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Investigation of Flavonoid Levels of Black Rice from Rice (*Oryza sativa L.*) on Sumba Island

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Abstract: Rice (*Oryza sativa L.*) is Indonesia's main cereal crop and is classified into three types based on color: white, red, and black. Among these, black rice has the highest antioxidant content. As a secondary metabolite, antioxidant levels vary depending on regional characteristics. On Sumba Island in East Nusa Tenggara, black rice known as Wojalaka grows across four districts with distinct environmental conditions. This study aimed to evaluate the flavonoid content of Wojalaka black rice from these districts. Using UV-Vis spectrophotometry, ground rice samples were extracted via methanol maceration and analyzed through linear regression. The flavonoid levels found were West Sumba (Lamboya) 74.03 µg/L; Southwest Sumba (Kodi) 67.67 µg/L; Central Sumba (Umbu Ratunggai) 31.65 µg/L; and East Sumba (Matawai) 132.16 µg/L. The highest flavonoid concentration was observed in Matawai at 132.16 µg/L, while the lowest was in Umbu Ratunggai at 31.65 µg/L. These results indicate that flavonoid content depends on habitat conditions the drier the environment where the rice grows, the higher its flavonoid levels.

Keywords: Black rice, flavonoids, wojalaka, Sumba.

Introduction

Black rice (*Oryza sativa L.*) is a rice variety that has high nutritional value and bioactive components. (Das et al., 2023) . The black color of rice is caused by the content of anthocyanins and flavonoids which act as antioxidants. (Iswanti et al., 2024) . The content in black rice is able to ward off free radicals by donating hydrogen atoms or through its ability to chelate metals. Specifically, anthocyanins and flavonoids have the ability to prevent cardiovascular disease by reducing cholesterol and widening blood vessels. (Cañizares et al., 2024) . These two compounds also play a role in preventing type 2 diabetes by reducing inflammation and increasing insulin sensitivity , so that the body can manage sugar more optimally. (Haruni et al., 2024) .

Another benefit that can be obtained is its ability to prevent cancer, especially colorectal cancer, leukemia, and ovarian cancer, by reducing inflammation and fighting free radicals that risk causing the growth of cancer cells. (Thepthanee et al., 2022) . Anthocyanins and flavonoids also have positive effects on brain cognitive function by protecting brain cells from damage, increasing blood flow to the brain, and preventing the buildup of beta-amyloid plaques commonly found in Alzheimer's patients. (Minocha et al., 2022) these two compounds also have the ability as antimutagenic, antihypertensive, and can help prevent liver dysfunction. (Ciumărnean et al., 2020). Flavonoids in black rice have several special properties that make them superior to flavonoid sources from other plants (Liang et al., 2023).

Black rice (*Oryza sativa L*) has been an important part of Asian culinary culture and is now gaining global attention as a functional food. Recent studies have shown that black rice contains five times higher levels of flavonoids than white rice, with total free flavonoids ranging from 3,462-12,061 mg/100g dry weight. This high flavonoid content makes black rice a potential source of natural antioxidants. (Mapoung et al., 2023) . Another special feature is that flavonoids in black rice work synergistically with anthocyanins which give a purplish black color, thus producing stronger antioxidant activity in counteracting free radicals. (Suryanti et al., 2020) .

The distribution of bioactive compounds in black rice is concentrated in the outermost layer of the grain, especially in the aleurone and bran. Studies show that 84.8% of phenolic compounds, 77.5% flavonoids and 73.9% anthocyanins are found in the outermost 2% layer of black rice grains. The total flavonoid content in black rice bran reaches 13,106.16 mg/100 g for free flavonoids and 902.25 mg/100g for bound flavonoids (Cañizares et al., 2024) . This content variation is influenced by genetic factors, geographical conditions, and cultivation techniques. (Maulani R et al., 2019) .

