The effect of polishing time on the proximate and antioxidant properties of MARDI Warna 98 rice

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ABSTRACT

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| MARDI Warna 98 (MW 98) is coloured rice, released in 2018 by the Malaysia Agricultural Research and Development Institute (MARDI), specifically as nutrient-dense rice. It has the potential to substitute traditional coloured varieties in East Malaysia, where most coloured rice is consumed. The bran of MW 98 is red, and the pigment would give an undesirable taste that most people refuse to consume, although the healthy properties lie in the bran. A study was carried out to determine the bran removal degree that may affect the proximate and antioxidant properties of MW 98 rice. The bran removal degree depends on polishing time, where a longer time removes more bran. The bran was removed at four (4) polishing times (10, 20, 30 and 60 seconds), and unpolished rice was used as a control. The proximate content evaluation was carbohydrate, protein and fat content. At the same time, the antioxidant properties measured were total phenolic content (TPC), total flavonoid content (TFC), total anthocyanin content (TAC) and 2, and 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities. Antioxidant parameters were measured spectrophotometrically. The study proves the accumulation of health benefits of rice is in the bran, that unpolished rice significantly showed higher TPC (220.46 mg GAE/100g), TFC (943.30 mg CAE/100g), TAC (73.42 mg CYE/100g), as well as the protein (8.6g) and fat (2.3g) content. Whereas the carbohydrate content showed a significant increase with a longer polishing time (82.6g). Therefore, rice with the bran intact was highly recommended for a healthy diet. |

*Keywords: Antioxidant; coloured rice; polishing time; total flavonoid content; total phenolic content; total anthocyanin content; proximate*

1. INTRODUCTION

The high prevalence of non-communicable diseases (NCD) such as diabetes, cancer and cardiovascular diseases has gained a lot of attention among researchers and nutritionists. Studies from all over the world recommend nutrient-dense food to reduce the severity of NCD. For Asian people, rice is the main source of carbohydrates, which contributes 55 -80% to the total calorie intake of people (Bhattacharjee et al. 2002), thus, several types of rice, either genetically bred or through processing with high nutrients, are produced as an alternative to white rice. Among them are brown (unpolished) or pigmented rice (red, black and purple rice), which constitutes high proteins, dietary fibre, vitamins and essential minerals, located in the rice bran (Butsat & Siriamornpun, 2010; Laokuldilok et al. 2013). Besides that, pigmented rice is also rich in bioactive compounds such as total phenolic and flavonoid, as well as radical scavenging activity (Thitipramote et al. 2016). In addition to that, grains with darker pericarp colour (red and black rice) have been positively associated with the antioxidant activity, with potential benefits on health, such as prevention of cancer (Hudson et al. 2000; Hu et al. 2003; Hyun & Chung, 2004; Chen et al. 2006), control of blood lipids, which may help in the prevention of cardiovascular problems (Ling et al. 2001) and diabetes complications (Morimitsu et al. 2002).

Malaysia Agricultural Research and Development Institute (MARDI) produced coloured rice, namely MARDI Warna 98 (MW 98), on 12 February 2018, specifically as nutrient-dense rice and to substitute traditional coloured varieties in East Malaysia, where most coloured rice is consumed (Fig.1). The bran of MW 98 is red. Nutrients analysis shows the rice in 100 g is high in magnesium (116.9 mg), a source of protein (8.9 g), iron (3.5 mg), potassium (323.7 mg) and dietary fiber (5.6 g), as well as a low-fat (1.9 g) content and free of sodium (2.2 g) (Guideline to nutrition labeling and claim, Food Safety & Quality Division, Ministry of Health, 2010). Besides its healthy properties, the maturity period of MW 98 is also shortened to 109 – 112 days, which is typically traditional coloured rice has a longer maturation time (more than 150 days) (Zaki et al. 2019). The short maturity period allows rice cultivation twice a year, as practised in West Malaysia, thus increasing the self-sufficiency level (SSL) of rice.

Primary processing of rice consists of drying, milling (dehusking, polishing and grading) and storage. These processes might be different from widely consumed white rice because the physicochemical properties of rice, especially the antioxidant properties, must be preserved. A suitable drying temperature that preserves the quality traits of MW 98 is reported at 46 - 50°C (Hanisa et al. 2022). After drying, the paddy husk is removed through dehulling to produce brown rice, where the bran layer is intact on the rice kernel. The subsequent polishing removes the bran layer and produces different degrees of white rice. A combination of several operations converts paddy to well-milled white rice, mostly preferred by consumers for its superior cooking quality. For pigmented rice, the bran is kept intact to preserve the health-promoting compounds in the bran. The red bran, however, would give an undesirable taste that most people refuse to consume, although the healthy properties lie in the bran. The eating quality could be enhanced by removing the bran. The amount of bran removal from the brown rice kernel is positively correlated with polishing time. Higher polishing time means more bran removal, and it is critical in pigmented rice as the antioxidant properties are mostly present in the bran layer. A suitable polishing time for milling rice is important without adversely affecting the nutrient value. Therefore, the study was undertaken to determine the suitable polishing time of MW 98 red rice that would preserve the antioxidant properties.



