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Chemical Compositions, Phytochemicals, and Antioxidant Capacity of Rice Bran, Rice Bran Layer, and Rice Germ

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Abstract

In the present study, chemical compositions, phytochemicals, and antioxidant activity of rice bran (contained bran layer and rice germ) rice bran layer (without germ), and rice germ of four indica rice cultivars (waxy and non waxy) were investigated. The yield of rice bran, bran layer, and rice germ of all rice types were ranged between 17.59 -21.75%, 14.63-17.14%, and 2.81- 3.19% of the whole kernel, respectively. Rice germ fraction was high in protein, lipid, and fiber, whilst rice bran layer fraction was a good source of carbohydrate and ash. In the phytochemical compositions and antioxidant activity study, rice germ contained highest amount of α-tocopherol, γ-tocopherol, and indicated the strongest antioxidant activity. The rice bran layer showed highest level of γ-oryzanol with the amount ranged from 5.07 mg/g in RD 6 to13.55 mg/g in black waxy rice.

Keywords: rice germ, antioxidant activity, rice bran, pigmented rice, colored rice

1. Introduction

Rice bran is a by-product from milling process of paddy rice to produce polished rice. In general, it contains 12-20 % of total kernel weight including pericarp, seed coat, nucellar layer, aleurone layer, embryo, and outer portion of the starchy endosperm. Rice bran is the most nutritious part of rice and a good source of

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bioactive phytochemicals such as γ-oryzanol, tocopherols, and tocotrienols; which have health beneficial properties and antioxidant activity. In recent years, rice bran has been extensively studied for its antioxidative and disease-fighting properties in disorders such as lowering the risk of cancer formation, coronary hearth diseases, and lower of cholesterol [1], having anti-inflamatory activity, and inhibition cholesterol oxidation [2], [3]. However, commercial rice bran, commonly, is a mixture of rice bran layer and rice germ (embryo) while the difference in the chemical compositions, phytochemical contents, and antioxidant activity between rice bran, rice germ, and rice bran layer of different rice cultivars has not been well-documented; therefore, the study was carried out.

2. Materials and methods

2.1. Sample preparation

Paddy rice of non waxy rice (Kao Dok Mali 105 (KDML) and Red rice) and waxy rice (RD6, and Black rice) were purchased from a local milling in Mahasarakham province, Thailand. All paddy rice samples were de-hulled and polished using rice de-husker and rice milling machine, set at 8% degree of milling, to obtain the rice bran, whereas rice germ samples were separated from brown rice by hands and then the remaining parts of kernels were polished to obtain bran layer. All rice bran fractions were stabilized at 115°C for 5 min before used for analyses.

2.2. Chemical composition analysis

The proximate composition was determined using standard methods of [4]; moisture by drying in an oven at 105 ℃ until constant weight; ash contents by muffle furnace temperature of 550 ℃; crude protein by Kjeldahl method, using 5.95 as the conversion factor; crude fat by the Soxhlet extraction method using petroleum ether as a solvent. Total carbohydrate content was calculated by difference.

2.3. Phytochemical analysis

2.3.1 Determination of total phenolic content

The total phenolic content of bran extracts was determined using the Folin–Ciocalteu reagent method reported by [5]. Gallic acid was used as a standard and results were calculated as gallic acid equivalents GAE (mg)/g of bran.

2.3.2 Determination of phytic acid

The phytic acid content was measured using the method described by [6].

2.3.3 Determination of α- tocopherol and γ-tocopherol, and γ-oryzanol content

Rice bran preparation was done by followed the method reviewed by [7] until the bran extracts were obtained. The analysis gamma oryzanol and alpha tocopherol was performed using the reversed phase high performance liquid chromatography (RP-HPLC) according to the method reported by [8] with some modifications. The Shimadzu HPLC system (model L-6200A) equipped with a Photo diode array detector and a computer system was applied. Detection was operated at 292 and 325 nm, simultaneously. The spectra from 250 to 600 nm were recorded for all peaks. The samples were injected through a guard-column and separated on C18 column (4.60 x 150mm, 4 μm,) (Phenomenex, USA). Gradient elution at ambient temperature was used, mobile phase A was methanol, mobile phase B was water, and mobile phase C was butanol. The gradient was as follows: 0-12 min 92%A, 4%B and 4%C: 12-25 min linear gradient from 4%B to 3%B and 4%C to 5%C with flow rate of 1.5 ml/min and injection volume of 20 μl.. The tocopherols were detected at 292 nm and gamma-oryzanols was detected at 325 nm. Chromatograms were recorded, and peak areas were

used to calculate the content of gamma-oryzanol and tocopherols compared with those of standards.

