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Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan

Shahid Iqbal [a,*], M.I. Bhanger [a], Farooq Anwar [b]

[a] Free Radical Research Laboratory, National Center of Excellence in Analytical Chemistry, University of Sindh, Jamshoro 76080, Pakistan
[b] Department of Chemistry, University of Agriculture, Faisalabad 38040, Pakistan

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Abstract

The antioxidant activity of five indigenous rice bran varieties, i.e. Rice bran-Super kernel (RB-kr), Rice bran-Super 2000 (RB-s2), Rice bran-Super Basmati (RB-bm), Rice bran-Super-386 (RB-86) and Rice bran-Super fine (RB-sf), collected from the same agricultural plots, was evaluated. The order of antioxidant activity was evaluated by measurement of total phenolic content, antioxidant activity in linoleic acid system, reducing power, metal chelating ability, scavenging capacity by DPPH radical and ABTS cation radical and conjugated dienes. Determination of major antioxidant components reported in rice bran, i.e. tocopherols, tocotrienols and γ-oryzanol, was made using reverse phase HPLC. However, for comparison of tocopherol content, quantification was also done by voltammetry. The overall order of antioxidant activity was RB-kr > RB-s2 > RB-bm > RB-86 > RB-sf. However, according to the chelating activity and conjugated dienes assays the antioxidant efficacy of RB-sf was higher than RB-bm and RB-86. Antioxidant power was correlated with growth period and irrigation water demand by a particular variety. A strong correlation of these two parameters with antioxidant activity was observed. RB-kr has the longest growth period and takes the least amount of water out of the series and exhibits highest antioxidant activity. Strongly reverse behavior was observed in case of RB-sf.
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Keywords: Antioxidant activity; Rice bran; Phenolics; Tocopherols; HPLC; Voltammetry

1. Introduction

Increased concern over the safety of synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Razali, Norhaya, & Norasimah, 1997; Tsuda et al., 1998) has lead to an increased interest in exploration of effective and economical natural antioxidants. There is increasing evidence that changing one's diet to an increased intake of food 'relatively high' in selected natural antioxidants, such as plant polyphenols, vitamin C or flavonoids, can reduce the incidence of chronic and degenerative diseases (Laandrault et al., 2001; Shahidi, 2000; Wilson, 1999). Several sources of natural antioxidants have been investigated, including plants and microorganisms (Arai et al., 2002; Bandoniene, Pukalskas, Venskutomis, & Gruzdiene, 2000). Cereals and legumes containing a wide range of phenolics have been claimed to be a good source of natural antioxidants (Krings, El-saharty, El-Zeany, Pabel, & Berger, 2000). The best-known natural antioxidants that have proven important in the food industry and in human health are tocopherols, vitamin C and carotenoids (Shahidi, 1997). Antioxidant activities of some ferulic acid sugar esters have been reported to be stronger than that of free ferulic acid in a microsomal lipid peroxidation system (White & Xing, 1997).

Rice bran is one of the most abundant co-products produced in the rice milling industry. Although, it has

* Corresponding author. Tel.: +92 221 771379/+92 300 3079516; fax: +92 221 771560.
E-mail address: ranashahid313@yahoo.com (S. Iqbal).

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been recognized as an excellent source of vitamins and minerals, it has been under-utilized as a human food and has traditionally been used primarily in animal foods. Research conducted in last two decades has shown that it contains a unique complex of naturally occurring antioxidant compounds (Moldenhauer, Champagne, McCaskill, & Guraya, 2003). Current research has shown that rice bran may contain even 100 different antioxidants, as new ones are being discovered (Bidlack, 1999). Among the most powerful of these are oryzanols, tocopherols and tocotrienols (Godber & Wells, 1994).

Antioxidant compounds in rice bran or rice bran oil have professed health benefits as well as their antioxidant characteristics for improving the storage stability of foods. Also, it has been determined that the amount of cholesterol lowering occurs to a greater extent than expected from the fatty acid composition of the oil, suggesting that besides fatty acids, other components in the oil were responsible for the cholesterol lowering effect (Rogers et al., 1993). Oryzanol has been reported to be used in the cure of nerve imbalance and disorders of menopause (Rogers et al., 1993).

