



Flavonoid Content and Antioxidant Activity of Brown Rice: Method Temperature and Time

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ABSTRACT

Background: Brown rice contains bioactives, including flavonoids and carotenoids, which function as antioxidants. Polyphenols are flavonoid molecules that are widely found in plant life and have antioxidant properties. Antioxidants can help suppress or prevent oxidation of substrates. The body needs antioxidants to ward off free radicals.

Aims: This study evaluated the antioxidant and flavonoid activities of brown rice extract, using ethanol for extraction in an ultrasonic bath. Each of the first and second Erlenmeyer flasks received 150 ml of ethanol for extraction.

Methods: An ultrasonic bath operating at 47 kHz and 45 °C was used for further extraction for either 20 or 30 minutes on the primary flask. The Erlenmeyer jar was also removed at 55 °C after 20 or 30 minutes. Flavonoid quantifications began with dissolving 10 mg of brown rice extract in ethanol to reach a concentration of 1000 ppm. The content was homogenized until uniform, incubated, and the absorbance measured at 415 nm. To prepare an extract solution from 25 mg of extract, ethanol was added to make a total of 25 ml. The resulting extraction solution contained concentrations of 100, 200, 300, 400, and 500 ppm, with 1 mL of 0.4 mM DPPH solution added to each concentration.

Results: Variations in extraction time and temperature did not significantly affect flavonoid levels ($p>0.05$), increasing extraction temperatures resulted in diminished antioxidant efficacy.

Conclusion: The study confirmed the direct correlation between antioxidant capacity and flavonoid concentration, emphasizing that lower extraction temperatures yield better results.

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1. Introduction

Billions globally rely on rice, with brown rice (*Oryza rufipogon*) offering numerous advantages over white rice (Boukaew *et al.*, 2022; Cheirsilp *et al.*, 2023; Ferrari *et al.*, 2021; Jansom, 2020). Key bioactive components of brown rice include pigments and flavonoids, which are characterized by their

various colors such as red, blue, and purple (anthocyanins) or creamy-white to reddish-orange and bluish-purple (flavonoids) (Chima & Fasuan, 2021; Hasan *et al.*, 2023; Zhou *et al.*, 2023; Zhu, 2021). The structure of flavonoids features a C6-C3-C6 chain, which is a combination of phenyl-propane units, stemming from phenylalanine and a C6 unit derived from a polyethylene pathway. This configuration leads to a carbon base structure consisting of 15 carbon atoms, where two benzene rings (C6) are linked by a propane chain (C3) (Noer *et al.*, 2018).

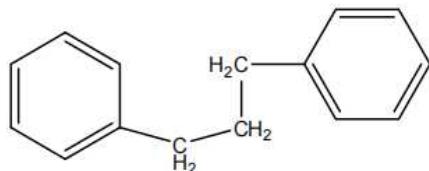


Figure 1. Basic Structure of Flavonoids (Noer *et al.*, 2018).

Flavonoids, including flavonols, catechins, and anthocyanins, play significant roles as antioxidants, with sources including both synthetic agents like tocopherols and natural ones like fresh fruits and vegetables (Singh *et al.*, 2021; SubbuThavamurugan *et al.*, 2023). Antioxidants protect cells from free radical damage, which can disrupt lipids, proteins, and DNA, increasing oxidative stress linked to various diseases, including diabetes mellitus, cardiovascular conditions, and cancer (Hwang *et al.*, 2020; Hwang & Moon, 2021; Liu *et al.*, 2022; Susiloringrum *et al.*, 2024). The Ultrasound-Assisted Extraction (UEA) method, characterized by ultrasonic waves in the frequency range of 20 to 2000 kHz, offers advantages over traditional extraction techniques by generating concentrated extracts with higher yields and facilitating faster processes through enhanced cell wall breakdown and permeability (Andriani *et al.*, 2019; Susiloringrum *et al.*, 2024). Recent studies, including research by Han *et al.* (2024), have explored the impact of temperature and extraction duration on the flavonoid content and antioxidant activity of Sirsak leaves. The findings revealed that optimal extraction conditions specifically at 45°C for 20 minutes—resulted in 903.90 mg QE/g total phenolic content, corresponding to 19.14% flavonoid extraction and an IC50 value of 258.155 mg/L. Similarly, a study by Kristina *et al.* (2022) on duwet leaves indicated an IC50 of 99.74 ppm and a phenolic content of 445.78 mg GAE/g, while Susiloringrum and Sari (2023) examined the antioxidant and sunscreen properties of *Zingiber purpureum* rhizome using UEA, emphasizing the importance of temperature on extraction outcomes. *Overall, the research underscores the beneficial effects of antioxidants extracted through UEA and the necessity of optimizing extraction conditions to enhance antioxidant activity in various plant materials.*