2.4. Antioxidant activity

The study on antioxidant activity of rice bran, rice bran layer, and rice germ was evaluated through three different test methods as follows. (1) **DPPH radical scavenging activity**: The antioxidant activity of the extracts, on the basis of the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by [9]. (2) **Total antioxidant capacity**: Total antioxidant capacity assay was carried out using the method reported by [9].The antioxidant activity is expressed as the number of equivalents of BHT. (3) **Linoleic acid emulsion system-thiocyanate method**: Lipid peroxidation inhibition activity of rice bran fractions was determined through the linoleic acid emulsion system-thiocyanate method by following the method reported by [10].

2.5. Statistical analysis

The means and standard deviations of all measurements were reported from triplicate determinations for each sample. One-way ANOVA in CRD was used and the significant difference between treatments were performed using Duncan's new multiple range test (DMRT) at $p < 0.05$.

3. Results and discussion

3.1. Yield and chemical compositions

Yield and chemical compositions of each rice bran fraction are indicated in table 1. The yields of rice bran (contained both rice bran layer and germ), rice bran layer, and rice germ of all four cultivars were ranged from 17.59 to 21.75%, 14.63 to 17.14, and 2.81 to 3.19, respectively. For the determination of crude fat content, the results revealed that rice germ contained highest amount of fat ranged from 20.16 to 22.56% whereas the bran layer had lowest level of fat content. Rice germ fraction also had highest values of crude protein in all cultivars while crude fiber contents were highest in pigmented rice, as detailed in table 1. For the measurement of ash and carbohydrate, the highest amounts were observed in bran layer fraction. These results, especially the chemical compositions of rice bran, were closed to those reviewed by [11].

3.2. Phytochemical study

Total phenolic contents (TPC) of each rice bran fraction and other phytochemicals, determined in this study, are showed in table 1. The results showed that rice bran layer, in all rice cultivars, contained the greatest level of TPC, phytic acid, and γ-oryzanol followed by rice bran and rice germ. Moreover, it was found that the rice bran layer of pigmented rice contained higher level of TPC than that in white rice. In contrast, α-tocopherol and γ- tocopherol were highest in rice germ. These results were similar to those studied by [12] and [13].

3.3. Antioxidant activity

3.3.1. DPPH radical scavenging activity

The results were expressed as the concentration of the extract to inhibit 50% of DPPH radical (IC_{50}). As antioxidants donate protons to this radical, the absorption decreases. The lower value of IC_{50} indicates a

higher antioxidant activity [18]. The strongest of antioxidant activity was 1.03 and 1.15 mg/ml, observed in rice germ of black rice and red rice, respectively (Fig.1) whereas the IC_{50} of α-tocopherol standard was 0.29 mg/ml. These results were similar to that studied by [14], [15].

Table 1 Chemical compositions and phytochemical content of bran layer, rice bran, and germ (%dry weight)

