GRASAS Y ACEITES, 57 (3),
JULIO-SEPTIEMBRE, 328-335, 2006,
ISSN: 0017-3495

Evaluation of the antioxidant activity of rice bran extracts using different antioxidant assays

By **Shahzad Ali Shahid Chatha, Farooq Anwar*, Maleeha Manzoor** and **Jawad-ur-Rehman Bajwa**

Department of Chemistry, University of Agriculture, Faisalabad.-38040, Pakistan.
Email: fqanwar@yahoo.com

RESUMEN

Evaluación de la actividad antioxidante de extractos de salvado de arroz mediante differentes ensayos

Se evalúa la actividad antioxidante diferentes extractos (100% metanol, 80% metanol, 100% acetona and 80% acetona) de salvado de arroz -var. Super Kernel- mediante diferentes ensayos y utilizando aceite de girasol como substrato. Los ensayos utilizados fueron la estimación del % de inhibición de la peroxidación en sistemas con ácido linoleico, el contenido total en compuestos fenólicos y la pérdida de β-caroteno en sistemas con ácido linoleico. Adicionalmente, los concentrados de extractos de salvado de arroz se añadieron a aceite de girasol y las muestras se almacenaron a temperatura ambiente. La extensión de la oxidación se evaluó mediante el índice de peróxidos, el índice de p-anisidina, así como la formación de dienos y trienos conjugados.

El orden de la eficacia antioxidante de los extractos de salvado de arroz fue el siguiente: 80 % metanol> 100 % metanol > 80 % acetona > 100% acetona. Los resultados demostraron que los extractos del salvado de arroz de la variedad Super Kernel del arroz indígena de Paquistán son una fuente viable de antioxidantes naturales y podrían ser explotados como alimentos funcionales.

PALABRAS-CLAVE: Antioxidantes- Ensayos de actividad antioxidante - Fenoles totales- Oxidación - Porcentaje de inhibición.

SUMMARY

Evaluation of the antioxidant activity of rice bran extracts using different antioxidant assays

In the present work the antioxidant activity of different solvent (100% methanol, 80% methanol, 100% acetone, 80% acetone) extracts of rice bran was evaluated following different antioxidant assays and using sunflower oil as oxidation substrate. The rice bran extracts were evaluated from the estimate of % inhibition of peroxidation in linoleic acid system, total phenolics content (TPC) and loss of β-carotene in a linoleic acid system. Additionally, crude concentrated rice bran extracts were added into the sunflower oil samples and stored under ambient conditions. The extent of oxidative deterioration was followed by the measurement of peroxide-, *p*-anisidine-, conjugated diene-, and triene- values. The general order of antioxidant efficacy of rice bran extracts as determined by various antioxidant assays was 80% methanolic extract > 100% methanolic extract > 80% acetone extract > 100% acetone extract. The results of the present comprehensive analysis demonstrate that rice bran extracts of the Super Kernel variety indigenous to Pakistan are a viable source of natural antioxidants and might be exploited for functional foods and nutraceutical applications.

KEY-WORDS: Antioxidant activity - Antioxidant assays - Oxidation substrate - Percent inhibition - Total phenolics.

1. INTRODUCTION

Synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyle gallate (PG) and tertiary butyle hydroquinone (TBHQ) have been in use as food additives for a long time. However, recent reports have revealed that the use of these compounds may be related to some health risks, including cancer and carcinogenesis (Hou, 2003; Prior, 2004). Growing concerns over the safety of synthetic antioxidants has lead to an increased interest in the exploration of effective and economical natural antioxidants (Jeong *et al.,* 2004; Rababah *et al.,* 2004).

In recent years there has been increasing evidence that an intake of food "relatively high" in selected natural antioxidants such as plants polyphenols, vitamin C and flavonoids reduces the incidence of degenerative diseases e.g. cancer, CVD and aging (Anwar *et al.,* 2003; Laandrault *et al.,* 2001; Wu *et al.,* 2004). There is growing interest among food scientists to identify antioxidants that are safe and of natural origin and in this area a diverse group of plant compounds called natural antioxidants has been the focus of intense research.

Several sources of natural antioxidants have been investigated, including plants and microorganism. Plants are rich sources of natural antioxidants, the best known are tocopherols, carotenoids, vitamin C and different phenolics (Vichi *et al.,* 2001). Leafy green vegetables and beans contain phytochemicals that are chemo preventive. Most of them contain antioxidant substances e.g., indoles, carotenoids, vitamin C, and phenolics. Many spices and herbs are added to food not only for flavor but also for

preservation and suppression of rancidity along with the autoxidation of fats. Cereals and legumes contain a wide range of phenolics and are a good source of natural antioxidants. Phenolic acids occur in the grain of cereals primarily in bound form as conjugates with sugars, fatty acids, or proteins which also act as natural antioxidants (Shahidi, 1997[a]).