Other physiological effects include decrease of hepatic cholesterol biosynthesis and plasma cholesterol. Oryzanol has been accredited for its ability to reduce cholesterol absorption (Rong, Ausman, & Nicolosi, 1997). The complete oryzanol group is unique to rice bran oil, but the exact composition of oryzanol depends on the rice cultivar. Crude rice bran oil can contain $\leqslant 2\%$ (V/V) oryzanol (Norton, 1995). Tocotrienols have been reported to be involved in anticancer activity (Komiyama, Hayashi, Cha, & Yamaoka, 1992). Significant differences in the levels of tocotrienols and oryzanols from commercially available rice bran oil have been reported (Nicolosi, Rogers, Ausman, & Orthoefer, 1994). Diack and Sakska (1994) found that on separating vitamin E and oryzanol compounds from rice bran oil, the individual concentrations varied substantially according to the origin of the rice bran.

Lowering serum cholesterol levels in blood, specifically the low-density lipoprotein (LDL) fraction, aids in cardiovascular health and tends to lessen gallstone formation (Babcock, 1987). Animal and human studies show cholesterol lowering from a hypercholesterolemic status with rice bran. Reductions usually occur in the LDL fraction (Wells, 1993). Thus rice bran is a potential source of antioxidants for the food, pharmaceutical and cosmetic industries.

Rancidity has been a major problem with rice bran due to microbial activity generally associated with raw rice bran (Godber, Martin, Shin, Setlhako, Tricon & Gervais, 1993; Moldenhauer et al., 2003) as well as due to enzymatic deterioration known as 'hydrolytic degradation' (Sayre, 1988). Heating the rice bran in the presence of moisture permanently denatures lipolytic enzymes (Loeb & Mayne, 1952) and destroys lipolytic microbes. As long as the bran remains sterile, the enzyme activity will not increase (Lloyd, Siebenmorgen, & Beers, 2000).

The composition of rice bran varies depending on the source of bran, the milling techniques used (Malekian et al., 2000) and the stabilization techniques used. Lloyd et al. (2000) have grouped rice bran on the basis of grain size: long, medium, or short grain. Due to temperate growing conditions, the best-suited varieties cultivated in upper Punjab (Pakistan) are medium grain. However, no antioxidant activity evaluation study has been conducted on these indigenous varieties. This report describes the antioxidant activity of different varieties of rice bran as well as the determination of the main components responsible for antioxidant activity. Efforts have been made to study the effect of irrigation water quantity and growth period on antioxidant activity of these varieties.

2. Materials and methods

2.1. Samples and reagents

Freshly milled bran samples of five medium grain Rice varieties; Super kernel (RB-kr), Super-2000 (RB-s2), Super-basmati (RB-bm), Super-386 (RB-86) and Super-fine (RB-sf) were obtained from Chattha Rice Mills, Gujranwala, Pakistan. Freshly milled bran samples were collected directly from the milling system in polyethylene bags (Samples were collected from the same agricultural plot and same milling system). These bags were made air tight and stored at 4 °C in a cooler. All reagents (analytical and HPLC) used were procured from E. Merck or Sigma–Aldrich unless stated otherwise.

2.2. Stabilization of rice bran

Stabilization of rice bran was carried out according to the method of Malekian et al. (2000). A microwave oven with 550 W output power was used for the stabilization of rice bran. 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, i.e. until analyses.

2.3. Extraction of total antioxidants

Extraction was carried out following the method reported by Zuo, Chen, and Deng (2002). Bran samples (5.0 g) were ground to pass 1-mm sieve and extracted

thrice with 25 ml of 80% methanol for 3 h in an electrical shaker at room temperature. The contents of the flasks were further extracted twice with 20 ml of 80% methanol containing 0.15% HCl under the same set of conditions. The extracts were combined and filtered through a 0.45 μm of Nylon membrane filter. The extracts were evaporated to dryness under reduced pressure at 45 °C by a rotary evaporator and stored under freezer at −18 °C until used for further analysis.