Compositions	Bran layer	Rice bran	Germ
Khao dok mali 105 (Non-waxy rice)			
Yield (% of whole grain)	14.89±0.47^{b}	17.64±0.72^{a}	2.81±0.11^{c}
Color	Light brown	Light brown	Light brown
Fat	12.45±0.23^{c}	18.80±0.51^{b}	21.58±0.92^{a}
Protein	10.90±0.09^{c}	13.66±0.14^{b}	15.27±0.32^{a}
Carbohydrate	45.31±1.00^{a}	40.63±0.08^{b}	30.96±0.61^{c}
Fiber	13.51±2.08	12.48±0.12	9.52±1.73
Ash	12.18±0.10^{a}	10.65±0.28^{b}	6.94±0.05^{c}
Phenolic compounds (mg/g)	1.64±0.11^{a}	1.57±0.07^{a}	0.40±0.04^{b}
Phytic acid (mg/g)	63.88±0.34^{a}	50.68±0.86^{b}	37.92±0.37^{c}
γ-oryzanols (mg/g)	5.5±0.08^{a}	3.5±0.03^{b}	1.75±0.01^{c}
α – tocopherol (μg/g)	32.77±0.75^{c}	46.12±1.42^{b}	62.52±2.02^{a}
γ – tocopherol (mg/g)	46.63±1.52^{c}	40.94±1.82^{b}	51.59±1.66^{a}
RD6 (Waxy rice and white in color)			
Yield (% of whole grain)	15.67±0.47^{b}	19.51±0.80^{a}	2.90±0.34^{c}
Color	Light brown	Light brown	Light brown
Fat	11.62±0.64^{c}	16.96±0.41^{b}	20.16±0.22^{a}
Protein	10.73±0.06^{c}	12.07±0.23^{b}	17.40±0.81^{a}
Carbohydrate	47.56±0.82^{a}	42.54±0.62^{b}	26.29±0.41^{c}
Fiber	10.97±0.07	11.77±0.49	11.67±0.55
Ash	13.87±1.02^{a}	10.78±0.33^{b}	7.57±0.04^{c}
Phenolic compounds (mg GAE/g)	1.88±0.24^{a}	1.96±0.04^{a}	0.81±0.01^{b}
Phytic acid (mg/g)	61.76±3.00^{a}	48.12±2.22^{b}	35.01±1.04^{c}
γ-oryzanols (mg/g)	5.07±0.52^{a}	1.52±0.10^{b}	1.60±0.02^{b}
α – tocopherol(μg/g)	32.80±1.22^{c}	41.36±0.72^{b}	60.61±2.40^{a}
γ – tocopherol(μg/g)	24.11±0.57^{c}	37.97±1.35^{b}	48.72±1.54^{a}
Black rice (Waxy and dark purple in color)			
Yield (% of whole grain)	17.14±0.85^{b}	21.75±0.62^{a}	3.19±0.39^{c}
Color	Dark purple	Dark purple	Dark purple
Fat	11.04±0.14^{c}	15.85±0.47^{b}	21.83±0.36^{a}
Protein	11.73±0.07^{c}	13.27±0.05^{b}	20.04±0.45^{a}
Carbohydrate	47.86±1.12^{a}	45.06±2.11^{a}	28.74±0.60^{b}
Fiber	11.95±0.15^{b}	12.68±0.79^{b}	17.42.67±0.04^{a}
Ash	12.77±0.42^{a}	9.72±0.12^{b}	6.31±0.07^{c}
Phenolic compounds (mg GAE/g)	7.14±0.60^{a}	6.65±0.93^{a}	1.35±0.01^{b}
Phytic acid (mg/g)	39.26±1.48^{a}	35±1.02^{b}	31.87±1.01^{c}
γ-oryzanols (mg/g)	13.55±0.70^{a}	9.12±0.73^{b}	1.41±0.07^{c}
α – tocopherol(μg/g)	37.44±0.66^{c}	43.57±1.77^{b}	71.09±3.42^{a}
γ – tocopherol(μg/g)	36.70±0.49^{b}	35.31±1.11^{b}	45.61±2.06^{a}
Red rice (Non waxy and red-brown in color)			
Yield (% of whole grain)	14.63±0.63^{b}	17.59±0.41^{a}	2.87±0.45^{c}
Color	Brownish red	Brownish red	Brownish red
Fat	10.80±0.08^{c}	17.32±0.69^{b}	22.56±0.22^{a}
Protein	10.01±0.61^{c}	12.93±0.70^{b}	19.14±0.41^{a}
Carbohydrate	49.96±1.34^{a}	41.23±0.34^{b}	29.09±0.78^{c}
Fiber	10.16±0.19^{b}	12.11±1.76^{b}	14.07±1.48^{a}
Ash	14.07±0.11^{a}	11.41±0.04^{b}	6.55±0.04^{c}
Phenolic compounds (mg GAE/g)	4.01±0.04^{b}	4.39±0.09^{a}	1.18±0.01^{c}
Phytic acid (mg/g)	41.22±0.02^{a}	39.91±1.91^{b}	39.54±1.30^{b}
γ-oryzanols (mg/g)	10.07±0.06^{a}	8.58±0.02^{b}	1.60±0.06^{c}
α – tocopherol(μg/g)	36.80±2.07^{c}	44±1.05^{b}	69.77±3.15^{a}

γ – tocopherol(μg/g)	21.06±1.38[c]	25±0.17[b]	34.71±1.53[a]

Means in horizontal lines followed by same letter do not differ between each other ($p < 0.05$)

3.3.2. Total antioxidant capacity

The total antioxidant capacity of each rice bran fraction was expressed as the number of equivalent of the antioxidant standards (Butylated hydroxytoluene; BHT) (Fig.1). In this study, the total antioxidant capacity of rice germ of black rice was highest, followed by that of rice germ from red rice, rice bran, and bran layer from black rice, whilst the lowest total antioxidant capacity values were found in all fractions from white rice (KDML and RD6). The rice germ and rice bran exhibited electron-donating ability and thus they may act as radial chain terminators transforming reactive free radical species into more stable non-reactive products [16].

