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Inovasi Fermentasi: Meningkatkan Kandungan Antioksidan pada Bekatul Menggunakan *Aspergillus Oryzae*

Innovation in Fermentation: Enhancing Antioxidant Content in Rice Bran Using *Aspergillus Oryzae*

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Article Info	ABSTRAK
<p>Article history:</p> <p>Received 11 13, 2023 Revised 11 29, 2023 Accepted 11 30, 2023</p>	<p>Penelitian ini bertujuan menguji aktivitas antioksidan ekstrak fermentasi bekatul sebagai pendekatan inovatif dalam meningkatkan kandungan senyawa bioaktif. Bekatul kaya senyawa fenolik dan γ-oryzanol, namun penggunaan fermentasi enzimatik untuk meningkatkan bioaktivitasnya masih terbatas. Studi ini mengeksplorasi aktivitas antioksidan ekstrak fermentasi bekatul, menawarkan wawasan baru dalam memanfaatkan potensi antioksidan bahan alami yang belum terexplorasi. Bekatul difermentasi dengan <i>Aspergillus Oryzae</i> selama 7, 14, dan 21 hari. Produk fermentasi diekstraksi menggunakan etanol 70%, dan aktivitas antioksidannya ditentukan secara in-vitro dengan metode DPPH. Hasil menunjukkan fermentasi 14 hari memiliki aktivitas antioksidan tertinggi (AAI 1,47), kategori aktivitas kuat; sedangkan untuk bekatul non-fermentasi dan fermentasi 7 hari, AAI masing-masing 1,09 dan 1,05. Fermentasi 21 hari menunjukkan aktivitas terendah (AAI 0,55), kategori sedang. Temuan ini memberikan kontribusi pada pemahaman pengaruh durasi fermentasi terhadap aktivitas antioksidan bekatul, mendukung potensinya sebagai sumber antioksidan.</p>
<p>Kata kunci</p> <p>Fermentasi bekatul Aktivitas antioksidan <i>Aspergillus Oryzae</i> Metode DPPH</p>	
<p>Keywords:</p> <p>Fermented rice bran Antioxidant activity <i>Aspergillus oryzae</i> DPPH method</p>	
	<p>ABSTRACT</p> <p>6 This study aims to assess the antioxidant activity of fermented rice bran extract as an innovative approach to enhance the content of bioactive compounds. Although rice bran is known for its richness in phenolic compounds and γ-oryzanol, the use of enzymatic fermentation to increase its bioactivity remains limited. The research explores the antioxidant activity of fermented rice bran extract, providing fresh insights into harnessing the untapped potential of natural antioxidants. Rice bran was fermented with <i>Aspergillus oryzae</i> for 7, 14, and 21 days. The fermented product was then extracted using 70% ethanol, and its antioxidant activity was determined in vitro using the DPPH method. The results indicated that a 14-day fermentation exhibited the highest antioxidant activity (AAI 1.47), categorized as strong antioxidant activity. For non-fermented rice bran and 7-day fermentation, AAI values were 1.09 and 1.05, respectively. Fermentation for 21 days showed the lowest antioxidant activity (AAI 0.55), categorized as moderate. These findings contribute to understanding the impact of fermentation duration on the antioxidant activity of rice bran, supporting its potential as a valuable source of antioxidants.</p>

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1. PENDAHULUAN

Exploring natural antioxidants has become imperative, considering the diversity of diseases triggered by free radicals. Rice bran, a byproduct of the rice milling process, has garnered significant attention in recent decades as a potential source of bioactive compounds. Phenolic compounds and γ -oryzanol in rice bran are crucial antioxidants for human health [1]–[5]. Various factors, such as rice varieties, milling methods, and storage conditions, can influence the content of bioactive compounds in rice bran [2], [4], [6]. To maximize the antioxidant potential of rice bran, an enhancement in the content of bioactive compounds is essential.

One promising approach is through the fermentation process. Fermentation has been recognized as an effective method to enhance the content of bioactive compounds in various food materials [7], [8]. In this context, this research aims to explore the impact of fermentation on the antioxidant activity of rice bran extract. The uniqueness of this study lies in the utilization of *Aspergillus oryzae* as a fermentation agent, a rarely employed element in rice bran fermentation.

