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The analysis of nutritional value, total phenolic and flavonoid contents, and antioxidant activities from the ethanolic extracts of the roasted broken brown rice powder

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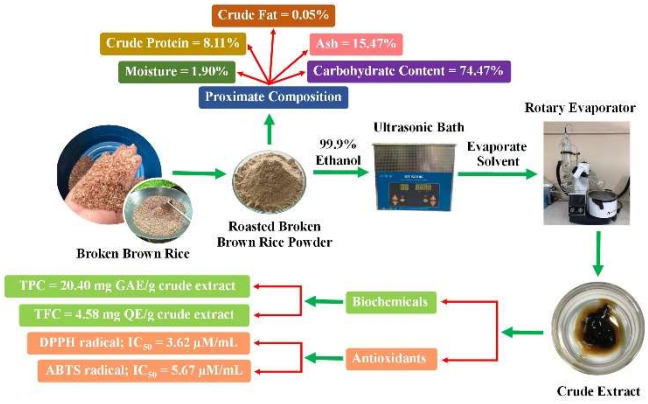
Abstract

The objective of this study was to determine the nutritional value, total phenolic and flavonoid contents, and antioxidant activities of the roasted broken brown rice powder. The roasted broken brown rice powder was dried at 60 °C for 3 h in a hot air oven and extracted with absolute ethanol by using ultrasonic technique. Then, the sample was filtered to separate the residue and mixture solution and dried to remove the solvent by rotary evaporator for obtaining the ethanolic crude extract. Finally, the crude extract was collected and determined proximate composition, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity. The results revealed that the percentage crude extract of the extraction yield was 0.47% by weight with crude carbohydrate (74.47% w w^{-1}), crude protein (8.11% w w^{-1}), and fat (0.05% w w^{-1}), respectively. The TPC and TFC were found at an average of 20.40 ± 0.37 mg GAE g^{-1} crude extract and 4.58 ± 0.19 mg QE g^{-1} crude extract, respectively. Moreover, the sample has highly efficient antioxidants activity similar to a standard Trolox solution. Therefore, it was reasonably concluded that the roasted broken brown rice powder product was rich in beneficial nutrients for the health effects and represented adding value to a by–product of rice production.



Keywords: Roasted broken brown rice powder; Ethanolic extracts; Total phenolic and flavonoid contents; Antioxidant activities; Nutritional value

1. Introduction

Rice is a common staple food for a large segment of half the world population. Many varieties of rice grown in the world have several colors, shapes, and sizes. Belong to the species *Oryza sativa* which has its origin in Asia and occupies the great majority of all global rice production and consumption as white rice or brown rice. White rice is a commonly consumed type because the refined grain is also polished to appear more palatable and increase its shelf life but the process removes much of its nutrition [1, 2].

Brown rice, a whole grain, is also a popular option that contains all parts of the grain.

This rice is comparatively more nutritious, containing more fiber, carb-rich endosperm, proteins, lipids, minerals, vitamins, and several bioactive compounds such as γ-aminobutyric acid (GABA), phenolic, flavonoid, and antioxidants [3, 4]. It consists of favorable content and benefit for many health benefits such as prevention of cancer and chronic diseases like type 2 diabetes, reduction on the risk of heart disease, obesity, and control of blood lipids [5, 6]. "Broken Rice" is a by-product during grain polishing from rice mills, which is the fragments of rice that are not completely broken. Deshelling is the first step in rice processing and yields brown rice then the polishing or whitening process. Some quality losses occur during milling which is the major problem of the rice industry [7]. The market value with the broken grain is much less than that for whole grains and abandoned from the whole grains while absolutely nothing inferior with this rice than the other grains. It contains abundant nutrients and chemical composition but it is not aesthetically pleased to consumers. They perceived the broken rice as lower grade rice and sold it at a lower price than whole grain rice of the same quality. Therefore, it is most often used for making animal fodder, especially for very young animals, and the raw material of flour can be used as an important ingredient for many food products [8 − 11]. It is also suitable for baby food and cereals for health lover because it is naturally gluten-free and easy to use with high in calories [12, 13].