2.4. Determination of total phenolic content

The total phenolic content of bran extracts was determined using the Folin–Ciocalteu reagent (Osawa & Namiki, 1981; Singleton & Rossi, 1965). The reaction mixture contained 200 μl of diluted bran extracts, 800 μl of freshly prepared diluted Folin–Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The final mixture was diluted to 7 ml with deionized water. Mixtures were kept in dark at ambient conditions for 2 h to complete the reaction. Then the absorbance at 765 nm was measured on a Perkin–Elmer Lambda-2 Spectrophotometer, with a 1 cm cell. Gallic acid was used as a standard and results were calculated as gallic acid equivalents (g/100 g) of bran. The reaction was conducted in triplicate and results were averaged.

2.5. Antioxidant activity determination in linoleic acid system

The antioxidant activity of sample extracts was determined following a reported method of Osawa and Namiki (1981). Sample extracts were added to a solution mixture of linoleic acid (0.13 ml), 99.8% ethanol (10 ml), and 0.2 M sodium phosphate buffer (pH 7.0, 10 ml). The total volume was adjusted to 25 ml with distilled water. The solution was incubated at 40 °C and the degree of oxidation was measured according to the thiocyanate method (Yen, Duh, & Tsai, 1993) with 10 ml of ethanol (75%), 0.2 ml of an aqueous solution of ammonium thiocyanate (30%), 0.2 ml sample solution and 0.2 ml of ferrous chloride ($FeCl_2$) solution (20 mM in 3.5% HCl) being added sequentially. After 3 min of stirring, the absorption values of mixtures measured at 500 nm were taken as peroxide contents. A control was performed with linoleic acid but without the extracts. Synthetic antioxidants, BHT and α-tocopherol were used as positive control. The percent inhibition of linoleic acid peroxidation, 100 − [(Abs. increase of sample at 360 h/Abs. increase of control at 360 h) × 100], was calculated to express antioxidative activity.

2.6. Determination of reducing power

The determination of reducing power was performed by the method used by Yen et al. (1993). Extracts (0.2, 0.4, 0.6, 0.8, 1.0, 1.2 mg) were mixed with phosphate buffer (5.0 ml, 2.0 M, pH 6.6) and 1% potassium ferricyanide (5 ml), and the mixtures were incubated at 50 °C for 20 min. About 5 ml of 10% trichloroacetic acid were added and the mixture was centrifuged at 650*g* for 10 min. The upper layer of the solution (5 ml) was mixed with distilled water (5 ml) and 0.1% ferric chloride (1 ml) and absorbance was measured at 700 nm. The experiment was conducted in triplicate and results were averaged.

2.7. Chelating activity

Fe^{2+} chelating activity was measured by a 2,2′-bipyridyl competition assay (Re et al., 1999). The reaction mixture contained 0.25 ml of 1 mM $FeSO_4$, 1 ml of Tris–HCl buffer (pH 7.4), 0.25 ml of extract, 0.4 ml of 10% hydroxylamine–HCl, 1 ml of 2,2′-bipyridyl solution (0.1% in 0.2 M HCl) and 2.5 ml of ethanol. The final volume was made up to 6.0 ml with water. The absorbance at 522 nm was measured and used to evaluate Fe^{2+} chelating activity using disodium ethylenediaminetetracetate (Na_2EDTA) as a standard.

2.8. $ABTS^{\cdot +}$ scavenging assay

ABTS radical cation scavenging assay was carried out following a modified method of Re et al. (1999) ABTS radical cation was prepared by passing a 5 mM ABTS aqueous solution through the oxidizing reagent, manganese dioxide, on Fisher Brand P8 filter paper. Excess manganese dioxide was removed from the filtrate by passing the solution through a 0.2 mm Fisher Brand membrane. The extracts were diluted in 5 mM phosphate buffered saline (PBS, pH 7.4), to an absorbance of about 0.700 (±0.020) at 734 nm in a 1 cm cell. 1.0 ml of each of the extracts was added to 5 ml $ABTS^{\cdot +}$ solution and the absorbance reading were taken 10 min after the initial mixing at room temperature. PBS was used as the blank. The calibration curve of Trolox standard was plotted. The antioxidant activities of bran extracts were expressed as Trolox equivalent content in 1.0 g of extract.