Despite numerous studies evaluating the potential of fermentation to enhance antioxidant activity in food products [9], [10], research on the impact of fermentation on the antioxidant activity of rice bran is still limited. Most previous studies have predominantly focused on rice as a potential source of bioactive compounds. Therefore, this research is distinctive in applying a fermentation method using *Aspergillus oryzae* to rice bran, aiming to augment its antioxidant activity.

Aspergillus oryzae is recognized as a fungus that prolifically produces enzymes. This fungus holds advantages over other microbes as the enzymes it produces are widely utilized in food processing, and it has Generally Recognized as Safe (GRAS) status. Additionally, the enzymes produced exhibit extracellular properties [11]. *Aspergillus oryzae* contains septate mold, free and irregular conidiophores, does not form sexual spores, and possesses clean and colorless mycelium and branches [12].

This research is anticipated to provide fresh insights into the utilization of *Aspergillus Oryzae* in rice bran fermentation, expanding the understanding of the impact of fermentation on antioxidant activity in rice bran. The outcomes of this study are expected to establish a robust scientific foundation for harnessing rice bran as a potential source of bioactive compounds with heightened antioxidant activity in the food industry.

2. METODE

Materials and Equipment

The tools utilized in this study include a UV-VIS Spectrophotometer and a set of glassware commonly used in chemical and biological laboratories. The materials employed in this research encompass 5 kg of Mentik Wangi rice bran, a culture of *Aspergillus oryzae*, 70% ethanol, analytical-grade ethanol (e-merch), analytical-grade DPPH (e-merch), and sterilized distilled water (aqua sterile).

Procedure

Rice Bran Fermentation

One hundred fifty grams of rice bran powder in a 1,000 ml Erlenmeyer flask with 20 ml of sterilized distilled water was autoclaved at 121°C for 30 minutes. A 30% (v/w) spore suspension was added, followed by incubation for 7, 14, and 21 days of varying durations. The success of the fermentation process was assessed based on changes in pH, color, aroma, and texture [13].



Preparation of Ethanol Extract from Fermented Rice Bran

The extract was prepared using the maceration method with a ratio of 1:10 with 70% methanol. The samples subjected to extraction included non-fermented rice bran and rice bran fermented for 7, 14, and 21 days.

Antioxidant Testing with DPPH method

Antioxidant activity was assessed in vitro using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The results of the testing allowed for the calculation of the IC50 value using the following equation [14]:

$$\%I = \frac{A_0 - A_t}{A_0} \times 100\% \dots\dots\dots \text{(Equation 1)}$$

The percentage inhibition (%I) at each sample concentration can be formulated to determine the IC50 value. The AAI value is calculated using the equation [15]:

$$AAI = \frac{\text{Konsentrasi DPPH} (\frac{\mu\text{g}}{\text{ml}})}{IC_{50} (\frac{\mu\text{g}}{\text{ml}})} \dots\dots\dots \text{(Equation 2)}$$

3. RESULTS

Mentik Wangi rice was determined at the Herbal Materia Medica Laboratory, Batu. The certificate with the number 074/629/102.20-A/2022 confirms that the plant used in this study is Mentik Wangi rice (*Oryza sativa* L.). The identification of *Aspergillus oryzae* was carried out macroscopically using Lacto phenol cotton blue staining, revealing elongated hyphae with an apical extension, conidial heads bursting with more than four nuclei, and vesicles forming a semi-circle, as depicted in **Figure 1**.

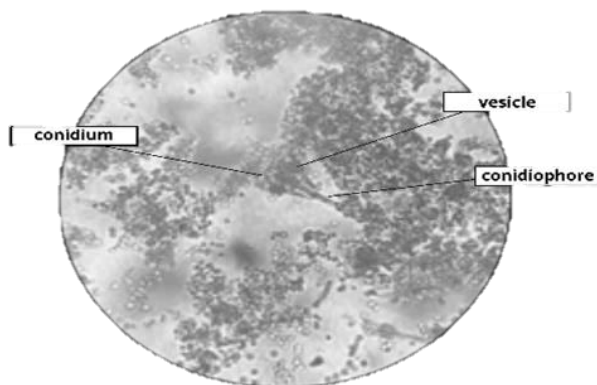


Figure 1. Results of Aspergillus Oryzae Identification.