The roasted broken brown rice powder used as raw material in this study was obtained from Ban Khok Sa–At, Um–Chan subdistrict, Kusuman district, Sakon Nakhon province. There was less economic value (low–cost but high potential utilization) as a resource for value–added products. The use of roasted broken brown rice powder from broken rice products could increase the utilization of broken rice grains which reduced losses incurred normally from the locally processed rice grains. This study focus on analyzing the nutritional value, total phenolic, and flavonoid contents, and antioxidant activities from the ethanolic extracts of roasted broken brown rice powder as a resource for value–added products. It is essential for optimizing quality and economic return.

2. Materials and Methods

Materials

The roasted broken brown rice powder as a raw material in this study was obtained from Ban Khok Sa–At, Um–Chan subdistrict, Kusuman district, Sakon Nakhon province as presented in Fig. 1(a – c). Absolute ethanol (99.99%) as an organic solvent for extraction of the broken brown rice sample was perched from QRëC, New Zealand. Folin – Ciocalteu reagent, Trolox, gallic acid, quercetin, 2,2–Diphenyl–1–picrylhydrazyl (DPPH$^{\bullet}$), 2,2–azino–bis (3–ethylbenzothiazoline–6–sulfonic acid)(ABTS) were bought from Sigma, Germany. Other chemicals used in this work were the analytical grade.

Sample preparation and extraction

The roasted broken brown rice powder as a raw material was dried at 60 °C for 3 h in a hot air oven. After drying, the obtained sample of approximately 50 g was extracted with absolute ethanol at a ratio of 1 : 3 (w v^{-1}) by using the ultrasonic technique for 1 h and controlled temperature at 40 °C. Then, the sample was filtered to separate the residue and mixture solution. In the subsequent step, the mixture solution was carefully dried to remove the solvent by rotary evaporator for obtaining the ethanolic crude extract. The final step, the crude extract as shown in Fig. 1(d) was collected in a reagent bottle and then kept at – 20 °C until testing time. All of the methods for the roasted broken brown rice powder sample extraction process were repeated three times to study the

$$\text{Percentage of Yield} = \frac{\text{crude extract of the broken brown rice (g)}}{\text{initial weight of the broken brown rice (g)}} \times 100 \quad (1)$$

(a) **(b)** **(c)** **(d)**

Fig. 1 Showing (a) the broken brown rice, (b) the roasting process of the broken brown rice, (c) The roasted broken brown rice powder as a raw material in this study, and (d) the crude extract from the roasted broken brown rice powder.

percentage yield of crude extract which was calculated by the following Eq. (1):

Determination of total phenolic content (TPC)

The total phenolic content (TPC) of the crude extract sample was determined by using the Folin – Ciocalteu assay method following the published reports from Ti *et al.* [14], Chen *et al.* [15], and Peanparkdee *et al.* [16]. The experiment briefly explains the crude extract of 5 mg was diluted with 10 mL of absolute ethanol. After that, the obtained mixture was pipetted as the considerable quantity of 0.50 mL and it was reacted against freshly 10% Folin Ciocalteu of 2.50 mL. The resulting mixture was incubated at room temperature for 5 min and then neutralized with 7.50% (w v^{-1}) Na_2CO_3 of 2 mL. The final reaction mixture was centrifuged at 3,000 rpm for 10 min, incubated at room temperature for 10 min, and analyzed by using a UV – visible spectrophotometer (UV – 1800, Shimadzu, Japan) with a wavelength of 740 nm. The results concentration of a specific TPC compound in the crude extract of the sample was reported as milligram (mg) of Gallic acid equivalent which was used as a standard per gram (g) of the sample extract.

Determination of total flavonoid content (TFC)

The crude extract of the roasted broken brown rice powder was evaluated for the total flavonoid content (TFC) according to the experimental method reported by Zhang *et al.* [4], Chu *et al.* [17], and Chua *et al.* [18]. In the first step, 5 mg of the crude extract was diluted with absolute ethanol of 10 mL. Then, the 0.50 mL mixing solution was pipetted and the absolute ethanol of 1.50 mL was added. Then, the mixing solution was pipetted as an amount of 0.50 mL, added the absolute ethanol of 1.50 mL and 0.10 mL of 10% $AlCl_3$, respectively. After that, the resulting mixture was added 0.10 mL of 1 M CH_3COOK, and gradually filled with distilled water of 2.80 mL. Finally, the reaction mixture was gently shaken to carefully mix and incubated at room temperature for 30 min. The mixed solution was carefully measured absorbance at the specific wavelength of 437 nm. In this work, quercetin was applied as a standard and the TFC of the crude extract was evaluated in terms of milligram (mg) quercetin equivalent (QE) per gram (g) of the crude sample extract.

