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Research note

Determination of antioxidant component and activity of rice bran extract

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Abstract Rice bran is one of the most important co-products in the rice milling. In this research, antioxidant activity of two Iranian rice bran varieties, Fajr and Tarem, extracted by three different solvents (methanol, ethanol and ethyl acetate) was evaluated. The order of antioxidant activity was evaluated by measurement of total phenolic content, antioxidant activity in linoleic acid system, reducing power and scavenging capacity by DPPH radical. The methanolic extract of Fajr rice bran showed a total phenolic content of 3.31 mg Gallic acid/gr rice bran and a DPPH free radical-scavenging activity of 93.91%, achieved at 50 mg/ml concentration, and a percent inhibition of linoleic acid peroxidation of 68.01% with reducing power. These results indicated that the methanolic components of the rice bran extracts might potentially be natural antioxidants.

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1. Introduction

Rice bran is a rich source of natural antioxidants which can be used as free radical scavengers. It is widely recognized that many of the today's diseases are due to the oxidative stress that results from an imbalance between formation and neutralization of free radicals. The rice bran oil antioxidants are very efficient in reducing low density lipoprotein and total serum cholesterol. Vitamin E is a generic term for a group of four tocopherols (α , β , γ and δ) and four tocotrienols (α , β , γ and δ), of which α -tocopherol has the highest biological activity [1]. Rice bran oil contains about 0.1–0.14 vitamin E components and 0.9%–2.9% oryzanol; the concentrations can vary substantially, according to the origin of the rice. Oryzanol is

a mixture of esters of ferulic acid with sterols and triterpene alcohols, and has similar antioxidant properties with vitamin E vitamins [2]. It provides hydrogen for the neutralization of free radicals. A number of studies showed that vitamin E functions as a chain-breaking antioxidant that prevents the propagation of free radical reactions. Tocotrienols have been reported to be involved in anticancer activity. Safety concerns over synthetic antioxidants have led to an increasing interest in identifying natural antioxidants. The replacement of synthetic antioxidants by antioxidants from natural sources has many benefits due to their health benefits [3]. Recent studies on the phytochemicals and antioxidant properties of rice bran involve using various solvents such as hexane, methanol, acidic 80% methanol, isopropanol and ethyl acetate [4]. The object of this study was to investigate the antioxidant activity of two varieties of rice bran, which were extracted by three different solvents, to provide information on functional food for commercial practices.

2. Materials and methods

2.1. Material

Folin–Ciocalteu reagent, sodium carbonate, ferrous chloride, ammonium thiocyanate, potassium ferricyanide, trichloroacetic acid, ferric chloride and methanol were purchased from Merck, Gallic acid from Fluka 2,2-diphenyl-1-picrylhydrazyl (DPPH) and linoleic acid from Sigma-Aldrich.

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2.2. Methods

2.2.1. Preparation of rice bran

Rice bran powders of two varieties of Iranian rice, namely Fajr and Tarem, were obtained by milling rice grain in a local grinding mill in Babolsar, followed by sieving to separate grain from rice bran. Stabilization of rice bran was carried out in a microwave oven with 550 W output power. One hundred grams of each sample was packed in a polyethylene microwave-safe bag and subjected to microwave heating in a preheated oven for 3 min at 120 °C, and then cooled down at room temperature overnight. This procedure was repeated three times to ensure the stabilization. Then the samples were placed in cooler at 4 °C for one week until analyses [5].

2.2.2. Extraction of total antioxidants

Five grams of stabilized rice bran were extracted with 20 ml of methanol (MeOH), ethyl acetate (EtOAc) and ethanol (EtOH) at room temperature for 3 h in an electrical shaker. The residue was re-extracted twice and filtered through Whatman No. 1 filter paper. The extracts were combined and dried under vacuum, using a rotary evaporator (50 °C), and weighed immediately.