The yield of extract weight to powder weight for each sample is presented in **Table 1**.

Table 1. Powder to Extract Yield

Sample	Powder Weight (g)	Extract Weight (g)	Yield b/b (%)
Non Fermented	300	32	10.67
7-Day Fermentation	300	47	15.67
14-Day Fermentation	300	33	11.00
21-Day Fermentation	300	30	10.00

Identification was carried out using the tube test method to determine the compounds present in the extract. The results of compound identification testing can be observed in **Table 2**.



Table 2. Identification of Compound Contents

Golongan senyawa	Ekstrak			
	NF	F1	F2	F3
Flavonoid	+	+	+	+
Steroid	-	-	-	-
Triterpenoid	-	-	-	-
Saponin	+	+	+	+

Explanation:

NF : Non Fermentation

F1 : 7-Day Fermentation

F2 : 14-Day Fermentation

F3 : 21-Day Fermentation

"+" Sign : Presence of the compound

"-" Sign : Absence of the compound

The free radical scavenging activity of non-fermented and fermented rice bran extract was measured using the DPPH method, employing a UV-Vis spectrophotometer at a maximum wavelength of 516 nm. The results of the measurements are recorded in

Table 3.**Table 3. Results of Antioxidant Activity**

Sample	IC ₅₀ (PPM)	AAI	Category
NF	145,0684	1,09	Kuat
F1	149,5527	1,05	Kuat
F2	107,0728	1,47	Kuat
F3	285,0715	0,55	Sedang

Explanation:

NF : Non Fermentation

F1 : 7-Day Fermentation

F2 : 14-Day Fermentation

F3 : 21-Day Fermentation

4. DISCUSSION

Fermentasi Bekatul

The extract yield in this study reflects the proportion of bioactive compounds successfully extracted from rice bran. Under non-fermentation conditions, the yield of rice bran extract reached 10.67%, while during a 7-day fermentation, a significant increase was observed, reaching 15.67%. This indicates that the early stages of the fermentation process can enhance the extraction efficiency of desired compounds [16]. Although a decrease in yield occurred after 14 days of fermentation to 11%, and the yield remained at 10% after 21 days of fermentation, these values are still relatively high overall.

The increased yield during the 7-day fermentation can be associated with the heightened enzymatic activity during the fermentation process. Enzymes produced by *Aspergillus oryzae* may contribute to breaking down the cell walls of rice bran, facilitating the extraction of bioactive compounds [17]. With more extended fermentation periods, degradation of some compounds or changes in chemical composition may occur, leading to a decrease in yield [18]. The extract yield results reflect the initial stages of understanding the potential bioactive compounds of fermented rice bran.

The presence of flavonoid and saponin content was detected in non-fermented (NF) and all fermented samples (F1, F2, F3). Flavonoids, known as antioxidant compounds [5], were present in all samples, indicating the potential antioxidant benefits of rice bran that are enhanced through fermentation. The presence of saponins is also noteworthy, as these compounds have been associated with anti-inflammatory and



immunomodulatory properties [19]. This qualitative analysis provides further context for interpreting yield results and helps elucidate the contributions of each compound to the antioxidant potential and health benefits of fermented rice bran extract. However, further analysis of specific compound contents is needed for a more comprehensive understanding.