Determination of antioxidant activity with the scavenging effects on DPPH• radical

The DPPH• assay was a follow–up to the capacity of the extracted samples which could give electrons to DPPH• radicals and this was a color change process of DPPH• radicals from purple to yellow. Generally, the absorbance at 517 nm was properly applied to determine the reduction value of the DPPH• radical and the

Trolox compound was mostly used as the standard solution for performance reference Khunchalee *et al.* [19], Hobanthad *et al.* [20], Jandaruang *et al.* [21]. In this study, a weighing 5 mg of crude extract was diluted in absolute ethanol and carefully adjusted the volume to 10 mL to prepare the stock sample solution. An examination of the antioxidant content was carried out by mixing the 300 µL of the obtained sample solution with the 2,700 µL of 0.06 mM $DPPH^{\bullet}$ solution. Next, the reaction mixture was thoroughly mixed, kept in the dark, and incubated for 30 min at room temperature. Finally, the resulting reaction mixture was taken to measure the absorbance by using a UV-visible spectrophotometer at a specific wavelength of 517 nm. The obtained report data would present the relationship between the concentrations of the sample and IC_{50} value which was compared versus the standard Trolox solution in terms of the percentage of antioxidants capacity (%inhibition) using the following equation:

$$\%\,\text{inhibition} = \frac{\text{Abs of the blank (ethanol} + DPPH^{\bullet}) - \text{Abs of the sample (extract} + DPPH^{\bullet})}{\text{Abs of the blank (ethanol} + DPPH^{\bullet})} \times 100 \quad (2)$$

All of this method was repeated in the experiment of 3 replicates and the experimental results show the data reliability consists of the mean value and standard deviation.

Determination of antioxidant activity with the scavenging effects on ABTS radical

An investigation of the antioxidant activity of the crude extract of the roasted broken brown rice powder by using the ABTS radical scavenging activity was carried out following the reports of Wongklom *et al.* [22], Xu *et al.* [23], and Seong *et al*. [24]. The crude extract solution was carefully prepared by weighing 5 mg and adjusted with the absolute ethanol to the volume of 10 mL. While ABTS reagent was prepared by the 7 mM of ABTS solution and mixed with 2.45 mM of $K_2S_2O_8$ solution at a ratio of 1 : 1 and incubated at – 20 °C for 12 h. The absolute ethanol was used to dilute the obtained ABTS solution so that the absorbance value was within the range of 0.70 – 0.90 at a specific wavelength of 734 nm. After that, the reaction mixture was prepared by adding 150 µL of crude sample solution and mixed with 2,850 µL of the obtained ABTS solution. Then, the generated mixture was incubated in the dark condition at room temperature for 2 h. The ABTS radical scavenging potential of the sample extracts was analyzed by using a UV-visible spectrophotometer at a specific wavelength of 734 nm. The Trolox compound was used as a standard solution for comparing the efficiency of antioxidant activity. The percentage of the ABTS radical scavenging potential was calculated as following:

$$\%\,\text{ABTS radical scavenging potential} = \frac{\text{Abs of the blank} - \text{Abs of the sample}}{\text{Abs of the blank}} \times 100 \quad (3)$$

The Abs of the blank was the absorbance of the ethanol mixing with ABTS solution and the Abs of the sample was the absorbance of the crude extract solution mixing with ABTS solution. The obtained result of this experiment for the ABTS scavenging activity was presented as an IC_{50} value.