**Fig. 1. The red rice of MARDI Warna 98 (MW 98)**

2. material and methods

**2.1 Rice samples**

A red rice variety, MARDI Warna 98 (MW 98) was planted for the experimental study at MARDI Headquarters in Serdang, Selangor, Malaysia.

**2.2 Drying**

As a previous study by Hanisa et al. (2022) found, rice was dried at the recommended temperature of 46 -50°C. Drying was stopped when the moisture content reached a safe storage level of 13 – 14%.

**2.3 Milling of rice**

Paddy MW 98 was dehulled (Satake THU-35B) to remove the husk and produce brown rice. After that, the brown rice was subjected to different polishing times, which were 0-sec (unpolished), 10, 20, 30 and 60-sec. The selected highest polishing time of 60 seconds was based on the standard practice of commercial rice millers for white rice. The low range of polishing time was for producing brown rice (undermilled rice).

**2.4 Proximate analysis**

The proximate analysis of MW 98 at each polishing time tested has been carried out using AOAC (2000). Data obtained from proximate analysis were moisture content (MC), protein, ash, fat and fibre.

**2.5 Preparation of rice extract for antioxidant analyses**

One gram of rice sample was ground to superfine flour before being extracted with 25 ml of methanol-absolute containing 1% HCl according to the method described by Shen et al. (2009) with slight modifications. The extract was shaken for 24 hours by using an orbital shaker at 200 rpm (WiseShake, SHO-2D) in the dark. The mixtures were then centrifuged at 4000 g for 15 minutes, and the supernatant was filtered using filter paper (Whatman No. 1) and collected into a 10 ml amber bottle. The extracted solution was kept at 4°C until further analysis.

* + 1. **Total phenolic content (TPC)**

The total phenolic content was assayed using the Follin-ciocalteu calorimetric method with slight modification (Shen et al. 2009). Briefly, an aliquot (300 µL) of the extract was introduced into a measuring cylinder containing freshly diluted (2.25 ml) 10-fold Folin-Ciocalteu reagent (Merck) and allowed to stand at room temperature for 5 mins. Then, the reaction was neutralized with 2.25 ml saturated sodium carbonate (60 g/L) before being kept in the dark for 90 mins at ambient temperature until the sample turned blue. Absorbance was measured at 765 nm using a UV-visible spectrophotometer (Cary 60 UV-VIS). The total phenolic content was calculated based on the calibration curve of gallic acid and expressed as mg of gallic acid equivalent per 100 g of the rice flour (mg GAE/100g) of dry weight.

* + 1. **Total flavonoid content (TFC)**

The total flavonoid content was determined by a colourimetric method (Shen et al. 2009) with minor modifications. An aliquot (0.5 ml) of the appropriately diluted extract was pipetted into test tubes containing 2.25 ml deionized water and reacted with 0.15 ml of 5% sodium nitrite (NaNO2). After incubation for 6 mins, 0.3 ml 10% aluminium chloride hexahydrate (AlCl3.6H2O) solution was added, and the mixture was allowed to stand for another 5 mins before 1 ml 1M sodium hydroxide (NaOH) was added. The reaction solution was mixed thoroughly by using a vortex (Vortex Mixer VM-300) and measured immediately at an absorbance of 510 nm with a UV-visible spectrophotometer (Cary 60 UV-VIS). Total flavonoid content was calculated using the standard catechin curve prepared using (+)-catechin hydrate (SIGMA) and expressed as mg catechin equivalent (CAE) per 100 g dry weight of the sample.

* + 1. **Total anthocyanin content (TAC)**

The total anthocyanin content was measured by the pH differential method, which is based on the structural changes in anthocyanin chemical forms and absorbance at pH 1.0 and 4.5 (Lee et al. 2005). Briefly, 0.5 ml of the extracted solution was transferred into a test tube containing 3.5 ml of 0.025 M potassium chloride (KCl) buffer at pH 1.0 and kept in the dark for 15 minutes at room temperature. The absorbance was measured at 515 nm and 700 nm, respectively. The extracts then followed the same procedure with 0.025 M sodium acetate (CH3COONa) buffer at pH 4.5 against solvent extraction. The absorbance was measured using a UV-visible spectrophotometer (Cary 60 UV-VIS). The difference in absorbance between pH values and wavelength was calculated as follows:

*A= (A520nm -A700nm) pH1.0-(A520nm -A700nm) pH4.5*

The total anthocyanin concentration was calculated and expressed as cyanidin-3-glucoside equivalents as follows:

Anthocyanin (mg/L) = [A x MW x DF x 1000] / ε x 1

Where A = absorbance, MW = molecular weight for cyanidin-3-glucoside (449.2 g/mol), DF = dilution factor, ε = molar extinction coefficient (26,900 L/cm mol), 1 = path length in cm, and 1000 is the factor for conversion from g to mg.