2.9. $DPPH^{\cdot}$ scavenging assay

Free radical scavenging activities of bran extracts was determined by using a stable 2,2′-diphenyl-1-picrylhydrazyl radical ($DPPH^{\cdot}$) following a previously reported method (Heinonen, Lehtonen, & Hopia, 1998). Briefly, a freshly prepared solution of $DPPH^{\cdot}$ (5.0 ml) was added to 1.0 ml of bran extracts. The decrease in absorbance, measured at different intervals, i.e. 0, 0.5, 1, 2, 5 and 10 min, (up to 50%) at 515 nm was determined with a Hitachi UV–Vis Model U-2000 spectrophotometer. The remaining concentration of $DPPH^{\cdot}$ in

the reaction medium was calculated from a standard calibration curve. The absorbance measured at 5 min of the antioxidant–DPPH radical reaction was used to compare the DPPH radical scavenging capacity of each bran extract.

2.10. Measurement of conjugated dienes

Measurement of conjugated dienes was carried out by the method of Xu and Godber (1999) with some modifications. The methanolic extracts were added to 1.0 g of methyl linoleate (MeLo), followed by evaporation of methane under Argon atmosphere. Oxidation of methyl linoleate was carried out in dark at 40 °C. Analysis was carried out after every 24 h by dissolving 10 mg of sample in 5 ml of *n*-hexane. Conjugated dienes content was determined by measuring absorbance spectrophotometrically at 234 nm with a Perkin–Elmer lambda-2 spectrophotometer using *n*-hexane as a blank. Analysis was carried out in triplicate and averaged.

2.11. Determination of γ-oryzanol content

Hexane extracts of all the rice bran varieties were obtained by Soxhlet apparatus. In the oils from individual varieties, γ-oryzanols were determined by a previously reported reverse phase HPLC method (McBride & Evans, 1973). HPLC unit model Hitachi L-6200, specifications are as: column (C 18, 150 × 2.1 mm, 5 μm), UV–Vis detector at 330 nm, flow rate 1.4 ml/min, Rheodyne 7125 six-port injector, Sample loop 50 μl and mobile phase was methanol, acetonitrile, dichloromethane and acetic acid (50:44:3:3 by volume). The total analysis time was approximately 22 min and γ-oryzanol peaks appeared around retention time of 16–19 min. A Hitachi Chromatointegrator model D-2500 with a built-in computer program was used for data handling and quantification. Concentrations of all the components of γ-oryzanol were calculated individually from the signal recorder. The total concentration of γ-oryzanol was achieved by summing up concentrations of all the components.

2.12. Quantification of tocopherols and tocotrienols by reverse phase HPLC

The HPLC procedure of Rogers et al. (1993) was used for the determination of tocopherols and tocotrienols with some modifications. Tocopherols and tocotrienols were measured by a HPLC system connected with fluorescent detector at Ex = 298 nm and Em = 328 nm. A Hewlett–Packard 200 × 2.1 mm narrow-bore analytical column packed with 5-m ODS (C18) Hypersil Silica was used for the separation of tocols. The mobile phase, i.e. acetonitrile/methanol/isopropanol/water (45:45:5:5 by volume), was programmed to acetonitrile/methanol/isopropanol (50:45:5) within 10 min. These conditions were maintained for 15 min before returning to the original conditions. Total HPLC run time was 26 min. A fine separation was achieved for tocopherols but bands were broad for tocotrienols. The results for tocopherols were also investigated by voltammetry for comparison.

Samples were quantified by comparing the retention times/peak areas with those of standards obtained from E. Merck (Germany) and Sigma Chemical Company for tocopherols and tocotrienols, respectively. Total tocopherol and tocotrienol contents were determined by summing up the α, β, γ and δ isomers.

