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Antioxidants and *in vitro* starch digestibility of coloured rice, and its effect on blood sugar and malonaldehyde in streptozotocin-nicotinamide-induced diabetic rats

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Abstract

Diabetes mellitus (DM) is an autoimmune disease caused by abnormal regulation of blood sugar, with type 2 DM representing 90 - 95% of total DM incidence. One of the preventive measures to improve blood sugar control is the regulation of eating patterns. The purposes of the present work were therefore (1) to determine the proximate composition, carotene and anthocyanin contents, starch digestibility, and antioxidant capacity of Baturraden white rice, Baturraden organic brown rice, and Sirampog black rice; and (2) to determine the effect of feeding these rice on fasting blood sugar and malondialdehyde (MDA) production in diabetic rats. Sirampog black rice had higher levels of carotene and anthocyanin, and higher radical scavenging activity than Baturraden organic brown and white rice. In diabetic rats, Sirampog black rice (Δ = 153.80 mg/dL) could reduce fasting blood sugar more effectively than Baturraden organic brown rice (Δ = 124.48 mg/dL) and Baturraden white rice (Δ = 14.62 mg/dL). Diabetic rats treated with Sirampog black rice also presented the lowest MDA levels of 2.62 nmol/mL when compared with that of Baturraden organic brown rice (3.96 nmol/mL) and Baturraden white rice (10.14 nmol/mL). Based on these results, patients with DM are advised to consume Sirampog black rice. In the future, it is necessary to perform trials in patients with DM to determine the effect of Sirampog black rice on fasting blood sugar and diabetic weight loss.

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Introduction

The number of patients with diabetes mellitus (DM) continues to increase. According to the International Diabetes Federation (IDF), 536.6 million people had DM in 2021, and by 2045 this number is expected to increase by 46% to 783.2 million (Ogurtsova *et al*., 2022). Eighty per cent of people with DM live in low- and middle-income economies (Bruno *et al*., 2017). Patients with DM adjust their diet to prevent an increase in blood sugar levels (Huang *et al*., 2018). One technique of control is to eat meals high in fibre and antioxidants, and with a low glycaemic index (Maghsoudi *et al.*, 2016; Aini *et al*., 2022).

When compared with other carbohydrate sources, rice represents the most important source of calories for most of the population in Indonesia. It is estimated to contribute 60 - 80% of calories and 45 - 55% of protein to the average Indonesian diet (Sami *et al*., 2017). According to Hu *et al.* (2012), increased consumption of white rice is associated with an increased risk of type 2 DM. Whilst special types of rice such as brown and black rice are also available in Indonesia, white rice remains the main staple food (Fatchiyah *et al*., 2020; Yuliana and Akhbar, 2020).

When compared with white rice, brown and black rice are more advantageous to health because they contain high anthocyanin levels – 0.33 - 1.39 mg/100 g in brown rice (Qi *et al*., 2019) and 19.4 - 140.8 g/100 g in black rice (Pratiwi and Purwestri, 2017). Anthocyanins have radical scavenging activity and the ability to suppress oxidative stress (Pedro *et al*., 2016). Increased radical scavenging activity might help prevent clinical complications of DM. Indeed, research in experimental animals has proved that antioxidants can inhibit the early stages of DM (Chaiyasut *et al.*, 2016).

Another advantage of brown and black rice is that both forms contain higher levels of fibre (*i.e.*, 3.3 and 5.8%, respectively) than white rice, which only contains about 0.75% fibre (Pengkumsri *et al.*, 2015).

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Dietary fibre consumption may have a positive effect on blood glucose levels in patients with type 2 DM because it is capable of slowing the process of gastric emptying and glucose absorption by the small intestine (Fujii *et al*., 2013). According to Schwingshackl *et al.* (2017), foods that raise blood glucose levels quickly have a high glycaemic index (GI), whereas those that raise blood sugar levels more slowly have a low GI. Food GI values can be grouped into three classes, namely low GI (< 55), medium GI (55 - 70), and high GI (> 70). Prasad *et al.* (2019) indicated that the GI of black rice is quite low at 42.3.

Brown and black rice also contain fat, which is mainly found in the aleurone/bran parts. In addition to containing natural oil (γ-oryzanol), the aleurone/bran (rice bran) layer may contain bioactive components such as tocopherol and β-carotene (Shao *et al*., 2014). According to Laokuldilok *et al.* (2011), the main antioxidants in rice bran are γ-oryzanol (62.9%) and phenolic acid (35.9%). As a natural antioxidant, γ-oryzanol is very effective in preventing oxidation and the formation of free radicals (even more than vitamin E) (Huang and Lai, 2016). An experimental animal study showed that γ-oryzanol significantly reduced plasma low-density lipoprotein (LDL) cholesterol levels (Kozuka *et al*., 2013). They found that the bioactive component of the food is a natural oil, the amount of which depends on the rice processing methodology. As broken rice (which still has the bran layer), brown and black rice has a higher bioactive content than white rice (the latter generally being polished, so the bran part is removed and thus wasted).

Several parts of Indonesia have become centres of local rice production including areas in the Brebes regency, Banyumas regency, and Baturraden district. The local varieties of Baturraden rice are brown and white rice, whereas the variety of Brebes rice is Sirampog black rice (Pratiwi and Purwestri, 2017). Whilst it is known that different environmental conditions may affect the physical, chemical, and functional characteristics of rice, there have been no studies specifically on the characteristics and functional properties of Sirampog black rice, Baturraden white rice, and Baturraden organic brown rice.

The present work therefore had two main objectives. The first was to determine the proximate composition, carotene and anthocyanin contents, the starch digestibility, and antioxidant capacity of Sirampog black rice, Baturraden organic brown rice, and Baturraden white rice. The second was to determine the effect of feeding these rice on fasting blood sugar and malondialdehyde (MDA) production in diabetic rats.

Materials and methods

Materials

The samples were Sirampog black rice from the Sirampog village, Brebes regency, Central Java province, Indonesia, and Baturraden white rice and organic brown rice from the Baturraden village, Banyumas regency, Central Java, Indonesia. Wistar rats reared on Comfeed AD II were used as the model organism.

Chemicals used included gallic acid standard, 2,2-diphenyl-1-picryl-hydrazine-hydrate (DPPH), quercetin standard (all from Sigma Aldrich, Germany), and other chemicals for analysis.

Characterisation of chemical and functional properties

Chemical characterisation

The first phase of the present work involved determining the chemical properties of the different rice varieties. This included characterising the moisture content (AOAC, 2005), ash content (AOAC, 2005), protein content (AOAC, 2005), fat content (AOAC, 2005), carotene content (Renuka *et al*., 2016), anthocyanin content (Pedro *et al*., 2016), and carbohydrate content (by difference).

