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*Research article*

# Bio-active compounds, their antioxidant activities, and the physicochemical and pasting properties of both pigmented and non-pigmented fermented de-husked rice flour

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**Abstract:** The aim of this study was to determine the effect of both the Solid State Fermentation (SSF) technique and the use of *Rhizopus oligosporus* on the physicochemical changes of fermented de-husked rice flour. Three varieties of de-husked rice, i.e., Mentik Wangi Susu (non-pigmented), red Cempo Merah, and black Jowo Melik (pigmented) were fermented using *Rhizopus oligosporus.* Fermentation was performed at room temperature with a fermentation time of 0, 24, 48, and 72 hours. The analyzed parameters were proximate composition, bio-active compounds, and pasting profile. The results showed an increase in flour pasting profile, ash, protein, and fat content after the fermentation. The total availability of the total phenolic content (TPC) and antioxidant capacity were also increased. The highest TPC (0.37 mg GAE/g) and antioxidant capacity (1.43 mg TEAC/g) were obtained in the Jowo Melik variety at 72 hours of fermentation. In contrast, anthocyanin and carbohydrate contents decreased as fermentation time increased. The highest anthocyanin content of 0.53 mg/g (after 24-hour fermentation) was obtained in the Jowo Melik variety. In conclusion, 72-hour-fermented black rice flour (Jowo Melik) has a higher potential to be developed as a functional food.

**Keywords:** anti-oxidant; de-husked rice; fermentation; rhizopus oligosporus; pigmented rice

## 1. Introduction

Brown rice consists of de-husked rice grains, in which the inedible hull is removed but the bran and germ remain intact. In general, the organoleptic sensory value de-husked rice, including color, taste, and texture, is favored less. However, it is well known that de-husked rice has a higher nutritional value and more bio-active compounds compared to milled rice. The content of the bioactive compounds of de-husked rice varies depending on their variety, genetic factors, and environmental conditions. De-husked rice can be categorized based on their pigmented outer layer as brown, red, black, and sometimes violet. The content of pigments on the surface of rice influences the content of bio-active compounds [1]. Black and red rice contain phenolic content which is twice as high as brown rice (from which white rice is derived) [2].

The majority of phenolic acid (68.3%) is covalently attached to the compounds found within cell wall structures, such as cellulose, hemicellulose, and lignin [3], which means their antioxidant activity is not optimal. Several techniques could be applied to increase the antioxidant activity, including breaking the phenolate bonds with physical, chemical, and enzymatic methods. Chemical methods pose a risk of retaining chemical residues in the product, and physical methods are not a suitable choice either. The physical method is economically feasible, but it could cause a decrease in several phenolic compounds. The enzymatic method is mainly preferable due to its safety and feasibility, with Solid State Fermentation (SSF) as one of the preferred processes.

During the fermentation process, microbe fermentation produces enzymes that can release phenolic compounds from the substrate. The success of the SSF technique in increasing antioxidant activity depends on the type of bond between the phenolics and other compounds, the enzymes produced by microbes, and the fermentation conditions. *R. oligosporus* fungi is commonly applied in SSF techniques due to their effectivity in increasing the number of bio-active compounds [4–6]. *R. oligosporus* produces xylanase, β-glycosidase, and α-amylase enzymes during fermentation [6,7]. Glucosidase enzymes hydrolyze glycosidic bonds to release phenolic aglycone groups and glycosides containing disaccharides and oligosaccharides.

The high water content problem lies in the fermented de-husked rice, which causes the rice to have a shorter shelf-life. The fermented rice also has a different shape and texture, due to the tight bond with the fungi which is difficult to break. This problem could be addressed by turning the rice into a more processed product: flour. Fermented rice flour is a potential solution to create products, which are not yet available on the market, with the high nutritional value and bio-active compounds of pigmented and non-pigmented de-husked rice. Therefore, the aim of this study was to determine the effect that SSF using the fungus *R. oligosporus* has on the increase in bio-active compounds and antioxidant activity of pigmented and non-pigmented fermented de-husked rice flour and to characterize the fermented de-husked rice flour as a functional food.

## 2. Materials and methods

### *2.1. Data collection*

This research was conducted from March 2019 to October 2019. The main materials used in this study were 3 varieties of de-husked rice, namely non-pigmented Mentik Wangi Susu from local farmers, a pigmented red Cempo Merah variety from Sawangan village in the Sawangan sub-district,

and a black pigmented Jowo Melik variety from Kleteran village in the Grabak sub-district, Magelang regency, Yogyakarta, Indonesia. The white rice (Mentik Wangi Susu) and red rice (Red Cempo Merah) were harvested on 25 February 2019, while the black rice (Jowo Melik) was harvested on 28 February 2019.

### *2.2. Fermentation of de-husked rice (white, red, and black)*

The freshly harvested rice was dried under the sun and stored at room temperature. Then, the dry paddy grain was milled (de-husked) using a grinding machine (a Yanmar HW-60A, Japan) to obtain de-husked rice. Fermentation was carried out with a single culture of *R. oligosporus* strain F74 from the Indonesian Culture Collection (ICC) Research Center for Biology, LIPI in Cibinong. The fermentation method applied to the de-husked rice was derived from a modified method from Hayat et al. [8]. The de-husked rice (200 g) was washed and steamed with distilled water at 100 ℃ for 10 minutes. It was steamed and cooled in an environment at room temperature. The number of inoculated spores was 1% ($1.5 \times 10^6$) of the de-husked rice's (white, red, and black) weight. Then the rice was fermented in aerobic conditions at room temperature with fermentation times of 0, 24, 48, and 72 hours and packed in aerobic conditions into a 18 cm × 14 cm polyethylene container that was 1.5 cm thick and which had been perforated along 2 cm × 2 cm segments with holes 1–2 mm in diameter. It was then incubated at room temperature. The fermented rice was then freeze dried (using a Labconco, USA freeze drier) for 72 hours. The rice was mashed using a blender and filtered using a 100 mesh sieve; then it was stored at a temperature of −18 ℃ for further analysis. The analyzed parameters in this research were proximates, total anthocyanin, total phenolic compounds, antioxidant capacity, and pasting profile with two replications.

