Research article

Antioxidant activity and inhibition of α-amylase and α-glucosidase in fermented black rice bran-based analog rice

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Abstract: Analog rice is an alternative food that can also be a functional food. Analog rice has the same shape as rice grains, can be made from non-rice flour, and can be consumed like white rice. The purpose of this study is to determine the effect as an antidiabetic of the addition of fermented black rice bran (FBB) and non-fermented black rice bran (NFBB) on analog rice based on in vitro assays. This research was conducted in three stages: analog rice was made from the raw materials of sorghum, yellow soybean, black soybean, FBB and NFBB; analysis of the phytochemical characteristics of analog rice; evaluation of DPPH-radical scavenging; and analysis of the inhibitory effects of agents α-amylase and α-glucosidase. Increased phenol, flavonoid, and anthocyanin content were found in analog rice with the addition of FBB. In addition, analog rice with the addition of FBB also had antioxidant activity and higher inhibition of α-amylase and α-glucosidase activity with a range of 54.50–65.52%, 63.16–65.51% and 60.27–62.09% respectively compared to analog rice with the addition of NFBB. The results of this study indicate that analog rice with the raw materials of sorghum, beans and the addition of FBB has potential as an antidiabetic food.

Keywords: antihyperglycemic; digestive enzyme; functional food; radical-scavenging
1. Introduction

Diabetes is a disease chronic caused by non-infectious metabolic disorder, characterized by hyperglycemic conditions or high blood glucose levels [1]. The most common diabetes is type 2 diabetes (T2D) which is caused by lifestyle factors and diet that causes obesity [2]. Postprandial hyperglycemia can be reduced by modulating the absorption of glucose in the body as it reduces the hydrolysis of carbohydrates. Pancreatic α-amylase is an enzyme in the initial stages of starch hydrolysis, and α-glucosidase subsequently hydrolyzes di- and oligosaccharides into glucose in the intestine [3]. Some bioactive components act as inhibitors that can inhibit the activity of the enzymes α-amylase and α-glucosidase, which can reduce the absorption rate of glucose and postprandial hyperglycemia. Digestive enzyme inhibitors are generally found in functional foods that contain many bioactive components such as polyphenols [4]. The condition of hyperglycemia will also encourage the formation of free radicals that trigger a state of oxidative stress in the body. Oxidative stress will reduce insulin secretion and inhibit glucose uptake in muscle cells and adipose [5], because oxidative stress can trigger different expressions of some genes involved [6]. Therefore the body needs antioxidant-rich foods to prevent oxidative stress. Testing the activity of antioxidants and inhibitors of α-amylase and α-glucosidase enzymes can be used as an approach to evaluate the antidiabetic potential of a functional food.

Analog rice is an artificial rice which has the shape of white rice granules. It can be made from non-rice flour, cooked and consumed as white rice, and has high functional properties [7]. Therefore, analog rice can be consumed as a functional food and as an alternative staple food. Analog rice made from a mixture of corn, sago, yellow soybeans, and rice bran contains phenols, flavonoids, resistant starches, high food fiber and has a low IG value [8–11]. Analog rice from sorghum, red palm oil, yellow soybeans, and rice bran has been reported to have properties that inhibit colon cancer [12].

In this study, analog rice was made using the raw materials of sorghum, yellow soybeans, black soybeans, and rice bran which are still underutilized. The selection of raw materials was based on the results of previous studies that show that sorghum [13], yellow soybeans [14], black soybeans [15], and rice bran [16] have antioxidant activity, are able to inhibit α-amylase and α-glucosidase enzymes, reduce blood glucose levels, increase insulin levels and regenerate β-pancreatic cells. Rice bran, which is a byproduct of hulling rice, still contains many bioactive components, food fiber and phytochemicals, so it can be used as a raw material for food products such as bakery products, drinks, and meatballs [17]. Adding rice bran to food products can also prevent chronic diseases such as cancer and cardiovascular disease [18]. Fermentation by the solid state fermentation (SSF) method can increase the amount of phenolic and antioxidant activity of rice bran which has been widely reported by several researchers [17,19,20]. Bran fermented for 4 days can increase phenolic compounds, flavonoids, γ-orizanol, α-tocopherol, and have antioxidant activity and inhibition of α-amylase [21]. There have been several studies on diabetes that have focused on bioactive components in functional foods. However, there have been no studies on antidiabetic activity of analog rice. Therefore this study was conducted to determine the effect of adding fermented black rice bran (FBB) and non-fermented black rice bran (NFBB) to analog rice on its potential as an antidiabetic. The results of this study are expected to provide scientific information about the potential of analog rice derived from local Indonesian raw materials as an antidiabetic.
2. Materials and methods