Starch digestibility

The digestibility of starch was analysed *via* spectrophotometry (Chen *et al*., 2017). First, a maltose standard curve was generated by adding 1 mL of standard maltose solution to prepare 0.0, 0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL concentrations in closed test tubes before adding 2 mL of dinitro salicylic acid (DNS) solution to each tube. The solutions were heated in boiling water for 12 min, and then immediately cooled (under running water). Thereafter, 10 mL of distilled water was added to each solution which had been mixed until homogeneous. The sample absorbance was measured at 520 nm using a UV-vis spectrophotometer.

For sample analysis, 100 mg of the sample was put into a test tube and supplemented with 10 mL of distilled water. The test tube was covered with aluminium foil, vortexed for 30 s, and heated in a water bath at 90°C for 30 min, then cooled. The sample was subsequently calibrated and dissolved in a 100-mL volumetric flask with the addition of distilled water. Thereafter, 1 mL of the sample solution was aliquoted into a test tube, and supplemented with 1.5 mL of distilled water and 2.5 mL of 0.1 M Na-phosphate buffer solution (at a pH of 7.0). This setup was performed in duplicate (with one replicate serving as a blank). The capped test tubes were incubated at 37°C for 15 min.

The sample and blank solutions were supplemented with 2.5 mL amylase enzyme solution (1 mg/mL in phosphate buffer solution [pH 7.0]) and were incubated once more at 37°C for 30 min before being transferred to a closed test tube containing 2 mL of DNS solution. The obtained solutions were heated in boiling water for 12 min, and then immediately cooled under running water. A total of 10 mL of distilled water was added to each solution, which had been mixed until homogeneous (using a vortex). The absorbances of the sample and blank solutions were measured at 520 nm with a UV-vis spectrophotometer. The digestibility of starch was calculated using Eq. 1:

Starch digestibility =

$$\frac{Maltose\ of\ sample\ after\ enzymatic\ reaction}{Maltose\ of\ pure\ starch\ after\ enzymatic\ reaction} \times 100\% \quad \text{(Eq. 1)}$$

DPPH radical scavenging activity

The radical scavenging activity was carried out using DPPH (Septiana and Asnani, 2013). The extract solution was prepared by dissolving the extract in methanol to obtain concentrations of 125, 250, 500, 1,000, and 2,000 ppm. DPPH was dissolved in 2 mL of methanol. Then, the mixture was vortexed for 1 min, and left to settle for 30 min before the absorbance was measured at 517 nm. A decrease in absorbance indicated an increase in the ability to capture DPPH radicals. DPPH radical scavenging activity was calculated using Eq. 2:

DPPH (%) =

$$\left(\frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control}\right) \times 100 \quad \text{(Eq. 2)}$$

Analysis of fasting blood sugar levels and antioxidative stress in diabetic rats fed with rice

This phase involved evaluating fasting blood sugar and antioxidative stress levels (MDA) in diabetic rats that received rice treatments. Before analysing the factors, the rats were fed with different rice preparations.

Sample preparation

Rice samples were ground and sifted through a 60 mesh to obtain rice flour. It was dissolved in pure water, and collected using a probe needle. Feeding was carried out based on the body weight of the rats (3.6 g rice flour/200 g rat body weight).

Animal treatments

Animal treatments consisted of three phases, namely an adaptation period, induction period, and intervention period.

Adaptation period

In the 1-week adaptation period, 24 male Wistar rats (aged 8 - 12 weeks and weighing 175 - 200 g) were housed in individual cages. During the adaptation period, rats were fed Comfeed AD II and could drink water *ad libitum*. On the last day of the adaptation period, the body weight of the rats was determined (serving as the initial measurement before entering the induction period).

Induction period

During the induction period, a solution containing streptozotocin (45 mg/kg), nicotinamide (110 mg/kg), and citrate buffer (3 mL/200 g) was delivered intraperitoneally until fasting blood sugar levels of the Wistar rats reached ≥ 200 mg/dL. The induction period lasted for 3 d, after which the time for fasting blood sugar levels and body weight were recorded. During the induction period, the rats were provided with standard feed *ad libitum*.

Intervention period

During intervention period, there were four groups of rats: normal rats fed with a standard ad libitum feed (Group 1), diabetic rats fed with Baturraden white rice (Group 2), diabetic rats fed with Baturraden organic brown rice (Group 3), and diabetic rats fed with Sirampog black rice (Group 4). Treatments for each group were repeated six times (*i.e.*, 24 Wistar rats in total across the four groups). Each group was given rice based on the recommended amount of rice consumption in humans (with a human body weight of 70 kg being equal to a rat weight of 200 g; Chaiyasut *et al.*, 2016). A dosage of 3.6 g rice feed/200 g rat body weight was given per day. The intervention period lasted for 14 d, after which the time of fasting blood sugar, MDA levels, and rat body weight were recorded.

Analysis of blood sugar

Blood sugar levels were determined using the enzymatic glucose oxidase-phenol aminophenazone (GOD-PAP) method. Blood samples (pre- and post-treatment) were collected from test animals in each treatment group. As much as 0.5 mL of blood was obtained *via* the eye canthus (under the eyeball), and centrifuged at 4,000 rpm for 15 min in an Eppendorf tube. Thereafter, supernatant (0.01 mL) was transferred to a test tube and supplemented with 1 mL of GOD-FS reagent. The contents were mixed and allowed to stand for 20 min, after which absorbance was measured at 500 nm with a UV-vis spectrophotometer. Blood sugar levels were calculated using Eq. 3:

$$\frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \times Concentration\ of\ standard\ (100\ mg/dL) \qquad \text{(Eq. 3)}$$

Analysis of body weight

Changes in the body weight of the experimental animals were recorded (using analytical balances). The observational time intervals were as follows: at the beginning and end of the induction period and on day 14 (before the experimental animals were euthanised).

Analysis of MDA levels

The thiobarbituric acid reactive substance (TBARS) method, based on the ability of TBA and MDA to form pink complexes, was used to measure MDA levels. A total of 1 g of liver tissue was homogenised with 9.0 mL of 1.15% KCl solution, and a total of 0.2 mL of liver homogenate was supplemented with 0.2 mL of 8.1% sodium dodecyl sulphate, 1.5 mL of 20% acetic acid solution (pH 3.5), and 1.5 mL of 0.8% TBA solution. The obtained mixture was added to 4.0 mL of water, heated at 100°C for 60 min, and then cooled using an ice bath. After cooling, the mixture was supplemented with 1.0 mL water and 5.0 mL of an *n*-butanol:pyridine mixture (15:1 v/v). After mixing, the samples were centrifuged at 4,000 rpm for 10 min. The organic layer was removed, and the absorbance was measured at 532 nm using a UV-vis spectrophotometer.