The plants of *gramineae* family are well known to contain natural antioxidant compounds like ferulic acid, caffeic acid, gallic acid, para-cumaric acid and flavonoids. Some of the gramineae plants like oat and wheat have been evaluated for their antioxidant principals (Shahidi, 1997[b]).

Rice (*Oryza sativa*) is a member of gramineae family. Rice bran is a huge agricultural waste of the rice polishing industry which is usually used in Pakistan as animal feed. No other useful purpose for rice bran has been detected. Rice bran is produced from the outer layer of brown rice. It contains high valued protein, fat and nutritional dietary fiber. In addition to phytonutrients, rice bran contains vitamins and minerals. Aside from a significant oil concentration, rice bran is reported to inherently contain a high level of medicinally important antioxidant gama oryzanol, a natural mixture of ferulic esters (Anwar *et al*, 2005).

Although a significant number of reports are available on the nutritional attributes of rice bran, relatively little is reported in the scientific literature regarding its antioxidant potential. As Pakistan is a big rice producer, a huge quantity of rice bran is produced annually as agricultural waste. In view of the beneficial uses and vital role the natural antioxidants can play in human health, a great demand exists to exploit additional plants and agricultural wastes as potential sources of natural antioxidants. The present research work was aimed to investigate the antioxidant activity of locally available rice bran of the Super Kernel variety using different antioxidant assays and sunflower oil as oxidation substrate.

2. MATERIALS AND METHODS

2.1. Samples and reagents

Freshly milled rice bran samples were collected directly from (Chaudhary Rice Mills, Jaranwala, Pakistan) the milling system in polyethylene bags. These bags were made air tight and stored at 4 °C. Refined, bleached and deodorized (RBD) Sunflower oil was obtained from United Industries Pvt. Limited, Kashmir Road Faisalabad, Pakistan. All reagents and chemicals used were from E. Merck or Sigma-Aldrich.

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, power was used for the stabilization of rice bran. One hundred grams of rice bran sample were packed in a polyethylene microwave-safe bag and subjected to microwave heating in a preheated oven for 3 min at 120 °C and cooled down at room temperature overnight. This procedure was repeated three times to ensure stabilization.

2.3. Extraction of rice bran

The powdered sample of rice bran (20 g) was individually mixed with 200 mL of extraction solvents; 100% methanol (pure methanol), 100% acetone (pure acetone), 80% methanol (methanol: water, 80:20 v/v) and 80 % acetone (acetone: water, 80:20 v/v) in conical flasks. Extraction was executed in a shaker for 48 hours. Each of the extracts was filtered and freed from moisture with Na_2SO_4. The solvents were distilled off under reduced pressure using a rotary evaporator (EYELA, Rotary Vacuum Evaporator, N.N. Series, equipped with an Aspirator and a Digital Water Bath SB-651, Japan). The crude concentrated extracts were stored under refrigeration until used for further analysis (Perumal and Becker, 2003).

2.4. Antioxidant Activity Evaluation

A preliminary evaluation of antioxidant activity of the crude concentrated rice bran extracts was made using the following tests.

2.4.1. Estimate of total phenolic contents (TPC)

TPC of rice bran extracts was determined using the Folin-Ciocalteu reagent method (Osawa and Namiki, 1981). The reaction mixture contained 200µl of diluted (with deionized water) bran extract, 800 µl of freshly prepared diluted Folin-Ciocalteu reagent and 2 ml of sodium carbonate solution (7.5%). The final mixture was diluted to 7ml with deionized water. Mixtures were kept in the dark at room temperature for 2 hrs to complete the reaction. Then the absorbance at 765 nm was measured using a Hitachi U-2001 spectro-photometer, model 121-0032, with a 1cm cell. Gallic acid was used as a standard and results were calculated as gallic acid equivalents (mg/100g) of bran. The reaction was conducted in triplicate and results were averaged.

2.4.2. Antioxidant activity determination in linoleic acid system

The antioxidant activity of bran extracts was determined following the reported method of Osawa and Namiki (1981). Extracts (5 mg) of each rice bran sample 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, 10 ml). The total mixture was diluted 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 *et al*., 1993) with 10 ml of ethanol (75%), 0.2 ml of an aqueous solution of ammonium thiocyanate (30%), 0.2 ml of 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 500nm were taken as peroxide contents. A control was performed with linoleic acid but without the bran extracts. Synthetic antioxidants; BHT and AA (200 ppm) were used as positive controls. The maximum peroxidation level observed as 360 h (15 days) in the sample that contained no antioxidant component was used as a test point. 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 antioxidant activity.