The proximate analysis of the roasted broken brown rice powder

The proximate compositions of the roasted broken brown rice powder which consist of moisture, ash, crude protein, and crude fat were evaluated by using a standard method from the official methods of the Association of Official Analytical Chemists International (AOAC; 2004)

and following the reports of Zubair *et al.* [25], Oko *et al.* [26], Vunain *et al.* [27], and Verma and Srivastav [28]. Additionally, the total percentage of the carbohydrate content in the sample was calculated by subtracting the percentage value of the moisture, ash, protein, and fat from the total of 100% following the reports from Akalu and Geleta [29], and Eze [30].

3. Results and Discussion

The percentage yield extraction of the roasted broken brown rice powder

According to the sample preparation and extraction by using ultrasonic technique and applied the absolute ethanol as an organic solvent, the experimental results found that the physical characteristics of the obtained crude extract were dark brown and very sticky as presented in Fig. 1(d). In addition, the calculation of the extraction yield showed the percentage crude extract of 0.47% by weight compared with the weight of the roasted broken brown rice powder sample. Since this research focuses on the study of crude extracts which naturally contained phenolic and flavonoid compounds, absolute ethanol was used as an organic solvent for the extraction of these important compounds because of a polar group of them. For these reasons, the amount of the obtained crude extract was quite a few. This result accorded to the research of Khunchalee *et al.* [19] which has described involving the solvent as the important factor to obtain the extraction yield from the sample.

Quantification of total phenolic content (TPC) and total flavonoid content (TFC) from the crude extract

As shown in Fig. 2, the TPC and TFC from the crude extract of the roasted broken brown rice powder were found at an average of 20.40 ± 0.37 mg GAE g^{-1} crude extract and 4.58 ± 0.19 mg QE g^{-1} crude extract, respectively. This study was performed in triplicate and the resulting data has a little discrepancy. These data were compared with the published report of Hansakul *et al.* [31] who researched the TPC and TFC of Thai rice extract and they presented that the TPC of Dawk Mali 105 and Sangyod were approximate of 18 – 189 mg GAE g^{-1} rice extract. While TFC both of Dawk Mali 105 and Sangyod was about of 19 – 46 mg rutin hydrate eq g^{-1} rice extract. In addition, a report from Teeranachaideekul *et al.* [32] showed TPC and TFC compounds of the rice Sangyod, Munpoo, Rice berry, Homnin, and Luempua in the range of 128 – 322 mg GAE g^{-1} extract and 86 – 341 mg CE g^{-1} extract, respectively. All of this published report used rice grain samples that have not gone through any process. On the other hand, the roasted broken brown rice powder as a raw material for this work was roasted to be processed into innovative products from rice waste. In this case, it was highly probable that some of the TPC and TFC in the roasted broken brown rice powder was decomposed from heat which directly affected the quantity of the TPC and TFC which is less than those reports reference.

The antioxidant capacity of crude extract

The scavenging activity data of $DPPH^{\bullet}$ radical in terms of the specific percentage of antioxidants capacity (%inhibition) both of a standard Trolox solution and crude extract solution obtained from the roasted broken brown rice powder was displayed in Fig. 3. The experimental data found that the standard Trolox solution had the IC_{50} value of 3.45 µM while the crude extract solution demonstrated the IC_{50} value of 3.62 µM. Besides, the obtained result of the ABTS radical scavenging activity assay was also explained in Fig. 4. The presented data indicated that both a standard Trolox solution and crude extract solution

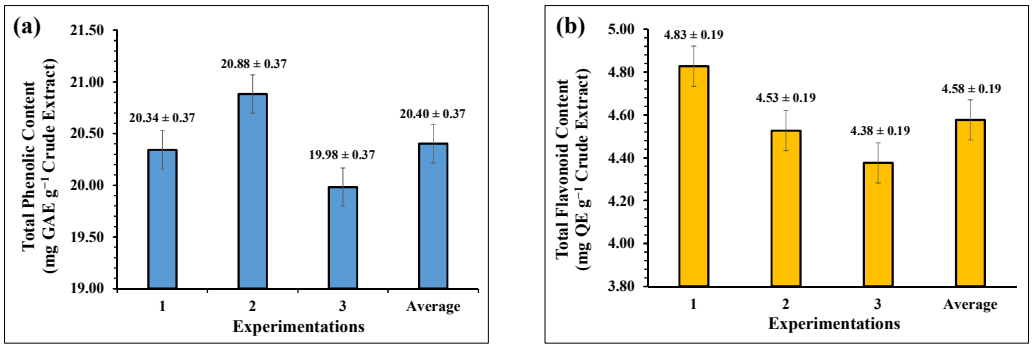


Fig. 2 (a) total phenolic content (TPC) and (b) total phenolic content (TFC) from the crude extract of the roasted broken brown rice powder.