2.13. Tocopherols determination by voltammetry

Tocopherol was determined by voltammetry following a slightly modified method (Yen & Duh, 1993). Samples were prepared by dissolving 1.0 g of each extract in 5 ml of 0.12 M H_2SO_4 solution in 2:1 ethanol–benzene mixture. A glassy carbon electrode was used at a scan rate of 20 mV/s. Peak heights and peak shapes were used to investigate the reproducibility of the results.

3. Results and discussion

3.1. Reducing power

Literature reports (Siddhuraju, Mohan, & Becker, 2002; Yildirim, Oktay, & Bilaloglu, 2001) are evident that the reducing power of bioactive compounds is associated with antioxidant activity. Thus, a relation should be located between reducing power and the antioxidant effect. The reducing power of methanolic extracts was measured and found to increase with increasing amount of extracts. BHA was used to compare the reducing power of these extracts. The reducing power of different varieties and reference, i.e. BHA, RB-kr, RB-s2, RB-bm, RB-86 and RB-sf, at a concentration of 1.0 mg was 2.42, 1.75, 1.73, 1.61, 1.56 and 1.65, respectively, as shown in Fig. 1.

The highest reducing power out of these varieties was observed in RB-kr, which was in agreement with TPC, radical and radical cation scavenging ability. While lowest reducing power was observed for RB-sf, which had far less TPC and scavenging ability than those of other varieties. Amazingly, the reducing power pattern is similar to the result of the conjugated dienes measurement, while contrary to the pattern observed in all other assays. However, for all the varieties the reducing power was less than that for BHA. Although some researchers (Awika, Rooney, Wu, Prior, & Zevallos, 2003) report that the antioxidative effect is concomitant with the development of reducing power. As results for all the varieties except Rb-sf were in agreement with TPC and scavenging activities. Therefore, reducing power evalua-

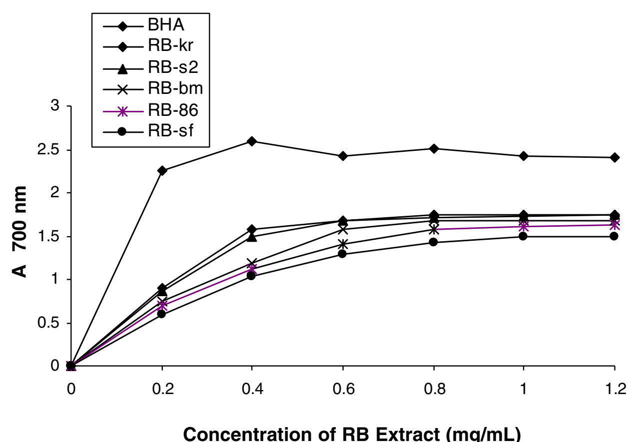

Fig. 1. Reducing capacity of bran extracts. *Legends key*: RB (Rice bran), kr (kernel), s2 (Super-2000), bm (Super basmati), 86 (Super-86), sf (Super fine).

tion may be taken as an important parameter for the assessment of antioxidant activity. A large variation in reducing power was observed among the varieties but this difference went on decreasing with the increase in the dose of extracts. RB-kr is the variety demanding longest growth period with least quantity of water while RB-sf has shortest growth period with largest quantity of water out of these. The results can be exploited in terms that varieties with long growth period and demanding less quantity of water have greater reducing power and vice versa.

3.2. 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 (Table 1). The phenolic compounds may contribute directly to antioxidative action (Awika et al., 2003). Significant differences were observed for TPC among the varieties. TPC was in the range of 2.51–3.59 mg/g of bran extracts. Highest TPC was observed for RB-kr while lowest for RB-sf. RB-kr and RB-s2 had nearly equal and far greater than that for the RB-sf variety. These values are higher than those of "Akron"(Yu, Perret, Harris, Wilson, & Haley, 2003) and "Trego" (Zhou & Yu, 2004) wheat bran varieties, which have been reported as potent sources of antioxidants, suggesting exploitation of rice bran as a viable source of antioxidant for nutraceuticals and functional foods. Same effect of growth period and quantity of irrigation water on TPC was observed as in case of reducing power. No correlation was found among the TPC and chelating activity of bran extracts, however, a linear correlation of TPC with scavenging activity was observed with slight variations.