2.4.3. Antioxidant activity determination in a β-carotene linoleic acid system

Two mL of a solution of β-carotene in chloroform (1 mg/mL) were added to a flask containing 40 mg of linoleic acid and 400 mg of tween 40. The chloroform was removed using a rotary evaporator (EYELA, Rotary Vacuum Evaporator, N.N. Series, equipped with an Aspirator and a Digital Water Bath SB-651, Japan) at 45 °C for 4 min, and 100 mL of distilled water was added slowly to the semisolid residue with vigorous agitation (manual,1 min), to form an emulsion. 5 mL aliquot of the emulsion was added to a tube containing 0.2 mL of the antioxidant solution (500 mg/L) and the absorbance was measured at 470 nm using a Hitachi U-2001 spectrophotometer, model 121-0032, with a 1cm cell, immediately, against a blank, (consisting of the emulsion without β-carotene). The tubes were placed in a water bath at 50 °C, and the absorbance measurements were conducted again at 15 min intervals up to120 min. . All measurements were carried out in triplicate. The antioxidant activity (A.A) of the extracts was evaluated in terms of loss of beta-carotene using the following formula:

$$AA = [1-(A_0-A_t) / (A'_0-A'_t)]\ 100$$

Where A_0 and A_t are the absorbance values measured at zero time of incubation for test sample and control, respectively and A'_0 and A'_t are the absorbance measured in the test sample and control, respectively, after incubation for 120 min. (Perumal and Becker; 2003).

2.5. Measurement of antioxidant activity using SFO as oxidation substrate

The crude concentrated rice bran extracts were added to 100 g RBD sunflower oil samples at a concentration of 600 mg/Kg and stored in the dark at room temperature (i.e. 30-35 °C) for 75 days in sealed polyethylene teraphthalate (PET) bottles. 10 samples (5 samples in duplicate) of each group were stored. The oxidative deterioration level was followed by the measurement of peroxide value (PV), conjugated dienes and trienes contents and *p*-anisidine value. The analysis was carried out after every 15 days. Determination of PV was made according to the IUPAC standard method (IUPAC, 1987). Specific extinctions at 232 and 268 nm were determined using a Hitachi, U-2001, model 121-0032 spectrophotometer. Samples were diluted with iso-octane to bring the absorbance within limits (0.2-0.8) and ($^{1\%}\varepsilon_{1cm\ (\lambda)}$), which were calculated following the standard method of IUPAC (1987). The *p*-anisidine value was calculated according to the IUPAC standard method (IUPAC, 1987) by measuring absorbance at 350nm using a Hitachi, U-2001, model 121-0032 spectrophotometer.

All determinations were carried out in triplicate and data is recorded as mean ± standard deviation of duplicate samples. Significant differences ($p < 0.05$) were calculated using Duncan's multiple range test, following a previously reported method (Steel and Torrie, 1980).

3. RESULTS AND DISCUSSION

3.1. Preliminary antioxidant activity evaluation

In the present work different antioxidant assays were employed to evaluate the antioxidant activity of rice bran extracts. Table 1 shows the percentage yield of rice bran extracts in different solvents ranging from 11.21–16.71%. The highest yield was obtained in 80% methanol and the lowest in 100% acetone. The highest yield of rice bran extract as established in the present analysis employing 80% methanol might be due in part to the high polarity and efficacy of the methanol:water (80:20v/v) solvent system. Methanol is usually recommended for the extraction of antioxidant compounds (Iqbal *et al*., 2005) and its effectiveness could be improved by adding water as co-solvent, particularly, in the protocols, where the extraction of antioxidant compounds of a multifarious nature is mandatory. Shahidi, (1997[b]) reported that cereals like wheat, rice and oat etc., contained free, soluble esters, insoluable, bound and total phenolics.

Fig.1 shows the decrease in absorbance of β-carotene in the presence of different solvent

Table 1
Determination of Rice bran extract yield

Solvent	Yield (%)
100 % Methanol (pure methanol)	13.60 ± 0.70
80 % Methanol (methanol:water, 80:20 v/v)	16.71 ± 0.64
100 % Acetone (pure acetone)	10.02 ± 0.82
80 % Acetone (acetone:water 80:20 v/v)	11.21 ± 0.50

Values (mean ± SD) are average of duplicate samples analyzed individually in triplicate (n =2 x 3), ($P < 0.05$).

