

Antioxidant capacities and gamma oryzanol content of color rice bran oil

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Abstract

Rice berry and purple rice of a Thai rice variety collected from Phitsanulok province were analysed to determine of gamma oryzanol content and their antioxidant capacity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH^o) radical scavenging assay. The rice sample was extracted with hexane. The bran oil of purple rice sample showed higher values of antioxidant activity based on the DPPH^o assay and total phenolic content based on Folin-Ciocalteu method than the rice berry oil sample. In addition, the bran oil of purple rice sample showed highest values of gamma oryzanol content than the rice berry oil sample. The total phenolic content of purple rice bran oil had the highest value (2.9-3.2 mg/GAE/g) whilst rice berry had the lowest total phenolic content (2.7-2.9 mg/GAE/g). The average content of gamma oryzanol of rice berry and purple rice were found to be 0.017 and 0.028 µg/mg respectively. This study demonstrates that the color rice bran oil can be considered as valuable sources of bioactive compounds with high antioxidant properties.

Keywords: Rice berry, Purple rice, Antioxidant capacity, Total phenolic content, Gamma oryzanol

1. Introduction

Rice is a major cereal crop in the developing world. It is consumed as a staple food by over one-half of the world's population with approximately 95% of production in Asia (Bhattacharjee, Singhal, & Kulkarni, 2002). Although widely consumed as white rice, there are many special cultivars of rice that contain colour pigments such as black rice, red rice and brown. Their name refers to the kernel colour (black, red or purple) which is formed by deposits of anthocyanins (Sompong, Siebenhandl-Ehn, Linsberger-Martin & Berghofer, 2011). Rice bran is one of the valuable by products of the rich

milling process, particularly rich in dietary fibers and contains of starch, protein, vitamins and dietary minerals. Rice bran oil extracted from the germ and inner husk of the rice, is rich in gamma-oryzanol (Patel & Naik, 2004). Gamma-oryzanol (Fig. 1), a group of ferulic acid esters of phytosterols and triterpene alcohols, is a naturally rich source of antioxidants and unsaturated fatty acids. It is used in a wide range of applications in food, cosmetic and pharmaceutical industries (Siripairoj, Kaewchada & Jaree, 2014). However, there is limited published information available about antioxidant capacities and gamma oryzanol content of the color rice bran oil.

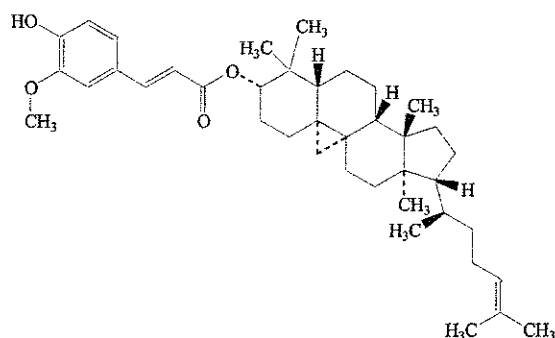


Fig. 1 Structure of gamma-oryzanol

2. Materials and method

2.1 Chemical and reagent

The compounds 2,2-diphenyl-1-picrylhydrazyl (DPPH^o), 2,6-di-tert-butyl-4-methylphenol (BHT), Folin-Ciocalteu's reagent and standard of gallic acid were purchased from Sigma-Aldrich Fine Chemicals (st. Louis, MO). Gamma-oryzanol (98% purity) was purchased from Tokyo Chemical (Japan). Methanol, ethanol and acetonitrile were purchased from Merck (Germany). Cetyl trimethylammonium bromide (CTAB) was purchased from Fluka (Buchs, Switzerland). All chemical reagents were analytical grade.