2.1. Analog rice preparation

The materials used in this study are Numbu cultivar sorghum from a local farmer in Gadog, Indonesia, Grobogan cultivar yellow soybean from BB Biogen, Indonesia, Detam 1 cultivar black soybeans from Balitkabi in Malang, Indonesia, and Cempo Ireng cultivar black rice bran prepared from a paddy which was bought from local farmers in Yogyakarta, Indonesia. The skin of the black rice was polished by rice mill processing (Satake Grain Testing Mill, Japan). The FBB was fermented by the SSF method using Rhizopus oligosporus [21].

2.2. Analog rice

Analog rice production was carried out following [7] with modifications to the temperature of extrusion. The analog rice was made in three stages: (1) mixing the raw material (flour) for 10 minutes with a dry mixer, (2) extruding process using the twin screw extruder (Berto BEX-DS-2256) at 100 °C, (3) the final product of analog rice granules was dried at 60 °C for 3 hours. Four analog rice formulas were made (the formulas are subject to a patent application): AR1 analog rice from a mixture of sorghum, yellow soybean, and NFBB; AR2 analog rice from a mixture of sorghum, black soybean, and NFBB; AR3 analog rice from a mixture of sorghum, yellow soybean, and FBB; and AR4 analog rice from a mixture of sorghum, black soybean, and FBB.

2.3. Sample extraction

Analog rice was ground in a blender (Maspion, Indonesia), and passed through an 80 mesh sieve. A sample extraction was prepared [22] with slight modifications. Analog rice and 70% ethanol (1:10) were mixed for 24 hours, and then filtered with number 41 filter pads. The residue was re-extracted with 70% ethanol. All supernatants were collected and centrifuged at 1207 xg for 20 minutes. The ethanolic extract was evaporated using a rotary vacuum evaporator at 50 °C for 1 hour, and then exhaled with N\textsubscript{2} gas until all the solvents were removed and stored at −18 °C until use. The extract was used for all analysis. All experiments were done in triplicate.

2.4. Analysis of phytochemical composition

The total phenolic content was determined using the Folin-Ciocalteu reagent [19]. Absorbance was measured at 765 nm using a UV spectrophotometer. Gallic acid was used as a standard. Results were expressed as milligrams equivalent of gallic acid per 100 grams of sample (mgGAE/100g sample).

The total flavonoid content was measured using Quercetin as a standard [23]. The absorbance was measured using a UV-VIS spectrophotometer at 432 nm. The results obtained were expressed in milligrams equivalent of quercetin per 100 grams sample (mgQE/100g sample).

Total anthocyanin content was measured based on the pH difference method [24]. The absorbance was measured at 510 and 700 nm. The absorbance value was calculated as $A = [(A510 − A700) \text{ at pH 1} − (A510 − A700) \text{ at pH 4.5}]$. The total anthocyanin was expressed as milligrams of...
cyanidin-3-glucoside (C3G) per gram of sample using a molar coefficient of 26.900 and a molecular weight of (BM) 449.

Analysis of γ-oryzanol was performed using high performance liquid chromatography (HPLC, Agilent Technologies 1200 series) [25]. As much as 20 μL of the standard and extract samples were injected into a C-18 column (Zorbax Eclipse XDB C-18 column 4.6 x 150 nm) with a flow rate of 1 mL/minute. A mixture of methanol and acetonitrile (35:65) was used during the mobile phase using the HPLC UV-VIS detector at a wavelength of 325 nm. Results were expressed as milligrams per grams of dried sample (mg/g db).

α-tocopherol was analyzed following the method 971.30 [26]. 20μL of extract was injected into HPLC (Agilent Technologies 1200 series) at 280 nm wavelength, then into a C-18 column (Zorbax Eclipse XDB C-18 column 4.6 x 150 nm), and a UV-VIS detector, methanol:isopropanol (98:2) as mobile phase, with a flow rate of 1 mL/minute. Results were expressed as milligrams per grams of dried sample (mg/g db).

