International Food Research Journal 26(3): 791-799 (June 2019)

Journal homepage: *http://www.ifrj.upm.edu.my*

Antioxidative evaluation of solvent extracts and fractions of oil refining steps from rice bran

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Article history

Received: 13 September, 2018
Received in revised form: 16 December, 2018
Accepted: 25 January, 2019

Keywords

Antioxidant activity
Rice bran oil
Refining process
Phenolic content

Abstract

The present work investigated the bioactive phytochemical contents (phenolics, flavonoids, oryzanol, β-carotene, tocols) and antioxidant activity of the methanolic (RBM), ethanolic (RBE), and acetone (RBA) extracts of rice bran or oil fractions from the refining steps of rice bran oil (RBO). The antioxidant activity was investigated by using five complementary *in vitro* methods: phosphomolybdenum assay, DPPH radical scavenging activity, H_2O_2 scavenging activity, hydroxyl radical scavenging activity, and reducing power. The antioxidant activity was found to increase as a function of extract concentration in all extracts and oil fractions. Of the solvents examined, the extract from methanol, which had the highest total phenolic content (TPC) and total flavonoid content (TFC), showed the highest antioxidant activity followed by RBE and RBA. However, the refining process of RBO reduced the bioactive components and antioxidant activity of RBO, in particular, a significant reduction was recorded after the neutralisation and deodorisation steps. The refining process caused about 68%, 75%, 49%, 68%, and 40% decrease in TPC, TFC, oryzanol, β-carotene, and total tocol contents, respectively, whereas about 50% reduction in antioxidant activity was found in almost all samples. FTIR spectral data revealed the existence of multiple functional groups in the samples. The order of antioxidant content matched well with that of the antioxidant activity of all samples. The present work thus recommends the use of rice bran or RBO for making various types of food materials, as it has high antioxidant activity that improves human health. In addition, the obtained data will help small entrepreneurs and farmers improve the refining process of RBO for better marketability..

Introduction

Rice bran is a by-product of the rice milling industry. It is gaining increasing commercial importance around the world because it contains many beneficial bioactive phytochemicals and positive biological effects. Rice bran contains a considerable amount of oryzanols, tocopherols, and tocotrienols, which have the strongest antioxidant activity (Perez-Ternero *et al*., 2017). Rice bran oil extracted from rice bran is a healthy generous gift from nature to mankind. It is used worldwide as cooking oil due to the presence of important micronutrients and its high smoke point, making it beneficial for use when deep-frying food stuffs. Rice bran oil is a popular oil to use nowadays because of its balanced fatty acid profile, antioxidant capacity, and cholesterol-lowering ability (Pal and Pratap, 2017). Other vegetable oils could improve their nutritive value through blending with RBO and thus take advantage of the presence of micronutrients in the oil. In modern society, crude oil extracted using a solvent, cannot be directly used without refining. This is because unrefined oils have unpleasant odours and contain residual solvent, free fatty acids, oxidation products, colour pigments, phospholipids, and metals. A refining process is thus essential to obtain odourless, bland, and oxidatively stable oil that is acceptable to consumers. The refining process consists of a series of purification steps, which may be carried out via chemical or physical process, with each step having specific functions. During refining, certain minor compounds present

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in the unsaponifiable fraction of crude oil that have pro-oxidant or antioxidant activity are removed or reduced (Fine *et al*., 2016).

Degumming, neutralisation, bleaching, and deodorisation are the common steps in the chemical refining of RBO (Mezouari and Eichner, 2007). Considering the above, the refining process should be optimised to obtain high-quality RBO with useful amounts of bioactive components for human consumption. However, refining RBO is more complicated than refining other vegetable oils due to the difference in the former's phytochemical composition (Yoon and Kim, 1994). Some research works on the oxidation tendency, biochemical composition, and properties of crude/refined RBO have been carried out elsewhere (Mezouari and Eichner, 2007; Pestana *et al*., 2008; De and Patel, 2010). In addition, Szydłowska-Czerniak and Łaszewska (2015) reported that the refining of crude rapeseed oils caused 60% and 80% reduction in the oil's antioxidant capacity and total phenolics, respectively. However, the influence of the refining process on the bioactive phytochemicals and antioxidant activity of fractions as a result of the refining steps of RBO has rarely been investigated. Accordingly, the present work was conducted to examine the influence of the steps in refining RBO on the bioactive components and antioxidant activity of oil fractions of RBO. In addition, the antioxidant activity of various solvent extracts of rice bran was also analysed, in relation to their phytochemical compositions.

Materials and methods

Sample and reagent

Freshly milled rice bran (5 kg, BRRI dhan 49) was directly collected from the milling system and placed in airtight polyethylene bags. The sample was packed in a polyethylene microwave-safe bag with moisture level adjusted to 21% (Malekian, 1992) and then subjected to microwave heating using a microwave oven (2450 MHz, output power 850 W) for 3 min. Next, the sample was cooled down to room temperature. The stabilised bran was immediately used for solvent extraction. DPPH (2,2-Diphenyl-1-picrylhydrazyl) and standards of phenolic compounds were purchased from Sigma Aldrich (Sigma Aldrich, St. Louis, MO, USA). Analytical grade solvents were used in the present work (Merck GmbH, Darmstadt, Germany or Mumbai, India).

Extraction and refining process

The stabilised rice bran was extracted using n-hexane with a solid-to-solvent ratio of 1:10 (w/v), and subsequently placed on a magnetic stirrer at room temperature for 3 h followed by filtering through a Whatman No. 4 filter paper. The residue was re-extracted twice with the same solvent. The filtrates were combined and dried under reduced pressure at 45°C. Extraction was conducted with methanol, ethanol or acetone from the defatted bran left after extraction with n-hexane, applying the same procedure as described above. The crude oil from the hexane extract and other solvent extracts were individually weighed to calculate the yield percentage and then stored in glass containers at 4°C prior to further analyses. The chemical refining process of crude RBO was carried out based on the conventional method (Mezouari and Eichner, 2007; Pestana *et al*., 2008). The oil was degummed and treated with sodium hydroxide solution at 80–90°C. Afterwards, the neutralised oil was washed and dried at 105°C. The oil was bleached at 90°C using 1% bleaching earth, filtered, and dewaxed. Finally, the oil was deodorised between 245°C and 257°C, and cooled to 45–48°C.