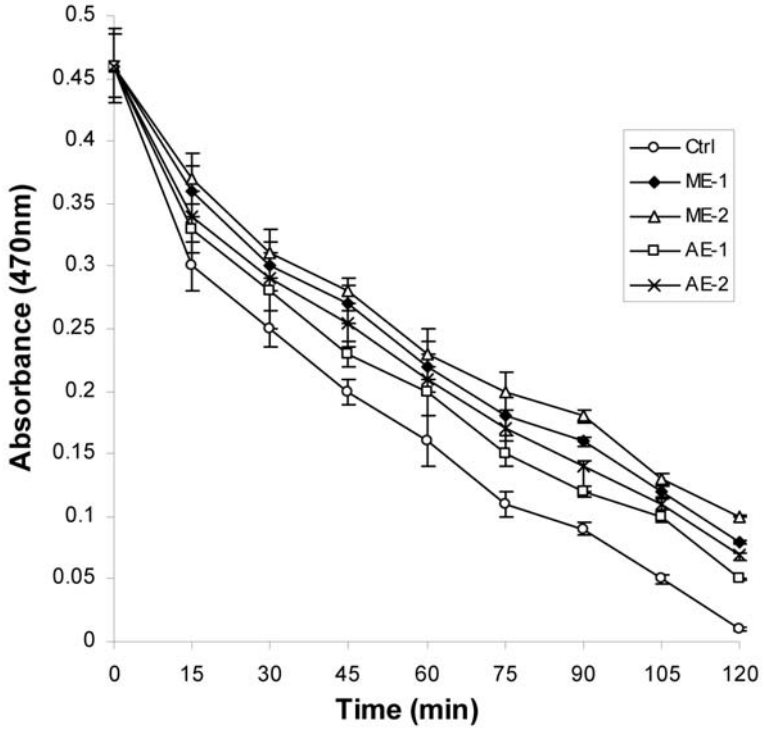

Figure 1
Antioxidant activity of extracts of rice bran in beta-carotene linoleic acid system

Ctrl	Control sample (without rice bran extract).
ME-1	100% methanol (pure methanol) extract of rice bran.
ME-2	80% methanol (methanol:water, 80:20 v/v) extract of rice bran.
AE-1	100% acetone (pure acetone) extract of rice bran.
AE-2	80% acetone (acetone:water, 80:20 v/v) extract of rice bran.

extracts of rice bran with the coupled oxidation of β-carotene and linolenic acid. The antioxidant activity of rice bran extracts was determined in terms of the loss of β-carotene. Different rates were observed for rice bran extracts and control. The decline in absorbance in case of control was found to be more rapid as compared with those of different solvent extrats of rice bran, indicating a significantly ($P < 0.05$) high antioxidant activity of rice bran. The overall decline rates in absorbance, among the investigated extracts were as follows; AE-1 > AE-2 > ME-1 > ME-2. In the present analysis, the highest antioxidant activity was exhibited by 80% methanolic rice bran extract, as indicated by the lowest decline in the absorbance of ME-2. The rapid decline in absorbance for AE-1 showed the least antioxidant activity of 100% acetone. As the ME-2 rice bran extract has exhibited greater antioxidant activity, it could be possible to declare that 80 % methanol is an effective solvent for the extraction of potent antioxidants from rice bran. The slower rate of oxidation of β-carotene in the case of ME-2 might be due to the high concentration of polyphenols, the natural antioxidants in rice bran extracts (Lu and Foo, 2000; Amarowicz *et al.,* 2001).

Fig.2 shows the contents of total phenolics (TPC) as determined by the Folin-Ciocalteu reagent method. The TPC of different rice bran extracts ranged from 250-397 mg/100g. The contents of total phenolics of 100% methanol (370 mg/100g) and 80% methanol (397 mg/100g) rice bran extracts was significantly ($P< 0.05$) higher than the values determined for 100% acetone (250 mg/100g) and 80% acetone (321 mg/100g) rice bran extracts. The phenolic acids seemed to be partly responsible for the antioxidant activity of the extracts. Iqbal *et al.,* 2005 reported that the estimate of TPC is a good measure of the antioxidant efficacy of the extracts. The phenolic compounds may contribute directly to antioxidant action (Awika *et al.,* 2003). It could be understood from the relative TPC of sample ME-2 that 80% methanol has been found to be the most efficient solvent system in extracting the phenolic antioxidants from rice bran as compared with those of other solvents. Iqbal *et al.,* (2005) reported the TPC of four varieties of rice bran indigenous to Pakistan ranging from 251-359 mg/100g. The TPC as determined in the present analysis of rice bran (super kernel) extracts were found to be higher than those of "Akron" wheat bran (Zhou and Yu, 2004), which was reported as a potent source of antioxidants, thus suggesting the exploitation of rice bran as a viable source of antioxidant for neutraceuticals and functional foods.

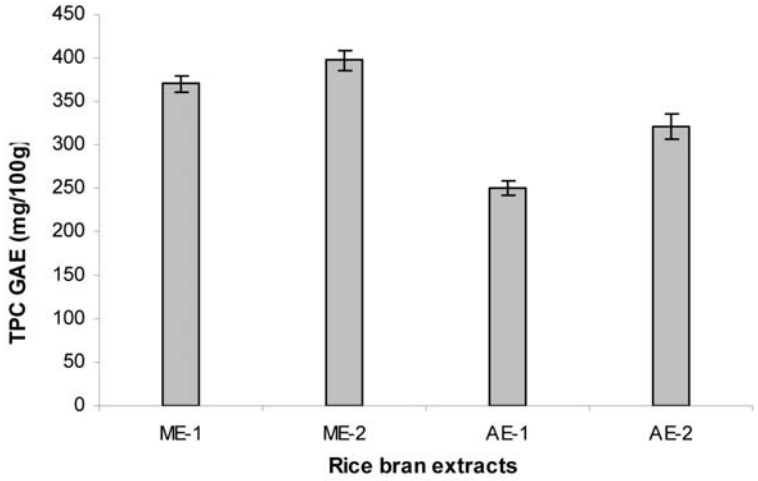

Figure 2
Total phenolic contents (TPC) of extracts of rice bran.
For abbreviations see Figure 1.