2.2 Rice sample and sample preparation

Rice berry and purple rice used in this study were collected from Phitsanulok province. The method of Li et al. (2012) was adapted to extract gamma-oryzanol from rice seeds. 2 g of rice seeds were homogenized with 10 mL of ethanol/hexane (4:3, v/v) for 5 min at 10,000 rpm, prior to being centrifuged for 20 min at 7600×g. The pellet was re-extracted twice with 10 mL of hexane and centrifuged. The supernatant were pooled and washed first with 10 mL distilled water and then with 5 mL of a 10% aqueous NaCl solution. The organic phase was retained and reduced to dryness under a gentle stream of nitrogen.

2.3 Determination of total phenolic content

The total phenolic content of crude extract was determined using the Folin-Ciocalteu method with some modifications (Liu & Yao, 2007). Briefly, 40 µl of crude extract solution was shaken for 1 min with 200 µl of diluted (1:10 with DI water) Folin-Ciocalteu reagent. After the mixture solution was shaken, 160 µl of 10% Na₂CO₃ was added, and final volume was made up to 1 ml with distilled water. After 2 h of reaction, the absorbance was read at 760 nm with micro-plate reader spectrophotometer and used to estimate the phenolic acid content using a standard curve prepared using standard of gallic acid.

2.4 Free-radical scavenging on DPPH^o

The free-radical scavenging capacity of crude extracts was evaluated according to the procedure of Liyana-Pathirana and Shahidi (2007) with some modifications. Briefly, 100 µl of crude extract was added to the freshly prepared 0.1 mM DPPH^o solution (900 µl), and the mixture was kept at room temperature in the dark room for 30 min. The absorbance was read at 517 nm with micro-plate reader spectrophotometer relative to the control (as 100%). The percentage of scavenging effect was expressed as $[1 - (A_{517} \text{ of sample} / A_{517} \text{ of control})] \times 100$. Radical scavenging activity was expressed as % inhibition of DPPH radicals. IC₅₀ values were also calculated. Tocopherol standard was taken as a referent compound.

2.5 HPLC determination of gamma oryzanol content

The ultraviolet-visible measurement was performed using a UV-1600 spectrophotometer (Shimadzu, Japan). The variable wavelength detector was operated at 330 nm for the determination of gamma-oryzanol concentration on adsorption

capacity study. High-performance liquid chromatography-diode array detection analyses were performed on a Shimadzu LC-10ADVP system (Shimadzu Corporation, Kyoto, Japan), equipped with degasser (DGU-14A), solvent delivery systems (LC-10ATVP), column oven, SPD-10A UV/Visible dual detector, and LC Lab-Solution software (Shimadzu Corporation, Kyoto, Japan). All analyses were achieved on Chromolith® Flash RP-18 column (25 mm×4.6 mm, 5µm). The mobile phase was a gradient binary phase consisting of 1.8 mM CTAB and methanol. Flow rate was kept at 1.0 ml min⁻¹ and the sample volume injected was set at 20 µl. The diode array detector was set to monitor at 330 nm for gamma-oryzanol. The column temperature was maintained at room temperature. Calibration curve was constructed with the external standard.

2.6 Statistical analysis

Antioxidant activity assay was carried out three times from the same crude extract in order to determine their reproducibility. Analysis of the variance was used to determine any difference in antioxidant activities resulting from these methods. Duncan's new multiple-range test was used to determine significant differences. Statistical significance was declared at $p < 0.05$.

3. Results and discussion

3.1 Total phenolic content

The total phenolic content of rice berry and purple rice bran oil at different location in Phitsanulok province are shown in Table 1. The total phenolic content of purple rice bran oil had the highest value (2.9-3.2 mg/GAE/g) whilst rice berry had the lowest total phenolic content (2.7-2.9 mg/GAE/g). All sample of rice bran oil from site 1 showed the greatest the total phenolic content compared to other sites.