2. Methods

The samples were obtained from a factory in Kuala Sebatu, Batang Tuaka, Indragiri Hilir, Indonesia and identified at the Biology Laboratory of Lancang Kuning University with identification number 140/Lab.Bio/B/IV/2024. In this process, we use the following ingredients: magnesium powder (Sigma®), concentrated HCl (Merck®), 10% NaOH solution, 2N HCl solution, ethanol pa (SmartLab®), aluminum(III) chloride 2% (Merck®), distilled water, quercetin, sodium acetate (Merck®), and DPPH (2,2-diphenyl-2-picrylhydrazil) (Merck®). Fifteen grams of brown rice grains were weighed on an analytical scale and then transferred to the Erlenmeyer pumpkin. Next, the cylinder is filled with 150 milliliters of 96% ethanol (1:10 w/v). Afterwards, the mixture is extracted in an ultrasonic bath at a frequency of 47 kHz and at a temperature of 45 °C and 55 °C for twenty and thirty minutes, respectively. Once the extraction is complete, the solution should be filtered with Whatman No. 1 paper. After that, the collected filtrate is disposed through the use of a rotary vacuum evaporator.

2.1 Implementation of research

Fifteen grams of brown rice grains were weighed on an analytical scale, and then transferred to the Erlenmeyer pumpkin. Next, the cylinder is filled with 150 milliliters of 96% ethanol (1:10 w/v). Afterwards, the mixture is extracted in an ultrasonic bath at a frequency of 47 kHz and at a temperature of 45 °C and 55 °C for twenty and thirty minutes, respectively. Once the extraction is complete, the solution should be filtered with Whatman No. 1 paper. After that, the collected filtrate is disposed through the use of a rotary vacuum evaporator.

2.2 Determination of Total Flavonoid Content

Ten mg of brown rice extract is inserted into a 10 mL volumetric vial, a 1000 ppm solution is produced by adding ethanol p.a. to the mark, resulting in a concentration of 1000 ppm. A 1000 ppm solution is transported to a 10 mL volumetric flask in one milliliter, and then 0.1 milliliters of 10% aluminum(III) chloride, 0.1 milliliter of 1 M sodium acetate solution, and ethanol are added to the flask mark. The mixture then mixed until homogenous. The sample solution is incubated at room temperature. Absorbance is calculated based on wavelength, which is replicated three times. A linear equation derived from the observed quercetin standard curve is used to calculate the concentration for each absorbance value (Vitalini *et al.* 2020).

2.3 Determination of Antioxidant Activity Content

The activity of free radical scavengers is measured by DPPH. 25 mg of extract is weighed and put into a 25 mL volumetric flask to make a 1000 ppm extract solution. Ethanol pa is then added to the mixture. Concentrations of 100, 200, 300, 400, and 500 ppm are also set. To reach the limit, 1 mL of 0.4 mM DPPH solution is added for each sample solution concentration. A UV-Vis spectrophotometer was used to measure absorption at a wavelength of 50 nm.

3. Results and Discussion

3.1 Flavonoid levels

Phytochemical screening tests were carried out on each 96% ethanol extract of brown rice to ensure the presence of flavonoid compounds (16,29). According to the data shown in Table 1, 96% brown rice ethanol extract contains flavonoids. The total flavonoid content of 96% ethanol extract of brown rice was determined by using quercetin as a standard in the reaction of AlCl₃ reagent (30–32). Flavonoid content is shown in Table 1 and determined through the AlCl₃ colorimetric method (33,34). The results of flavonoid content of 96% ethanol extract of brown rice and the results of quercetin DPPH reduction by 96% ethanol extract can be seen in Table 1.