2.5. DPPH radical-scavenging method

The DPPH-radical scavenging method was used to measure antioxidant activity [27]. The absorbance of the solution was measured at a wavelength of 517 nm using a UV-VIS spectrophotometer. DPPH-radical scavenging was expressed as a percentage calculated as \[1\] - (abs of sample/blank abs) X 100.

2.6. α-Amylase inhibition assay

Inhibition of the α-amylase was measured according to the method of Thalapaneni et al. [28]. Briefly, the extract sample was reacted with the amylase enzyme solution, then incubated at 37 °C for 10 minutes. 1% starch solution was added and re-incubated at 37 °C for 10 minutes. The 3,5-dinitrosalicylate (DNS) reagent was added, incubated for 5 minutes in boiling water, then as much as 5 mL of distilled water was added and the absorbance was measured using a UV-VIS detector spectrophotometer at 540 nm. The α-amylase inhibition was calculated as [(abs control - abs sample)/abs control] X 100.

2.7. α-Glucosidase inhibition assay

The α-glucosidase assay was carried out based on the method of Sancheti et al. [29]. A phosphate buffer solution 0.1 M (pH 7), 4-nitrophenyl α-Dglucopyranoside 0.5 mM, 10 μL extract sample and 25 μL α-glucosidase enzyme solution (1 mg/mL in 0.01 M phosphate buffer, pH 7) were reacted, and then incubated at 37 °C for 30 minutes. The reaction was stopped by adding sodium carbonate 100 μL (0.2 M). The inhibition of α-glucosidase was measured using a microplate reader at a wavelength of 410 nm. The percent inhibition was calculated as [1 - (abs sample / abs control)] X 100.

2.8. Statistical analysis

Data were analyzed using one way analysis of variance (ANOVA) and post hoc Duncan's multiple range test (p < 0.05). Data values were expressed as mean ± standard deviation (Mean ± SD).
3. Results and discussion

3.1. Total phenols, flavonoids and anthocyanins

Phenolic content in analog rice ranged from 429.31–758.61 mg GAE/100g (Table 1) and was significantly different (p < 0.05). Analog rice with the addition of FBB has a higher phenolic content compared to rice with the addition of NFBB in the same type of soybeans; AR4 > AR2 and AR3 > AR1. This result shows that the added FBB can increase the phenolic content of analog rice products. This is in accordance with the results of previous studies which showed that the phenolic content in FBB increased by 57.39% due to the fermentation process [21]. In this study it was found that analog rice using black soybeans with the addition of FBB and NFBB had a higher phenolic content, compared to yellow soybeans. This can be attributed to the higher phenolic content of black soybeans compared to yellow soybeans (258.02 mg GAE/100g versus 155.15 mg GAE/100g, data were not shown). This means that the phenolic content of analog rice is also strongly influenced by the raw materials used. This result was also supported by previous research [9] which stated that analog rice using raw materials of sorghum had a total phenolic content of 18 mgGAE/100g, while analog rice from composit sorghum and yellow soybeans had 24 mgGAE/100g.

The flavonoid content of analog rice ranged from 235.03–374.62 mgQE/100g and was significantly different (p < 0.05). The highest content was found in AR4 followed by AR2, AR3 and AR1. As with phenols, the flavonoid content of analog rice in the same type of soybeans with the addition of FBB is higher than with the addition of NFBB. Black soybeans in the AR4 and AR2 have a higher flavonoid content when compared to analog rice using yellow soybeans in AR3 and AR1. This is presumably because black soybeans have a higher flavonoid content. According to [30], the flavonoid content in black soybeans is 6 times higher (2.57 mg/g) than yellow soybeans (0.41 mg/g).

Anthocyanin content of analog rice is found to be higher with the addition of FBB than NFBB. The highest amount of anthocyanin obtained in this study was 78.88 mg/g (AR4) followed by AR3 (72.26 mg/g), then AR2 (70.89 mg/g) and AR1 (42.51 mg/g). The addition of FBB which has an anthocyanin content of 96.38 mg/g [21] contributed to the increase in anthocyanin levels of analog rice products. Black soybeans are also reported to contain 1.044 mg/g of anthocyanins [31], so AR2 using black soybeans and NFBB has high levels of anthocyanins as well (Table 1).