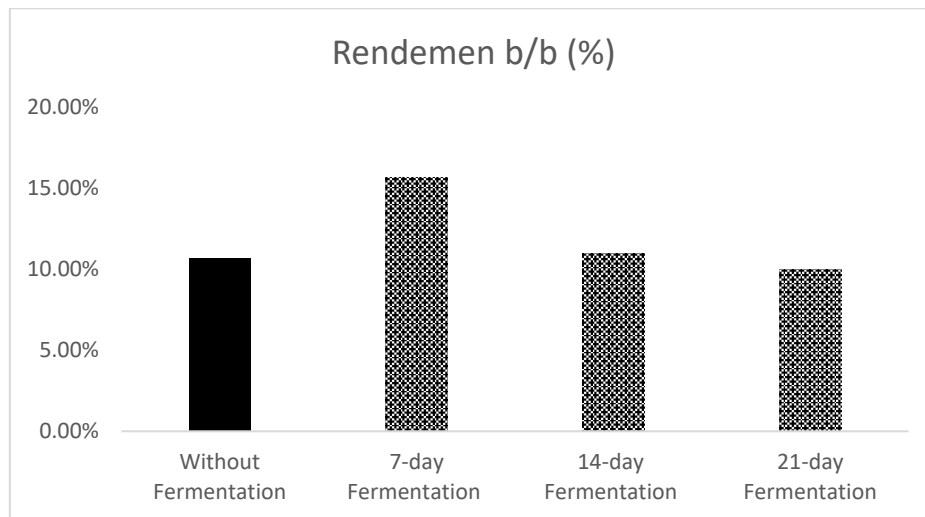


Image 1. Ethanol Extract Yield of Rice Bran

Previous research revealed that methanol extract from Menthikwangi variety rice bran had a total phenol content of $2794.28 \pm 181.83 \mu\text{g EAG/g}$ rice bran [6]. Another study on rice bran extract showed a yield of $37.79 \pm 3.89\%$, with a total phenol content of $2181.167 \pm 94.648 \text{ mg GAE/g}$ and a total flavonoid content of $132.00 \pm 31.75 \text{ mg quercetin/g}$ [20]. Another study compared yields in rice bran with ethanol extraction, where white, red, and black rice bran had 5.00, 4.67, and 4.18%, respectively [5], [19].

Antioxidant Activity

The results of antioxidant activity testing using the IC_{50} method on rice bran extracts show interesting variability in response to DPPH free radicals, as indicated in **Table 3**. In the non-fermented sample (NF), an IC_{50} value of 145.0684 ppm was found, indicating strong antioxidant activity (AAI: 1.09). This signifies an effective ability to capture free radicals under non-fermentation conditions.

In the 7-day fermentation (F1), although the IC_{50} value increased to 149.5527 ppm, the antioxidant activity category remained strong (AAI: 1.05). This indicates that the initial fermentation process does not significantly affect antioxidant capacity. Fermentation for 14 days (F2) showed a lower IC_{50} value of 107.0728 ppm, indicating an increase in antioxidant activity to a solid level (AAI: 1.47). This suggests that fermentation during this period positively influences the extract's ability to capture free radicals, achieving a higher level of antioxidant activity. In the 21-day fermentation (F3), there was a significant increase in the IC_{50} value to 285.0715 ppm, and the antioxidant activity category decreased to moderate (AAI: 0.55). This could imply that longer fermentation at this stage may result in a decrease in antioxidant activity.

Overall, the antioxidant activity test results indicate an increase in antioxidant activity during the 14-day fermentation, as indicated by a decrease in the IC_{50} value. However, it should be noted that longer fermentation at the 21-day stage may decrease antioxidant activity.