Table 1. Flavonoid content of brown rice.

	Flavonoid Content (mg QE/g)			
	Temperature 45°C		Temperature 55°C	
	20 minute	30 minute	20 minute	30 minute
Average ±SD	5.426 ± 0.01	4.412 ± 0.05	5.838 ± 0.04	5.810 ± 0.03

Based on the research of (Sholihah *et al.*, 2021), the UAE extraction method to obtain the most extraction has an optimal time of 25 minutes. This study uses a time of +- 5 minutes from the optimal time. The principle of this technique is that aluminum chloride forms a complex compound with a keto

group on the C-4 atom and a hydroxyl group on the C-3 or C-5 atom from the flavone and flavonol groups, as shown in Figure 1 ([Susiloningrum et al., 2024](#)). Flavonoids could be found in three replications. Brown rice extracted with ethanol at 45°C for 20 minutes yielded the highest flavonoid concentration is 5.426 ± 0.01 mg QE/g. Brown rice extracted with ethanol at 45°C for 30 minutes yielded the highest flavonoid concentration, with 4.412 ± 0.05 mg QE/g. Even after extraction at 45°C for 30 minutes, the flavonoid content was not significantly affected by the increase in temperature and extraction time. The results are in line with research conducted by [Xiong et al. \(2024\)](#) which involved the use of ultrasonics for soursop leaf extracts.

This is due to the greater likelihood that the material and solvent will interact with each other, resulting in better results until the saturation point of the solution is reached. The bioactive flavonoids undergo structural changes and produce insufficient extracts because they cannot withstand temperatures above 50 degrees Celsius. Shorter extraction time and very low temperature will result in a lower yield ([Munarko et al., 2021](#)). The best periods for temperature and extraction time should be considered, according to [Tyagi et al. \(2022\)](#).

Low extraction temperatures can lead to the extraction of only a portion of the active compounds from the material, leading to low levels of active compounds; conversely, extraction temperatures that are too high and longer extraction times can lead to evaporation of compounds in solution. This study showed that the temperature and duration for 96% ethanol extract of brown rice greatly affected its total flavonoid content. In three replications, extraction conditions performed at 45°C for 20 minutes resulted in the highest flavonoid concentration with 4.412 mg QE/g.

A temperature of 45°C proved to be effective enough to facilitate the release of flavonoid compounds from the brown rice matrix without causing thermal degradation, and the extraction time of 20 minutes ensured an efficient extraction time. Due to its polar nature, 96% ethanol as a solvent plays an important role in the solubility of flavonoids. Its polar properties allow for the most effective extraction of semi-polar compounds. These results show that the quality and quantity of flavonoid extraction results can be improved with controlled extraction conditions. Therefore, this extraction method is not only effective but can also be used as a reference to assess the flavonoid content of other foodstuffs. In addition, this method also provides a scientific basis for the manufacture of brown rice products that are rich in bioactive compounds.

3.2 The Antioxidant activity

The IC50 value of 96% ethanol extract of brown rice can be used to ensure its antioxidant activity. The percentage of inhibition and IC50 of anti free radicals was calculated with brown rice extract and quercetin ([Sulastri, 2018](#)). The degree of inhibition of free radical activity of a material is represented by the percentage of inhibition, which is proportional to the concentration of the sample. The IC50 value is a parameter for interpreting the DPPH results.

Alternatively, the analysis of quercetin antioxidant activity showed an IC50 value of 19.809 ppm, as indicated by the results shown in Table 2.

Table 2. Antioxidant levels of the DPPH method.