The addition of FBB to analog rice can increase total phenolic, flavonoid and anthocyanin content. The SSF technique is an alternative to increase the free phenolic compounds and antioxidant activity in food products [32]. Koji prepared from raw beans uses the SSF technique with Rhizopus sp., which has high antioxidant activity and is associated with increased total phenolic and anthocyanin content [33]. During the SSF process, hydrolysis of the glycosidic bonds into free aglycones occurred, which contributed to the high value of antioxidant activity [34].

3.2. The γ-oryzanol and α-tocopherol compound

The γ-oryzanol compound ranges from 2.84–3.36 mg/g (Table 1), not significantly different among the four analog rice formulas. The total amount of α-tocopherol obtained was significantly different for all four analog rice formulas. The highest amount of α-tocopherol was 126.16 µg/g in AR4 followed by AR3 which had 86.18 µg/g with added FBB, and the lowest content was found in AR2 (24.75 µg/g) and AR1 (14.11 µg/g) which contained added NFBB. The amount of γ-oryzanol
and α-tocopherol compounds obtained in analog rice are higher when compared to the content of γ-orizanol (0.041 mg/g) and α-tocopherol (3 µg/g) in white rice [35]. This is presumably due to the addition of black rice bran which contains a lot of γ-orizanol 1.85–4.21 mg/g [36] and α-tocopherol 43.57–150 µg/g [37]. According to [36] the addition of FBB to analog rice contributes to the increase of γ-orizanol and α-tocopherol. Enzyme activity during the fermentation process is also capable of releasing bound forms of γ-orizanol and α-tocopherol into free forms and have high bioavailability [39].

### Table 1. Chemical composition of analog rice* (db).

| Content                  | AR1           | AR2           | AR3           | AR4           |
|--------------------------|---------------|---------------|---------------|---------------|
| Total Phenol (mg AGE/100g) | 429.31 ± 0.72a | 636.56 ± 0.82c | 513.41 ± 1.06b | 758.61 ± 0.93d |
| Total Flavonoid (mg QE/100g) | 235.03 ± 0.96a | 354.97 ± 0.044c | 318.72 ± 1.24b | 374.62 ± 0.87d |
| Total Anthocyanin (mg C3G/g) | 42.51 ± 0.44a  | 70.89 ± 0.10b  | 72.26 ± 0.24c  | 78.88 ± 0.18d  |
| γ-Oryzanol (mg/g)         | 2.84 ± 0.42a   | 2.77 ± 0.36c   | 3.37 ± 0.37a   | 3.36 ± 0.39a   |
| α-Tocopherol (µg/g)       | 14.11 ± 0.29a  | 24.75 ± 3.16b  | 86.18 ± 0.25c  | 126.16 ± 0.75d |

*AR1: sorghum, yellow soybeans, and NFBB; AR2: sorghum, black soybeans and NFBB; AR3: sorghum, yellow soybeans and FBB; AR4: sorghum, black soybeans and FBB. Means ± standard deviations with the same letter on the same row differ not significantly at the test level of 5%.

γ-orizanol and α-tocopherol are antioxidant components, making up 38.7–41.1% and 1.2% of the total antioxidant components (respectively) found in bran. The γ-orizanol is the main antioxidant, and α-tocopherol is found in smaller amounts. The α-tocopherol can prevent oxidation of the body by donating hydrogen atoms from its hydroxyl groups to free radicals that often occur in diabetics [40].

### 3.3. Scavenging ability of DPPH radicals

Antioxidant activity in the analog rice was determined using the DPPH-radical scavenging method. The results showed that the scavenging ability of DPPH radicals ranged from 40.92–65.52% (Figure 1). The lowest antioxidant activity was found in AR1 and the highest was found in AR4. Antioxidants are naturally present in food, and can be increased by fermentation techniques [41]. Soy products fermented using the SSF technique have high antioxidant activity compared to non-fermented products [42]. This study also found that analog rice with the addition of FBB has high antioxidant activity. The phenolic aglycone obtained from fermentation has the highest antioxidant activity compared to the glycoside form [43]. It is also thought to be related to the number of free hydroxyl groups phenolic compounds and flavonoids obtained during SSF. The hydroxyl group can be a hydrogen donor for DPPH free radicals so antioxidant activity increases [44].