### *2.3. Determination of proximates and total anthocyanin content*

Analysis of moisture content, ash, protein, fat, and carbohydrate contents was achieved through the standard procedures of the Association of Official Analytical Chemists (AOAC) [9]. Total protein content was obtained by multiplying the nitrogen content with a protein conversion factor of 5.95, using the Kjeldahl method. The total carbohydrate content was determined by the difference method:

$$\text{Carbohydrate} = 100\% - (\text{moisture content} + \text{ash} + \text{protein} + \text{fat}) \quad (1)$$

Analysis of the total anthocyanin content was performed using the procedure by Giusti and Wrolstad [10]. Samples (1 g d.b) were dispersed with 10 mL of previously acidified methanol (methanol: 1 M HCl (85: 15, v/v), then were vortexed for 10 seconds and centrifuged at 3000 rpm for 20 minutes at room temperature. The process was repeated once. Analysis of anthocyanin was performed with a differential pH method. 100 μL sample extracts were each mixed with 5 mL potassium chloride buffer with a pH of 1.0 and with sodium acetate buffer with a pH of 4.5. The mixture was vortexed and then allowed to stand for 15 minutes. The absorbance of the samples was measured at 510 and 700 nm against distilled water using a UV-Vis spectrophotometer at the same time.

$$\text{Anthocyanin content (mg/g sample)} = A \times MW \times DF \times 100/(\varepsilon \times 1) \quad (2)$$

A = [(A510 − A700) at pH 1.0 − (A510 − A700) at pH 4.5]; MW = molecular weight of cyanidin-3glucoside (449.2); DF =dilution factor; ε = molar absorption of cyanidin-3-glucoside (26.900); 1 =

the equation presented above assumes a pathlength of 1 cm (width).

### *2.4. Total phenolic content and antioxidant capacity*

The preparation of extract samples was carried out using a modification of Reddy's method [11]. A sample (1 g) and 10 mL methanol (1: 10) were added together and then stirred for 3 hours in an orbital shaker (from Technico, India). The supernatant was separated using Whatman 42 filter paper after centrifugation at 3000 rpm for 20 minutes, and then it was stored at −18 ℃ for further analysis.

The analysis of total phenolic content (TPC) was carried out using the procedure by Razak et al. [4] and the Folin-Ciocalteu method. A methanolic extract sample of 1 mL was mixed with 5 mL Folin-Ciocalteu reagent and 4 mL of 7.5 % sodium carbonate solution for 2 hours at room temperature. The absorbance of the samples was measured at 765 nm using a UV-Vis spectrophotometer. The results were expressed as milligrams of gallic acid equivalent (GAE) per gram of sample (mg GAE/g sample). DPPH analysis was carried out by following a modified method from Brand-Williams [12]. Methanolic samples of 100 μL were reacted with 3.9 mL of DPPH solution in methanol (100 μmoL/L), and then they were incubated at room temperature for 2 hours. Absorbance of the samples was measured using a UV-Vis spectrophotometer at a wavelength of 515 nm. The control was a mixture of methanol and DPPH. Anti-oxidant activity was expressed as mg equivalent to Trolox.

### *2.5. Pasting profile*

The pasting profile of each fermented de-husked rice flour (white, red, and black) sample was measured using a Rapid Visco Analyzer (RVA; from Newport Scientific, Warriewood, Australia) following the procedure of the American Association for Clinical Chemistry (AACC) [13]. The moisture content of the sample was first measured. 3 g of the sample was put into a canister and 25 mL of distilled water was added. Samples were maintained at 50 ℃ for 1 minute and were then heated to 95℃ at a rate of 6 ℃/min. Then, temperature was reduced to 50 ℃ at the same 6 ℃/min rate and was then maintained at 50 ℃ for 5 min. The recorded parameters were pasting temperature, peak viscosity (PV), trough viscosity (TV), breakdown, setback, and peak time (PT).

### *2.6. Data Analysis*

The presented data were the average ± SD with two replications. Data were analyzed by Analysis of Variance (ANOVA) using IBM statistical software SPSS version 25 (Chicago, IL, USA). If there were differences in the ANOVA results, then a further Duncan's test was performed at 5% level.

## 3. Results and discussion

### *3.1. Proximate content*

Table 1 shows the proximate composition of non-pigmented and pigmented de-husked rice flour during the fermentation process. The results of this study showed an increase in moisture content up to 48-hour fermentation, i.e. 5.56%–7.50% in the Mentik Wangi Susu, 4.22%–7.57% in the Cempo Merah, and 4.96%–7.15% in the Jowo Melik. The increase in moisture content during the fermentation

process was apparently due to microbial activities, in which starch was converted into sugar, alcohol, and acids [14]. According to Chinsamran et al. [15], fermentation in rice starch causes the formation of holes in the starch granules. The increasing fermentation time causes a rough surface and enlarged pores [14]. On the other hand, Chu et al. [16] mentioned that the physical structure will become loose with moisture and more porous, thus contributing to water absorption capacity and retention. The moisture content found in this study was smaller than 10% on average. This means that de-husked rice flour can be stored for a long period. According to the Indonesian National Standard [17], the maximum moisture content of rice flour is 13%.