Estimation of oryzanol and β-carotene

The oryzanol concentration in all samples was spectrophotometrically obtained by determining the UV absorption at 315 nm of sample solution in hexane, using a specific extinction coefficient of 358.9 (Seetharamaiah and Prabakar, 1986). The carotene content in the oil samples were analysed via a spectrophotometer according to PORIM (PORIM, 1995) test methods no. p2.6. The sample was dissolved with n-hexane and the absorbance of the solution was recorded at 446 nm against n-hexane. Following that, the carotene content, calculated as β-carotene (mg/kg), was obtained.

Tocol analysis

The tocol content of the oil sample was determined using HPLC (Agilent 1100 series, Agilent Technologies, Wilmington, USA) composed of a column (250 × 4 mm) packed with 5 µm silica (Jones chromatography) and a fluorescence detector (Agilent Model G1321A, Massachusetts, USA) set at 292 nm and 330 nm excitation and emission wavelengths, respectively. The oil sample was dissolved with n-hexane (Merck, Darmstadt, Germany) before being injected into the HPLC. The mobile phase consisted of iso-propanol (Merck, Darmstadt, Germany) in n-hexane (0.5:99.5, v/v) with a flow rate of 1.4 mL/min. The tocols were analysed by comparing their retention times with those of standards.

Total phenolic and flavonoid compounds

Briefly, for the extraction of phenolic compounds, 1 g of oil and 1 mL of 70% methanol were mixed and agitated for 1 min. The mixture was then centrifuged at 3,000 rpm for 5 min and the upper methanolic phase taken. Next, 1 mL of fresh 70% methanol was added, and the extraction repeated for three times; the upper phases were combined and washed with three portions of 2 mL n-hexane to remove oil residues. The final methanolic solution was used for phenolic analysis (Durmaz and Gökmen, 2011). The TPC of the oil extract and other solvent extracts was measured using Folin and Ciocalteau's assay (Skerget *et al*., 2005). Gallic acid was employed as a calibration standard and the results were expressed as mg GAE (gallic acid equivalent)/g extract. The TFC of oil extract and other solvent extracts was determined following the method of Wu and Ng (2008). Catechin was taken as a calibration standard and the results were expressed as mg CE (catechin equivalent)/g extract. For the oil sample, TPC and TFC were expressed as mg GAE/100 g oil and mg CE/100 g oil, respectively.

Phosphomolybdenum assay (total antioxidant activity)

The total antioxidant capacity was estimated following the method of Adom *et al*. (2005). A mixture was prepared consisting of 0.3 mL of sample and 3 mL of reagent (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). This mixture was mixed in a test tube and incubated at 95°C for 90 min. After cooling to 25°C, the absorbance of the mixture was recorded at 695 nm against a blank on the spectrophotometer. The total antioxidant capacity, expressed as ascorbic acid equivalent (AE), was calculated, and the results were expressed as mg AE/g extract or mg AE/g oil.

1,1 Diphenyl 2 picrylhydrazyl (DPPH) assay

The DPPH radical scavenging assay was performed following the method of Negro *et al*. (2003). Briefly, 2 mL of 0.2 mM solution of DPPH in methanol was mixed with 2 mL of the samples dissolved in the extracting solvent at different concentrations. The reaction mixture was shaken and incubated in the dark at room temperature for 30 min, and the absorbance was read at 517 nm against the blank. The control solution contained equivalent pure methanol instead of the sample solution. DPPH free radical-scavenging activity was calculated according to Eq. 1:

$$\text{DPPH radical-scavenging activity (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100 \quad \text{(Eq. 1)}$$

Hydrogen peroxide scavenging activity

The H_2O_2 scavenging activity of the rice bran sample was determined following the method of Ruch *et al*. (1989). A solution of H_2O_2 (40 mM) was prepared in Na_2HPO_4–NaH_2PO_4 buffer solution (pH = 7.40, 0.2 mol/L). The extracts at different concentrations were added to hydrogen peroxide solution (0.6 mL). Absorbance of hydrogen peroxide at 230 nm was determined after 15 min against a blank solution containing phosphate buffer without hydrogen peroxide. The scavenging activity was calculated using Eq. 2:

$$\text{\% scavenging activity} = [(A_{control} - A_{sample}) / A_{control}] \times 100 \quad \text{(Eq. 2)}$$

Hydroxyl radical-scavenging activity

The hydroxyl radical-scavenging activities were determined following the method of Smirnoff and Cumbes (1989) with slight modifications. The following reagents were put into a reaction tube in the following order: 0.3 mL of 20 mM sodium salicylate, 1.0 mL of 1.5 mM FeSO4, 1.0 mL of various concentrations of sample solution, and 0.7 mL of 6 mM H_2O_2. The reagents were mixed immediately, and the reaction tubes put in a 37°C water bath for 1 h. Then, the absorbance of the mixture was recorded at 510 nm against a blank. The hydroxyl radical-scavenging ability was calculated using Eq. 3:

$$\text{Hydroxyl radical-scavenging activity (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100 \quad \text{(Eq. 3)}$$

Reducing power

The reducing power of the sample was determined following the method of Atmani *et al*. (2009). Different concentrations of extract in 2.0 mL of phosphate buffer (0.2 M, pH 6.6) were mixed with 2.5 mL of 1% potassium ferrocyanate. The mixture was incubated at 50°C for 20 min; 2.5 mL of 10% trichloroacetic acid was thenadded to the mixtures. A portion of the solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. The absorbance of the solution was recorded at 700 nm at a reaction time of 30 min. The increase in the absorbance of the reaction mixture indicated the reducing power of the sample.