* + 1. **DPPH radical scavenging activity**

The free-radical scavenging capacity of each extract was determined using a modified version of the procedure described by Somaratne et al. (2017), with some modifications. Briefly, 100 µL of extract solution was added to the freshly prepared 0.1 mM 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution (2.9 ml), and the mixture was kept at room temperature in the dark for 30 min. The absorbance was read at 517 nm using methanol-absolute as the blank with a UV-visible spectrophotometer (Cary 60 UV-VIS). The percentage of DPPH radical scavenging activity was expressed as follows:

*DPPH Scavenging Activity = (Abs Control -Abs sample) ÷ Abs control ×100*

All experiments were conducted in triplicate (n=3) and the results are expressed as mean ± standard error mean (SEM).

* 1. **Statistical analysis**

The experimental design was a Randomised Complete Block Design (RCBD). Analysis of variance was performed using Statistical Analysis Software (SAS 9.4). Treatment means were compared based on the LSD test at p≤0.05.

3. results and discussion

**3.1 Proximate composition**

The proximate compositions, protein, fat and carbohydrate are presented in Table 1. The protein content was found to be significantly higher in unpolished rice (4.61%) and at 10s polishing time (4.41%), compared to 30s (3.98%) and 60s (3.90%) of polishing. However, the indicated protein content was lower than that of other pigmented rice (unpolished or polished) (Chagam et al. 2017). This might be true because 57% relative amounts of protein were deposited in the rice grain's endosperm (Lamberts et al. 2007). Furthermore, the results obtained are consistent with findings by Sompong et al. (2011), who reported losses ranging from 2.5% to 10% in red and black rice varieties grown in Thailand and China.

A similar trend was found in fat content, where longer polishing time decreased the fat content. Fat content decreases significantly from 0, 10, 20, 30 and 60s with values of 2.27%, 1.87%, 1.00%, 0.82% and 0.35%, respectively. As reported by Chagam et al. (2017) also found that the content of various minerals, fats and proteins in three different pigmented rice varieties decreased significantly after polishing. Fats abundantly build up in the bran layers, and with the outer bran layer removal, fats also decreased (Roy et al. 2008). Furthermore, unpolished or brown rice presents the highest fat content, mainly from the bran layer's oil (Rosniyana et al. 2010).

On the contrary, carbohydrate content gradually increased with longer polishing times, reaching 82.59% at 60 seconds of polishing. A study by Puri et al. (2014) stated that carbohydrate was mainly concentrated in the endosperm of the rice kernel. Therefore, extended polishing increases the exposure of starch, leading to higher carbohydrate content. However, white rice generally has a carbohydrate content ranging from 77% and 80%. The results also showed that the carbohydrate content of unpolished rice (79.26%) is comparable to white rice. Therefore, the MW 98 rice can be a considerable source of carbohydrates, either in polished or unpolished form.

**Table 1. The proximate properties of MARDI Warna 98 rice at different polishing times**

|  |  |  |  |
| --- | --- | --- | --- |
| **Polishing time (s)** | **Protein (%)** | **Fat (%)** | **Carbohydrate (%)** |
| 0 (Unpolished) | 4.61±0.23a | 2.27±0.02a | 79.26±0.53c |
| 10 | 4.41±0.16a | 1.87±0.06b | 79.90±0.31c |
| 20 | 4.28±0.05ab | 1.00±0.04c | 81.29±0.07b |
| 30 | 3.98±0.10b | 0.82±0.08d | 81.63±0.12b |
| 60 | 3.90±0.12b | 0.35±0.07e | 82.59±0.09a |

Values are presented as mean±SEM of triplicates (n = 3). Values with different superscripts within the same column are significantly different (*p*<0.05).

**3.2 Antioxidant qualities**

The effect of polishing time on antioxidant capacities of MW 98 (TFC, TPC, TAC and DPPH radical scavenging) is presented in Table 2. Catechin served as the standard for determining TFC, and its calibration curve is shown in Fig.2. The TFC of MW 98 was found to be inversely proportional to polishing time, with TFC values decreasing as polishing time increased from 0s to 60s. The highest TFC was observed in brown rice (unpolished, 10s) at 943.6 mg CAE/100g, which dropped significantly to 147.73 mg/100g after 60s of polishing. Based on the data studied by Goufo & Trindade (2014) on red rice varieties, the TFC was found mostly located in the bran layer with TFC of 1,324.7 mg/100g, compared to only 352.2 mg/100g in the whole grain and inaccessible in rice endosperm as determined by the aluminium chloride method.