Experimental design

The study was carried out using a completely randomised design. The tested factor (*i.e.*, type of rice) consisted of three options, namely Baturraden white rice, Baturraden organic brown rice, and Sirampog black rice. The experiment was repeated six times so that 18 experimental units were obtained in total.

Statistical analysis

The data were analysed by analysis of variance (ANOVA) with the type I error level set at 5%. If there was a significant effect, Duncan's multiple range test was applied to determine group differences, with the type I error level set at 5%.

Ethical considerations

The present work was approved by the Research Ethics Commission, Faculty of Veterinary Medicine, Gadjah Mada University, Indonesia on January 13, 2020 with authorisation number 0002/EC-FKH/Eks/2020. The animals were housed individually at a controlled temperature (24°C), a 12-h photoperiod, and feed and water provided *ad libitum*.

Results and discussion

Chemical characteristics of Sirampog black rice, Baturraden organic brown rice, and Baturraden white rice

Moisture

The moisture content of Baturraden white rice (14.19%) was slightly higher than that of Baturraden organic brown rice (12.9%), with Sirampog black rice (12.59%) having the lowest moisture content (Table 1). The water content differences among the three types of rice may be a result of various factors including rice varieties, environmental conditions (*e.g.*, weather), nutrients, storage conditions, harvesting times, and postharvest factors (Yuliana and Akhbar, 2020). For example, native white rice from the Kapuas regency on Kalimantan, Indonesia had a moisture content of around 11.5%, together with the Siam Jurut (11.56%), Siam Pandak (11.72%), and Siam Palun (11.81%) varieties (Kamsiati *et al.*, 2018), whereas the water contents of local white rice from Indramayu, Cianjur, and Ciamis, West Java Province, Indonesia were 11.8, 11.7, and 12.5%, respectively (Indrasari *et al.*, 2008).

Table 1. Chemical and functional properties of Sirampog black rice, Baturraden organic brown rice, and Baturraden white rice.

Property	Sirampog black rice	Baturraden organic brown rice	Baturraden white rice
Moisture (%)	12.59[a]	12.9[a]	14.19[a]
Ash (%)	0.58[a]	0.98[a]	0.88[a]
Protein (%)	9.46[a]	6.71[c]	8.56[b]
Fat (%)	2.59[a]	1.97[a]	1.05[b]
Carbohydrate (%)	73.15[c]	77.61[a]	75.76[b]
Amylose (%)	23.67[a]	22.96[b]	23.73[a]
Dietary fibre (%)	8.05[a]	6.00[b]	4.775[c]
Starch digestibility (%)	72.48[a]	63.5[c]	66.87[b]
Carotene (µg/g)	9.655[a]	4.945[b]	0[c]
Antioxidant (%)	90.72[a]	91.71[a]	18.34[b]
Anthocyanin (mg/g)	15.7[a]	0.24[b]	0[c]

Means followed by different lowercase superscripts in the same row are significantly different ($p < 0.05$).

Similarly, the water contents of the Mandel Handayani and Segreng Handayani brown rice varieties from Gunung Kidul, Indonesia were 9.68 and 10.56%, respectively (Indriyani *et al.*, 2013). Furthermore, numerous brown rice types cultivated in Thailand, Sri Lanka, and China have a reported moisture content ranging from 9.28 to 13.13% (Pengkumsri *et al.*, 2015). Black rice has a somewhat lower moisture content, and has been shown to vary by region, with the Bantul variety of black rice having a moisture content of 4.23% (Pratiwi and Purwestri, 2017). Toraja black rice has been reported as having 10.5% moisture (Mangiri *et al*., 2016), and Tiara Dewata as having 7.08% (Widyawati *et al.*, 2015)

According to Indonesian National Standard (SNI 6128:2015), the maximum water content of rice is 14%. The levels recorded in the present work showed that Baturraden organic brown rice and Sirampog black rice were in line with this standard. However, Baturraden white rice had a higher water content (> 14%). According to Köten *et al*. (2020), excellent rice has a water content of no more than 14% – because increased levels of moisture make the rice more sensitive to fungi, bacteria, and other elements that cause harm. For example, these microorganisms can cause a rise in CO_2 levels and heat, making the rice more susceptible to damage and rotting.

Ash

The ash content did not differ significantly among the three types of rice (Table 1). The ash content of Baturraden white rice (at 0.88%) was not much different from that of IR-64 white rice (0.56%) (Yuliana and Akhbar, 2020), the Jasmine variety (0.50%) (Widyawati *et al*., 2015), and Inpari-21 (0.61%) (Purwani and Wardana, 2019). Baturraden organic brown rice had an ash content of 0.98%, which was lower than the Saodah variety (1.2%) (Widyawati *et al.*, 2015). Lastly, Sirampog black rice had an ash content of 0.58%, which was almost the same as the Toraja variety (0.4%) (Mangiri *et al.*, 2016). In contrast, another study on Enrekang black rice reported an ash content of 0.9% (Kereh *et al.*, 2016).

The ash content in rice should preferably be kept to a minimum. According to the Indonesian Food and Drug Supervisory Agency's guidelines, a maximum of 4% is allowed because ash contains minerals that might produce precipitate in the kidneys, posing a health risk. The ash concentration in rice bran reflects the mineral richness of the rice bran (Yamuangmorn *et al.*, 2021). Due to the semi-dry physical structure of rice, Sirampog black rice has a low ash concentration. Unlike Baturraden organic brown rice and Baturraden white rice, Sirampog black rice grains are clean and free of impurities. The presence of a considerable amount of germ and endosperm contaminants in grains may imply that the processing method is ineffective (because the obtained product still contains a lot of impurities and thereby results in impure ash contents).

Fat

The fat content of Baturraden white rice was lower than that of Baturraden organic brown rice and Sirampog black rice (Table 1). Baturraden organic brown rice had a fat content similar to SRI1 brown rice from Sri Lanka (1.15%) and CNR from China (2.35%), whereas Sirampog black rice had a fat level that was approximately the same as PK black rice from Thailand (3.72%) and CNB from China (2.85%) (Sompong *et al*., 2011). Baturraden white rice had a fat level of 0.91%, which was almost the same as that of Mentik white rice.

Baturraden organic brown rice and Sirampog black rice may have a relatively high fat content because only the husk layer is removed, leaving the bran (the outermost membrane layer with starchy endosperm and fat) (Köten *et al*., 2020). According to Hao *et al*. (2015), the aleurone part of rice contains 15 - 23% oil – with the three primary fatty acids, namely palmitic (12 - 18%), oleic (40 - 50%), and linoleic (30 - 35%), accounting for 90% of the total fatty acids in the aleurone layer.