**Table 1.** Proximate composition of fermented de-husked rice (dry weight).

| Fermentation time (h) | Moisture content (%) | Ash (% ) | Protein (% ) | Fat (% ) | Carbohydrate (%) |
|---|---|---|---|---|---|
| Mentik Wangi Susu | | | | | |
| 0 | $5.56 \pm 1.52^{abcd}$ | $2.05 \pm 0.01^{bc}$ | $10.19 \pm 0.20^{a}$ | $3.88 \pm 0.31^{bcd}$ | $78.32 \pm 1.96^{bc}$ |
| 24 | $5.99 \pm 0.59^{bcde}$ | $2.04 \pm 0.07^{bc}$ | $10.16 \pm 0.41^{a}$ | $3.85 \pm 0.45^{bcd}$ | $77.97 \pm 1.21^{bc}$ |
| 48 | $7.50 \pm 0.24^{ef}$ | $2.33 \pm 0.02^{d}$ | $12.18 \pm 0.36^{defg}$ | $5.17 \pm 0.41^{f}$ | $72.82 \pm 0.79^{a}$ |
| 72 | $8.73 \pm 2.15^{f}$ | $2.06 \pm 0.17^{bc}$ | $10.72 \pm 1.56^{ab}$ | $4.39 \pm 0.99^{cde}$ | $74.10 \pm 4.80^{a}$ |
| Cempo Merah | | | | | |
| 0 | $4.22 \pm 1.43^{ab}$ | $2.18 \pm 0.08^{cd}$ | $11.58 \pm 0.22^{bcde}$ | $3.03 \pm 0.24^{a}$ | $78.98 \pm 1.65^{c}$ |
| 24 | $3.81 \pm 0.30^{a}$ | $2.27 \pm 0.03^{d}$ | $12.72 \pm 0.32^{fg}$ | $3.48 \pm 0.29^{ab}$ | $77.71 \pm 0.90^{bc}$ |
| 48 | $7.57 \pm 2.07^{ef}$ | $1.96 \pm 0.21^{ab}$ | $12.36 \pm 0.73^{efg}$ | $4.51 \pm 0.05^{de}$ | $73.61 \pm 2.53^{a}$ |
| 72 | $6.37 \pm 0.62^{cde}$ | $2.01 \pm 0.08^{ab}$ | $12.95 \pm 0.66^{g}$ | $3.86 \pm 0.22^{bcd}$ | $74.81 \pm 1.32^{a}$ |
| Jowo Melik | | | | | |
| 0 | $4.96 \pm 1.36^{abc}$ | $1.91 \pm 0.03^{ab}$ | $10.92 \pm 0.66^{abc}$ | $3.81 \pm 0.27^{bc}$ | $78.40 \pm 0.51^{bc}$ |
| 24 | $4.97 \pm 0.25^{abc}$ | $1.85 \pm 0.05^{a}$ | $11.21 \pm 0.22^{bcd}$ | $4.17 \pm 0.35^{cde}$ | $77.80 \pm 0.27^{bc}$ |
| 48 | $7.15 \pm 0.19^{def}$ | $1.92 \pm 0.02^{ab}$ | $11.89 \pm 0.21^{cdef}$ | $4.79 \pm 0.30^{ef}$ | $74.26 \pm 0.32^{a}$ |
| 72 | $6.33 \pm 0.39^{cde}$ | $1.86 \pm 0.16^{a}$ | $11.70 \pm 0.54^{bcde}$ | $4.32 \pm 0.34^{cde}$ | $75.80 \pm 1.24^{ab}$ |

Values with different letters in the same column showed significant differences ($p < 0.05$); ($n = 2$).

The ash contents of Mentik Wangi Susu and Cempo Merah were significantly increased up to 48-hour fermentation, while it was not significantly different for Jowo Melik. The increase in ash content during the fermentation is apparently due to the phytase activation, which reduces phytic acid in de-husked rice [18]. According to Liang et al. [19], high levels of phytic acid in de-husked rice are distributed in the aleurone and bran layers. Minerals such as phosphorus, iron, and calcium are also in the aleurone layer and form phytate, while zinc is scattered throughout the inner endosperm as phytate [20]. Phytic acid was degraded during the fermentation process by the phytase enzyme produced by *R. oligosporus*. This result is in accordance with Suresh et al. [21], who found a phytase capacity in rice bran fermented by *R. oligosporus*. This study showed that the Mentik Wangi Susu variety with 48 hours of fermentation contained a higher ash content than the Cempo Merah and Jowo Melik varieties. A similar result was reported by Oduguwa et al. [22], wherein the ash content of de-husked rice fermented by *R. oryzae* also increased during the fermentation process.

As for the protein content, at 0–48-hour fermentation, there was an increase of 10.19%–12.18% in Mentik Wangi Susu, of 11.58%–12.36% in Cempo Merah, and of 10.92%–11.89% in Jowo Melik. An increase of protein content was found in the fermented de-husked rice flour and was apparently

due to the metabolic capacity of the fungi during the fermentation process. Benabda et al. [23] observed an increase in protease capacity during fermentation. This protease capacity caused degradation of complex protein–phytic acid bonds [21]. This increased protein content was also expected from the growth of the microbial cell biomass during the fermentation [22]. The Cempo Merah variety, after a 72-hour fermentation, achieved the highest protein content increase. Meanwhile, the Mentik Wangi Susu and Jowo Melik varieties experienced a decrease in protein content after 48-hour fermentation. This decrease was likely due to the degradation of protein molecules at the end of the 72-hour fermentation. According to Handoyo and Morita [24], protein is degraded into amino acids by fungi at a certain stage of fermentation to support their growth.