In Indonesia, especially in East Nusa Tenggara, there is a local black rice cultivar known as Wojalaka which has been cultivated for generations by the people of Sumba Island. (Basith et al., 2023). Sumba Island with its diverse geographical characteristics between districts allows for variations in flavonoid content in Wojalaka black rice cultivated in different regions. This research is important to conduct considering the limited scientific information regarding the potential of local Indonesian black rice, especially the Wojalaka cultivar, as a source of natural flavonoids that can be developed into functional foods.

Sumba Island with its diverse geographical characteristics between districts allows for variations in flavonoid content in Wojalaka black rice cultivated in different regions. Although Wojalaka black rice has long been utilized by the people of Sumba Island, East Nusa Tenggara Province, scientific information on its flavonoid content is still limited. This study is important to analyze and compare the flavonoid levels of Wojalaka black rice originating from four districts on Sumba Island, namely East Sumba, Central Sumba, West Sumba and Southwest Sumba. The results of this study are expected to provide scientific information on the potential of Wojalaka black rice as a functional food and become the basis for the development and standardization of its quality.

Material and Method

Time and Place

This research was conducted at the Bioscience Laboratory of Nusa Cendana University, Kupang, in October 2024. Rice samples (Oryza sativa L.) of the Woja Laka cultivar were collected from four districts on Sumba Island: West Sumba, Central Sumba, Southwest Sumba, and East Sumba.

Tools and Materials

The equipment used were dropper and volumetric pipettes, analytical balance, rotary evaporator, analytical balance, micropipette, blender, Erlenmeyer flask, funnel, test tube, beaker, measuring cup and UV-Vis spectrophotometry. The materials used were black rice samples from 4 districts on Sumba Island (West Sumba, Central Sumba, Southwest Sumba, and East Sumba), deionized water, aluminum foil, filter paper, methanol, quercetin standard solution, aluminum chloride (AlCl3) and Sodium nitrate ($NaNO_2$) .

Working Procedures

Samples were taken from 4 areas in each district on Sumba Island, namely Lamboya (West Sumba), Umbu Ratunggai (Central Sumba), Kodi (Southwest Sumba), and Matawai (East Sumba). The black rice samples that had been taken were then milled to separate the rice from the chaff before being analyzed in the Laboratory.

The next step is laboratory analysis. The process begins by preparing samples from each region, as much as 800 grams. Each sample is ground using a blender. After that, the sample is dried in an oven at a temperature of 60 ° C for 4 hours for extraction. The dried sample is reweighed as much as 400 grams, then extracted using the maceration method 3 times for 24 hours using 800 ml of 90% methanol solution. This process is carried out to obtain maximum extract results (Fitriyah et al., 2022) . The maceration soak (Figure 1) is then stored in a place protected from light (Najmah et al., 2023) .

Figure 1. Extract of black rice sample

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The maceration soak was then evaporated using a rotary evaporator at 40 °C to separate the extract and the solvent. The operating temperature was chosen at 40 °C to maintain good extract quality (Ridwanuloh & Syarif, 2019) . The following step is the determination of flavonoids using UV-Vis spectrophotometry and aluminum chloride solution ($AlCl_3$) as a reagent. The extract and standard solution of quercetin were each pipetted as much as 0.5 ml into a 15 ml test tube, then added 2 ml of deionized water. After that, it was added again with 5% sodium nitrite ($NaNO_2$) $_{solution}$ as much as 0.15 ml. The mixture was then left for 5 minutes, then 0.1 ml of 0.15% $AlCl_3$ solution. The mixture was again left for 5 minutes.

Measurements using a UV-Vis spectrophotometer were carried out at a wavelength of 415 nm. Determination of total flavonoids was based on the absorbance of the quercetin standard solution, namely 0; 12.5; 25.0; 37.5; 50.0; 62.5; 75.0; 87.5 and 100 µg/ml. The linear regression equation using the Lambert-Beer law, namely: Y = bx + a, where: y is the absorbance, x is the concentration (C), b is the slope, and a is the intercept (Febram Prasetyo et al., 2021) .

Data Analysis

The research data used the statistical method of analysis of variance (Analysis of variance/ANOVA). If there is a difference, the analysis is continued using the Duncan Test at a confidence level of 95% ($\alpha = 0.05$).