FTIR spectroscopy

The IR spectra of the solvent extracts and oil samples were recorded on a FTIR spectrometer (IRAffinity- 1S, Shimadzu Corporation, Kyoto, Japan) furnished with a high-sensitivity pyroelectric

detector (deuterated L-alanine doped triglycine sulphate). The spectra were acquired in the range of 4000–700 cm^{-1} with a spectral resolution of 2 cm^{-1} and 16 periodical scans.

Statistical analysis

All analyses were run in triplicate ($n = 3$) unless otherwise stated. The data, expressed as the mean and standard deviation (SD), were subjected to a One-Way Analysis of Variance (ANOVA). Mean values were compared at $p < 0.05$ significant level via Duncan's multiple range test using IBM SPSS 22 statistical software.

Results and discussion

As shown in Table 1, the yield of the rice bran extracts significantly varied with the solvents applied based on the following order: methanol (10.05%) > ethanol (7.80%) > acetone (5.64%). The yield of crude oil from rice bran was 17.08% (dry basis), which is similar to the value 17.35% (Hom-nin paddy variety) reported by Mingyai *et al*. (2017). However, significant loss (30.27%) in oil content was found in the refining process, where the highest loss was observed after the neutralisation step (17.75%) and the lowest was observed after the deodorisation step (3.79%). For the extract compositions, the TPC was significantly greater ($p < 0.05$) for the RBM (13.50 mg GAE/g) than that of RBE (12.25 mg GAE/g) and RBA (11.18 mg GAE/g) (Table 1). The TPC value of the RBM observed in the present work was lower than the value of TK9 rice bran reported elsewhere (15.70 mg GAE/g) (Lai *et al*., 2009). Some authors also noted that the methanolic extract of rice bran showed significantly ($p < 0.05$) higher TPC as compared to acetone (Chatha *et al*., 2006) and ethanolic (Arab *et al*., 2011) extracts. The RBO displayed a TPC of 22.23 mg GAE/100 g, which is similar to the TPC of walnut oil (22.00 mg GAE/100 g) reported by Gharibzahedi *et al*. (2013). The refining of RBO had led to a decrease of 68.02% in TPC content. The most significant change in TPC was found after the deodorisation step, with a decrease from 13.89 to 7.11 mg GAE/100 g. Chew *et al*. (2016) stated that the refining process reduced 64.50% of TPC in kenaf seed oil and the most marked change was observed after the deodorisation step. This might be due to the phenolic compounds becoming unstable during the oil refining process as a result of the applied heat treatment. In addition, the neutralisation step also removed a considerable amount of TPC. Phenolics are polar compounds and many of them are weak acids, so they can easily be removed from the oils with aqueous solutions during neutralisation with sodium hydroxide (Szydłowska-Czerniak *et al*., 2008). The rice bran solvent extracts contained total flavonoids ranging from 4.18 mg CE/g in RBM to 2.12 mg CE/g in RBA, whereas crude RBO contained 8.67 mg CE/100 g. As was the case with TPC, the refining process of the oil also reduced 75.89% of the flavonoid content with the highest reduction (55.62%) occurring after the deodorisation step (Table 1). Unfortunately, there has been no reference to variations in TFC being a result of the refining steps of RBO. Among the four solvents, methanol exhibited comparably high extraction efficiency of total phenolic and flavonoid substances. Ali *et al*. (2016) also reported that methanolic extract of groundnut exhibited the highest TPC and TFC. The oryzanol content in different extracts accounted for 0.87%, 0.84%, and 0.68% RBM, RBE, and RBA, respectively (Table 1). Lai *et al*. (2009) reported that the oryzanol content in the MeOH extract of TK9 rice bran amounted to 0.98%. However, RBO revealed an oryzanol content of 1.68% that was reduced to 0.85% after the deodorisation step. The oryzanol content was almost constant in crude and degummed oils but decreased significantly ($p < 0.05$) after the neutralization step. The loss of oryzanol to become part of the soap stock explains the decrease in total oryzanol content. The results of the present work concur with previously published results by Van Hoed *et al*. (2006), which reported that neutralisation caused significant reduction in oryzanol in RBO. In the present work, the refining process removed 49.40% of oryzanol, which is lower than the 83% value reported by Van Hoed *et al*. (2006) and the 51% value reported by Yoon and Kim (1994) for the same oil. As can be seen in Table 1, the amount of β-carotene in crude RBO was found to be 1.88 mg/kg. Al-Okbi *et al*. (2014) showed that the concentration of β-carotene in crude and refined RBO were 2.25 mg/kg and 0.49 mg/kg, respectively. The influence of oil refining on β-carotene content was almost similar to that of the other micronutrients depicted for RBO. The whole refining process of crude RBO removed 68.08% β-carotene, where the greatest loss of β-carotene was observed after the bleaching and deodorisation steps. Chew *et al*. (2016) found a 65.3% decrease in total carotenoid content when refining crude kenaf seed oil. Kreps *et al*. (2014) observed the greatest loss of β-carotene when bleaching degummed rapeseed oil. Moreover, this minor component is highly unsaturated and is easily degraded in the high temperatures of the deodorisation step (Suliman *et al*., 2013). Chew *et al*. (2016) revealed that carotenoids have a more

significant contribution to total antioxidant activity than phenolics. The present work revealed that the total tocol (sum of tocopherols and tocotrienols) content in RBO (844.50 ppm) was mainly affected by the neutralisation and deodorisation steps (Table 1). The neutralisation step caused 12.63% tocol reduction due to the instability of tocols in the presence of air and alkali (Tasan and Demirci, 2005). Tasan and Demirci (2005) showed that 15% of the total tocopherols in sunflower oil were reduced during the neutralisation step. Further reduction in total tocol content was observed during the bleaching step (7.65%), although the change was not that high. In the bleaching step, the decrease in tocol results in the adsorption and oxidation of these components (Zhu *et al*., 2016). Deodorisation significantly reduced (23.86%) the tocol content because the tocols might have distillated under the deodorising conditions (Van Hoed *et al*., 2006; Chew *et al*., 2016). Van Hoed *et al*. (2006) also observed 25% reduction of tocols after the deodorisation step of RBO. However, in the present work, about 40.67% of total tocol content disappeared with the refining of RBO. Mezouari *et al*. (2006) observed 38.98% reduction of total tocols during the chemical refining of RBO.