The results of Fig 3 depict that methanolic and acetone extracts of rice bran also exhibited good antioxidant activity in the linoleic acid peroxidation system in terms of the measurement of inhibition of peroxidation. At a concentration of 0.2 mg/mL, different rice bran extracts inhibited 75.0-92.0 % peroxidation of linoleic acid after incubation for 360 h (15days). Inhibition of peroxidation in linoleic acid was found to be 86.0, 92.0, 75.0, and 80.0% for samples ME-1, ME-2, AE-1 and AE-2, respectively. 80% methanollic extract of rice bran (ME-2) was found to show significantly (P < 0.05) higher inhibition (92.0%) of peroxidation in linoleic acid system, and thus reflected the highest antioxidant activity among the extracts. This percentage was comparable to that of the value obtained for BHT (93.0%), and statistically no significant differences were noted at P > 0.05. Whereas, 100% acetone rice bran extract (AE-1) exhibited the least antioxidant activity among the extracts as evident by the least inhibition of peroxidation by AE-1 (75.0%). On the other hand when compared to ascorbic acid, all the rice bran extracts were found to have significantly (P < 0.05) higher inhibition of peroxidation in a linoleic acid system. The % inhibition of peroxidation as exhibited by different

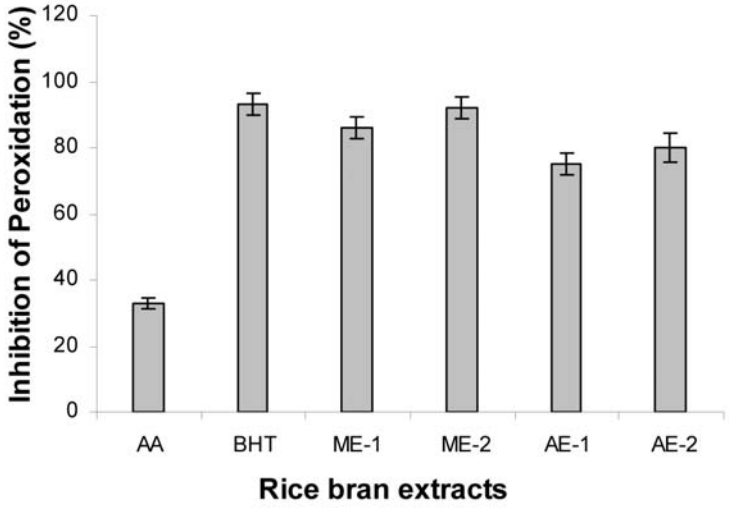

Figure 3
Antioxidant activity of rice bran and synthetic extract in linoleic acid system
AA Ascorbic acid.
BHT Butylated hydroxy toluene.
For abbreviations see Fig. 1

solvent extracts of rice bran might be attributed to the presence of various polyphenolics. Oryeneho and Hettiarachchy (1992) reported that the phenolic contents of various agriculture wastes contained potential antioxidant activity against the linoleic acid peroxidation system. Velioglu et al., (1998) also described that the total phenolic contents of fruits and vegetables contained potential antioxidant activities against the linoleic acid peroxidation system.

3.2. Antioxidant activity evaluation using sunflower oil as oxidation substrate

Table 2 presents the relative increase in the peroxide value (PV) of sunflower oil treatments under ambient storage. A typical pattern in the rise of PV was observed for almost all the sunflower oil (SFO) treatments. The control had the highest PV among all the oil treatments, showing a highest degree of oxidation. A significantly ($P < 0.05$) lower PV of SFO-1, SFO-2, SFO-3 and SFO-4 as compared with that of the control (SFO-0) clearly indicated a good antioxidant activity of rice bran extracts. 100 and 80% methanolic extracts of rice bran were found to be more effective in retarding the PV of sunflower oil as indicated by the slow rise in the formation of hydroperoxid products in SFO-1 and SFO-2 treatments as compared with those of acetone extracts of rice bran which retarded the PV to a lesser extent (as depicted by the formation of a high level of hydroperoxid products in SFO-3 and SFO-4 treatments). The results of PV specified that the 80% methanolic rice bran extract was more effective than the 100% methanolic extract. The PV, which measures hydroperoxid products in oils, is a good indicator of their primary oxidation state (McGinely, 1991).