Discussion

The sampling locations were selected based on information from the community about the black rice wojalaka commodity producing areas on Sumba Island, namely Matawai in East Sumba district, Umbu Ratunggai in Central Sumba district, Lamboya in West Sumba district and Kodi in Southwest Sumba district. These four areas have almost the same regional characteristics, namely air temperatures ranging from 22 - 27 ° C, soil types are regosol, mediterranean and lithosol, and on average are in alluvium geological formations. However, there is one significant difference, namely rainfall. Rainfall for Matawai in East Sumba is 1,123 mm/year, for Umbu Ratunggai in Central Sumba is 1,540 mm/year, for Lamboya in Southwest Sumba is 1,900 mm/year and for Kodi in West Sumba is 1,647 mm/year. Based on this, it is known that the driest area is Matawai in East Sumba district, while the most humid area is Lamboya 1,900 mm/year (World Encyclopedia, 2018) .

It is known that regosol, Mediterranean and lithosol soil types influence the formation of secondary metabolites (Chaouqi et al., 2023) . The drier the environmental conditions, the more flavonoid compounds the plants will produce as an adaptation mechanism to environmental stress (Laoué et al., 2022) . This is evident from the darker color of rice in drier areas, indicating a higher flavonoid content.

The drier soil conditions in Matawai cause plants to develop adaptation strategies by producing osmoregulators in the form of flavonoids to overcome water shortages. Other environmental factors such as sunlight intensity, altitude, and soil fertility also contribute to variations in flavonoid levels in Wojalaka black rice. This suggests that more extreme geographic conditions actually encourage increased production of bioactive compounds in plants (Alum, 2024) .

Based on differences in regional characteristics in the form of rainfall, there are differences in the color of Wojalaka black rice. The drier the area, the darker the color of the black rice produced (Basith et al., 2023; Hanas et al., 2017; Maulani R et al., 2019) where the darkest appearance of black rice comes from East Sumba district. A comparison of the appearance of Wojalaka black rice from 4 districts on Sumba Island is shown in Figure 2.

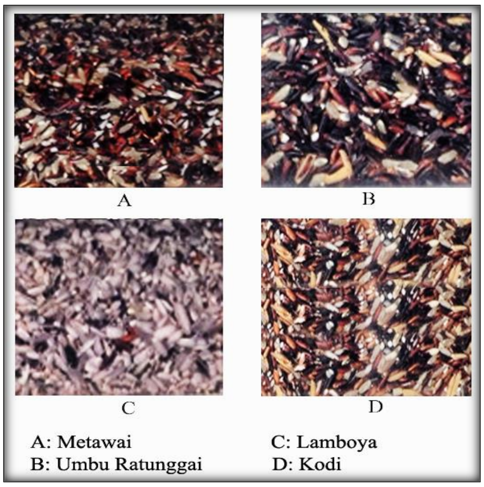

Figure 2. Wojalaka black rice from Sumba Island

Differences in regional characteristics, especially rainfall, will greatly affect the antioxidant content contained in black rice (Das et al., 2023) . Secondary metabolites are actually produced by plants to protect themselves from extreme environments, where these compounds are finally known to be useful for human health (Anggraeni

Putri et al., 2023) .

The differences in flavonoid compound content are proven by the results of qualitative analysis in identifying the presence of flavonoids from the four samples using the calorimetry method. The results of the analysis showed that the four samples had flavonoid content which was indicated by a color change when the sample extract was reacted with methanol plus $AlCl_3$ and $NaNO_2$, and aquadest (Najmah et al., 2023) . However, there are differences in color caused by the resulting reaction as presented in Figure 3.

The color of the Umbu ratunggai sample changed to yellowish orange, from Kodi it changed to yellowish red, from Lamboya it changed to yellowish brown, and from Matawai it changed to dark brown. This color change proves that there is flavonoid content (Kusnadi & Devi, 2017) . It is also known that the darker the color change in the sample indicates the higher the flavonoid content in the sample (Nurmila et al., 2019) . This is in accordance with previous research which states that this color difference is related to the amount of different flavonoid compounds (Anasthasia Pujiastuti et al., 2022) . Therefore, qualitatively it can be said that the less or drier the place where wojalaka black rice grows, the higher the flavonoid content in it.