The phosphomolybdenum method is being routinely used to evaluate the antioxidant capacity of extracts. The highest total activity was seen in RBM (15.62 AE mg/g), and conversely, the lowest in RBO (9.28 AE mg/g). A significant decrease in total antioxidant activity was observed upon refining of RBO (Table 1). After refining RBO, total antioxidant activity decreased from 9.28 to 2.87 AE mg/g. However, during this process, a large amount of the micronutrients and antioxidants such as polyphenols, oryzanol, tocols, etc. were lost, which in turn substantially reduced the antioxidant activity and nutritional value of RBO. In some cases, the present analytical data from the above antioxidants in rice bran differed from the literature values; these differences might be due to several factors, including genotype, growth period, milling technique, and stabilisation technique, all of which affect the level of antioxidant content (Lilitchan *et al*., 2008). Table 2 shows that the DPPH radical-scavenging activity (RSA) increased as the concentration of the solvent extract increased. At 31.25 µg/mL, the RBM, RBE, RBA, and RBO samples showed 44.47%, 39.25%, 32.13%, and 32.57% RSA, respectively, whereas the same solvent extracts at 500 µg/mL showed 84.62%, 80.40%, 74.74%, and 67.91% RSA, respectively. The highest RSA was exhibited by RBM at all concentrations. Baba *et al*. (2016) also observed the highest DPPH scavenging activity occurring in the methanol extract as compared to other solvent extracts. This might be due to the greater release of antioxidants from this extract. Pengkumsri *et al*. (2015) stated that hexane-extracted RBO exhibited the highest RSA as compared to hot-pressed, cold-pressed, and supercritical fluid extraction methods. RSA can be used to evaluate the antioxidative capacity of oily materials. However, the refining process of crude RBO has caused a decrease in the scavenging activity with the highest decrease being observed after the neutralisation and deodorization steps of RBO. Zhu *et al*. (2016) found that the RSA of peanut oil significantly decreased ($p < 0.05$) after the neutralisation step due to the removal of antioxidants. After full refining, depending on concentration, the RSA reduced by 42.23%–49.58% for RBO; lower than the range of 54.60%–60.50% for rapeseed oils, as reported by Szydłowska-Czerniak *et al*. (2015). An increase in antioxidant activity measured by hydroxyl radical scavenging was observed upon increasing the extract concentrations of rice bran extracts (Table 2). A similar trend was also reported for groundnut extracts by Ali *et al*. (2016). However, in the present work, the level of activity did not increase steadily with increasing concentrations. Methanol extracts showed better activity in comparison to other solvents; and this is consistent with the literature (Baba *et al*., 2016). This radical scavenging activity in RBO was reduced (40.40%–50.24%) due to the oil refining process; the reduction was most significant after the deodorisation step. In Table 3, the data shows that H_2O_2 scavenging activity increased in a dose-dependent manner, reaching a maximum value at 500 µg/mL. However, the activities observed in RBM and RBE were almost similar at all concentrations. Refining had a remarkable effect on the levels of H_2O_2 scavenging activity in the oil samples, especially after the neutralisation and deodorisation steps. After refining, at 500 µg/mL, the activity reduced from 53.31% in crude oil to 34.62% in fully refined oil—an approximately 35.06% reduction in activity. The absorbance, which reflects reducing power, was dose-dependent; increasing the concentration of extract caused a significant ($p < 0.05$) increase in all samples (Table 3). At all concentrations, the methanolic extract, which had the highest bioactive component content, showed the highest antioxidant activity. It is important to note that the refining process of RBO caused reducing power of crude RBO to fall (54.55%–62.50%), with significant reduction being recorded after the neutralisation and deodorisation steps. Szydłowska-Czerniak *et al*. (2015) reported that the highest decrease in reducing power of rapeseed oils took place during bleaching.

Table 1. Bioactive phytochemicals and total antioxidant activity of rice bran extracts, bran oil and oil fractions from refining steps.

Samples		Yield(%)	TPC (mg GAE/g)*	TFC (CE mg/g)*	Oryzanol (g/100 g)	β-Carotene (mg/kg)	Total tocols (mg/kg)	Total antioxidant activity (AE mg/g)
RBM		10.05 ± 0.12^{c}	13.50 ± 0.32^{c}	4.18 ± 0.04^{b}	0.87 ± 0.09^{b}	nd	nd	15.62 ± 0.13^{c}
RBE		7.80 ± 0.19^{b}	12.25 ± 0.49^{b}	4.014 ± 0.08^{b}	0.84 ± 0.06^{b}	nd	nd	15.16 ± 0.05^{b}
RBA		5.64 ± 0.13^{a}	11.18 ± 0.30^{a}	2.12 ± 0.12^{a}	0.68 ± 0.05^{a}	nd	nd	14.60 ± 0.05^{a}
Crude RBO		17.08 ± 0.45^{d}	22.23 ± 0.22^{e}	8.67 ± 0.19^{e}	1.68 ± 0.01^{b}	1.88 ± 0.02^{e}	844.5 ± 3.54^{e}	9.28 ± 0.04^{e}
Refining step	Degummed	16.11 ± 0.09^{c}	20.98 ± 0.19^{d}	7.11 ± 0.04^{d}	1.66 ± 0.01^{b}	1.73 ± 0.03^{d}	815.5 ± 7.78^{d}	8.72 ± 0.05^{d}
	Neutralised	13.25 ± 0.07^{b}	15.06 ± 0.05^{c}	5.89 ± 0.07^{c}	0.91 ± 0.04^{a}	1.62 ± 0.03^{c}	712.5 ± 6.36^{c}	5.82 ± 0.04^{c}
	Bleached	12.38 ± 0.13^{a}	13.89 ± 0.10^{b}	4.71 ± 0.10^{b}	0.88 ± 0.02^{a}	0.97 ± 0.02^{b}	658.0 ± 1.41^{b}	3.54 ± 0.03^{b}
	Deodorised	11.91 ± 0.20^{a}	7.11 ± 0.11^{a}	2.09 ± 0.10^{a}	0.85 ± 0.06^{a}	0.60 ± 0.03^{a}	501.0 ± 4.24^{a}	2.87 ± 0.08^{a}