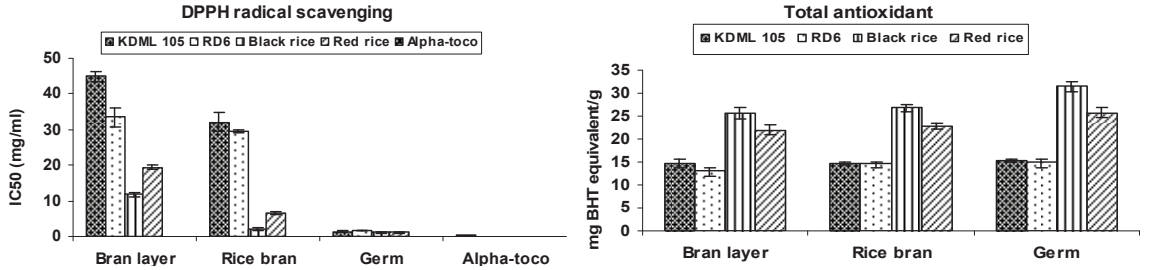

Fig. 1. DPPH radical scavenging activity and total antioxidant activity of bran layer, rice bran, and germ (IC50 of α-tocopherol standard, in the figure, is 0.29 mg/ml)

3.3.3. Linoleic acid emulsion system

The antioxidant effects of each rice bran fraction and cultivar on preventing peroxidation of linoleic acid are shown in Fig 2. The highest inhibition rate of almost all samples was indicated at the incubating time about 72 h. The BHT, α-tocopherol, and rice germ were likely to express more protective effect than rice bran and rice bran layer samples. If only rice cultivars were compared, all fractions of rice bran from pigmented rice indicated stronger activity than those of white rice. These may be due to the high content of phytochemicals, which corresponding to an antioxidant. After 72 h of the reaction, inhibition rate of samples was declined due to the oxidation products, hydroperoxides, decompose to secondary oxidation products. Antioxidant in the rice bran samples can slow down the peroxidation of linoleic acid. Hence, the ferric thiocyanate formation will be slow [10].

4. Conclusions

Rice germ was high in protein, lipid, and tocopherols; whilst rice bran layer was good source of carbohydrate, ash, and γ-oryzanol. Rice germ indicated the strongest antioxidant activity. These results could provide useful information for further studies and applications of each rice bran fraction to produce functional food or related products.

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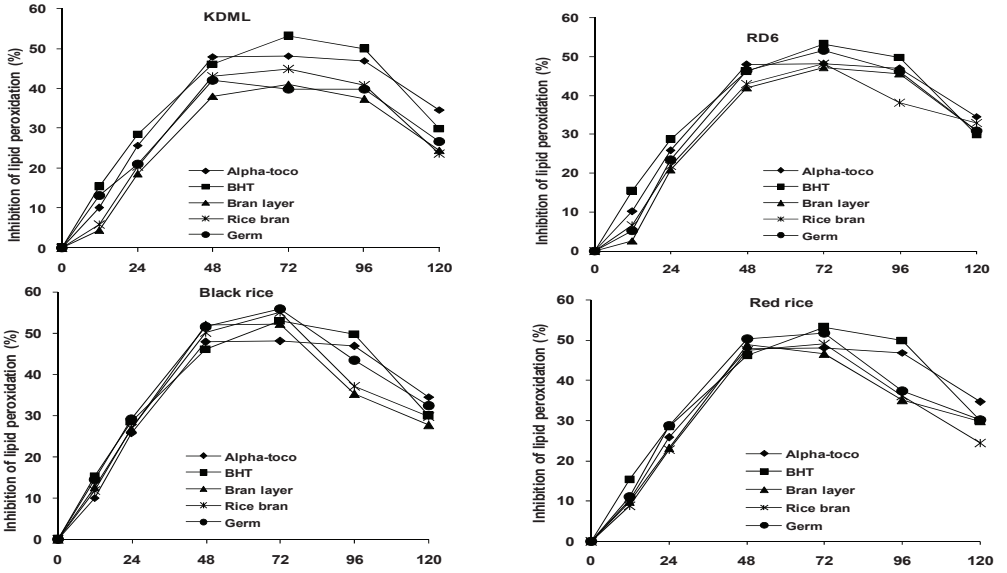

Fig. 2. Inhibition of lipid peroxidation (%) of different rice bran fractions, compared with BHT and α-tocopherol (75 ppm)

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