There was an increase in fat content up to 48 hours of fermentation of 3.88%–5.17% in the Mentik Wangi Susu, 3.03%–4.51% in the Cempo Merah, and 3.81%–4.79% in the Jowo Melik. The Mentik Wangi Susu at 48-hour fermentation had a higher fat content than the Cempo Merah and the Jowo Melik. According to a previous study, the differences in the fat content could be influenced by rice varieties [25]. The fat content was significantly increased ($p < 0.05$) at 48 hours of fermentation for the Mentik Wangi Susu and Cempo Merah varieties. The increase was likely due to the production of lipids by the fungus. On the other hand, the decrease in fat content at the end of the 72-hour fermentation was apparently due to the degradation of lipids by the very fungus that produced them. A similar finding was reported by Oliveira et al. [26], wherein a significant decrease in fat content was observed in rice bran fermented for 48 hours, and this reduction was thought to be from the utilization of lipids by *R. oryzae* to synthesize phospholipids as constituent compounds of the fungus cell membrane. The difference in fermentation time in correlation with the fat content decrease was due to the different types of substrates' availability and fermentation conditions.

The carbohydrate content of the three tested varieties was decreased up to 48-hour fermentation. The carbohydrate content decreased from 78.32% to 72.82% in the Mentik Wangi Susu, from 78.98% to 73.61% in the Cempo Merah, and from 78.40% to 74.26% in the Jowo Melik. The decrease is presumably because the fungus will degrade carbohydrates into simple sugars for its growth. According to Surojanametakul et al. [14], during fermentation, microbial activity occurs which will convert the starch in rice into sugar, alcohol, and acid. This result was mainly due to the carbohydrate degradation by fungi into simple sugars as a source of carbon to support its growth. This result was consistent with the research [27] which reported that the sugar content in rice bran fermented by *R. oryzae* continued to decrease during the fermentation process. A similar finding was reported by Ribeiro et al. [28] who recognized that there was a decrease in carbohydrates in rice bran fermented by *R. oryzae*. The proximate composition in this study was different from previous studies, possibly due to differences in particle size, varieties, and different growth conditions.

### *3.2. Bio-active compounds*

Changes in the total phenolic content during the fermentation process of non-pigmented and pigmented de-husked rice are presented in Figure 1. The results showed that the longer the fermentation process continued, the more the total phenolic content increased. These results are consistent with those previously reported [29]. They also showed that the highest total phenolic content was obtained at 72-hour fermentation for each rice variety. The Jowo Melik variety produced the highest total phenolic content of 0.37 mg Gallic Acid Equivalent (GAE)/g, followed by the Cempo Merah at 0.33 mg GAE/g, and then the Mentik Wangi Susu at 0.29 mg GAE/g.

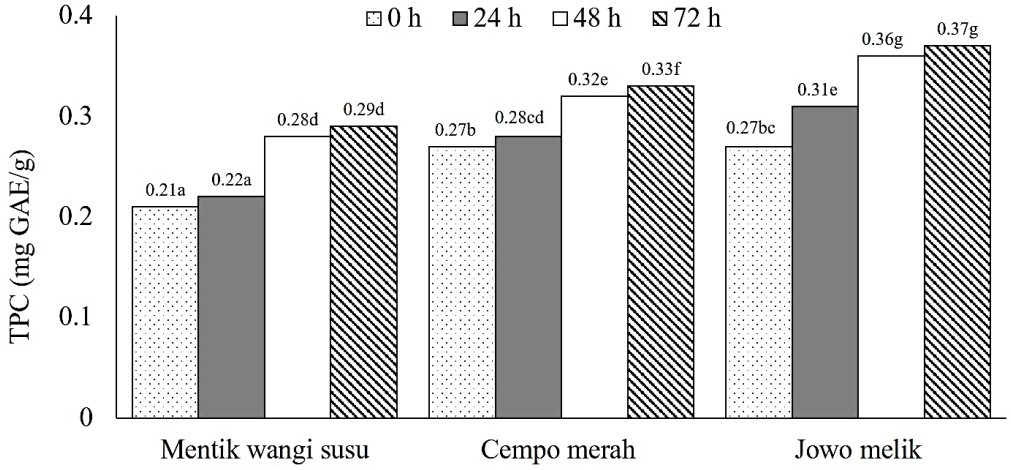


**Figure 1.** Total Phenolic Content (TPC) of fermented de-husked rice flour. Values followed by different letters showed significant differences ($p < 0.05$); n = 2.

Non-pigmented rice showed the least TPC in its native and fermented stages compared to the pigmented varieties. The increase in total phenolic content was caused by the degradation of the bond between lignocellulosic compounds and phenolic acids, which makes nore phenolic acid freely available. The degradation is carried out by some enzymes synthesized by fungi during the fermentation process in order to obtain polysaccharides, the constituent of the lignocellulosic compounds, to support its growth [30,31]. Several of the enzymes synthesized by fungi during the fermentation process were xylanase, β-glycosidase, and α-amylase [6,7]. In previous studies, the total phenolic content in fermented, red de-husked rice was 206.53 ± 8.45 mg GAE per 100 g dry weight. This amount is 5 times greater than the phenolic content of unfermented rice [29].