GAE: gallic acid equivalent; CE: catechin equivalent; AE: ascorbic acid equivalent. Data are mean of three replicates (n = 3) ± SD except for total tocols (n = 2). nd: not detected. Values within a column with the same lowercase letter are not significantly different at $p < 0.05$. RBO: rice bran oil; RBM: methanolic extract; RBE: ethanolic extract; RBA: acetone extract. *For oil samples, TPC and TFC are expressed as mg GAE /100 g oil and mg CE/100 g oil, respectively.

Table 2. DPPH and hydroxyl radical scavenging activity of rice bran extracts, bran oil and oil fractions from refining steps.

Sample/Sample concentration (µg/mL)		DPPH radical scavenging activity				
		31.25	62.50	125	250	500
RBM		44.47 ± 0.50^{gA}	57.81 ± 0.62^{gB}	67.40 ± 1.19^{hC}	76.41 ± 1.61^{fD}	84.62 ± 1.31^{hE}
RBE		39.25 ± 0.79^{fA}	52.30 ± 1.43^{fB}	63.74 ± 0.60^{gC}	74.07 ± 1.83^{eD}	80.40 ± 1.26^{gE}
RBA		32.13 ± 079^{eA}	38.19 ± 0.80^{dB}	50.00 ± 0.32^{fC}	63.97 ± 0.17^{dD}	74.74 ± 1.20^{fE}
Crude RBO		32.57 ± 0.97^{eA}	40.75 ± 0.53^{eB}	48.32 ± 1.13^{eC}	61.64 ± 0.65^{dD}	67.91 ± 0.83^{eE}
Refining step	Degummed	29.27 ± 1.10^{dA}	38.45 ± 0.55^{dB}	45.64 ± 0.85^{dC}	54.42 ± 2.43^{cD}	60.84 ± 0.75^{dE}
	Neutralised	24.26 ± 0.90^{cA}	32.36 ± 1.19^{cB}	37.85 ± 0.15^{eC}	46.18 ± 0.15^{bD}	52.74 ± 0.90^{cE}
	Bleached	21.09 ± 1.06^{bA}	30.79 ± 0.57^{bB}	35.07 ± 0.34^{bC}	45.70 ± 1.39^{bD}	49.72 ± 1.01^{bE}
	Deodorised	16.42 ± 0.27^{aA}	22.65 ± 0.37^{aB}	25.50 ± 0.17^{aC}	31.87 ± 0.51^{aD}	39.23 ± 0.58^{aE}
Sample/Sample concentration (µg/mL)		Hydroxyl radical scavenging activity				
		15.62	31.25	62.50	125	250
RBM		30.15 ± 0.50^{gA}	37.18 ± 0.14^{gB}	45.62 ± 1.14^{fC}	47.94 ± 1.18^{gD}	61.17 ± 1.07^{gE}
RBE		28.09 ± 0.70^{fA}	35.96 ± 0.78^{fB}	42.85 ± 0.69^{eC}	45.03 ± 0.89^{fD}	58.86 ± 0.91^{fE}
RBA		27.19 ± 0.10^{eA}	34.73 ± 1.32^{fB}	41.70 ± 0.28^{eC}	43.75 ± 0.88^{fD}	52.87 ± 0.57^{eE}
Crude RBO		25.37 ± 0.04^{dA}	31.27 ± 0.28^{eB}	38.08 ± 1.63^{dC}	41.65 ± 0.93^{eD}	48.15 ± 1.03^{dE}
Refining step	Degummed	24.93 ± 0.92^{dA}	29.90 ± 0.26^{dB}	37.99 ± 0.73^{dC}	39.88 ± 1.50^{dD}	46.62 ± 0.47^{eE}
	Neutralised	21.42 ± 0.55^{cA}	25.55 ± 1.36^{eB}	32.80 ± 0.54^{eC}	33.91 ± 0.06^{eC}	38.75 ± 0.85^{bD}
	Bleached	19.64 ± 0.19^{bA}	23.65 ± 0.60^{bB}	29.25 ± 0.31^{bC}	31.48 ± 0.39^{bD}	37.99 ± 0.21^{bE}
	Deodorised	13.88 ± 0.28^{aA}	15.56 ± 0.44^{aB}	22.16 ± 0.13^{aC}	24.82 ± 0.14^{aD}	28.56 ± 0.52^{aE}

Data are mean of three replicates (n = 3) ± SD. Values within a row with the same uppercase letters are not significantly different at $p < 0.05$. Values within a column with the same lowercase letters are not significantly different at $p < 0.05$. RBO: rice bran oil; RBM: methanolic extract; RBE: ethanolic extract; RBA: acetone extract.

Table 3. Hydrogen peroxide scavenging activity and reducing power of rice bran extracts, bran oil and oil fractions from refining steps.

