compared to the addition of NKL yeast separately (3.43-3.60). This addition produced the right amount of lactic acid and provided the conditions for growing bacteria, yeast, and mold from NKL. Therefore, it yields volatile compounds (aroma) favored by the panelists, such as, ethanol, acetic acid, and esters. The ethanol content of white glutinous rice and black glutinous rice in this study

was 1.30% and 1.79%, respectively (Yulianto and Pujimulyani, 2021). Utamingdyah et al. (2022) reported that tape from various types of rice (non-glutinous) inoculated with *Lactobacillus plantarum* Dad-13 produced lactic acid levels, acetic acid, and ethanol, respectively, at 0.21-0.53%, 0.14-0.35%, and 0.81-0.91%. Cronk et al. (1979) showed that *Amylomyces rouxii*, separately or in combination with different yeasts, yielded a higher range of alcohols, including isobutanol (2-methyl-1-propanol), 2-methyl-1-butanol and isoamyl alcohol (3-methyl - 1-butanol).

**Color.** The most preferred colors of white and black glutinous rice tape are the results of adding *Lactobacillus plantarum* Dad-13 and *Saccharomyces boulardii* simultaneously. For the

black glutinous rice tape, the most preferred color is black because the anthocyanin content and yellowness value ( $b^*$ ) are higher than the control tape (Yulianto and Pujimulyani, 2021). Meanwhile, the preferred white glutinous rice tape color tends to be yellowish-white and slightly lighter. In addition to the anthocyanin content, especially in black glutinous rice tape, the growth of yeasts such as *Saccharomyces cerevisiae*, *Candida*, and *Hansenulla* can affect the color intensity of glutinous rice tape.

**Taste.** The tape has a sweet, alcoholic, and slightly sour taste. Sweet taste is produced from sugar formed primarily from the hydrolysis of starch by amylase produced by fungi. Furthermore, alcohol is formed from the fermentation of glucose by yeast, and lactic acid is formed from the fermentation of sugar to lactate by lactic acid bacteria. Meanwhile, acetic acid is produced by the oxidation of alcohol by acetic acid bacteria. The fungi, yeast, and bacteria were on the tape from the NKL inoculum and the addition of the probiotic cells (*Lactobacillus plantarum* Dad-13 and *Saccharomyces boulardii*). Table 3 shows that the white and black glutinous rice tapes with the probiotic cells were favorites of the panelists with scores of 4.40 (like to very like). The sugar, alcohol, and acid ratio may have influenced the decision of this taste preference level. Furthermore, the total sugar content, ethanol, and pH of white and black glutinous rice tape with the inclusion of the two probiotic cells were 7.73%, 3.20%, 1.30%, 1.79%, 5.04, and 4.57, respectively (Yulianto and Pujimulyani, 2021).

**Texture.** The simultaneous addition of LAB (*Lactobacillus plantarum* Dad-13) with yeast (*S. boulardii*) increased the panelists' preference for texture features on white and black glutinous rice tape. Moreover, NKL inoculum containing fungi, especially those with high activity of amylase, plays a vital role in the hydrolysis of starch into sugar, which also impacts the softness of the glutinous rice tape texture produced. The combination of the LAB and yeast also gave a certain softness level, which the panelists may prefer. Meanwhile, only LAB or yeast alone did not provide the texture the panelists liked. This could be because the inclusion of the two probiotic

cells was sufficient to enable conditions for the growth of amylolytic fungus, resulting in a texture of glutinous rice tape that was neither too soft nor too hard. Panelists prefer probiotic glutinous rice tape with a relatively soft and watery texture.

**Overall.** The results of the level of preference test of the overall quality attributes of white and black glutinous rice tapes generally have a rhythmic relationship with the results of the preference level for each feature on aroma, color, taste, and texture quality. Furthermore, white glutinous rice tape and white glutinous rice were added with both probiotics simultaneously, and the panelists preferred this. The tape with the two types of probiotic cells had the highest overall preference rating (4.6 = like to very like), followed by *S. boulardii*, the control (NKL), and finally *L. plantarum* Dad-13. Moreover, adding only *L. plantarum* Dad-13 to the white glutinous rice tape production decreased the preference level of the panelists compared to the control. This was due to sufficient lactic acid formed, which causes the sour taste and is less liked by the panelists. Meanwhile, the panelists' preference for black glutinous rice tape that was only inoculated with *L. plantarum* Dad-13 or *S. boulardii* was not significantly different from the tape with NKL separately (control).

## CONCLUSION

The addition of *Lactobacillus plantarum* Dad-13 simultaneously with *Saccharomyces boulardii*, or separately in the production of white and black glutinous rice tape increased the number of lactic acid bacteria by 2 log cycles and adding *Saccharomyces boulardii* simultaneously with *Lactobacillus plantarum* Dad-13 increased yeast count by 1 log cycle than control (NKL). The addition of *Saccharomyces boulardii* simultaneously with *Lactobacillus plantarum* Dad-13 or separately resulted in higher anthocyanin levels in black glutinous rice tape than in white glutinous rice tape. The antioxidant activity of black glutinous rice tape (87.57-88.61 %RSA) was higher than that of white glutinous rice tape 15.58-51.22 (%RSA). The addition of *Lactobacillus plantarum* Dad-13 simultaneously with *Saccharomyces boulardii* increased the preference

level for aroma, color, taste, texture, and overall, as well as resulted in white glutinous rice tape and glutinous rice, which the panelists most favored. Glutinous rice tape added with *Lactobacillus plantarum* Dad-13 and *Saccharomyces boulardii* has the potential as a probiotic food.

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