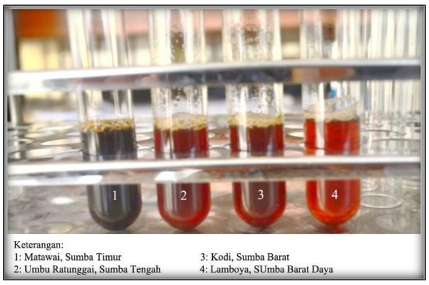

Figure 3. Color of Sample Extract after reaction with AlC_3 and $NaNO_2$

The mechanism of flavonoid biosynthesis under dry conditions involves a series of complex responses at the cellular, developmental, and physiological levels of the plant. (Kubra et al., 2021) . Under drought stress conditions, plants will activate the flavonoid biosynthesis pathway as a defense mechanism by increasing the expression of key genes such as PAL (phenylalanine ammonia lyase), CHS (chalcone synthase), and FLS (flavonol synthase). When plants experience drought stress, there is an increase in the production of Reactive Oxygen Species (ROS) which triggers oxidative stress. In response, plants increase the biosynthesis of flavonoids which act as antioxidants to neutralize ROS. The CHS gene, which is the entry point for the flavonoid biosynthesis pathway, will increase its expression as the duration of drought stress increases, which is then followed by an increase in the total flavonoid content (Baozhu et al., 2022) .

Dry conditions also trigger plants to develop adaptation strategies by producing osmoregulators in the form of flavonoids to overcome water shortages (L. Yang et al., 2020) . The flavonoids produced not only function as antioxidants, but also play a role in adjusting cell osmosis and protecting cell membranes from damage due to dehydration (Shomali et al., 2022) . This process involves the activation of transcription factors that regulate flavonoid biosynthesis, where these factors themselves are controlled by the altered redox status of cells due to drought stress (Yang et al., 2021) . To ensure the flavonoid content in Wojalaka black rice samples quantitatively, the test was carried out using a UV Vis spectrophotometer. The test results are presented in Table 1.

Table 1. Quantitative measurement of flavonoid levels

Sample	Test	Absorbance	Flavonoid Level (µg/L)	Average Flavonoid levels (µg/L)
West Sumba	1	0.6796	74.03	
(Lamboya)	2	0.6794	74.01	74.03
	3	0.6797	74.04	
Central Sumba	1	0.2897	31.65	
(Umbu Ratunggai)	2	0.2894	31.61	31.65
	3	0.2898	31.66	
Southwest Sumba	1	0.6205	67.67	
(Score)	2	0.6203	67.58	67.67
	3	0.6211	67.67	
East Sumba	1	1.2144	132.16	
(Matawai)	2	1.2147	132.19	132.16
	3	1.2143	132.15	

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To ensure the differences between the three samples, the quantitative test results were retested using the statistical method of analysis of variance (ANOVA). Based on the results of the analysis of variance, it is known that there are significant differences in flavonoid content between each cultivar. Each sample showed a significant difference, and after being continued with the Duncan test, the results showed that the Lamboya sample was significantly different from the Kodi sample, significantly different from the Umbu Ratunggai sample, and significantly different from the Matawai sample as presented in Table 2.

Table 2. Average flavonoid content of Wojalaka black rice from the Sumba area

Cultivar origin	Flavonoid Levels $\overline{X} \pm$ ***Std.* Dev**
The Spring of Ratunggai	31.65 ±0.51a
Score	67.67 ±0.26b
Lamboya	74.03 ±0.15c
Matawai	132.16 ±0.20d

Note: The same notation shows no significant difference in the DMRT test (P=0.05), while numbers with different notations show significantly different results in the DMRT test (P=0.05).