Sample/Sample concentration (µg/mL)	Hydrogen peroxide scavenging activity				
	31.25	62.5	125	250	500
RBM	31.89 ± 0.16^{fgA}	43.29 ± 1.00^{gB}	51.15 ± 0.38^{gC}	54.31 ± 1.13^{fD}	65.66 ± 0.42^{hE}
RBE	32.84 ± 0.64^{gA}	42.42 ± 0.32^{fB}	49.89 ± 0.94^{fC}	54.62 ± 0.40^{fD}	64.84 ± 0.48^{gE}
RBA	30.71 ± 0.35^{fA}	40.75 ± 0.0.43^{fB}	45.96 ± 0.65^{eC}	51.66 ± 1.23^{eD}	59.36 ± 0.89^{fE}
Crude RBO	29.37 ± 1.81^{eA}	38.38 ± 1.65^{eB}	44.98 ± 1.06^{eC}	48.65 ± 0.61^{dD}	53.31 ± 0.59^{eE}

Table 3 (Cont.)

Refining step	Degummed	27.33 ± 0.22^{dA}	36.10 ± 0.55^{dB}	42.07 ± 1.13^{dC}	46.87 ± 0.46^{cD}	51.73 ± 0.74^{dE}
	Neutralised	24.51 ± 0.23^{cA}	31.45 ± 0.37^{cB}	38.53 ± 0.77^{cC}	40.11 ± 1.44^{bD}	44.34 ± 0.45^{cE}
	Bleached	19.26 ± 0.59^{bA}	24.97 ± 1.77^{bB}	36.67 ± 0.65^{bC}	39.10 ± 0.08^{bD}	43.23 ± 0.32^{bE}
	Deodorised	13.83 ± 0.38^{aA}	19.02 ± 0.12^{aB}	22.76 ± 0.31^{aC}	30.83 ± 0.70^{aD}	34.62 ± 0.38^{aE}
Sample/Sample concentration (µg/mL)		Reducing power				
		31.25	62.5	125	250	500
RBM		0.49 ± 0.011^{gA}	0.63 ± 0.005^{fB}	0.72 ± 0.015^{eC}	0.80 ± 0.021^{gD}	0.86 ± 0.027^{fE}
RBE		0.43 ± 0.001^{fA}	0.54 ± 0.001^{eB}	0.64 ± 0.002^{dC}	0.73 ± 0.001^{fD}	0.79 ± 0.004^{eE}
RBA		0.33 ± 0.001^{dA}	0.45 ± 0.001^{dB}	0.55 ± 0.002^{cC}	0.63 ± 0.001^{eD}	0.68 ± 0.004^{dD}
Crude RBO		0.26 ± 0.001^{bcA}	0.37 ± 0.001^{cB}	0.56 ± 0.002^{cC}	0.61 ± 0.001^{dD}	0.66 ± 0.004^{cE}
Refining step	Degummed	0.37 ± 0.024^{eA}	0.47 ± 0.005^{B}	0.56 ± 0.026^{cC}	0.60 ± 0.007^{dD}	0.63 ± 0.002^{cE}
	Neutralised	0.29 ± 0.001^{cA}	0.34 ± 0.001^{bB}	0.38 ± 0.001^{bC}	0.42 ± 0.002^{cD}	0.43 ± 0.002^{bD}
	Bleached	0.26 ± 0.022^{bA}	0.32 ± 0.013^{bB}	0.36 ± 0.004^{bC}	0.41 ± 0.001^{bD}	0.42 ± 0.007^{bD}
	Deodorised	0.11 ± 0.004^{aA}	0.14 ± 0.003^{aB}	0.21 ± 0.007^{aC}	0.26 ± 0.009^{aD}	0.30 ± 0.004^{aE}

Data are mean of three replicates (n = 3) ± SD. Values within a row with the same uppercase letters are not significantly different at $p < 0.05$. Values within a column with the same lowercase letters are not significantly different at $p < 0.05$. RBO: rice bran oil; RBM: methanolic extract; RBE: ethanolic extract; RBA: acetone extract.

FTIR spectral features of rice bran extracts and its oil samples are shown in Figures 1 and 2, respectively. Distinctive bands referring to the phenol group were observed in the regions 3,419–3,385 cm^{-1} (attributed to the stretch of O-H) and 1,230–1,064 cm^{-1} (attributed to C-H stretch) (Batista *et al*., 2016), which were found in all the solvent extracts but not in RBO. To provide a relative ranking of extracts in terms of antioxidant activity, the hydroxyl functionality, which is an integral part of most of phenolic phytochemicals such as polyphenols and flavonoids, was noted. The small absorption at 3,008 cm^{-1} could be attributed to the oil samples, i.e. the C–H stretching vibration of the cis-double bond and conjugated double bonds present in the primary oxidation products, as is the case of the autoxidation of oleic and linoleic acids (Belitz and Grosch, 1999). The bands related to the aromatic ring were observed at the regions 2,926–2,854 cm^{-1} (attributed to the stretch of the C-H of aromatic ring), and at 1,647 cm^{-1} (attributed to C-C stretch of aromatic ring) (Batista *et al*., 2016). The acetone extract and oil samples did not show any absorption bands at around 1,647 cm^{-1} and 1,064 cm^{-1}. The noticeable peak (C=O stretch) at the regions 1,747–1,735 cm^{-1} can be related to the absorption due to the ester carbonyl functional group of the triglycerides present (Abbas Ali *et al*., 2017), while the weak bands at the frequency range of 1,465–1,456 cm^{-1} could be due to the carboxylic C-O band of polyphenols (Kiat *et al*., 2014). The weak band near 1,161 cm^{-1} was only observed in the oil samples, and has been proven to closely relate to the proportion of saturated acyl groups in the sample. The presence and distribution of these functional groups in the chemical structures of polyphenols may influence their antioxidant properties in fruits as well as their bioavailability.

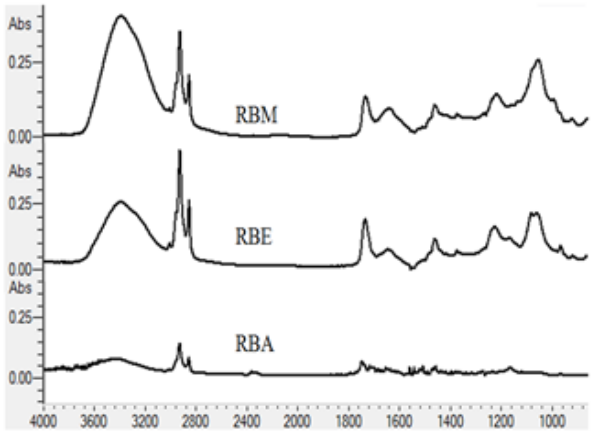

Figure 1. FTIR spectra of crude solvent extracts of rice bran. RBM: methanolic extract; RBE: ethanolic extract; RBA: acetone extract.