sample have very similar antioxidant values. The IC_{50} value of a standard Trolox solution and crude extract solution sample was 5.57 µM and 5.67 µM, respectively. All of the above data showed that the linearity (R^2) was greater than 0.9950. Hence, the correlation between the concentration of the substance and the percentage of inhibition from this experiment was highly reliable.

Both experimental results showed that the trend of antioxidant activity between a standard Trolox solution and crude extract solution sample was similar. The obtained results were consistent with the published reports of Jandaruang *et al.* [21], Hansakul *et al.* [31], Maisuthisakul *et al.* [33], and Sanwiriya mongkol *et al.* [34] who studied the antioxidant activity of various types of rice extracts from Thailand. They have described and discussed involving the antioxidant capacity of the extract from Thai rice that has high antioxidant potential due to the Thai rice sample consisting of a rich in antioxidants such as phenolic, flavonoid, GABA, anthocyanin, β–carotene, carotenoid, and other compounds. Moreover, several published articles reported the mineral content (e.g. Ca, Zn, Fe, Cu, Mn, and Mg), proximate compositions (e.g. protein, fat, and carbohydrate), and vitamins of rice which may help to inhibit free radicals as well [25, 27, 28, 35, 36]. Therefore, the roasted broken brown rice powder product not only contained TPC and TFC but may also have other important chemical compounds which were highly effective in antioxidants. These possible reasons supported the obtained experimental data on the antioxidant activity of both DPPH• and ABTS radical scavenging assay.

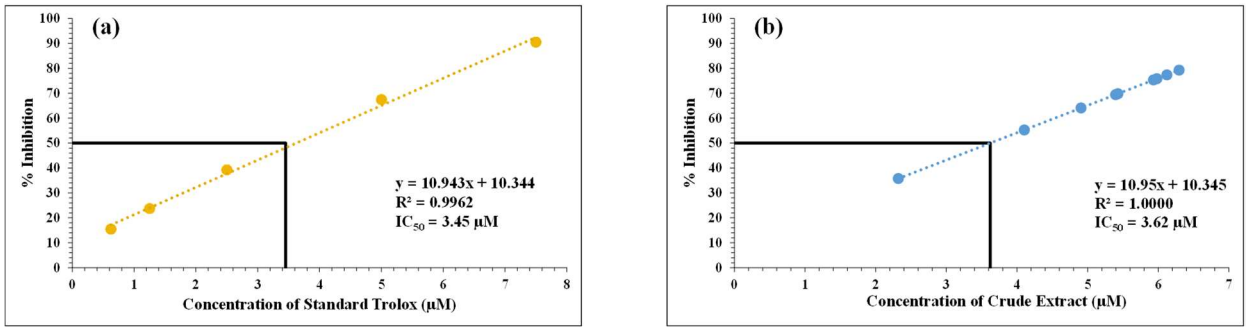


Fig. 3 (a) the results scavenging effects on DPPH• radical of standard Trolox solution in terms of the percentage of antioxidants capacity (%inhibition) and (b) the affected of the various concentration of crude extract solution on the percentage of antioxidants DPPH• radical capacity (%inhibition).