Paiva et al. [45] stated that antioxidant activity has a positive correlation with phenolic components. This study also found a positive correlation between phenolic components and antioxidant activity, namely r = 0.90. DPPH free radical scavenging activity is also associated with high anthocyanin content in analog rice. This is in accordance with research by Laokuldiilok et al. [40] which stated that pigmented rice bran showed high free radical scavenging activity–6 times that of white rice bran. In addition, it is also supported by a positive correlation value (r = 0.92) between anthocyanins and antioxidant activity.
Figure 1. Antioxidant activity of analog rice (100 µg/mL). Different letters indicate a significantly different difference by Duncan’s Multiple Range Test ($p < 0.05$). Description of analog rice refers to Table 1.

Figure 2. Inhibitory $\alpha$-amylase (A) and $\alpha$-glucosidase (B) in analog rice. Different letters indicate a significantly different difference by Duncan’s Multiple Range Test ($p < 0.05$). Description of analog rice refers to Table 1.
3.4. Inhibitory effects of analog rice against α-amylase and α-glucosidase

α-Amylase and α-glucosidase are enzymes that catalyze the degradation of starch into glucose for absorption by the body. Inhibition of these enzymes is useful for controlling excessive blood glucose and regulating hyperglycemia that causes diabetes [46]. Previous studies have reported that inhibition of the α-glucosidase enzyme by inhibitors can be one of the most effective ways to control type 2 diabetes [47].

The anti-amylase activity of analog rice is presented in Figure 2A. The percentage of inhibition of analog rice to α-amylase ranges from 53.03 ± 1.18% – 65.51 ± 0.51%. Analog rice with the addition of FBB significantly (p < 0.05) had higher anti-amylase activity ranging from 63.16–65.51%, compared to analog rice with NFBB only 53.03–55.20%. The inhibitory activity of analog rice to α-glukosidase ranged from 58.29–62.09% (Figure 2B). The highest inhibitory activity was obtained in AR4, which was made from black soybeans and FBB (62.09%), and it was significantly different (p < 0.05) from other analog rice.

The inhibitory activity of analog rice to α-amylase and α-glucosidase enzymes is thought to be related to the phenolic content in analog rice. Analog rice with the addition of FBB has higher total phenolic content due to the fermentation process being carried out. According to Sompong et al. [48] pigmented rice bran such as black rice bran is a rich source of phenolic components. The polyphenol and flavonoid content found in several foods is very effective at inhibiting α-amylase and α-glucosidase activity [49]. Phenolic compounds are able to inhibit α-amylase enzyme activity by binding to the α-amylase reactive side and changing its catalytic effect [50]. The polyhydroxyl group plays an important role in α-glukosidase inhibition [51]. In addition, polyphenols and flavonoids can mimic the pyranosil portion of α-glukosidase [52]. This study also found a positive correlation between total phenols and α-amylase and α-glucosidase inhibition \( r = 0.60 \) and \( r = 0.87 \). Previous studies also reported that there was a positive correlation between total phenols and anti-amylase activity \( r = 0.68 \) [53], and with α-glucosidase inhibitory activity [54]. The opposite has been reported by [11]; carrots, pumpkin, and radishes where phenolic content was not found, showed low inhibitory activity against α-amylase (<8.5%) and α-glucosidase (<2.5%).

4. Conclusions

The results of this study indicate that analog rice with the raw materials sorghum, black soybeans, and fermented black rice bran (AR4) has the highest amount of total phenols, flavonoids, anthocyanins, γ -oryzanol and α-tocopherol compared to the others. The analog rice also had the highest inhibitory activity against DPPH free radicals, α-amylase and α-glucosidase enzymes. The findings of this study show that analog rice made from fermented rice bran has potential as an anti-diabetes functional food.

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Conflict of interest

All authors declare no conflicts of interest.