The total anthocyanin content (TAC) showed a significant increase ($p < 0.05$) at 24-hour fermentation, i.e. 0.01–0.02 mg/g in the Mentik Wangi Susu, 0.04–0.07 mg/g in the Cempo Merah, and 0.42–0.53 mg/g in the Jowo Melik (Figure 2). The increase in anthocyanin content was due to the metabolic capacity of fungi during the fermentation process, which released the bonds of anthocyanin compounds. According to Zhang et al. [32], more than 99.5% of anthocyanin compounds in black rice bran were distributed in the free form but less than 0.5% were in the bonded form. This study suggested that *R. oilgosporus* released all bonds of anthocyanin compounds at the beginning of 24-hour fermentation. After 24-hour fermentation, anthocyanin content was decreased. This was due to the susceptibility of free-form anthocyanin to degradation by the β-glucosidase enzyme that is synthesized by *R. oligosporus* during the fermentation process. The result is in agreement with the research [33], which reports that the concentration of anthocyanin in rice bran decreases during the fermentation process with the increasing capacity of β-glucosidase. β-glucosidase degraded the anthocyanin bond by cutting the glycosidic bonds, releasing glucose and aglycone (anthocyanidin).

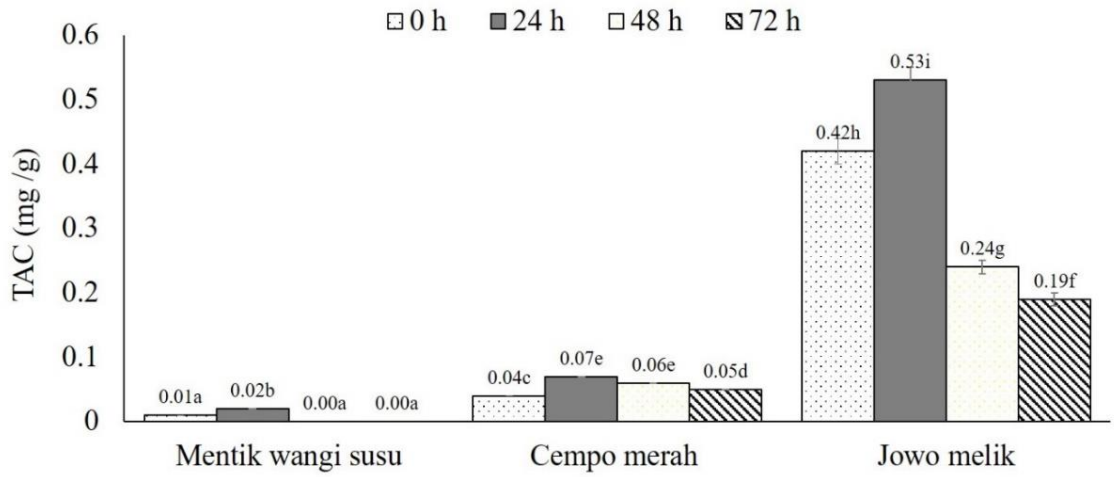


**Figure 2.** Total Anthocyanin Content of fermented de-husked rice flour. Values followed by different letters showed significant differences ($p < 0.05$); $n = 2$.

The result showed that total anthocyanin content was higher in the Jowo Melik variety of rice compared to the Cempo Merah and Mentik Wangi Susu varieties. This is in accordance with the results reported [34] which found that the anthocyanin compounds in black rice were 35 times higher than those in brown rice. Meanwhile, white rice was found to have a very low amount of anthocyanin compounds [35].

The antioxidant activities of fermented de-husked rice observed in this study using the DPPH method are presented in Figure 3. The results showed that fermentation time significantly affected the increase in antioxidant activity in the three rice varieties ($p < 0.05$). The highest antioxidant capacity was obtained by the Jowo Melik variety at 72-hour fermentation (1.43 mg TEAC/g), followed by the Cempo Merah variety (0.77 mg TEAC/g), and then the Mentik Wangi Susu variety (0.37 mg TEAC/g). The increase in antioxidant activity was thought to be affected by the large number of phenolic compounds that became free during the fermentation process, especially at 72 hours of fermentation (Figure 3). This was supported by a study [36] reporting that antioxidant activity is positively correlated with the presence of phenolic compounds. According to some studies, the free hydroxyl group of phenolic compounds and flavonoids was responsible as antioxidants [37,38]. The hydroxyl group plays a role in reducing radical action (peroxynitrite and peroxyl) by donating electrons or hydrogen atoms to the radicals and creating stable radical flavonoids [37].

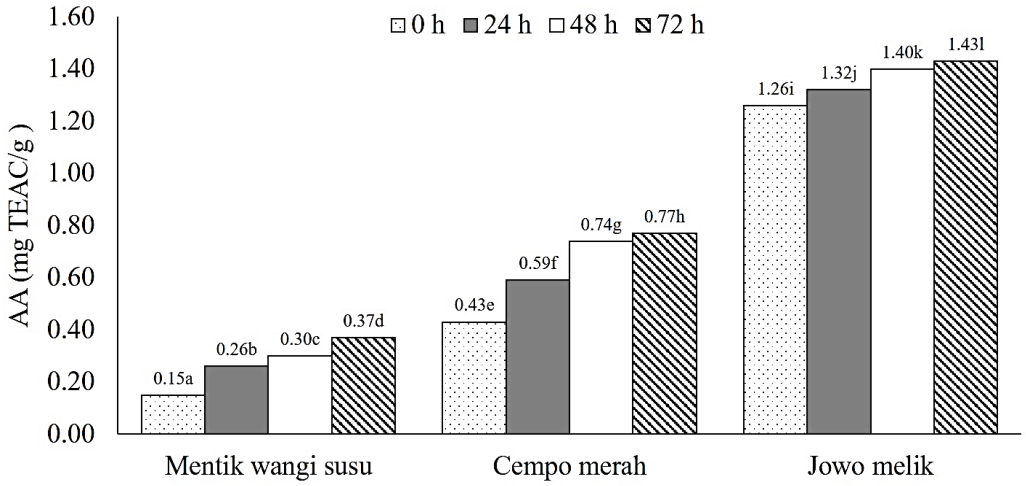


**Figure 3.** Antioxidant Activity (AA) of fermented de-husked rice flour. Values followed by different letters showed significant differences ($p < 0.05$); $n = 2$.