2.2.3. Determination of total phenolic content

The total phenolic content of bran extracts was determined, using the Folin–Ciocalteu reagent [6]. The reaction mixture contained 20 μ L of bran extracts was mixed with 100 μ L of the freshly prepared Folin–Ciocalteu reagent and a further 1.58 ml of distilled water. The mixture was shaken vigorously and 300 μ L of sodium carbonate (20% w/v) were added and the mixture was again shaken for 2 min. After the mixture was left to stand for 2 h at room temperature, the absorbance at 765 nm was measured by using a UV-vis spectrophotometer. Gallic acid was used as a standard, and results were calculated as Gallic acid equivalents (mg/g of bran).

2.2.4. Scavenging effect on DPPH radical

Free radical scavenging activities of bran extracts was determined by using a stable DPPH radical [7]. 0.1 ml of the extract solution was well mixed with 3.9 ml of methanol and 1.0 ml of DPPH solution. The mixture was kept at ambient temperature for 30 min prior to measurement of the absorbance at 517 nm. The scavenging effect was derived following Eq. (1):

$$\text{DPPH scavenging \%} = [1/(A_{517 \text{ nm, sample}} - A_{517 \text{ nm, control}})] \times 100. \quad (1)$$

2.2.5. Determination of reducing power

Extracts (10, 25, 50 mg/ml) were mixed with Phosphate Buffer (2.5 ml, 2.0 M, pH 6.6). The dilute sample was then mixed with 2.5 ml of 1% potassium ferricyanide and incubated at 50 °C for 20 min. About 2.5 ml of 10% trichloroacetic acid were added and the mixture was centrifuged for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and 1% ferric chloride (0.5 ml), and absorbance was measured at 700 nm [8].

2.2.6. Inhibition of lipid peroxidation (Ferric-Thiocyanate Method)

100 μ L of linoleic acid were dissolved in 4 ml of EtOH, 8 ml of 0.05 M phosphate buffer (pH 7.0) and 3.9 ml of distilled water. 50 μ L of sample (10, 25, 50 mg/ml) was added to 1.4 mL of the previously described linoleic acid solution. This mixture was kept in darkness and at 40 °C; the accelerated oxidation

Table 1: Yields and total phenolic content of Fajr rice bran with various solvents.

Solvent	Extract yield (%)	Total phenolic content (mg Gallic acid/g of bran)
Methanol	20.16	3.31 \pm 0.03
Ethanol	13.2	1.67 \pm 0.01
Ethyl acetate	15.95	1.29 \pm 0.03

Data are means \pm SD ($n = 3$), ($p < 0.05$), TPC, total phenolic content.

Table 2: Yields and total phenolic content of Tarem rice bran with various solvents.

Solvent	Extract yield (%)	Total phenolic content (mg Gallic acid/g of bran)
Methanol	8.8	2.01 \pm 0.04
Ethanol	5.94	1.05 \pm 0.02
Ethyl acetate	7.64	0.47 \pm 0.02

Data are means \pm SD ($n = 3$), ($p < 0.05$), TPC, total phenolic content.

of linoleic acid was measured after 24, 48, 72 and 96 h of thermal treatment. The determination of oxidation degree (as peroxides formation) was performed according to the ferric-thiocyanate method: 30 μ L of the reaction mixture was added to 2.91 ml of 75% ethanol, 30 μ L of 30% ammonium thiocyanate and 30 μ L of 0.02 M ferrous chloride in 3.5% hydrochloric acid. Mixtures were shaken and exactly after 3 min the absorbance was measured at 500 nm [9].

The percentage of LA peroxidation inhibition was calculated by Eq. (2):

$$\text{Inhibition on LA peroxidation \%} = [1/(A_{500 \text{ nm, sample}} - A_{500 \text{ nm, control}})] \times 100. \quad (2)$$

2.2.7. Statistical analysis

In this study, Media optimization for antioxidant activity was carried out by Response Surface Methodology (RSM). The Central Composite Design (CCD) was used [10]. It allows the determination of both linear and quadratic models. In general, a CCD for k factors, coded as (x_1, \dots, x_k) , consists of three parts: a factorial (or cubic) design containing a total of $n_{\text{fact}} = 2^k$ points with coordinates $x_i = -1$ or $x_i = +1$, for $i = 1, \dots, k$; an axial (or star) part formed by $n_{\text{ax}} = 2k$ points with all their coordinates null, except for the one that is set equal to a certain value α (or $-\alpha$), which usually ranges from 1 to \sqrt{k} ; and finally a total of n_c runs performed at the center point of the experimental region, where, of course, $x_1 = x_2 = \dots = x_k = 0$. A p value of < 0.05 was considered statistically significant.