Table 1
Total phenolic content and chelating activity of different rice bran varieties expressed as gallic acid equivalent and EDTA equivalent, respectively

Sample ID	TPC (Gallic acid eq.) (mg/g of bran)	Chelating activity (EDTA eq.) (μg/g of bran)
RB-kr	3.59 ± 0.02	683 ± 2.2
RB-s2	3.23 ± 0.03	715 ± 2.8
RB-bm	2.89 ± 0.02	623 ± 2.4
RB-86	2.73 ± 0.03	610 ± 3.9
RB-sf	2.51 ± 0.04	703 ± 3.4

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

3.3. Chelating activity

The chelating activity was measured against Fe^{2+} and reported as EDTA equivalents (Table 1). A large difference in chelating activity was observed among the species. The highest chelating activity was observed for RB-s2, followed by RB-sf, RB-kr, RB-bm and RB-86, respectively. The EDTA equivalent was 610–715 μg/g of extracts for different varieties. Chelation is an important parameter in the sense that iron is essential for life because it is required for oxygen transport, respiration and the activity of many enzymes. However, iron is an extremely reactive metal and will catalyze oxidative changes in lipids, proteins and other cellular components (Decker & Hultin, 1992; Smith, Halliwell, & Aruoma, 1992). However, the results from this parameter are not in agreement with those of others and a reverse effect of growth period and irrigation water demand was estimated among the varieties.

3.4. Radical and radical cation scavenging activity

The scavenging activity of crude methanolic extracts was determined by DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ assays as shown in Figs. 2 and 3, respectively. Both of these radicals are commonly used for assessment of antioxidant activity in vitro and are foreign to biological systems. All the varieties exhibited appreciable scavenging activity against both the radicals and the same order of scavenging was observed by both the assays. Maximum scavenging activity was observed for RB-kr, followed by RB-s2, RB-bm, RB-86 and RB-sf, respectively. By ABTS$^{\cdot+}$ assay, differences in antioxidant activity of all the varieties were not significant while by DPPH$^{\cdot}$, significant differences in scavenging activity were observed. Awika et al. (2003) has recently reported the superiority of the ABTS$^{\cdot+}$ assay over DPPH$^{\cdot}$, because ABTS$^{\cdot+}$ is operable over a wide range of pH, inexpensive and more rapid than that of the DPPH$^{\cdot}$ assay. However, a linear correlation between DPPH Radical scavenging activity and polyphenolic contents have been reported as variable ranges in different vegetables

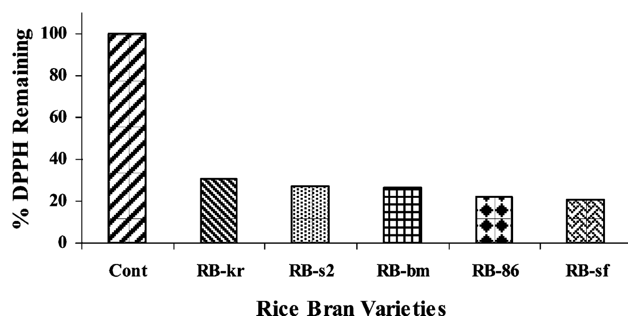

Fig. 2. Antioxidant activity determination by DPPH· assay.