Duncan's test results (level 5%) showed significant differences in the antioxidant activities of the three rice varieties which correlates the relationship of the antioxidant activity with the color of the rice. According to Anggraini et al. [39], the color of rice is influenced by the presence of anthocyanin compounds. The increasing color intensity shows the positively correlated increase in the anthocyanin content and its antioxidant activity. It was found that the Jowo Melik variety contained the highest amount of anthocyanin of those tested, followed much further behind by the Cempo Merah and the Mentik Wangi Susu varieties (Figure 2). This study also demonstrated that the highest antioxidant capacity was in the Jowo Melik variety compared to the other varieties. The results of previous work on unfermented and fermented, red de-husked rice showed antioxidants of 4.50–14.33 mg QE [29]. Different results were found in this study because the rice samples used were grown in different rice fields and with different harvesting times. Therefore, differences in total phenolic, anthocyanin, and antioxidant compounds are associated with different rice genotypes. As described in Butsat [40], the difference in total phenolic and antioxidant content in rice is due to different growing locations and genetic diversity [41].

### *3.3. Pasting profile*

The effect of the fermentation process on the pasting profile of non-pigmented and pigmented de-husked rice flour is presented in Figure 4. The results showed that the fermentation length used for the treatment affected the pasting profile. Pasting profiles include the Peak Viscosity (PV), Trough Viscosity (TV), and Final Viscosity (FV) fundamental parameters and the Breakdown viscosity (PV-TV), Setback viscosity (PV-PV) and total Setback (PV-TV) derived parameters. The Cempo Merah variety showed an additional decrease at 72 hours of fermentation. The fluctuation of pasting profiles during the fermentation process was determined by various factors. One of the reasons for the decreasing values in peak viscosity was the high amylose content and the low amylopectin content [42]. In a study by Patindol et al. [43], when the amylopectin peak area increases, amylose decreases, and it is possible that the

amylose molecule is located in the amorphous layer of starch grains so that it is more susceptible to enzymatic attack and other chemical changes than amylopectin located in the crystal layer. Amylose s able to inhibit the development of starch granules through the formation of amylose-lipid complexes while amylopectin can absorb water quickly causing the development of starch granules [42]. In addition, according to Surojanametakul et al. [14], peak viscosity, trough, breakdown, final viscosity, and setback will decrease in the fermentation process due to damage and changes in the starch structure.