References

1. Taha M, Alrashedy AS, Almandil NB, et al. (2021) Synthesis of indole derivatives as diabetic II inhibitors and enzymatic kinetics study of α-glucosidase and α-amylase along with their in-silico study. Intern J Biol Macromol 190: 301–318. https://doi.org/10.1016/j.ijbiomac.2021.08.207
2. Sanada H, Yokokawa H, Yoneda M, et al. (2012) High body mass index is an important risk factor for the development of type 2 diabetes. Intern Med 51: 1821–1826. https://doi.org/10.2169/internalmedicine.51.7410
3. Chiang YC, Chen CL, Jeng TL, et al. (2014) In vitro inhibitory effects of cranberry bean (Phaseolus vulgaris L.) extracts on aldose reductase, α-glucosidase and α-amylase. Int J Food Sci Techn 49:1470–1479. https://doi.org/10.1111/ijfs.12426
4. Nawaz M, Taha M, Qureshi F, et al. (2021) Synthesis α-amylase and α-glucosidase inhibition and molecular docking studies of indazole derivatives. J Biomol Struct Dyn 31: 1–11. https://doi.org/10.1080/07391102.2021.1947892
5. Hasim, Andrianto D, Ismail AI, et al. (2017) Antioxidant and α-glucosidase inhibitory activity of formulated ethanol extract of red yeast rice and rice bran. J Pharmacogn Phytochem 6: 1891–1893.
6. Behl T, Upadhyay T, Singh S, et al. (2021) Polyphenols targeting MAPK mediated oxidative stress and inflammation in rheumatoid arthritis. Molecules 26: 2–24. https://doi.org/10.3390/molecules26216570
7. Budijanto S, Yuliyanti (2012) Studi persiapan tepung sorgum (Sorghum bicolor L.Moench) dan aplikasinya pada pembuatan beras analog. J Tek Pert 13: 177–186.
8. Noviasari S, Kusnandar F, Budijanto S (2013) Pengembangan beras analog dengan memanfaatkan jagung putih. J Tek Ind Pangan 24: 195–201. https://doi.org/10.6066/jtip.2013.24.2.195
9. Noviasari S, Kusnandar F, Setiyono A, et al. (2015) Beras analog sebagai pangan fungsional dengan indeks glikemik rendah. J Gizi Pangan 10: 225–232.
10. Kurniawati M, Budijanto S, Yuliana ND (2016) Karakterisasi dan indeks glikemik beras analog berbahan dasar tepung jagung. J Gizi Pangan 11: 169–174.
11. Budijanto S, Andri YI, Faridah DN, et al. (2017) Karakterisasi kimia dan efek hipoglikemik beras analog berbahan dasar jagung, sorgum, dan sagu aren. J Agritech 37: 402–409. https://doi.org/10.22146/agritech.10383
12. Sadek NF, Yuliana ND, Prangdimurti E, et al. (2017) Plant sterol esters in extruded food model inhibits colon carcinogenesis by suppressing inflammation and stimulating apoptosis. J Med Food 20: 659–666. https://doi.org/10.1089/jmf.2016.3867
13. Kim M, Kim E, Kwak HS, et al. (2014) The ingredients in Saengshilk, a formulated health food, inhibited the activity of α-amylase and α-glucosidase as anti-diabetic function. Nutr Res Pract 8: 602–606. https://doi.org/10.4162/nrp.2014.8.5.602
14. Indranupakorn R, Sobharaksha P, Luangtana-anan M (2010) Antioxidant activities of the soybean extracts obtained by classical extraction. Int J Pharm Sci 6: 113–121.
15. El-Kordy EA, Alshahrani AM (2015) Effect of genistein, a natural soy isoflavone, on pancreatic β-cells of streptozotocin-induced diabetic rats: Histological and immunohistochemical study. *J Microse Ultrastruct* 3: 108–119. https://doi.org/10.1016/j.jmau.2015.03.005

16. Wahyuni AS, Munawaroh R, Da’i M (2015) Antidiabetic mechanism of ethanol extract of blackrice bran on diabetic rats. *Natl J Physiol Pharm Pharmacol* 6: 106–110. https://doi.org/10.5455/njpmp.2015.5.1111201590