3. Results and discussion

3.1. Total phenolic content

The Total Phenolic Content (TPC) was determined following a modified Folin–Ciocalteu reagent method, and results were expressed as gallic acid equivalents (Tables 1 and 2). Higher TPC was observed for Fajr, than Tarem variety. The yield of the Fajr rice bran extracts significantly varied with the solvents applied, in the order of MeOH (20.16%) EtOAc (15.95%) $>$ EtOH (13.2%). For the extract compositions, the total phenolic content was significantly greater for the Methanolic extract (3.31 gGAE/kg bran), than for the ethanol and ethyl acetate ($p < 0.05$). The total phenolic contents for Fajr rice bran extracts (1.27–3.31 g GAE/g bran) are close to that reported for Thailand long-grain

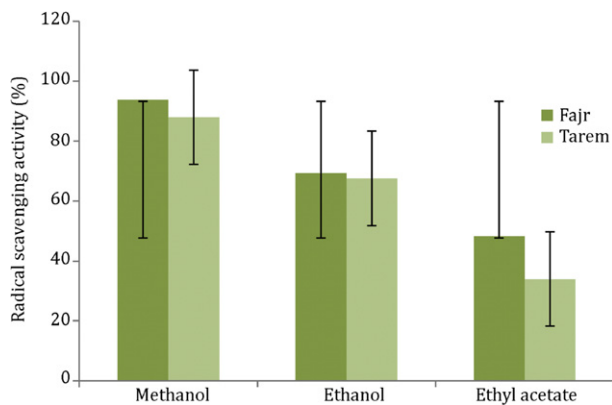


Figure 1: DPPH radical scavenging activity.

(LG) rice bran with MeOH extraction time of 12 h (2.2–3.2 g GAE/kg bran) and the rice flour extracts from 481 Chinese rice cultivars with acidic MeOH at RT for 24 h (1.1–2.5 g GAE/kg flour) [11–13]. They are in the range between those reported for Pakistan rice bran extract with acidic 80% MeOH at RT for 3 h (2.5–3.6 g GAE/kg bran).

3.2. Radical scavenging activity

Figure 1 demonstrates that, at the concentration examined (50 mg/ml), a general tendency of MeOH extract > EtOH extract > EtOAc extract was found for DPPH scavenging activity ($p < 0.05$). Maximum scavenging activity was observed for Fajr variety (93.91%). The reason why MeOH extract had superior antioxidant activities to the EtOH and EtOAc, which exhibited a comparatively greater amount of the antioxidant compositions, may be explained by the possibility of more polar phenolic compounds and lipids in the MeOH extract, than in the EtOH and EtOAc [13].

3.3. Reducing power

The reducing power of rice bran extracts are shown in Figures 2 and 3. The reducing power of rice bran extracts increased with increasing concentration. At 10 mg/ml, the absorbance values of brans extracted by methanol, ethanol and ethyl acetate at 700 nm were 0.989, 0.536, 0.482 for Fajr bran and 1.023, 0.671 and 0.308 for Tarem, respectively.

Reducing power in Fajr variety was higher than Tarem which was in agreement with TPC, and radical scavenging ability. The reducing power of rice bran extracts was related to the total content of phenolic compounds.

The highest values of absorption for reducing power in rice bran extracts were observed in methanolic extract of Fajr variety ($p < 0.05$).

The results demonstrate that some compounds of rice bran extracts such as tocopherols were electron donors which can terminate radical chain reactions [14].