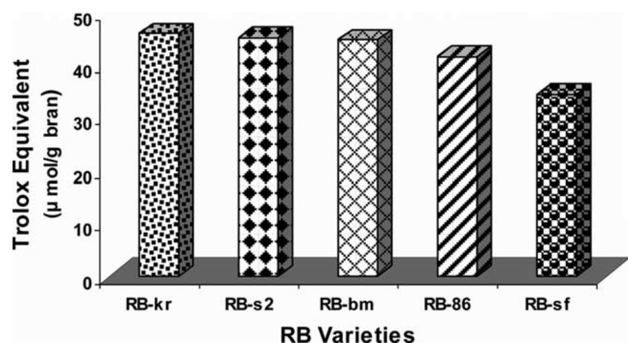

Fig. 3. Antioxidant activity determination by ABTS assay.

and fruits (Jimenez-Escrig, Rincon, Pulido, & Saura-Calixto, 2001; Von Gadow, Joubert, & Hansmann, 1997). Kinetic studies of DPPH·-extract reaction were carried out to estimate scavenging activity as a function of time. Scavenging activity was nearly the same at first minute of reaction and diverged with the increase in time until becoming similar near 10 min reaction time. Maximum difference among the varieties was observed at 5 min of reaction and the remaining amount (%) of DPPH radical at 5 min after initiation of reaction, as shown in Fig. 2, was 30.6, 27.2, 26.1, 22.5 and 20.6 for RB-kr, RB-s2, RB-bm, RB-86 and RB-sf, respectively. The same correlation of growth period, quantity of water needed and antioxidant activity was observed in all the assays with slight variations. The results among different parameters are strongly correlated.

3.5. Antioxidant activity in linoleic acid system

Antioxidant activity of all the bran extracts was observed in linoleic acid system and compared with BHA and α-tocopherol as shown in Fig. 4. Antioxidant activity of all the varieties was found to be higher than α-tocopherol but lower than BHA. However, no significant differences could be estimated in antioxidant activity among the varieties. Highest antioxidant activity was observed for RB-kr while lowest for RB-sf and the same order was exhibited. These results suggest that rice bran

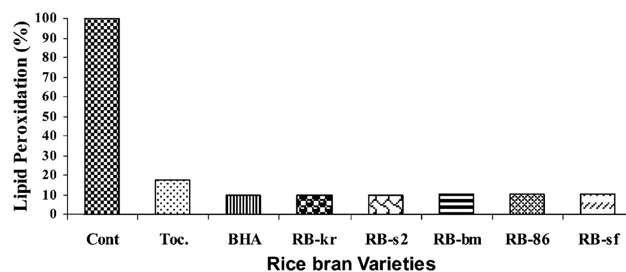

Fig. 4. Antioxidant activity determination in linoleic acid system.

may be a possible treatment for cardiovascular diseases, cholesterol lowering and other lipid peroxidation processes, being a more powerful antioxidant complex than tocopherol.

3.6. Conjugated dienes

Conjugated dienes (CD) are often measured as indicators of free radical production. Oxidation of unsaturated fatty acids results in the formation of CD. The CD provide a marker of the early stages of lipid peroxidation (Halliwell & Gutteridge, 1985). Measurement of conjugated dienes is a useful index of the early stages of peroxidation in studies with pure lipids and isolated lipoproteins (Dekkers, Van Doornen, & Kemper, 1996). Conjugated dienes were measured after every 24 h up to 4 days and were compared with α-tocopherol. Conjugated dienes for all the samples and standard went on increasing as a function of time in a linear fashion following the same kinetics order. All the samples exhibited less increase in conjugated dienes (Fig. 5), i.e. more antioxidant activity than that of α-tocopherol, suggesting exploitation of rice bran as a valuable source for suppression of oxidative stress and to control lipid peroxidation in various body organs. All the samples showed inhibition to oxidation strongly for the first 36 h. The order of antioxidant activity was RB-kr > RB-s2 > RB-sf > RB-bm > RB-86. Antioxidant activity of RB-sf was quite inconsistent relative to that exhibited by other assays. Up to 48 h, RB-sf has normal antioxidant activity but in the last two days, dienes content in

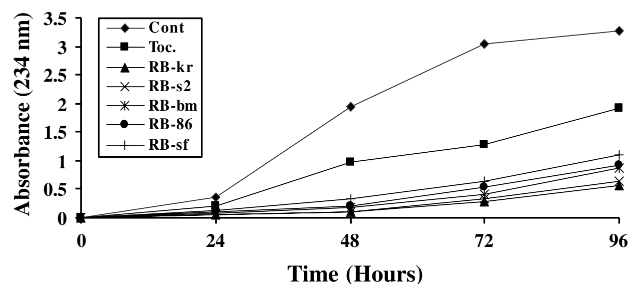

Fig. 5. Antioxidant activity evaluation by measurement of conjugated dienes.