17. Liu L, Zhang R, Deng Y, et al. (2017) Fermentation and complex enzyme hydrolysis enhance total phenolics and antioxidant activity of aqueous solution from rice bran pretreated by steaming with α-amylase. *Food Chem* 221: 636–643. https://doi.org/10.1016/j.foodchem.2016.11.126

18. Okarter O, Liu RH (2010) Health benefits of whole grain phytochemicals. *Crit Rev Food Sci Nutr* 50: 193–208. https://doi.org/10.1080/10408390802248734

19. Razak DLA, Rashid NYA, Jamaluddin A, et al. (2015) Enhancement of phenolic acid content and antioxidant activity of rice bran fermented with Rhizopus oligosporus and Monascus purpureus. *Biocatal Agric Biotechnol* 4: 33–38. https://doi.org/10.1016/j.bcab.2014.11.003

20. Noviasari S, Kusnandar F, Setiyono A, et al. (2019) Profile of bioactive compound, antioxidant and anti-amylase activity of fermented black rice bran with *Rhizopus oligosporus*. *Pertanika J Trop Agric Sci* 42: 489–501.

21. An JS, Bae IY, Han S, et al. (2016) In vitro potential of phenolic phytochemicals from black rice on starch digestibility and rheological behaviors. *J Cereal Sci* 70: 214–220. https://doi.org/10.1016/j.jcs.2016.06.010

22. Chang CC, Yang MH, Wen HM, et al. (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal* 10: 178–182.

23. Lee J, Durst RW, Wrolstad RE (2005) Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. *J AOAC Int* 88: 1269–1278.

24. Sabir A, Rafi M, Darusman LK (2017) Discrimination of red and white rice bran from Indonesia using HPLC fingerprint analysis combined with chemometrics. *Food Chem* 221: 1717–1722. https://doi.org/10.1016/j.foodchem.2016.10.114

25. AOAC (2006) Official Method 971.30 α-Tocopherol and α-Tocopheryl acetate in foods and feeds. In AOAC Official Method. The Association of Official Analytical Chemist, Washington, DC, USA: AOAC Inc.

26. Kubo I, Masuoka N, Xiao P, et al. (2002) Antioxidant activity of dodecyl gallate. *J Agric Food Chem* 50: 3533–3539. https://doi.org/10.1021/jf011250h