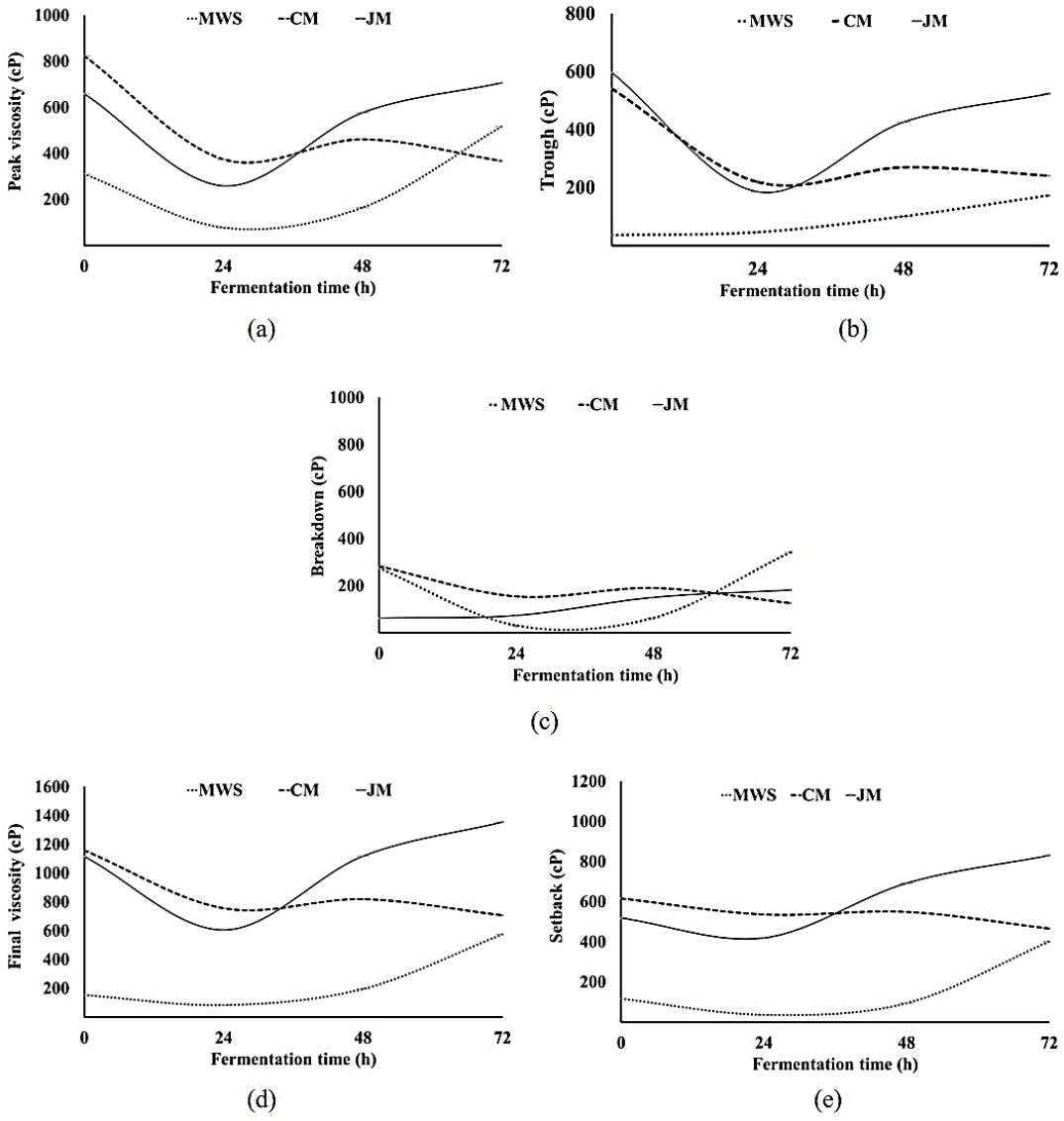


**Figure 4.** Pasting profile of fermented de-husked rice flour. Parameters for different rice varieties and periods of fermentation, (a) peak viscosity, (b) trough viscosity, (c) breakdown viscosity, (d) final viscosity, (e) setback viscosity. (n = 2). MWS = Mentik Wangi Susu; CM = Cempo Merah; JM = Jowo Melik.

It was suspected that, during the fermentation process, the amylose content decreased and amylopectin content increased. This was supported by the results in Olanipekun et al. [44], which reported that the amylose content decreased with increased fermentation time, and conversely, the

amylopectin content increased. A similar finding was reported by Balogun et al. [45] in velvet beans after fermentation with *R. oligosporus*.

In this study, the highest peak viscosity at 72-hour fermentation was obtained in the Jowo Melik variety at 706.75 ± 87.50 cP, followed by the Mentik Wangi Susu at 517 ± 110.31 cP, and the Cempo Merah at 367.50 ± 143.20 cP. High peak viscosity values indicated the ability to develop bigger starch granules before the viscosity breakdown [46].

The results of the breakdown viscosity test showed that the Mentik Wangi Susu variety had the highest breakdown at 72-hour fermentation with a value of 343.5 ± 189.98 cP, followed by the Jowo Melik variety at 182 ± 25.99 cP, and the Cempo Merah variety at 126.50 ± 60.11 cP. The high breakdown value of starch paste from the Mentik Wangi Susu indicated that the starch paste was increasingly unstable during the cooking process, which caused the loss of viscosity. High breakdown viscosity values tend to be undesirable due to the cohesive products [44]. On the other hand, the breakdown viscosity of starch paste from the Jowo Melik and Cempo Merah varieties was not significantly different ($p > 0.05$).

High breakdown viscosity values are related to shorter gelatinization peak times due to the earlier onset of starch gelatinization. This caused starch granules to withstand the pressure because of increased swelling in the granules for longer periods. The development of large granules caused a weakening bond in the granules which lead to breakdown [44]. This observation is reinforced with the results in this study, in which the peak viscosity of Mentik Wangi Susu paste was shorter than that of the Jowo Melik and Cempo Merah varieties (Table 2). In addition, the breakdown viscosity is also influenced by the amylose content. Varavinit et al. [47] reported that the rice starch paste, with a high breakdown viscosity value, had a low amylose content.

The Jowo Melik variety at 72-hour fermentation had the highest final viscosity value of 1355.50 ± 196.42 cP, followed by the Cempo Merah at 707.25 ± 244.61 cP, and the Mentik Wangi Susu at 575 ± 43.38 cP. Oloyede et al. [48] reported that the final viscosity value is positively correlated to the ability of starch to form a thick paste or gel during cooking and cooling. This result indicated that the Jowo Melik rice flour which was fermented for 72 hours could form a more stable gel compared to the Cempo Merah and Mentik Wangi Susu varieties.

The setback viscosity values reflected the retrogradation process during cooling [44]. Starch paste with a high setback value underwent a greater retrogradation. The results showed starch paste from 72-hour fermentation of the Jowo Melik produced a higher setback value of 830.75 ± 134.91 cP compared to the Cempo Merah at 466.25 ± 161.55 cP and the Mentik Wangi Susu at 401.5 ± 37.13 cP. This reveals that the starch paste from the 72-hour fermented Jowo Melik tended to harden more during cooling as a result of the reformation of the gelatinized starch crystal structure.

The interaction of proteins with starches such as amylose and open-chain amylopectin was reported to slow the retrogradation of starch paste through hydrogen bonds. This may influence the differences in the results of the setback viscosity values of the Mentik Wangi Susu, the Cempo Merah, and the Jowo MelikThe setback viscosity value of the Cempo Merah tended to decrease with increasing fermentation time, in contrast to that of the Mentik Wangi Susu and the Jowo Melik. This was presumably due to the increased protein content in the Cempo Merah variety during fermentation, resulting in more protein–starch complex molecules (Table 2). Synthesis of amylase enzymes by *R. oligosporus* during fermentation was also suggested as one cause of the breakdown of hydrogen protein–starch bonds, as fungi require simple sugars for their growth.