Protein

When compared with Baturraden white rice and Baturraden organic brown rice, Sirampog black rice had the highest protein content (9.46%) (Table 1). This protein concentration is nearly identical to that of other black rice with a protein content of 9.05% (Widyawati *et al.*, 2015) and local Enrekang black rice (8.2%) (Kereh *et al*., 2016). Baturraden white rice had a protein content of 8.54%, which was also nearly the same as other white rice varieties with a protein content of 8.17% (Kamsiati *et al.*, 2018). Baturraden organic brown rice had a protein content of 6.71%, which was similar to that of other brown rice varieties (ranging from 6.61 to 7.96%).

The different protein levels in rice varieties may be due to rice grinding, during which time the husk are separated (meaning that the protein, fat, vitamins, and minerals on the exterior are lost). Another factor that impacts rice protein content is the state of the soil where the rice had been cultivated, which is influenced by the genetic character of the variety (Kamsiati *et al*., 2018). Rice grown in soil that is high in nitrogen will have a high protein level (Fatchiyah *et al*., 2020).

Carbohydrate

Baturraden organic brown rice had the highest carbohydrate content when compared with Baturraden white rice and Sirampog black rice (Table 1). The Baturraden organic brown rice carbohydrate content was also higher (77.61%) when compared with brown rice from Cianjur, West Java, Indonesia (73.88%) (Purwani and Wardana, 2019). In contrast, Baturraden white rice had a carbohydrate content of 75.76%, which was lower than IR-64 white rice (88%) and Cianjur white rice (77.03%). Sirampog black rice had a carbohydrate content of 73.15%, which was almost the same as black rice from Tangerang, West Java (75.10%) and Toraja black rice (85%) (Mangiri *et al*., 2016). The high carbohydrate content in Baturraden organic brown rice may be because of its low ash, protein, and fat contents. Meanwhile, Sirampog black rice likely had the lowest carbohydrate content because it also had the highest ash, protein, and fat contents.

Most rice carbohydrates can be found in the form of starch (with significantly lower levels of pentosan, cellulose, hemicellulose, and sugars). Starch makes up between 85 and 90% of the dry weight of rice, whereas pentosan only makes up 2.0 - 2.5% of the weight of broken husked rice, and sugar makes up 0.6 - 1.4% (Fatchiyah *et al.*, 2020). Because starch is the predominant ingredient, the physicochemical qualities of rice are mostly determined by starch properties. Carbohydrate levels are directly tied to the GI, with carbohydrates in low-GI foods being broken down slowly, and resulting in slower glucose release and thus more stable blood glucose levels (Wang *et al.*, 2021). Low-GI foods have been demonstrated to improve glucose and fat levels in patients with high blood sugar, as well as patients with insulin resistance. Many carbohydrate-rich items (including rice, potatoes, and bread) can be digested and absorbed quickly, thus resulting in elevated blood glucose levels.

Dietary fibre

The dietary fibre content of Sirampog black rice was 8.05%, higher than Baturraden white rice (4.78%) and Baturraden organic brown rice (6%). The difference in dietary fibre is influenced by the manner of processing: Baturraden organic brown rice and Sirampog black rice still contained broken skin, thus had more dietary fibre in their aleurone, whereas Baturraden white rice had undergone a peeling process and as such contained only a small amount of dietary fibre. According to Fujii *et al*. (2013), food is said to be high in fibre if it contains at least 6% dietary fibre. Based on this, Baturraden organic brown rice and Sirampog black rice could be classified as food sources of dietary fibre.

Baturraden white rice, however, still has a greater dietary fibre level than Tasikmalaya white rice (0.40%) (Hernawan and Meylani, 2016). Similarly, the fibre content of Baturraden organic brown rice was higher than Tasikmalaya brown rice (1.62%) and Mendel-Handayani brown rice (0.25%) (Indriyani *et al.*, 2013). Sirampog black rice had more fibre than IAC 600 black rice (5.67%) (Salgado *et al.*, 2010) and Tasikmalaya black rice (4.2%) (Hernawan and Meylani, 2016).

High-fibre foods may reduce body weight because the food will stay in the digestive tract for a relatively short time, thus resulting in reduced absorption of nutrients. Furthermore, foods with high fibre content could make you feel fuller, thus resulting in less food consumption. Dietary fibre-rich foods are typically high in calories, low in sugar, and aid in weight loss. Dietary fibre can also decrease the absorption of glucose, thus helping to regulate blood sugar and slow the rise in blood sugar levels (Fujii *et al*., 2013).

Carotene

Brown and black rice had 4.92 and 9.66 g/g carotene, respectively, whereas Baturraden white rice had no β-carotene. According to Indriyani *et al.* (2013), brown rice generally has carotenoid levels of 0.14 - 0.77 g/g. Carotenoids, pigments that give foods the colours that range from yellow to orange to red, include β-carotene, astaxanthin, lycopene, lutein, zeaxanthin, cryptoxanthin, and fucoxanthin, among others. In general, carotenoids also act as antioxidants, and help prevent the body from being damaged by free radicals. As a result of their many health benefits, carotenoids are also used in nutraceutical goods.

Anthocyanin

While white rice does not have anthocyanins, the anthocyanin content of the Baturraden organic brown rice was 0.24 mg/g, which was much lower than that of Sirampog black rice (15.7 mg/g). These anthocyanin levels were higher than those reported by Widyawati *et al*. (2015): 0.00247 and 0.02314 mg/g for Saodah brown rice and Java black rice, respectively. Therefore, black rice had the most anthocyanin, followed by brown and white rice. Black rice is usually rich in flavonoids (*e.g.*, quercetin, dihydromyricetin, naringin, trifolin, and protocatechuic acid), with levels five times that of white rice. If the anthocyanin level is high, then the antioxidant activity will also be high.

Radical scavenging activity

Baturraden white rice had lower radical scavenging activity (18.34%) than Baturraden organic brown rice (91.71%) and Sirampog black rice (90.91%). This is because pigmented rice is rich in functional compounds including anthocyanins, carotenoid tocopherols, tocotrienols, and γ-oryzanol, which are distributed in the pericarp, germ, and aleurone layers (Prasad *et al*., 2019). Previous data on radical scavenging activity in rice reported 95.05% for brown rice (Qi *et al*., 2019), 66.27% for black rice, and 18.40% for white rice (Pang *et al.*, 2018). Similarly, Mangiri *et al*. (2016) reported 94.14% radical scavenging activity for brown rice and 48.77% for black rice. Widyawati *et al*. (2015) reported the radical scavenging activity as 0.90 mEq Vit E/g for Saodah organic red rice, 0.43 mEq Vit E/g for Java black rice, and 0.02 mEq Vit E/g for Jasmine white rice.

Based on the radical scavenging activity, the inhibitory ability of phytochemical compounds in the organic brown rice extract was the highest when compared with the white and black rice extracts. This is because brown rice had the highest levels of total phenols and total flavonoids. Phenolic compounds and flavonoids can donate hydrogen atoms to the purple DPPH free radical to form a yellow compound (Ciulu *et al*., 2018).