The lowest content was detected in the Umbu Ratunggai cultivar rice, which was 31.65 ±± 0.51 µg/L, while the highest was found in the Matawai cultivar rice at 132.16 ±± 0.20 µg/L. The difference in levels is related to the place where the rice is grown. Upland rice has a significantly higher flavonoid content than lowland rice (Jin et al., 2024) . The results of observations before visual sampling showed that the Matawai area had drier soil conditions, followed by the Lamboya and Kodi areas. This happened because the field conditions led to drought stress conditions, so that plants developed adaptation strategies to overcome water shortages. One of the strategies developed by plants including rice is the production of osmoregulators, one of which is flavonoids. Other factors that are thought to influence the flavonoid content in this rice are climate, soil fertility, rainfall, light intensity, and altitude (Sarma et al., 2023) .

The results of the study on the flavonoid levels of Wojalaka black rice can be compared with several similar studies from various regions. In Indonesia, a study on 11 black rice cultivars showed flavonoid levels ranging from 50 - 600 mg / 100 g, much higher than the findings in Wojalaka black rice which ranged from 31.65 - 132.16 µg / L (Basith et al., 2023) . A study on black rice in Thailand showed that the total flavonoid content in black rice bran reached 13,106.16 mg / 100g for free flavonoids and 902.25 mg / 100g for bound flavonoids (Cañizares et al., 2024) . Meanwhile, a study in India on Kavuni black rice showed a strong correlation between flavonoid content and antioxidant activity with an r value = 0.82 (Thanuja & Parimalavalli, 2020) .

The significant difference in flavonoid levels between Wojalaka black rice and other varieties may be caused by different environmental conditions where they grow, where the drier the habitat where the rice grows, the higher the flavonoid content, according to research results on Wojalaka black rice. Certain communities on Sumba Island consume black rice because it is healthier, resistant to hunger, can reduce the risk of disease, and accelerate the healing of other diseases.

Flavonoids are one of the secondary metabolites found in plants. This compound can be used in the medical field as an antimicrobial, wound infection medicine, antifungal, anti-virus, anti-cancer, and anti-tumor. In addition, flavonoids can also be used as antibacterial, antiallergic, cytotoxic, and antihypertensive. There are certain types of flavonoids which are active components of plants that are traditionally used to treat liver dysfunction and treat capillary fragility in humans (Das et al., 2023) .

The findings of this study have several important practical implications for the development of Wojalaka black rice cultivation. Drier environmental conditions with low rainfall such as in Matawai (1,123 mm/year) have been shown to produce higher flavonoid levels (132.16 µg/L) compared to areas with high rainfall. This suggests that to optimize flavonoid content, Wojalaka black rice should be cultivated in dry land or fields rather than paddy fields.

Farmers can adjust cultivation techniques by reducing the frequency of irrigation to create controlled drought stress conditions, thus triggering plants to produce more flavonoids as a defense mechanism. The selection of planting locations also needs to consider land characteristics such as regosol, Mediterranean and lithosol soil types with optimal temperatures. In addition, the development of Wojalaka black rice can be focused on areas with high sunlight intensity because these conditions support the formation of flavonoid compounds (Haruni et al., 2024) . For quality standardization, it is necessary to develop cultivation guidelines that include location selection, irrigation arrangements, and determining the right harvest time to produce black rice with optimal flavonoid content. This will help farmers produce high-quality Wojalaka black rice that can be marketed as functional food with higher economic value.

Conclusion

Based on the results of the research that has been conducted, it can be concluded that the flavonoid levels found in rice (Oryza sativa L.) cultivar Woja Laka on Sumba Island, West Sumba Regency (Lamboya) 74.03 µg/L, Southwest Sumba (Kodi) 67.67 µg/L, Central Sumba (Umbu Ratunggai) 31.65 µg/L, and flavonoid levels in East Sumba (Matawai) 132.16 µg/L and the highest and lowest flavonoid levels in rice (Oryza sativa L.) cultivar Woja Laka are the highest in Matawai at 132.65 µg/L and the lowest in Umbu Ratunggai at 31.65 µg/L. Flavonoid levels depend on the habitat, the drier the habitat where the rice grows, the higher the flavonoid levels contained.

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