**Table 2.** Pasting profile of fermented de-husked rice flour.

| Fermentation time (h) | PV (cP) | TV (cP) | BD (cP) | FV (cP) | SB (cP) | Peak time (minutes) | PT (°C) |
|---|---|---|---|---|---|---|---|
| Mentik Wangi Susu | | | | | | | |
| 0 | 310.75 ± 52.25$^{bcd}$ | 36.25 ± 4.35$^{a}$ | 274.50 ± 47.92$^{de}$ | 153.00 ± 38.70$^{ab}$ | 116.75 ± 34.36$^{a}$ | 7.47 ± 0.00$^{a}$ | 83.54 ± 1.09$^{c}$ |
| 24 | 77.50 ± 4.80$^{a}$ | 46.50 ± 3.51$^{a}$ | 31.00 ± 8.16$^{a}$ | 83.25 ± 4.99$^{a}$ | 36.75 ± 8.50$^{a}$ | 8.47 ± 0.00$^{b}$ | 80.56 ± 0.62$^{ab}$ |
| 48 | 164.75 ± 17.73$^{ab}$ | 101.00 ± 6.93$^{ab}$ | 63.75 ± 24.62$^{ab}$ | 195.75 ± 2.63$^{ab}$ | 94.75 ± 9.54$^{a}$ | 8.70 ± 0.07$^{bc}$ | 80.29 ± 1.16$^{a}$ |
| 72 | 517.00 ± 110.31$^{defg}$ | 173.50 ± 79.77$^{ab}$ | 343.50 ± 189.98$^{e}$ | 575.00 ± 43.38$^{bc}$ | 401.50 ± 37.13$^{b}$ | 8.46 ± 0.62$^{b}$ | 82.14 ± 2.12$^{bc}$ |
| Cempo Merah | | | | | | | |
| 0 | 823.75 ± 344.58$^{h}$ | 541.00 ± 376.45$^{d}$ | 282.75 ± 38.92$^{de}$ | 1157.75 ± 743.93$^{de}$ | 616.75 ± 367.52$^{bc}$ | 9.57 ± 0.20$^{de}$ | 89.38 ± 1.67$^{d}$ |
| 24 | 374.50 ± 137.263$^{bcde}$ | 219.50 ± 74.18$^{ab}$ | 155.00 ± 63.29$^{bc}$ | 756.00 ± 287.49$^{cd}$ | 536.50 ± 213.73$^{b}$ | 9.56 ± 0.58$^{de}$ | 92.86 ± 1.02$^{efg}$ |
| 48 | 461.00 ± 243.76$^{cdef}$ | 270.25 ± 124.48$^{bc}$ | 190.75 ± 119.31$^{cd}$ | 819.50 ± 419.99$^{cd}$ | 549.25 ± 295.52$^{b}$ | 9.25 ± 0.12$^{cd}$ | 93.18 ± 1.50$^{fg}$ |
| 72 | 367.50 ± 143.20$^{bcde}$ | 241.00 ± 83.15$^{abc}$ | 126.50 ± 60.11$^{abc}$ | 707.25 ± 244.61$^{cd}$ | 466.25 ± 161.55$^{b}$ | 9.23 ± 0.09$^{cd}$ | 93.66 ± 1.17$^{g}$ |
| Jowo Melik | | | | | | | |
| 0 | 659.50 ± 13.87$^{fgh}$ | 597.00 ± 10.52$^{d}$ | 62.50 ± 3.70$^{ab}$ | 1116.00 ± 2.31$^{de}$ | 519.00 ± 11.02$^{b}$ | 10.10 ± 0.09$^{e}$ | 91.93 ± 0.22$^{efg}$ |
| 24 | 259.75 ± 117.08$^{abc}$ | 18.00 ± 79.33$^{ab}$ | 73.75 ± 38.22$^{abc}$ | 605.75 ± 294.70$^{bc}$ | 419.75 ± 216.58$^{b}$ | 9.60 ± 1.04$^{de}$ | 92.66 ± 1.27$^{efg}$ |
| 48 | 577.00 ± 99.64$^{efg}$ | 426.25 ± 66.03$^{cd}$ | 150.75 ± 33.81$^{bc}$ | 1118.50 ± 210.36$^{de}$ | 692.25 ± 144.52$^{bc}$ | 9.31 ± 0.06$^{cd}$ | 91.55 ± 0.60$^{ef}$ |
| 72 | 706.75 ± 87.50$^{gh}$ | 524.75 ± 61.51$^{d}$ | 182.00 ± 25.99$^{bcd}$ | 1355.50 ± 196.42$^{e}$ | 830.75 ± 134.91$^{c}$ | 9.38 ± 0.08$^{d}$ | 91.04 ± 0.02$^{de}$ |

Note: Peak viscosity (PV), trough viscosity (TV), breakdown viscosity (BD), setback viscosity (SB), peak time, and pasting temperature (PT) values with different letters in the same column showed significant differences ($p < 0.05$); $n = 2$.

## 4. Conclusion

This study showed that de-husked rice flour of the Jowo Melik variety with a fermentation time of 24 hours obtained the highest total anthocyanin content, which then was decreased up to 72 hours of fermentation. Meanwhile, the fermentation time (0–72 hours) of the Jowo Melik resulted in a lower proximate composition (ash, protein, and fat) compared to the Cempo Merah and the Mentik Wangi Susu varieties. The pasting profile of the non-pigmented Mentik Wangi Susu variety showed the highest breakdown, indicating that the starch paste was increasingly unstable during the cooking process, causing it to lose its viscosity compared to pigmented de-husked rice (the Jowo Melik and Cempo Merah varieties). Therefore, the best result was obtained by the black de-husked rice flour (the Jowo Melik), with a fermentation time of 72 hours producing the highest total phenolic compounds. The high antioxidant capacity of the Jowo Melik showed that its thickness and flour properties remained stable as it was heated.

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## Conflict of interest

No authors have any conflicts of interest.

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