**Table 2. The antioxidant properties of MARDI Warna 98 at different polishing times**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Polishing**  **time (s)** | **TFC**  **(mg/ CAE 100 g)** | **TPC**  **(mg/ GAE 100 g)** | **TAC**  **(mg/100g)** | **DPPH (%)** |
| 0 | 943.60±0.21a | 220.46±0.43a | 73.42±0.52a | 91.68±0.12b |
| 10 | 825.33±1.20b | 182.70±0.14b | 70.30±0.37b | 93.86±0.14a |
| 20 | 459.96±0.45c | 93.51±0.07c | 27.55±0.04c | 94.14±0.07a |
| 30 | 329.56±0.15d | 71.13±0.04d | 20.45±0.00d | 94.16±0.11a |
| 60 | 147.73±0.20e | 16.19±0.24e | -2.84±0.01e | 95.31±0.08a |

Values aree presented as mean ± SEM of triplicates (n = 3). Valeus with different superscript within the same column are significantly different (*p*<0.05).

**Fig. 2. The calibration curve of catechin used to estimate TFC in red rice of MARDI Warna 98**

The trend of the total phenolic content (TPC) was similar to the flavonoid content. Gallic acid served as the standard for determining the TPC, and its calibration curve is shown in Fig. 3. The TPC of MW98 significantly (p<0.05) decreased with increasing polishing time, from 220.46 mg GAE/100g, 182.70 mg GAE/100g, 93.52 mg GAE/100g, 71.13 mg GAE/100g and 16.19 mg GAE/100g at 0, 10, 20, 30 and 60s of polishing, respectively. Similar to flavonoid content, phenolic compound is deposited in the outer layer of the rice grain, where polishing (removal of bran) can reduce the phenolic concentration. Walter & Marchesan (2011) stated that approximately 70-90 % of phenolic compounds are deposited in the bran of brown pericarp rice grain. In addition, Goufo & Trindade (2014) reported that the TPC in red rice was in the order of bran>whole grain>endosperm, 3,509.9 mg GAE /100g, 500.3 mg GAE /100g and 64.8 mg GAE /100g, respectively, as determined using the Folin-Ciocalteu assay. Previous studies have reported that the total polyphenolic compound is tremendously higher in red and black pigmented rice than in light brown rice or non-pigmented white rice (Somaratne et al. 2017).

**Fig. 3. The calibration curve of gallic acid used to estimate TPC in red rice of MARDI Warna 98**

Similar findings were shown in TAC, as the Cyanidine-3-glucoside (CYE) equivalent of MW 98 served as the standard. The TAC showed a great extent, ranging from unpolished to polished rice, corresponding to polishing time. The highest TAC was found in brown rice at 73.42 mg CYE/100 g, and decreased significantly to less than -2.84 mg CYE/100 g at 60 sec polishing time. Approximately 85% of anthocyanins appear to be mainly associated with the bran layer of black pericarp grains (Hu et al. 2003) and are almost not deposited in the endosperm of rice. Milling the whole grain significantly reduces the anthocyanin concentration and colour of the rice grain (Hirawan et al. 2011). Additionally, the present finding also proved colour of the rice grain correlated with the anthocyanin content since the colouration of rice derived from the accumulation of anthocyanin in pericarp or bran rice (Goufo & Trindade, 2014). The milling process reduces nutritional components, including antioxidant activity (Ma et al.2020; Xu et al.2021).

DPPH radical scavenging activities show slightly high readings as the polishing time increases, ranging from 91% to 95%. Studies by Franca Finocchiaro et al. (2007) stated antioxidants of pigmented rice were highly correlated with TPC, suggesting phenolics were the main compound responsible for free radical scavenging activity in rice. However, not only phenolic compounds contributed to the antioxidant capacity of rice grain, but also other phytochemicals such as carotenoids, tocols and γ-oryzanol. Besides, the DPPH activity not only depends on rice bran, but also on genetic diversity and climatic variation (Choi el al. 2007).

4. Conclusion

Coloured rice possessed antioxidant activity, but variations are observed mainly due to genotype, pericarp colour and processing. Polishing time affects the antioxidant properties of the MARDI Warna 98 (MW 98) variety, which reduces the total flavonoid, phenolic, and anthocyanin contents with higher polishing time. However, the DPPH radical scavenging activity was not affected by polishing time, where the scavenging activity increased with a longer duration of polishing. Thus, to get a higher content of antioxidant properties, consumption of unpolished MW 98 red rice is recommended. Further research on the cooking characteristics and sensory evaluation is strongly suggested to determine the palatability of MW 98 at different polishing times.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (Chatgpt, COPILOT, etc) and text-to-image generators have been used during the writing or editing of this manuscript.

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