IC50 (ppm)	Temperature 45°C		Temperature 55°C	
	20 minute	30 minute	20 minute	30 minute
Average \pm SD	381.102 ± 0.01	406.673 ± 0.04	388.351 ± 0.02	408.744 ± 0.01

The IC50 values of brown rice extract showed 381.767 and 394.371 ppm at 45°C for twenty minutes and thirty minutes, and 295.046 and 404.093 ppm at 55°C for twenty minutes and thirty minutes, respectively. The IC50 value shows that 96% ethanol extract from brown rice has weak antioxidant activity. This is because extracts with IC50 values exceeding 200 ppm are often considered weak antioxidants. The antioxidant activity of brown rice extract decreases along with the increase in extraction temperature, this is in line with the study (Danastry *et al.*, 2021; Hradaya & Husni, 2021) that the antioxidant activity of *E. spinosum* extract decreases along with the increase in extraction temperature.

The results showed that the brown rice extract with the lowest IC50 value was produced by extracting for twenty minutes at 45°C, which resulted in a concentration of 381.102 ppm (Hajas *et al.*, 2022). The IC50 value of brown rice extract, however, is greater than that of brown rice extract, according to (Tyagi *et al.*, 2021). Brown rice extract has an IC50 of 295.046 ppm. Although this brown rice extract has relatively low antioxidant activity, the correlation between flavonoid content and IC50 value suggests that higher levels of extracted flavonoids indicate higher antioxidant activity. As a result, the ideal extraction temperature at 45°C for 20 minutes results in higher biological activity and aids in the release of flavonoids (Chima & Fasuan, 2021; Singh *et al.*, 2021). The extraction conditions and total flavonoid content of brown rice affects the antioxidant activity of 96% ethanol extract of brown rice, which is assessed through IC50 value. From the three replications, the brown rice extract showed the lowest IC50 value of 406.673 ppm at 45°C for 20 minutes, 388.351 ppm at 55°C for 20 minutes, and 408.744 ppm at 55°C for 20 minutes (Liu *et al.*, 2022; SubbuThavamurugan *et al.*, 2023). However, IC50 values above 200 ppm are usually classified as low which indicates that brown rice extract has weak antioxidant activity.

These results suggest that there is a link between total flavonoid levels and antioxidant activity: the more flavonoid content the better the extract is at fighting free radicals. After twenty minutes of extraction at 45°C, the highest flavonoid concentration (6.492 mg QE/g) and the lowest IC50 value were produced. This shows a direct effect of flavonoid levels on antioxidant activity (Hwang & Moon, 2021; Źy  lewicz, 2018). Thus, it can be concluded that, although the antioxidant activity of brown rice extract is lacking, the optimization of the extraction process can increase the content of flavonoids that help its biological activity. These results provide important insights into the process of developing brown rice products as a natural ingredient that has additional health benefits.

The UAE method is one of the modern extraction techniques that utilizes ultrasonic waves to accelerate and increase the efficiency of the extraction process of bioactive compounds from natural materials (Kristina *et al.*, 2022). The method used in this study obtained an average flavonoid content of 5.8 mgEQ / mg, this is lower than the study optimization of anthocyanin extraction from Sirampog Black Rice (Sholihah *et al.*, 2021). This study used brown rice, not crushed or brown rice flour, so that some flavonoid compounds were still left in the brown rice which could cause the lower flavonoid content. The average antioxidant compound in this study was obtained at 408,774 ppm, this result is higher than the study the antioxidant activity of red rice ethanol extract (*Oryza Rufipogon*), this is because of the use of 96% ethanol as solvent. The concentration of solvents and the size of sample particles greatly affect the results of the flavonoids and antioxidants produced.

4. Conclusions

This study demonstrates that ultrasonic-assisted extraction (UAE) of brown rice can produce extracts with appreciable antioxidant activity and flavonoid content, although the overall antioxidant capacity remains relatively low. The findings indicate that lower extraction temperatures, particularly 45 °C, are more favorable for preserving flavonoids and enhancing antioxidant activity, as both parameters tend to decrease with increasing temperature. These results suggest that optimizing particle size (for example using brown rice flour) and extraction conditions could improve the recovery of bioactive compounds

and support the development of functional food products based on brown rice. Nevertheless, the present work is limited to a single variety and a narrow range of process conditions, so caution is required when extrapolating the conclusions to other types of rice or extraction systems. Future studies are recommended to explore different solvents, particle sizes, and extraction techniques, as well as to assess the stability and biological efficacy of the extracts in real food matrices and in vivo models.

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