Table.3 & 4 show the conjugated dienes (CD) and conjugated trienes (CT) contents for stabilized and control samples of sunflower oil. It was observed that CD and CT contents went on increasing as a function of time in a distinctive manner. Almost all the stabilized sunflower oil samples showed the formation of CD and CT to a significantly ($P < 0.05$) lower level as compared with that of the control, thus reflecting the antioxidant efficacy of the investigated rice bran extracts. The order of antioxidant activity of the rice bran extracts was found to be as follows; 80% methanol > 100% methanol > 80% acetone > 100% acetone as indicated by the formation of CD and CT (2.70 & 1.89, 2.76 & 1.92, 2.91 & 2.30 and 3.15 & 2.59) in the samples SFO-2, SFO-1, SFO-4 and SFO-3

Table 2
Relative increases in PV (meq / Kg) of sunflower oil stabilized with rice bran extracts at ambient storage

IPD	SFO-0	SFO-1	SFO-2	SFO-3	SFO-4
0	0.50 ± 0.03	0.50 ± 0.04	0.50 ± 0.02	0.50 ± 0.03	0.50 ± 0.03
15	1.00 ± 0.09	0.79 ± 0.06	0.73 ± 0.05	0.91 ± 0.06	0.85 ± 0.08
30	2.09 ± 0.12	1.39 ± 0.10	1.28 ± 0.09	1.67 ± 0.10	1.56 ± 0.11
45	3.27 ± 0.10	1.92 ± 0.09	1.79 ± 0.09	2.36 ± 0.12	2.27 ± 0.12
60	4.36 ± 0.13	2.43 ± 0.12	2.32 ± 0.11	2.97 ± 0.11	2.93 ± 0.11
75	5.46 ± 0.20	3.20 ± 0.11	2.90 ± 0.10	4.50 ± 0.13	4.36 ± 0.12

Values (mean ± SD) are average of duplicate samples analyzed individually in triplicate ($n = 2 \times 3$), ($P < 0.05$).

IPD. Incubation Period (days).
SFO-0. Controlled sunflower oil sample (with out rice bran extract).
SFO-1. Sunflower oil sample stabilized with 100% methanol extract of rice bran.
SFO-2. Sunflower oil sample stabilized with 80% methanol extract of rice bran.
SFO-3. Sunflower oil sample stabilized with 100% acetone extract of rice bran.
SFO-4. Sunflower oil sample stabilized with 80% acetone extract of rice bran.

Table 3
Relative increase in conjugated dienes ($\varepsilon_{1cm}(\lambda_{232\ nm})$) of sunflower oil stabilized with rice bran extracts at ambient storage

IPD	SFO-0	SFO-1	SFO-2	SFO-3	SFO-4
0	1.40 ± 0.05	1.40 ± 0.04	1.40 ± 0.04	1.40 ± 0.01	1.40 ± 0.10
15	1.80 ± 0.12	1.41 ± 0.10	1.37 ± 0.09	1.55 ± 0.10	1.52 ± 0.11
30	2.41 ± 0.13	1.74 ± 0.11	1.70 ± 0.10	1.94 ± 0.11	1.85 ± 0.10
45	3.02 ± 0.20	2.09 ± 0.15	2.03 ± 0.12	2.34 ± 0.14	2.20 ± 0.15
60	3.65 ± 0.17	2.40 ± 0.17	2.36 ± 0.15	2.77 ± 0.13	2.55 ± 0.12
75	4.28 ± 0.12	2.76 ± 0.13	2.70 ± 0.13	3.15 ± 0.12	2.91 ± 0.21

Values (mean ± SD) are average of duplicate samples analyzed individually in triplicate ($n = 2 \times 3$), ($P < 0.05$).
For abbreviations see Table 2.

treatments, respectively at the end of analytical period. CD and CT are often measured as indicators of free radical production. The oxidation of unsaturated fatty acids results in the formation of CD and CT. The CD and CT provide a marker of the early stages of lipid peroxidation (Halliwell and Gutteridge, 1985). The measurement of CD and CT is a useful index of the early stages of peroxidation in studies of pure lipids and isolated lipoprotein (Dekkers *et al.,* 1996).

Table 4 shows the relative increase in the *p*-anisidine values of sunflower oil treatments and the control. It could be noted that the control had significantly (P< 0.05) higher values of *p*-anisidine as compared with those of stabilized sunflower oils. The 80% methanolic rice bran extract was found to be the most effective among other extracts, as indicated by the lowest rise in *p*-anisidine values of SFO-2 treatment. The highest *p*-anisidine value of SFO-3 treatment demonstrated the least antioxidant potential of 100% acetone extract of rice bran. Saddiq *et al.*,(2005) also reported the highest antioxidant activity of 80% methanolic extracts of *M.oleifera* leave in terms of the measurement of PV, CD,CT and *P*-anisidine values under accelerated storage of stabilized sunflower oil samples.