3.4. Antioxidant activity in linoleic acid system

Measurement of lipid hydroperoxides is an essential part of understanding lipid oxidation processes. During the oxidation process, peroxide was decomposed into lower molecular weight compounds. In this study, the FTC method was used to measure the peroxides formed during initial stages of lipid

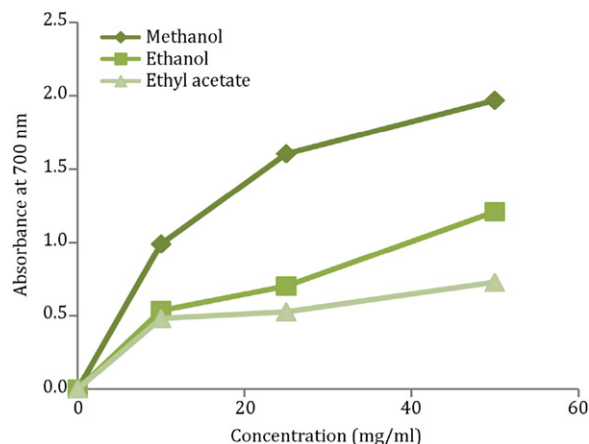


Figure 2: Reducing capacity of Fajr bran extracts.

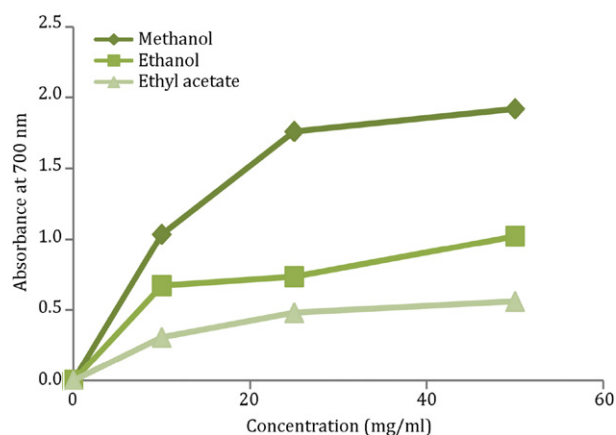


Figure 3: Reducing capacity of Tarem bran extracts.

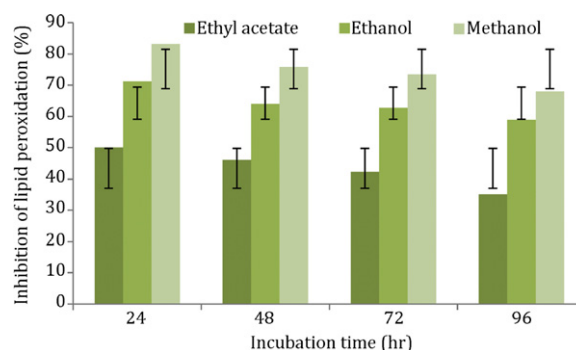


Figure 4: Inhibition of lipid peroxidation% of Fajr bran at 50 (mg/ml) concentration.

oxidation. The antioxidative effect of rice bran extracts through linoleic acid peroxidation, during accelerated lipid peroxidation at 40 °C for 96 h at three different concentrations (10, 25, 50), are shown in Figures 4–6 for Fajr bran and Figures 7–9 for Tarem. The inhibition of lipid peroxidation decreased with incubation time, because of formation of peroxide.

As it is shown, lipid peroxidation increased with increasing the concentration of extracts. At 50 mg/ml concentration and after 96 h incubation, methanolic extract of Fajr and Tarem exhibited strong inhibition of lipid peroxidation (68.01% and 65.5%, respectively), and the lowest inhibition was observed in

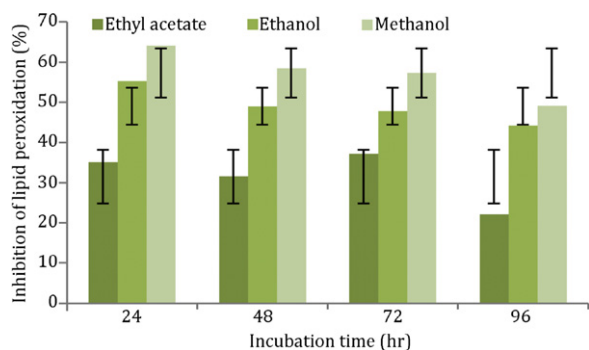


Figure 5: Inhibition of lipid peroxidation% of Fajr bran at 25 (mg/ml) concentration.