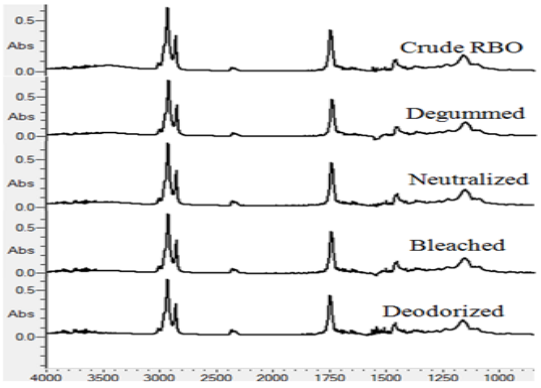

Figure 2. FTIR spectra of bran oil and oil fractions from refining steps of rice bran oil (RBO).

Conclusion

Among the pure solvents, methanol was the most efficient for extraction of antioxidant compounds, followed by ethanol, acetone, and hexane. However, the methanolic extract displayed strong ability to act as an antioxidant as compared to other oil fractions or crude extracts, which is probably due to its high phenolic content. The samples showed dose-dependent antioxidant activities. Moreover, it was confirmed that each subsequent step of refining caused decreased bioactive phytochemical content. The most marked reduction in antioxidants and their activities was observed after the neutralisation and deodorisation steps of RBO refining. The instability of phenolic compounds and other antioxidants in the presence of air and alkali and the thermolabile nature of these compounds might have been responsible for their reduction and the consequent decline in antioxidant activity in the refined oil. With a few exceptions, the FTIR spectra of the studied samples showed similar spectral characteristics of the functional group region, which indicates similar components present in the samples. However, the intensity of the band differed with the samples, suggesting unequal levels of phenolic content in the samples. Therefore, the analytical data generated from the present work can be employed by oil-processing industries for assessing the antioxidants and their activities in crude extracts or oil fractions as a result of refining phases, and thus assist them in modifying the refining process.

References

Abbas Ali, M., Anowarul Islam, M., Othman, N. H. and Noor, A. M. 2017. Effect of heating on oxidation stability and fatty acid composition of microwave roasted groundnut seed oil. Journal of Food Science and Technology 54(13): 4335-4343.

Adom, K. K., Sorrells, M. E. and Liu, R. H. 2005. Phytochemicals and antioxidant activity of milled fractions of different wheat varieties. Journal of Agricultural and Food Chemistry 53(6): 2297-2306.

Ali, A., Islam, A. and Pal, T. K. 2016. The effect of microwave roasting on the antioxidant properties of the Bangladeshi groundnut cultivar. Acta Scientiarum Polonorum Technologia Alimentaria 15(4): 429-438.

Al-Okbi, S. Y., Ammar, N. M., Mohamed, D. A., Hamed, I. M., Desoky, A. H., El Bakry, H. F. and Helal, A. M. 2014. Egyptian rice bran oil: chemical analysis of the main phytochemicals. Rivista Italiana Delle Sostanze Grasse 91(1): 47-58.

Arab, F., Alemzadeh, I. and Maghsoudi, V. 2011. Determination of antioxidant component and activity of rice bran extract. Scientia Iranica 18(6): 1402-1406.

Atmani, D., Chaher, N., Berboucha, M., Ayouni, K., Lounis, H., Boudaoud, H., … and Atmani, D. 2009. Antioxidant capacity and phenol content of selected Algerian medicinal plants. Food Chemistry 112(2): 303-309.

Baba, W. N., Rashid, I., Shah, A., Ahmad, M., Gani, A. Masoodi, F. A., … and Wani S. M. 2016. Effect of microwave roasting on antioxidant and anticancerous activities of barley flour. Journal of the Saudi Society of Agricultural Sciences 15(1): 12-19.

Batista, N. N, de Andrade, D. P., Ramos, C. L., Dias, D. R. and Schwan, R. F. 2016. Antioxidant capacity of cocoa beans and chocolate assessed by FTIR. Food Research International 90: 313-319.

Belitz, H. and Grosch, W. 1999. Food Chemistry (2nd ed). Heidelberg, Germany: Springer-Verlag.

Chatha, S. A. S, Anwar, F., Manzoor, M. and Bajwa, J. R. 2006. Evaluation of the antioxidant activity of rice bran extracts using different antioxidant assays. Grasas y Aceites 57(3): 328-335.

Chew, S.-C., Tan, C.-P., Long, K. and Nyam, K.-L. 2016. Effect of chemical refining on the quality of kenaf (*Hibiscus cannabinus*) seed oil. Industrial Crops and Products 89: 59-65.

De, B. K. and Patel, J. D. 2010. Effect of different degumming processes and some nontraditional neutralizing agent on refining of RBO. Journal of Oleo Science 59(3): 121-125.

Durmaz, G. and Gökmen, V. 2011. Changes in oxidative stability, antioxidant capacity and phytochemical composition of Pistacia terebinthus oil with roasting. Food Chemistry 128(2): 410-414.

Fine, F., Brochet, C., Gaud, M., Carre, P., Simon, N., Ramli, F. and Joffre, F. 2016. Micronutrients in vegetable oils: The impact of crushing and refining processes on vitamins and antioxidants in sunflower, rapeseed, and soybean oils. European Journal of Lipid Science and Technology 118(5): 680-697.

Gharibzahedi, S. M. T., Mousavi, S. M., Hamedi, M., Rezaei, K. and Khodaiyan, F. 2013. Evaluation of physicochemical properties and antioxidant activities of Persian walnut oil obtained by several extraction methods. Industrial Crops and Products 45: 133-140.