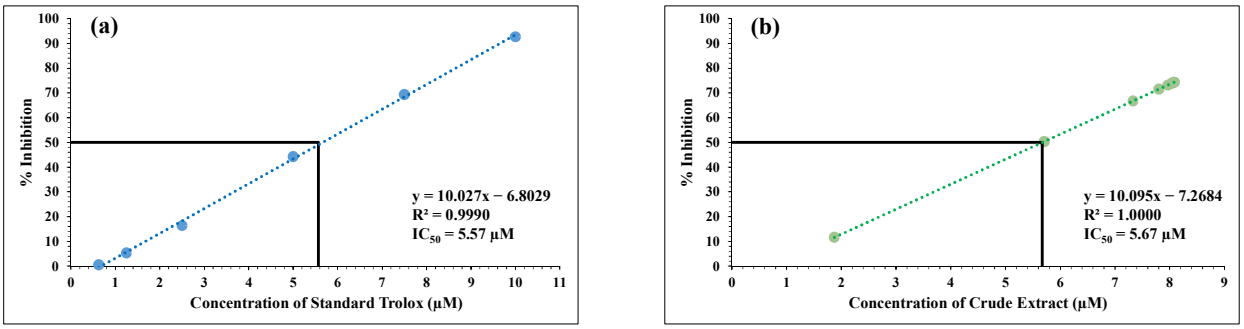


Fig. 4 The results scavenging effects on ABTS radical in terms of the percentage of antioxidants capacity (%inhibition) of (a) standard Trolox solution and (b) crude extract solution at various concentrations.

The proximate composition of the roasted broken brown rice powder

The percentage proximate composition of the roasted broken brown rice powder product sample was illustrated in Fig. 5. The obtained experimental results indicated that the major compositions of the sample were crude carbohydrates which has higher than 74% (w w^{-1}). While crude protein and fat were approximately 8.11% and 0.05% (w w^{-1}), respectively. The results of this experiment were similar to the research report of Zubair *et al.* [25], Verma *et al.* [28], Wongpriaw [35], and Chooklin *et al.* [37] who have researched and reported the nutritional values of the selected rice varieties sample. However, the moisture value of the roasted broken brown rice powder sample had lower than 2% (w w^{-1}) when compared with the previous reports displayed the percentage of moisture about 8 – 12% (w w^{-1}). This may be the rice samples used in this work undergoing a roasting process to remove moisture.

Moreover, this research work has also analyzed the percentage of ash value which was determined together with the fiber values. The data found that ash value was up to about of 15% (w w^{-1}). The high value of ash content in the sample may due to the mineral composition in the rice sample which was dependent on the soils and the water used for cultivation [25, 28, 35, 37]. There is also another possible reason to explain the high ash content of rice samples. Because the rice sample was heat–roasted, the moisture content was low. Furthermore, the analyzed carbohydrate and protein content were also relatively high value even after the rice sample went through the heating process. From this supporting information, residual ash content can be inferred as a crude fiber of the roasted broken brown rice powder product that will have a positive effect on the excretory system of consumers. Hence, all of the obtained experimental results indicated that the roasted broken brown rice powder product not only has nutritional benefits but also contains powerful antioxidants.

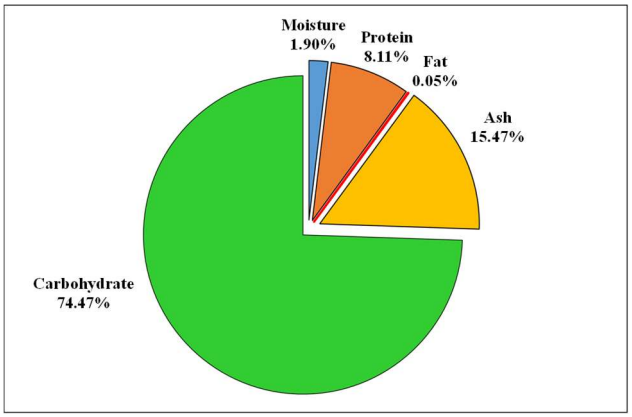


Fig. 5 The percentage proximate composition of the roasted broken brown rice powder.

4. Conclusion

The overall experimental results suggested that the roasted broken brown rice powder product containing the TPC and TFC compounds has highly efficient antioxidants activity similar to a standard Trolox solution. Furthermore, the obtained results of proximate composition analysis by following standard of the official methods of the Association of

Official Analytical Chemists International (AOAC; 2004) found that the nutritional value of the roasted broken brown rice powder sample was comparable to the selected rice varieties sample from several published reports. Therefore, it was reasonably concluded that the roasted broken brown rice powder product was rich in beneficial nutrients for the health effects. Thus, the roasted broken brown rice powder sample represented an innovative product from processing and adding value to a by–product of rice production in the rural area of Sakon Nakhon province.

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