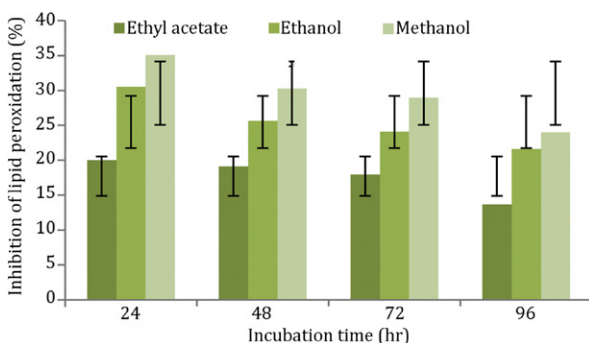


Figure 6: Inhibition of lipid peroxidation% of Fajr bran at 10 (mg/ml) concentration.

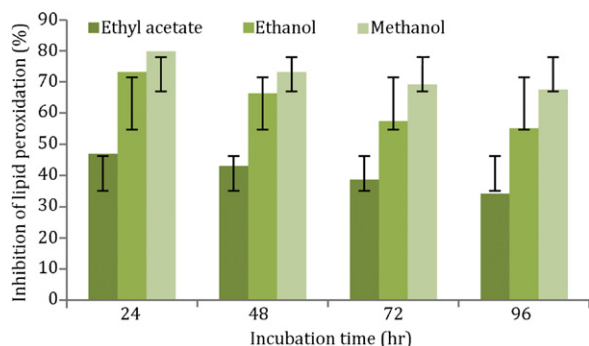


Figure 7: Inhibition of lipid peroxidation% of Tarem bran at 50 (mg/ml) concentration.

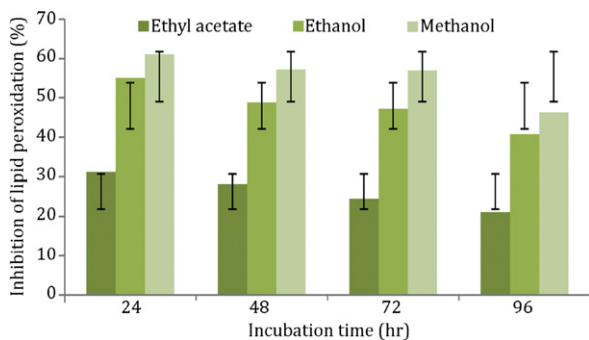


Figure 8: Inhibition of lipid peroxidation% of Tarem bran at 25 (mg/ml) concentration.

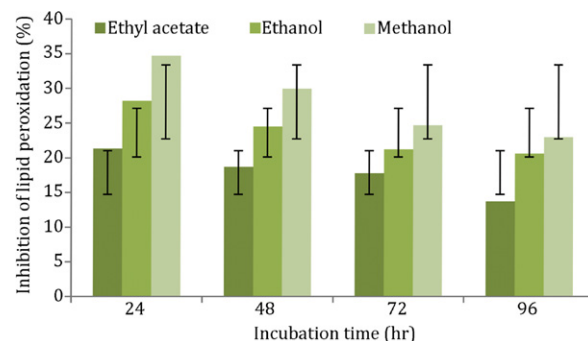


Figure 9: Inhibition of lipid peroxidation% of Tarem bran at 10 (mg/ml) concentration.

ethyl acetate extracts. These results suggest that rice bran may be a possible treatment for cardiovascular diseases, cholesterol lowering and other lipid peroxidation processes [15].

4. Conclusions

Extraction of antioxidant phytochemicals from Fajr rice bran with MeOH produced a significantly greater yield and total content in phenolic compounds than ethanol and ethyl acetate solvents, which lead to high antioxidant activities of extracts in inhibiting linoleic acid peroxidation, DPPH radical scavenging ability and reducing power. The strong antioxidative activity of rice bran extracts might be due to the presence of mainly tocotrienols or the synergistic effect of tocopherols and tocotrienols.

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