3.2 DPPH^o radical scavenging activity

The free-radical scavenging activity of the bran oil extracts of rice berry and purple rice were evaluated using the DPPH^o assay method (Table 1). The DPPH radical scavenging assay is rapid and sensitive way to survey the antioxidant activity of a specific compounds or plant extracts. The DPPH radical scavenging capacity in the study was reported after 10 min reaction time for all samples investigated. The parameter used to measure the radical scavenging activity of the extracts and fractions evaluated is IC₅₀ value. The results obtained were shown in Table 1. Purple rice oil sample (IC₅₀ = 5.69-6.36 µg/ml) of hexane extract showed the highest antioxidant potential when compared to other extracts. The value is comparable with that of standard α -tocopherol (IC₅₀ = 3.5±0.2 µg/ml).

Table 1

Total phenolic content, % inhibition DPPH^o and IC₅₀ of rice berry and purple rice bran oil

Sites	Rice bran oil	Total phenolic content (mg GAE/g)	% inhibition DPPH ^o	IC ₅₀ (mg/ml)
1	Rice berry	2.7±0.1	56.27	11.08
	Purple rice	3.2±0.2	72.02	5.69
2	Rice berry	2.5±0.1	67.20	11.32
	Purple rice	2.9±0.2	78.14	6.36

3.3 The content of gamma oryzanol

The proposed reversed-phase high-performance liquid chromatography-diode array detection was applied to the determination of gamma oryzanol in rice berry and purple rice of a Thai rice variety collected from Phitsanulok province. The sample was prepared according to sample preparation (see Experimental) and the

content of gamma oryzanol in each sample solution was determined using the optimum conditions. The samples gave well-defined peaks. There is no interference peak present in either sample. The average content of gamma oryzanol of rice berry and purple rice were found to be 0.017 and 0.028 $\mu\text{g}/\text{mg}$ respectively.

Table 2

The gamma oryzanol content of rice berry and purple rice bran oil

Sites	Rice bran oil	Gamma oryzanol content ($\mu\text{g}/\text{mg}$)	% Recovery
1	Rice berry	0.019 ± 0.11	107.64
	Purple rice	0.032 ± 0.05	118.45
2	Rice berry	0.015 ± 0.03	101.22
	Purple rice	0.024 ± 0.09	112.52

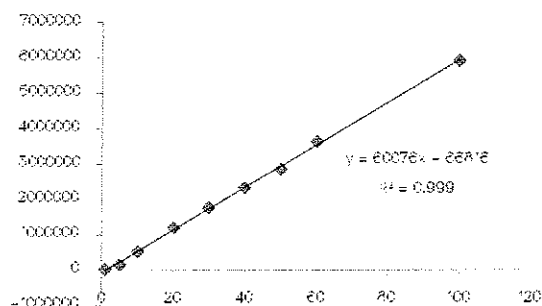


Fig. 2 Calibration curve of gamma oryzanol standards

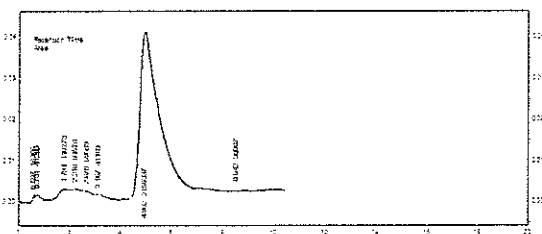


Fig. 3 Chromatogram of gamma oryzanol in purple rice bran oil sample

4. Conclusions

This study demonstrates that the color rice bran oil can be considered as valuable sources of bioactive compounds with high antioxidant propoties. The concentration of bioactive constituents, including phenolic acid and gamma oryzanol were found in greater amounts in bran oil. The bran oil of purple rice sample showed higher values of antioxidant activity based on the DPPH^o assay and total phenolic content based on Folin-Ciocalteu method than the rice berry oil sample. In addition, the bran oil of purple rice sample showed highest values of gamma oryzanol content than the rice berry oil sample.

Acknowledgements

We are grateful to the Rajabhat Pibulsongkram University for their financial support. We would also like to thank Associate Professor Dr. Boonsom Liawruangrath for her valuable suggestions and comments with the preparation of the manuscript.

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