By coupling the results of different antioxidant assays with the oxidation parameters of stabilized sunflower oil in the present study, it is understandable that both the methanolic and acetone extracts of rice bran have exhibited good antioxidant activity. However, the antioxidant activity of the 80% methanolic extract was found to be significantly higher than acetone extracts, which might be attributed to the combined effect and high polarity of the methanol-water mixture. The results of the present study reveal that rice bran is a potent source of natural antioxidants, containing a unique mixture of phenolic compounds, suggesting its use in neutraceutical and functional food applications.

REFERENCES

Amarowicz R, Karamac M and Chavan U. 2001. Influence of the extraction procedure on the antioxidative activity of lentil seed extracts in a beta-carotene-linoleate model system. *Grasas Aceites*. **52** (2): 89-93.

Anwar F, Bhanger M I, Yasmeen S. 2003. Antioxidant activity of some natural extracts in corn oil. *Advanced Research of Plant Lipids*, Proceeding of 15-ISPL, May 12-17, 2002, Okazaki, Japan. Eds. N. Muarata, M.Yamada, I.Nishida et al., Kluwer Publishers Netherlands, pp. 24-27.

Anwar F, Anwer T and Mahmood Z. 2005. Methodical characterization of rice (*Oryza sativa*) bran oil from Pakistan. *Grasas Aceites*. **56** (2): 126-135.

Awika JM, Rooney LW, Wu X, Prior RL and Zevallos LC. 2003. Screening methods to measure antioxidant activity of Surghum (*Surghum bicolor*) and Surghum products. *Journal of Agriculture and Food Chemistry*. **51**, 6657-6662.

Table 4
Relative increase in conjugated triene (ε_{1cm} ($\lambda_{268\ nm}$)) of sunflower oil stabilized with rice bran extracts at ambient storage

IPD	SFO-0	SFO-1	SFO-2	SFO-3	SFO-4
0	0.10 ± 0.02	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01
15	0.85 ± 0.06	0.71 ± 0.02	0.70 ± 0.02	0.79 ± 0.04	0.76 ± 0.04
30	1.36 ± 0.05	1.01 ± 0.08	1.00 ± 0.02	1.24 ± 0.10	1.15 ± 0.04
45	1.88 ± 0.11	1.31 ± 0.10	1.29 ± 0.07	1.69 ± 0.08	1.54 ± 0.11
60	2.39 ± 0.14	1.61 ± 0.09	1.59 ± 0.10	2.14 ± 0.12	1.92 ± 0.09
75	2.90 ± 0.11	1.92 ± 0.10	1.89 ± 0.09	2.59 ± 0.11	2.30 ± 0.10

Values (mean ± SD) are average of duplicate samples analyzed individually in triplicate (n = 2 x 3), (P < 0.05).
For abbreviations see Table 2.

Table 5
Relative increases in *p* - anisidine value (ε_{1cm} ($\lambda_{350\ nm}$)) of sunflower oil stabilized with rice bran extracts at ambient storage.

IPD	SFO-0	SFO-1	SFO-2	SFO-3	SFO-4
0	0.70 ± 0.04	0.7 ± 0.05	0.7 ± 0.03	0.7 ± 0.04	0.7 ± 0.03
15	2.66 ± 0.15	1.56 ± 0.12	1.40 ± 0.11	2.30 ± 0.10	1.90 ± 0.13
30	3.90 ± 0.17	2.46 ± 0.10	2.26 ± 0.16	3.43 ± 0.13	3.06 ± 0.11
45	5.03 ± 0.34	3.40 ± 0.20	3.20 ± 0.21	4.50 ± 0.12	4.03 ± 0.23
60	7.80 ± 0.30	4.33 ± 0.19	4.13 ± 0.20	6.73 ± 0.18	5.13 ± 0.20
75	10.10 ± 0.26	5.76 ± 0.28	5.30 ± 0.32	8.16 ± 0.32	7.36 ± 0.30

Values (mean ± SD) are average of duplicate samples analyzed individually in triplicate (n = 2 x 3), (P < 0.05).
For abbreviations see Table 2.

Dekkers JC, Van Doornen LJP and Kemper HCG.1996. The role of antioxidant vitamins and enzymes in the prevention of exercise-induced muscles damage. *Sports Medicine*, **21**, 213-238.

Halliwell B and Gutteridge JMC.1985. The chemistry of oxygen radicals and other oxygen derived species. In B. Halliwell and J. M. C. Gutteridge (Eds.), *Free radicals in biology and medicine* (pp. 20-64). New York: Oxford University Press.

Hou, D. X. 2003. Potential mechanism for cancer chemoprevention by anthocyanine. *Current Advancements in Molecular Medicines*.**3:** 149-159.