Bioactive compounds in rice such as antioxidants include phenolic compounds, flavonoids, anthocyanins, proanthocyanins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid (Chay *et al.*, 2017). The antioxidant activity of anthocyanins depends on their chemical structures (where the basic structure orientation in the ring determines the ease with which the hydrogen atoms in the hydroxyl group are donated to free radicals). According to Moko *et al.* (2019), the colour density of rice extract is directly proportional to the anthocyanin content in the rice (*i.e.*, the darker the red colour, the higher the anthocyanin content). Because

Sirampog black rice is so rich in natural pigments, namely anthocyanins, its dark purple colour is close to black.

Starch digestibility

The *in vitro* digestibility of starch shows the ease with which it may be hydrolysed by human digestive enzymes (Pang *et al.*, 2018). The higher the digestibility value *in vitro*, the easier it is for starch to be digested into simple sugars by amylolytic enzymes. Based on the enzymatic hydrolysis mechanism, amylose is hydrolysed by α-amylase, whereas amylopectin is hydrolysed by α-amylase and β-amylase (glucoamylase). This causes amylopectin to be digested over a longer period of time than amylose.

Hypoglycaemic and antioxidative stress effects in diabetic rats

The hypoglycaemic effect refers to the ability to suppress or lower blood sugar levels. Hyperglycaemia is a condition characterised by blood glucose levels that are higher than normal (Diaz-Canul *et al.*, 2021). According to Surono *et al.* (2021), the blood glucose levels in normal rats should be between 50 and 135 mg/dL. However, all streptozotocin-induced (DM) rats in the current study showed a 200 mg/dL increase in fasting blood sugar levels. The process of determining the effect of treatment on fasting blood sugar levels is depicted in Figure 1.

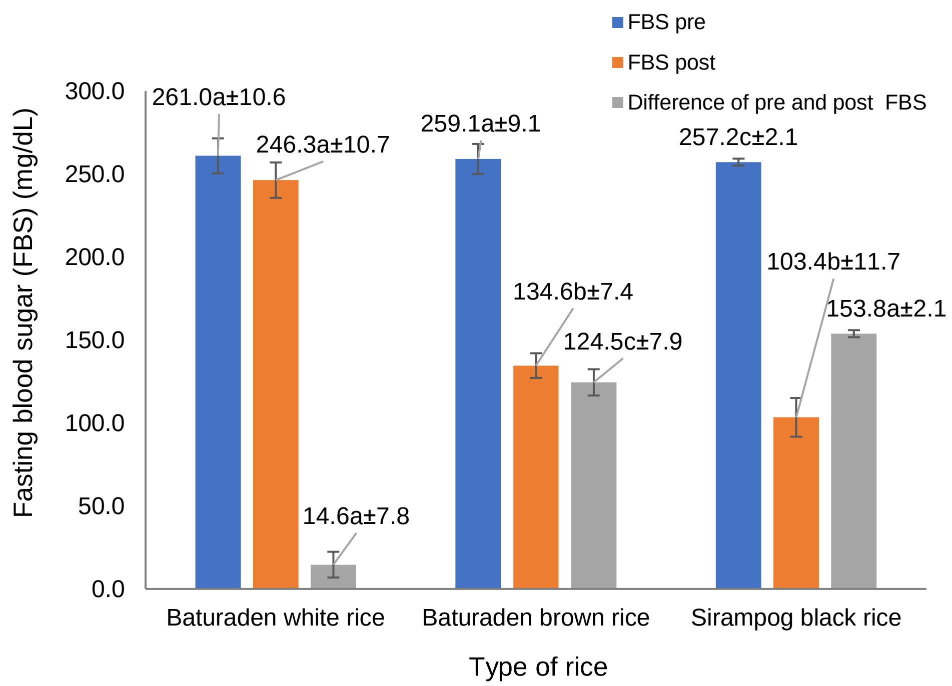

Figure 1. Fasting blood sugar (FBS) levels in diabetic rats before and 14 days after feeding with various types of rice. Means with different lowercase letters are significantly different ($p < 0.05$).

Fasting blood sugar levels decreased in all groups of DM rats after treatment for 2 w. More specifically, a significant decrease in fasting blood sugar occurred in rats treated with Sirampog black rice (Δ = 153.80 mg/dL) followed by Baturraden organic brown rice (Δ = 124.48 mg/dL) and Baturraden white rice (Δ = 14.62 mg/dL). These findings may be due to the high dietary fibre content in Sirampog black rice and Baturraden organic brown rice (8.05 and 5.97%, respectively).

Dietary fibre consists of the plant polysaccharide lignin, which is resistant to hydrolysis *via* human digestive enzymes (Gong *et al.*, 2018). Therefore, fibre consumption has a positive effect on blood glucose levels in people with type 2 DM because fibre that enters with food (especially soluble fibre) will absorb a lot of fluid in the stomach, and make the food more viscous. Foods that are more viscous slow down the digestive process so that the absorption of nutrients such as glucose occurs slowly (Jiang *et al.*, 2021). Slow glucose absorption means that blood glucose levels decrease. According to Jia *et al.* (2020), high fibre consumption is associated with decreased fasting blood glucose levels and 2-h postprandial glucose levels in patients with type 2 DM. As such, good fibre consumption would be beneficial for people with DM (with a recommended fibre consumption of 20 - 35 or 25 g/day; Bruno *et al.*, 2017).

In the present work, DM rats treated with Sirampog black rice presented an MDA level of 2.62 nmol/mL, whereas DM rats treated with Baturraden organic brown rice or Baturraden white rice had higher MDA levels of 3.96 and 10.14 nmol/mL, respectively (Figure 2). Since the MDA level can describe the activity of free radicals in cells, it is used as one of the parameters to indicate the occurrence of oxidative stress due to free radicals (Xie *et al.*, 2020). According to Schwingshackl *et al.* (2017), an increase in oxidative stress is directly proportional to the formation of MDA. In a previous study by Khoo *et al.* (2017), the MDA level was 1.599 nmol/mL in normal (non-diabetic) rats and 5.693 nmol/mL in diabetic rats. In the present work, Sirampog black rice showed the greatest potential to maintain MDA levels in diabetic rats. The ability to maintain MDA levels was more likely influenced by the antioxidant, anthocyanin, and carotenoid contents of Sirampog black rice. Ayala *et al.* (2014) stated that a high accumulation of free radicals, unaccompanied by antioxidants in the body, causes oxidative stress.