Kiat, V. V., Siang, W. K., Madhavan, P., Chin, J. H., Ahmad, M. and Akowuah, G. A. 2014. FT-IR profile and antiradical activity of dehulled kernels of apricot, almond and pumpkin. Research Journal of Pharmaceutical, Biological and Chemical Sciences 5(2): 112-120.

Kreps, F., Vrbiková, L. and Schmidt, S. 2014. Influence of industrial physical refining on tocopherol, chlorophyll and beta-carotene content in sunflower and rapeseed oil. European Journal of Lipid Science and Technology 116(11): 1572-1582.

Lai, P., Li, K. Y., Lu, S. and Chen, H. H. 2009. Phytochemicals and antioxidant properties of solvent extracts from Japonica rice bran. Food Chemistry 117(3): 538-544.

Lilitchan, S., Tangprawat, C., Aryusuk, K., Krisnangkura, S., Chokmoh, S. and Krisnangkura, K. 2008. Partial

extraction method for the rapid analysis of total lipids and γ-oryzanol contents in rice bran. Food Chemistry 106(2): 752-759.

Malekian, F. 1992. Functional, nutritional and storage characteristics of rice bran as affected by microwave heat and extrusion stabilization methods. USA: Louisiana State University, PhD thesis.

Mezouari, S. and Eichner, K. 2007. Comparative study on the stability of crude and refined rice bran oil during long-term storage at room temperature. European Journal of Lipid Science and Technology 109(3): 198-205.

Mezouari, S., Eichner, K., Parkash Kochhar, S., Brühl, L. and Schwarz, K. 2006. Effect of the full refining process on rice bran oil composition and its heat stability. European Journal of Lipid Science and Technology 108(3): 193-199.

Mingyai, S., Kettawan, A., Srikaeo, K. and Singanusong, R. 2017. Physicochemical and antioxidant properties of rice bran oils produced from colored rice using different extraction methods. Journal of Oleo Science 66(6): 565-572.

Negro, C., Tommasi, L. and Miceli, A. 2003. Phenolic compounds and antioxidant activity from red grape marc extracts. Bioresource Technology 87(1): 41-44.

Pal, Y. P. and Pratap, A. P. 2017. Rice bran oil: A versatile source for edible and industrial applications. Journal of Oleo Science 66(6): 551-556.

Pengkumsri, N., Chaiyasut, C., Sivamaruthi, B. S., Saenjum, C., Sirilun, S., Peerajan, S., ... and Kesika, P. 2015. The influence of extraction methods on composition and antioxidant properties of rice bran oil. Food Science and Technology 35(3): 493-501.

Perez-Ternero, C., de Sotomayor, M. A. and Herrera, M. D. 2017. Contribution of ferulic acid, gamma-oryzanol and tocotrienols to the cardiometabolic protective effects of rice bran. Journal of Functional Foods 32: 58-71.

Pestana, V. R., Zambiazi, R. C., Mendonça, C. R. B, Bruscatto, M. H., Lerma-García, M. J. and Ramis-Ramos, G. 2008. Quality changes and tocopherols and γ-orizanol concentrations in rice bran oil during the refining process. Journal of the American Oil Chemists' Society 85(11): 1013-1019.

Palm Oil Research Institute of Malaysia (PORIM). 1995. PORIM Test Methods. Malaysia: PORIM.

Ruch, R. J., Cheng, S. J. and Klaunig, J. E. 1989. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 10(6): 1003-1008.

Seetharamaiah, G. S. and Prabakar, J. V. 1986. Oryzanol content of Indian rice bran oil and its extraction from soapstock. Journal of Food Science and Technology 23: 270-273

Skerget, M., Kotnik, P., Hadolin, M., Rizner-Hras, A., Simonic, M. and Knez, Z. 2005. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chemistry 89(2): 191-198.

Smirnoff, N. and Cumbes, Q. J. 1989. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry 28(4): 1057-1060.

Suliman, T. E. M. A., Jiang, J. and Liu, Y. F. 2013. Chemical refining of sunflower oil: effect on oil stability, total tocopherol, free fatty acids and colour. International Journal of Engineering Science and Technology 5(2): 449-454.

Szydłowska-Czerniak, A. and Łaszewska, A. 2015. Effect of refining process on antioxidant capacity, total phenolics and pro-oxidant contents in rapeseed oils. LWT - Food Science and Technology 64(2): 853-859.

Szydłowska-Czerniak, A., Karlovits, G., Dianoczki, C., Recseg, K. and Szłyk, E. 2008. Comparison of two analytical methods for assessing antioxidant capacity of rapeseed and olive oils. Journal of the American Oil Chemists' Society 85(2): 141-149.

Tasan, M. and Demirci, M. 2005 Total and individual tocopherol contents of sunflower oil at different steps of refining. European Food Research and Technology 220(3-4): 251-254.

Van Hoed, V., Depaemelaere, G., Vila Ayala, J., Santiwattana, P., Verhé, R. and De Greyt, W. 2006. Influence of chemical refining on the major and minor components of rice bran oil. Journal of the American Oil Chemists' Society 83(4): 315-321.

Wu, S.-J. and Ng, L.-T. 2008. Antioxidant and free radical scavenging activities of wild bitter melon (*Momordica charantia* Linn. var. *abbreviata* Ser.) in Taiwan. LWT-Food Science and Technology 41(2): 323-330.

Yoon, S. H. and Kim, S. K. 1994. Oxidative stability of high-fatty acid rice bran oil at different stages of refining. Journal of the American Oil Chemists' Society 71(2): 227-229.

Zhu, M., Wen, X., Zhao, J., Liu, F., Ni, Y., Ma, L. and Li, J. 2016. Effect of industrial chemical refining on the physicochemical properties and the bioactive minor components of peanut oil. Journal of the American Oil Chemists' Society 93(2): 285-294.