Iqbal S., Bhanger MI and Anwar F. 2005. Antioxidant properties and components of some commercially available varieties of rice bran in Pakistan. *Food Chemistry*, **93**: 265- 272.

IUPAC, standard method for the analysis of Oils, Fats and Derivatives, Sixth Edition, Pergamon Press, Oxford, New York, Sydney, 1987. p. 20 – 118.

Jeong SM, Kim SY, Kim DR, Jo SC, Man KC, Ahn DU and Lee SC. 2004. Effect of heat treatment on the antioxidant activity of extracts from citrus peals. *J. Agric. Food Chem*., **52**: 3389-3393.

Laandrault N, Pouchert P, Ravel P, Gase F, Cros G and Teissedro PL.2001. Antioxidant activities and phenolics level of French wines from different varieties and vintages. *J. Agric. Food Chem*. **49**, 3341-3343.

Lu Y and Foo LY.2000. Antioxidant and radical scavenging activities of polyphenols from apple pomace. *Food Chem*. **68**, 81-85.

Malekain F, Rao RM, Prinyawiwatkul W, Marshall WE, Windhauser M and Ahmedna M.2000. Lipase and lipoxygenase activity, functionality and nutrient losses in rice bran during storage (pp. 1-68), Bulletin No. 870. Baton Rouge: LSU Agriculture center, Louisiana Agricultural experiment Station.

Mc Ginely L. 1991. Analysis and quality control for processing and processed fats. In: Analysis of Oilseeds, Fat and Fatty Food, Eds. Rossell JB; Printed, JLR, Elsevier Applied Science, London, New York, 1991, pp. 460-470.

Oryeneho SN and Hettiarachchy NS. 1992. Antioxidant activity of durum wheat bran. *J. Agri. Food Chem*.**49**: 1496-1500.

Osawa T and Namiki M.1981. A novel type of antioxidant isolated from leaf wax of eucalyptus leaves. *Agriculture and Biological Chemistry*, **45**, 735-739.

Perumal S. and Becker K. 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three agroclimatic origins of Drumstick tree leaves. *J. Agri. Food Chem.* **51**: 2144-2155.

Prior RL.2004. Absorption and metabolism of anthocyanine: potential health effects. In M. Meskin, W.R. Bidlack, A. J. Devies, D. S. Lewis and R. K. Randolph (Eds.), *Phytochemicals: mechanism of action* (pp 1-9). Boca Raton, FL: CRC Press.

Rababah T. M., Hettiarachchy N. S. and Horex R. 2004. Total phenolics and antioxidant activities of fenugreek, green tea, black tea, grape seed, ginger, rosemary, gotu kola and ginkgo extracts, vitamin E and *tert*. Butylhydroquinone. *J. Agri. Food Chem.* **52**: 5183-5086.

Sadddiq A, Anwar F, Manzoor M, Fatima A. 2005. Antioxidant activity of different solvent extracts of *Moringa oleifera* leaves under accelerated storage of sunflower oil. *Asian J. of Plant Sci.* 4(6): 630-635.

Shahidi F. 1997[a]. Natural antioxidants: An over view. In: F. Shahidi (Ed.), Natural antioxidant, Chemistry, Health Effects and Applications, (pp. 1-10). Champaign, IL, USA: AOCS press.

Shahidi F. 1997[b]. Natural antioxidants: An over view. In: F. Shahidi (Ed.), Natural antioxidant, Chemistry, Health Effects and Applications, (pp. 44-46). Champaign, IL, USA: AOCS press.

Steel R. G. D. and Torrie, J. H. (1980). Principles and procedures of statistics. London: McGraw Hill.

Velioglu Y. S., Mazza G., Gao L. and Oomah B. D. 1998. Antioxidant activity and total phenolics in selected fruits, vefetables ans grain products. *J. Agri. Food Chem.* **46**: 4113-4117.

Vichi S, Zitterl-Eseer, Jugl K and Franz MC. 2001. Determination of the presence of antioxidants derived from sage and organic extracts added to the animal fat by means of assessment of the radical scavenging capacity by photochemilumenescence analysis. *Nahrung*. **45** (2): 101-104.

Wu X., Gu L., Holden J., Haytowitz D. B., Gebhardt S E., Beecher G. and Prior R. L. 2004. Development of a database for total antioxidant capacity in foods: a preliminary study. *J. Food Composition and Analysis*. **17:** 407- 422.

Yen GC, Duh PD and Tsai CL. 1993. Relationship between antioxidant activity and maturity of peanut hull. *Journal of Agriculture and Food Chemistry*. **41**, 67-70.

Zhou, K. and Yu, L. 2004. Antioxidant activities of bran extracts from "Trego" wheat grown at different locations. *Journal of Agriculture and Food Chemistry*. **52**, 1112-1117.

Recibido: Julio 2005
Aceptado: Abril 2006