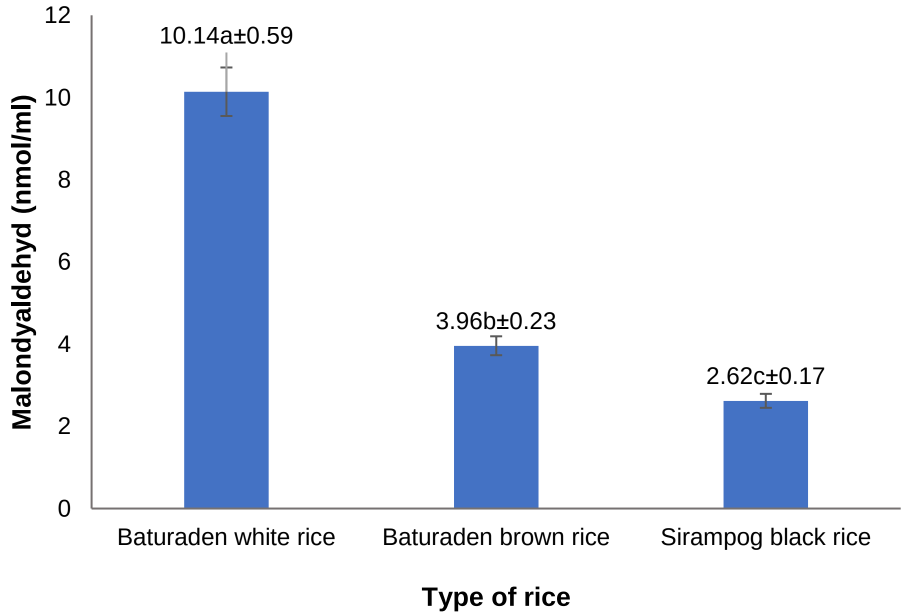

Figure 2. Malondialdehyde (MDA) levels in diabetic rats after 14 days of feeding with various types of rice. Means with different lowercase letters are significantly different ($p < 0.05$).

Baturraden organic brown rice had slightly higher antioxidant levels than Sirampog black rice (91.71 and 90.72%, respectively), although the values were not significantly different. In addition, the anthocyanin (15.7 mg/g) and carotene (9.66 g/g) contents of Sirampog black rice were higher than that of Baturraden organic brown rice (0.24 mg/g anthocyanin and 4.92 g/g carotene). Anthocyanins are the flavonoids that act as pigments, and have antioxidant properties. In addition, carotenoids have antioxidant functions that can protect the body from free radicals (Fatchiyah *et al.*, 2020).

Antioxidants, or reducing agents, function to prevent oxidation or to neutralise compounds that have been oxidised by donating hydrogen and/or electrons (Kadiri, 2017). In the body, there is an endogenous antioxidant or anti-free radical mechanism whereby free radicals are neutralised *via* the defence system between antioxidant enzymes (such as catalase, superoxide dismutase, and glutathione peroxidase) and a number of non-enzyme antioxidants, including vitamins A, C, and E; glutathione; ubiquinone; and flavonoids (Sivamaruthi *et al.*, 2018).

In addition to lowering fasting blood sugar and MDA levels in diabetic rats, rice consumption also increased the body weight of experimental rats (Figure 3). The body weight of diabetic rats that were treated with the three rice feeds increased almost the same amount as that of normal rats receiving the control feed. The body weight increase was most significant for rats treated with Baturraden organic brown rice (Δ = 13.3 g pre-post average), followed by Sirampog black rice (Δ = 11.8 g), and finally with a much lower weight increase, Baturraden white rice (Δ = 3.2 g). This weight gain in Wistar rats indicated that rice consumption could have a positive impact on patients with DM because this group generally experiences drastic weight loss (*i.e.*, burning off fat stores because of the body's inability to provide glucose that can be metabolised into energy; Sivamaruthi *et al.*, 2018) This phenomenon had been suggested by Chay *et al.* (2017), whose findings indicated that the inability of tissues to utilise blood glucose causes the liver to utilise more fatty acids and protein as an energy source instead.

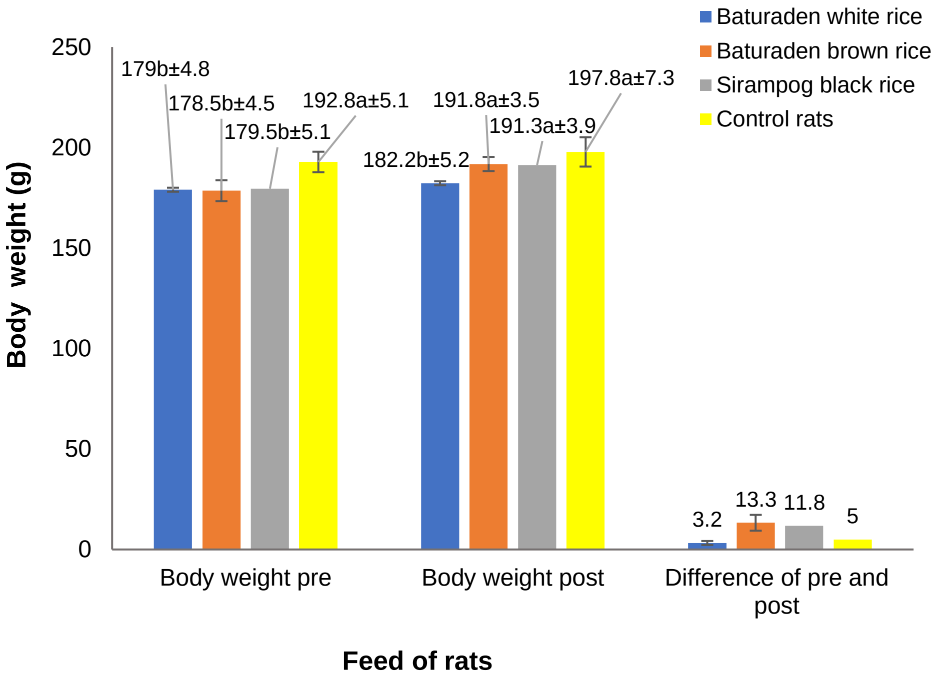

Figure 3. Body weight of diabetic rats before and after 14 days of feeding with various types of rice. Means with different lowercase letters are significantly different ($p < 0.05$).

Conclusion

Sirampog black rice had higher carotene, anthocyanin, and antioxidant levels than Baturraden organic brown and white rice. Sirampog black rice could also lower fasting blood sugar levels more effectively (Δ = 153.80 mg/dL) than Baturraden organic brown rice (Δ = 124.48 mg/dL) and Baturraden white rice (Δ = 14.62 mg/dL). Furthermore, DM rats fed with Sirampog black rice had the lowest MDA content (2.62 nmol/mL) when compared with Baturraden organic brown rice (3.96 nmol/mL) and Baturraden white rice (10.14 nmol/mL). Sirampog black rice is recommended for patients with DM, although its impact on fasting blood sugar and diabetic weight reduction in humans must first be tested in these patients. However, the present work had limitations because the levels of polyphenols and flavonoids and their composition were not determined; so, further research is warranted.