27. Xu BJ, Chang SKC (2007) A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *J food Sci* 72: S159–S166.
31. Zhou R, Cai W, Xu B (2017) Phytochemical profiles of black and yellow soybeans as affected by roasting. *Int J Food Prop* 20: 3179–3190. https://doi.org/10.1080/10942912.2017.1280678
32. Oliveira MDS, Cipolatti EP, Furlong EB, et al. (2012) Phenolic compounds and antioxidant activity in fermented rice (*Oryza sativa*) bran. *J Food Sci Technol* 32: 531–537. https://doi.org/10.1590/S0101-20612012005000071
33. Lee IH, Hung YH, Chou CC (2008) Solid state fermentation with fungi to enhance the antioxidative activity, total phenolic and anthocyanin contents of black bean. *Int J Food Microbiol* 121: 150–156.
34. Huynh NT, Camp JV, Smagghe G, et al. (2014) Improved release and metabolism of flavonoids by steered fermentation processes: a review. *Int J Mol Sci* 15: 19369–19388. https://doi.org/10.3390/ijms151119369
35. Torimitsu M, Nagase R, Yanagi M, et al. (2010) Replacing white rice with pre-germinates brown rice mildly ameliorates hyperglycemia and imbalance of adipocytokine levels in type 2 diabetes model rats. *J Nutr Sci Vitaminol* 56: 287–292. https://doi.org/10.3177/jnsv.56.287
36. Yu S, Nehus ZT, Badger TM, et al. (2007) Quantification of vitamin E and gamma-oryzanol components in rice germ and bran. *J Agric Food Chem* 55: 7308–7313. https://doi.org/10.1021/jf071957p
37. Saunders RM (1990) The properties of rice bran as a foodstuff. *Cereal Foods World* 35: 632–635.
38. Massarolo KC, de Souza TD, Collazzo CC, et al. (2017) The impact of *Rhizopus oryzae* cultivation on rice bran: Gamma-Oryzanol recovery and its antioxidant properties. *Food Chem* 228: 43–49. https://doi.org/10.1016/j.foodchem.2017.01.127
39. Gani A, Wani S, Masoodi F, et al. (2012) Whole-grain cereal bioactive compounds and their health benefits: a review. *J Food Process Technol* 3: 146–156. https://doi.org/10.4172/2157-7110.1000146
40. Laokulidilok T, Shoemaker CF, Jongkaewwattana S, et al. (2011) Antioxidants and antioxidant activity of several pigmented rice brans. *J Agric Food Chem* 59: 193–199. https://doi.org/10.1021/jf103649q
41. Duhan JS, Mehta K, Sadh PK, et al. (2016) Bio-enrichment of phenolics and free radicals scavenging activity of wheat (WH-711) fractions by solid state fermentation with Aspergillus oryzae. *Af J Biochem Res* 10: 12–19. https://doi.org/10.5897/AJBR2015.0854
42. Singh HB, Singh BN, Singh SP, et al. (2010) Solid-state cultivation of Trichoderma harzianum NBRI-1055 for modulating natural antioxidants in soybean seed matrix. *Bioresour Technol* 101: 6444–6453. https://doi.org/10.1016/j.biortech.2010.03.057
43. Heim KE, Tagliaferro AR, Bobilya DJ (2002) Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J Nutr Biochem* 13: 572–584. https://doi.org/10.1016/s0955-2863(02)00208-5
44. Dey TB, Kuhad R (2014) Upgrading the antioxidant potential of cereals by their fungal fermentation under solid-state cultivation conditions. *Lett Appl Microbiol* 59: 493–499. https://doi.org/10.1111/lam.12300
45. Paiva FF, Vanier NL, Berrios JDJ, et al. (2016) Polishing and parboiling effect on the nutritional and technological properties of pigmented rice. *Food Chem* 191: 105–112. https://doi.org/10.1016/j.foodchem.2015.02.047
46. Phoboo S, Shetty K, Elobeid T (2015) In vitro assays of anti-diabetic and anti-hypertensive potential of some traditional edible plants of Qatar. *J Med Active Plants* 3: 22–29. https://doi.org/10.7275/R59P2ZK6

47. Islam S (2006) Sweet potato (*Ipomea batatas* L.) leaf: its potential effect on human health and nutrition. *J Food Sci* 71: R13–R18. https://doi.org/10.1111/j.1365-2621.2006.tb08912.x

48. Sompong R, Siebenhandl-Ehn S, Linsberger-Martin G, et al. (2011) Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lanka. *Food Chem* 124: 132–140. https://doi.org/10.1016/j.foodchem.2010.05.115

49. Isbilir SS, Tuncay D (2014) A study on bioactive content, antioxidant activity, and α-amylase inhibition of black rice grown in Turkey. *Eur Int J Sci Technol* 3: 51–60.

50. Kunyanga CN, Ilmungi JK, Okoth MW, et al. (2012) Total phenolic content, antioxidant and antidiabetic properties of methanolic extract of raw and traditionally processed Kenyan indigenous food ingredients. *LWT-Food Sci Technol* 45: 269–276. https://doi.org/10.1016/j.lwt.2011.08.006

51. Kim JS, Kwon CS, Son KH (2000) Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. *Biosci Biotechnol Biochem* 64: 2458–2461. https://doi.org/10.1271/bbb.64.2458

52. Wongsa P, Chaiwarit J, Zamaludien A (2012) In vitro screening of phenolic compounds, potential inhibition against α-amylase and α-glucosidase of culinary herbs in Thailand. *Food Chem* 131: 964–71. https://doi.org/10.1016/j.foodchem.2011.09.088

53. Premakumara GAS, Abeysekera WKSM, Ratnasooriya WD, et al. (2013) Antioxidant, anti-amylase and anti-glycation potential of brans of some Sri Lankan traditional and improved rice (*Oryza sativa* L.) varieties. *J Cereal Sci* 58: 451–456. https://doi.org/10.1016/j.jcs.2013.09.004

54. Apostolidis E, Lee CM (2010) In vitro potential of ascophyllum nodosum phenolic antioxidant-mediated alpha-glucosidase and alpha-amylase inhibition. *J Food Sci* 75: H97–H102. https://doi.org/10.1111/j.1750-3841.2010.01544.x

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