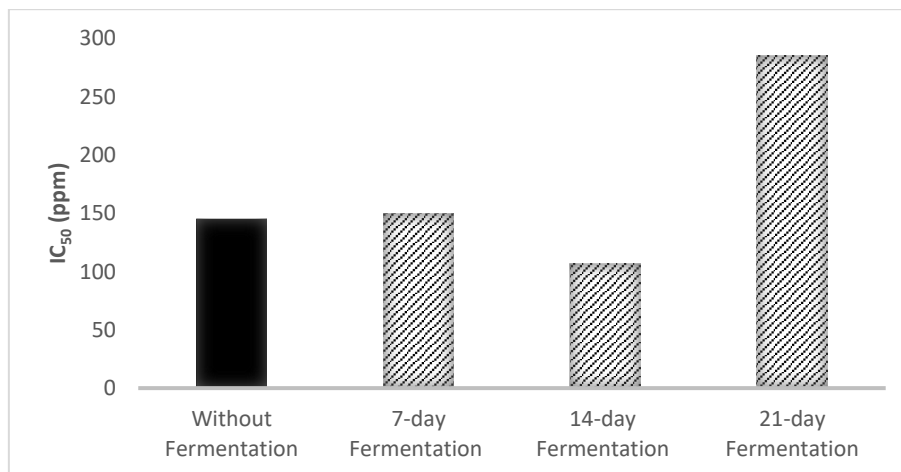
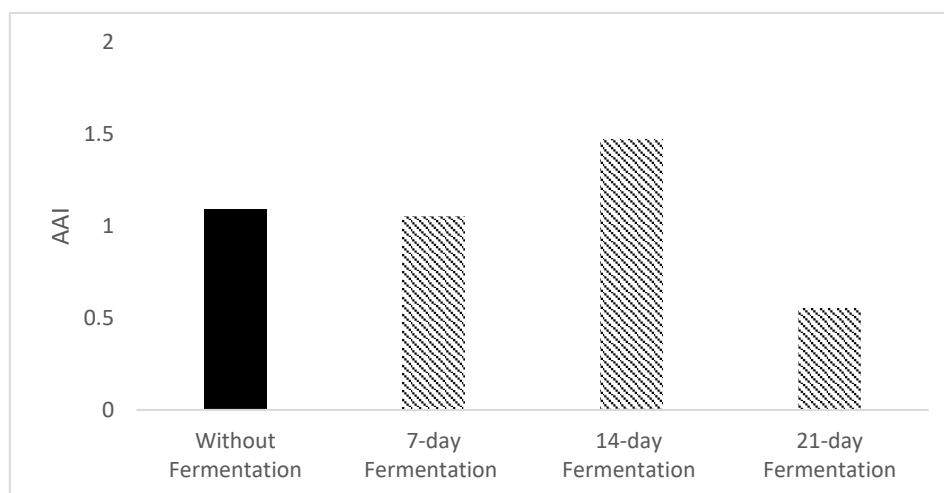


Image 2. The Effect of Fermentation on IC₅₀ Values

Previous research revealed that methanol extract from Mentik Wangi variety rice bran had a DPPH antioxidant activity of $41.28 \pm 0.60\%$ [6]. In comparison, our study shows variability in results, especially in antioxidant activity. Nevertheless, differences may be attributed to genetic variations among varieties and differences in extraction methods. Another study on rice bran extract reported an antioxidant activity of 90.470 ± 0.658 [20]. These results highlight significant variations between rice bran types and extraction methods, providing further insight into the potential variability in the bioactive properties of rice bran. Another study compared antioxidant activity in rice bran with ethanol extraction, where white, red, and black rice bran had antioxidant activities of 49.14, 62.41, and 85.62 ppm, respectively [5], [19]. This comparison indicates significant differences in yield and antioxidant activity depending on the type of rice bran.



Gambar 3. Pengaruh fermentasi pada Antioxidant Activity Index

The antioxidant activity of rice bran is intrinsically linked to its total phenolic content (TPC). Phenolic compounds, such as those found in phenol and γ -oryzanol, play a significant role in imparting antioxidant properties [21]. This study observed that fermentation of rice bran with *Aspergillus oryzae* is key in enhancing antioxidant activity. The results indicate that fermentation for 14 days yielded the highest antioxidant activity with an AAI value of 1.47, categorized as strong antioxidant activity. This finding is



consistent with the view that enzymatic fermentation can increase the content of bioactive compounds, including phenolics, directly contributing to antioxidant activity [7], [8], [10].

The research findings are consistent with a study on solid-state fermentation of rice bran using *Aspergillus awamori* and *Aspergillus oryzae* for five days, increasing rice bran's antioxidant activity. The study explained that fermentation enriched the content of phenolic compounds, especially protocatechuic acid and ferulic acid, which have been proven to enhance antioxidant activity [16]. These results support the effectiveness of fermentation as an approach to strengthen the antioxidant properties of natural resources.

Overall, this research contributes significantly to understanding the influence of the fermentation duration of rice bran with *Aspergillus oryzae* on its antioxidant activity. These findings not only reinforce the potential of rice bran as an antioxidant source through fermentation but also provide new insights into the duration of fermentation in enhancing antioxidant activity. The implications of this study strengthen the scientific foundation for utilizing rice bran as a potential source of antioxidant compounds.

5. CONCLUSION

The results indicate that the fermentation of Mentik Wangi rice bran with *Aspergillus oryzae* for 14 days yields the best outcome with strong antioxidant activity.

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