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References

Aini, N., Sustriawan, B., Wahyuningsih, N. and Mela, E. 2022. Blood sugar, haemoglobin and malondialdehyde levels in diabetic white rats fed a diet of corn flour cookies. Foods 11(12): 1819.

An, J. S., Bae, I. Y., Han, S. I., Lee, S. J. and Lee, H. G. 2016. *In vitro* potential of phenolic phytochemicals from black rice on starch digestibility and rheological behaviors. Journal of Cereal Science 70: 214-220.

Association of Official Agricultural Chemists (AOAC). 2005. Official methods of analysis of the AOAC International. Journal of AOAC International 41: 12.

Ayala, A., Muñoz, M. F. and Argüelles, S. 2014. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative Medicine and Cellular Longevity 2014: 360438.

Bruno, R., Petrella, E., Bertarini, V., Pedrielli, G., Neri, I. and Facchinetti, F. 2017. Adherence to a lifestyle programme in overweight/obese pregnant women and effect on gestational diabetes mellitus: A randomized controlled trial. Maternal and Child Nutrition 13(3): e12333.

Chaiyasut, C., Sivamaruthi, B., Pengkumsri, N., Keapai, W., Kesika, P., Saelee, M. and Lailerd, N. 2016. Germinated Thai black rice extract protects experimental diabetic rats from oxidative stress and other diabetes-related consequences. Pharmaceuticals 10(4): 3.

Chay, C., Hurtada, W. A., Dizon, E. I., Elegado, F. B., Norng, C. and Raymundo, L. C. 2017. Total phenolic, antioxidant activity and physic-chemical properties of waxy pigmented and non-pigmented rice in Cambodia. Food Research 1(1): 9-14.

Chen, M. H., Bergman, C. J., McClung, A. M., Everette, J. D. and Tabien, R. E. 2017. Resistant starch: Variation among high amylose rice varieties and its relationship with apparent amylose content, pasting properties and cooking methods. Food Chemistry 234: 180-189.

Ciulu, M., Cádiz-Gurrea, M. L. and Segura-Carretero, A. 2018. Extraction and analysis of phenolic compounds in rice: A review. Molecules 23(11): 1-20.

Diaz-Canul, C., Coop-Gamas, F. Y., Ávila-Escalante, M. L., Betancur-Ancona, D. A. and Aranda-González, I. I. 2021. Evaluation of minor steviol glycosides effect on insulin resistance, serum triglycerides, and antioxidant capacity of diabetized Wistar rats. International Food Research Journal 28(2): 342-349.

Fatchiyah, F., Sari, D. R. T., Safitri, A. and Cairns, J. R. K. 2020. Phytochemical compound and nutritional value in black rice from Java Island, Indonesia. Systematic Reviews in Pharmacy 11(7): 414-421.

Fujii, H., Iwase, M., Ohkuma, T., Ogata-Kaizu, S., Ide, H., Kikuchi, Y. and Kitazono, T. 2013. Impact of dietary fiber intake on glycemic control, cardiovascular risk factors and chronic kidney disease in Japanese patients with type 2 diabetes mellitus. The Fukuoka Diabetes Registry. Nutrition Journal 12(1): 159.

Gong, L., Cao, W., Chi, H., Wang, J., Zhang, H., Liu, J. and Sun, B. 2018. Whole cereal grains and potential health effects: Involvement of the gut microbiota. Food Research International 103: 84-102.

Hao, J., Zhu, H., Zhang, Z., Yang, S. and Li, H. 2015. Identification of anthocyanins in black rice (*Oryza sativa* L.) by UPLC/Q-TOF-MS and their *in vitro* and *in vivo* antioxidant activities. Journal of Cereal Science 64: 92-99.

Hernawan, E. and Meylani, V. 2016. Analysis of physicochemical characteristics of white rice, brown rice and black rice. Jurnal Kesehatan Bakti Tunas Husada 15(1): 79.

Hu, E. A., Pan, A., Malik, V. and Sun, Q. 2012. White rice consumption and risk of type 2 diabetes: Meta-analysis and systematic review. BMJ 344: 7851.

Huang, F., Nilholm, C., Roth, B., Linninge, C., Höglund, P., Nyman, M. and Ohlsson, B. 2018. Anthropometric and metabolic improvements in human type 2 diabetes after introduction of an Okinawan-based Nordic diet are not associated with changes in microbial diversity or SCFA concentrations. International Journal of Food Sciences and Nutrition 69(6): 729-740.

Huang, Y. P. and Lai, H. M. 2016. Bioactive compounds and antioxidative activity of colored rice bran. Journal of Food and Drug Analysis 24(3): 564-574.

Indrasari, S. D., Purwani, E. Y., Wibowo, P. and Jumali, J. 2008. Glycemic index value of rice of several varieties of rice. Penelitian Pertanian Tanaman Pangan 27(3): 127-134.

Indriyani, F., Nurhidajah, N. and Suyanto A. 2013. Physical, chemical and organoleptic characteristics of brown rice flour based on variations in drying time. Jurnal Pangan dan Gizi 4(8): 116231.

Jia, M., Yu, Q., Chen, J., He, Z., Chen, Y., Xie, J. and Xie, M. 2020. Physical quality and *in vitro* starch digestibility of biscuits as affected by addition of soluble dietary fiber from defatted rice bran. Food Hydrocolloids 99: 105349.

Jiang, G., Feng, X., Wu, Z., Li, S., Bai, X., Zhao, C. and Ameer, K. 2021. Development of wheat bread added with insoluble dietary fiber from ginseng residue and effects on physiochemical properties, *in vitro* adsorption capacities and starch digestibility. LWT – Food Science and Technology 149: 111855.

Kadiri, O. 2017. A review on the status of the phenolic compounds and antioxidant capacity of the flour: Effects of cereal processing. International Journal of Food Properties 20: S798-S809.

Kamsiati, E., Dharmawati, E. and Haryadi, Y. 2018. Physical and chemical properties of indigenous rice from tidal swamp land in Central Kalimantan. Jurnal Pangan 27(2): 107-116.

Kereh, B. C., Mayulu, N. and Kawengian, S. E. 2016. Description of the content of nutrients in black rice (*Oryza sativa* L.) of the Enrekang variety. Jurnal E-Biomedik 4(1): 1-7.

Khoo, H. E., Azlan, A., Tang, S. T. and Lim, S. M. 2017. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food and Nutrition Research 61(1): 1361779.

Köten, M., Ünsal, A. S. and Kahraman, S. 2020. Physicochemical, nutritional, and cooking properties of local Karacadag rice (*Oryza sativa* L.)-Turkey. International Food Research Journal 27(3): 435-444.