RB-sf increased over RB-bm and RB-86. This parameter is, however, rarely used for estimation of antioxidant activity in food systems; nevertheless, diene conjugation methods are still useful for isolated lipids.

3.7. Contents of tocopherols, tocotrienols and oryzanols

Tocols and oryzanols are the main antioxidants present in the rice bran. Antioxidant activity of oryzanols is almost 10 times higher than that of tocopherols, while tocotrienols have 40–60 times greater antioxidant power than those of tocopherols in different biological systems (Abdel-Aal & Hucl, 1999). A significant quantity of tocopherols (378–503 ppm; voltammetry; 392–512 ppm, HPLC) (Table 3), tocotrienols (343–478 ppm) (Table 3) and oryzanols (511–802 ppm) (Table 2) was estimated from all the varieties suggesting its potential uses in the food and pharmaceutical industries. Trends were in good agreement with other assays except in the case of tocopherols, where an anomaly was observed that indicated the quantity of tocopherols for RB-sf was higher than that of RB-86. However, oryzanol and tocotrienol contents of RB-86 were higher than that of RB-sf, which are more significant than tocopherols in terms of antioxidant activity. Tocotrienols eluted before tocopherols and order of elution was $\alpha > \beta > \gamma > \delta$ for tocopherols. β and γ isomers eluted over a very narrow range of retention time; therefore, for comparison, the voltammetric technique was used, which also demonstrated a very small difference in reduction potential of β and γ isomers. Tocotrienols are believed to counter the ravages of chronic diseases such as heart diseases, cancer and the degenerative effects of aging. Tocotrienols get incorporated into cellular lipid membranes, where it effectively inhibits the peroxidation chain of lipids (Burton & Ingold, 1981). In this way rice bran, being a unique complex of oryzanols and tocols, may be a good source of compounds for the inhibition of lipid peroxidation.

Table 2
HPLC data on quantitative determination of γ-oryzanol content from different rice bran varieties

Sample ID	γ-Oryzanol content (ppm)
RB-kr	802 ± 9.2
RB-s2	789 ± 10.2
RB-bm	698 ± 8.2
RB-86	655 ± 5.2
RB-sf	511 ± 7.8

Values are means ($n = 3$) ± SD ($n = 3$), ($p < 0.05$).

Table 3
HPLC and voltammetric data on quantification of tocopherol and HPLC data for tocotrienols from different rice bran varieties

Sample ID	Tocopherols (ppm)		Tocotrienols (ppm)
	Polarography	HPLC	HPLC
RB-kr	503 ± 3.1	512 ± 2.7	478 ± 2.4
RB-s2	472 ± 4.2	481 ± 5.2	452 ± 4.4
RB-bm	451 ± 7.3	459 ± 6.4	389 ± 6.1
RB-86	378 ± 4.1	392 ± 7.4	364 ± 5.4
RB-sf	423 ± 3.2	419 ± 11.3	343 ± 3.1

Values are means ($n = 3$) ± SD ($n = 3$), ($p < 0.05$).

3.8. Conclusions

From the present work, it could be concluded that growth period and quantity of irrigation water needed for cultivation of a variety has a significant effect on its antioxidant properties, provided, other environmental factors, i.e. humidity, soil texture, quality of water etc., are the same as in the present analysis. Antioxidant potential differs among the varieties up to a significant extent. Rice bran is a potent source of antioxidants, containing a unique mixture of tocols and oryzanols, suggesting its use in nutraceuticals and functional food industries. Moreover, its detailed in vivo studies are recommended for treatment of coronary heart diseases, cancer and aging.

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