Kozuka, C., Yabiku, K., Takayama, C., Matsushita, M., Shimabukuro, M. and Masuzaki, H. 2013. Natural food science based novel approach toward prevention and treatment of obesity and type 2 diabetes: Recent studies on brown rice and γ-oryzanol. Obesity Research and Clinical Practice 7: e165-e172.

Laokuldilok, T., Shoemaker, C. F., Jongkaewwattana, S. and Tulyathan, V. 2011. Antioxidants and antioxidant activity of several pigmented rice brans. Journal of Agricultural and Food Chemistry 59(1): 193-199.

Maghsoudi, Z., Ghiasvand, R. and Salehi-Abargouei, A. 2016. Empirically derived dietary patterns and incident type 2 diabetes mellitus: A systematic review and meta-analysis on prospective observational studies. Public Health Nutrition 19(2): 230-241.

Mangiri, J., Mayulu, N. and Kawengian, S. E. S. 2016. An overview of the nutrient content of black rice (*Oryza sativa* L.) Pare Ambo cultivar, South Sulawesi. Jurnal E-Biomedik 4(1): 2-6.

Moko, E. M., Ngangi, J. and Rahardiyan, D. 2019. Oxidative stability of crude extract of red variety rice bran from Minahasa, north Sulawesi on lipid-based food products. Food Research 3(3): 249-257.

Ogurtsova, K., Guariguata, L., Barengo, N. C., Ruiz, P. L. D., Sacre, J. W., Karuranga, S., … and Magliano, D. J. 2022. IDF diabetes atlas: Global estimates of undiagnosed diabetes in adults for 2021. Diabetes Research and Clinical Practice 183: 109118.

Pang, Y., Ahmed, S., Xu, Y., Beta, T., Zhu, Z., Shao, Y. and Bao, J. 2018. Bound phenolic compounds and antioxidant properties of whole grain and bran of white, red and black rice. Food Chemistry 240: 212-221.

Pedro, A. C., Granato, D. and Rosso, N. D. 2016. Extraction of anthocyanins and polyphenols from black rice (*Oryza sativa* L.) by modeling and assessing their reversibility and stability. Food Chemistry 191: 12-20.

Pengkumsri, N., Chaiyasut, C., Saenjum, C., Sirilun, S., Peerajan, S., Suwannalert, P. and Sivamaruthi, B. S. 2015. Physicochemical and antioxidative properties of black, brown and red rice varieties of northern Thailand. Food Science and Technology 35(2): 331-338.

Prasad, B. J., Sharavanan, P. S. and Sivaraj, R. 2019. Health benefits of black rice – A review. Grain and Oil Science and Technology 2(4): 109-113.

Pratiwi, R. and Purwestri, Y. A. 2017. Black rice as a functional food in Indonesia. Functional Foods in Health and Disease 7: 182-194.

Purwani, E. Y. and Wardana, I. P. 2019. Physico-chemical characteristics of specialty rice for innovative food. Jurnal Penelitian Pertanian Tanaman Pangan 2(3): 165.

Qi, X., Cheng, L., Li, X., Zhang, D., Wu, G., Zhang, H. and Wang, Y. 2019. Effect of cooking methods on solubility and nutrition quality of brown rice powder. Food Chemistry 274: 444-451.

Renuka, N., Mathure, S. V., Zanan, R. L., Thengane, R. J. and Nadaf, A. B. 2016. Determination of some minerals and β-carotene contents in aromatic indica rice (*Oryza sativa* L.) germplasm. Food Chemistry 191: 2-6.

Salgado, J. M., de Oliveira, A. G. C., Mansi, D. N., Donado-Pestana, C. M., Bastos, C. R. and Marcondes, F. K. 2010. The role of black rice (*Oryza sativa* L.) in the control of hypercholesterolemia in rats. Journal of Medicinal Food 13(6): 1355-1362.

Sami, W., Ansari, T., Butt, N. S. and Hamid, M. R. A. 2017. Effect of diet on type 2 diabetes mellitus: A review Introduction. International Journal of Health Sciences 11(2): 65.

Schwingshackl, L., Hoffmann, G., Lampousi, A. M., Knüppel, S., Iqbal, K., Schwedhelm, C. and Boeing, H. 2017. Food groups and risk of type 2 diabetes mellitus: A systematic review and meta-analysis of prospective studies. European Journal of Epidemiology 32(5): 363-375.

Septiana, A. T. and Asnani, A. 2013. Antioxidan activity of *Sargassum duplicatum* seaweed extract. Jurnal Teknologi Pertanian 14(2): 79-86.

Shao, Y., Xu, F., Sun, X., Bao, J. and Beta, T. 2014. Phenolic acids, anthocyanins, and antioxidant capacity in rice (*Oryza sativa* L.) grains at four stages of development after flowering. Food Chemistry 143: 90-96.

Sivamaruthi, B., Kesika, P. and Chaiyasut, C. 2018. Anthocyanins in Thai rice varieties : distribution and pharmacological significance. International Food Research Journal 25(5): 2024-2032.

Sompong, R., Siebenhandl-Ehn, S., Linsberger-Martin, G. and Berghofer, E. 2011. Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lanka. Food Chemistry 124(1): 132-140.

Surono, I. S., Wardana, A. A., Waspodo, P., Saksono, B. and Venema, K. 2021. Effect of taro starch, beet juice, probiotic, and/or psicose on gut microbiota in a type 2 diabetic rat model: A pilot study. Journal of Nutrition and Metabolism 2021: 1825209.

Wang, Z., Zhang, M., Liu, G., Deng, Y., Zhang, Y., Tang, X. and Wei, Z. 2021. Effect of the degree of milling on the physicochemical properties, pasting properties and *in vitro* digestibility of Simiao rice. Grain and Oil Science and Technology 4(2): 45-53.

Widyawati, P. S., Maya Suteja, A., Putut Suseno, T. I., Monica, P., Saputrajaya, W. and Liguori, C. 2015. Effect of pigment color difference in organic rice on antioxidant activity. Jurnal Agritech 34(4): 399.

Xie, F., Lei, Y., Han, X., Zhao, Y. and Zhang, S. 2020. Antioxidant ability of polyphenols from black rice, buckwheat and oats: *In vitro* and *in vivo*. Czech Journal of Food Sciences 38(4): 242-247.

Yamuangmorn, S., Sreethong, T., Saenchai, C., Rerkasem, B. and Prom-u-thai, C. 2021. Effects of roasting conditions on anthocyanin, total phenolic content, and antioxidant capacity in pigmented and non-pigmented rice varieties. International Food Research Journal 28(1): 73-82.

Yuliana, N. D. and Akhbar, M. A. 2020. Chemical and physical evaluation, antioxidant and digestibility profiles of white and pigmented rice from different areas of Indonesia. Brazilian Journal of Food Technology 23: 1-13.